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March 31, 2020

Division of Animal Feeds (HFV-220)
Office of Surveillance and Compliance
Center for Veterinary Medicine
Food and Drug Administration
7519 Standish Place
Rockville, MD 20855

Re: Notice of GRAS exemption for use of phytase 50104, an enzyme preparation expressed in the bacteria *Pseudomonas fluorescens* (strain BD50104), to increase the availability of phytin-bound phosphorus in poultry diets

Dear Mr. Wong:

Pursuant to 21 CFR Part 570, Subpart E, this submission is intended to notify FDA of BASF Enzymes LLC's determination – based on scientific procedures – that the above-referenced phytase 50104 enzyme preparation is generally recognized as safe when used to increase the availability of phytin-bound phosphorus in poultry diets at a recommended level of 250 to 2000 U/kg of feed. Accordingly, and as with all GRAS substances, such phytase 50104 enzyme preparation – when used as described above and in the attached information – is exempt from the premarket approval requirements applicable to food additives set forth in Section 409 of the Food, Drug, and Cosmetic Act and that section's implementing regulations.

The phytase 50104 enzyme preparation that is the subject of the notice will be marketed in two forms under the names CIBENZA®[1] PHYTAVERSE® L10 Phytase Enzyme and CIBENZA® PHYTAVERSE® G10 Phytase Enzyme. CIBENZA® PHYTAVERSE® L10 Phytase Enzyme is a liquid formulated product, and CIBENZA® PHYTAVERSE® G10 Phytase Enzyme is a granulated product. BASF Enzymes LLC and Novus International, Inc. collaborated to co-develop the phytase 50104 enzyme preparation.

- **BASF Enzymes LLC** is responsible for the manufacture of PHYTAVERSE® L44 Liquid Concentrate, the formulated liquid concentrate that is used to manufacture the products of commerce (i.e., CIBENZA® PHYTAVERSE® L10 Phytase Enzyme and CIBENZA® PHYTAVERSE® G10 Phytase Enzyme), and the product of commerce, CIBENZA® PHYTAVERSE® L10 Phytase Enzyme.
- **Novus International, Inc.** is responsible for the manufacture of the granulated product of commerce (i.e., CIBENZA® PHYTAVERSE® G10 Phytase Enzyme) and for the marketing of both products of commerce (i.e., CIBENZA® PHYTAVERSE® L10 Phytase Enzyme and CIBENZA® PHYTAVERSE® G10 Phytase Enzyme).

[1] ®CIBENZA and PHYTAVERSE are trademarks of Novus International, Inc. and are registered in the United States and/or other countries.

BASF Enzymes LLC
3550 John Hopkins Court
San Diego, CA 92121
www.basf.us

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In support of said notice, please find attached a discussion of the information relied upon by BASF Enzymes LLC when determining that phytase 50104 enzyme preparation is GRAS. Such discussion is set forth in the format required by proposed 21 CFR § 570 Subpart E. (81 FR 54960 – 8/17/2016)

After you and your colleagues have had an opportunity to review and consider the attached information, if you should have questions or need additional information, please let me, contact information is found below, know. All of the information that serves as the basis for this GRAS determination can be sent – upon request – to FDA and is available for review and copying at reasonable times.

Roxanna Van Dorn
Senior Regulatory Affairs Specialist
BASF Enzymes LLC
3550 John Hopkins Court
San Diego, CA 92121
Office Telephone: 858-481-8590
Mobile Telephone: 858-349-7339
Email: roxanna.vandorn@basf.com

Thank you in advance for your and your colleagues' efforts on behalf of this notice.

Sincerely,

Roxanna Van Dorn

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Use of Phytase 50104 Enzyme Preparation to Increase the Availability of Phytin-Bound Phosphorus in Poultry Diets

Filed by

BASF Enzymes LLC
3550 John Hopkins Court
San Diego, CA 92121

March 31, 2020

BASF Enzymes LLC
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PART 1: SIGNED STATEMENTS AND CERTIFICATION

A. Claim Regarding GRAS Status

This GRAS Notice is submitted in accordance with 21 CFR Part 570, Subpart E – Generally Recognized as Safe (GRAS) Notice. BASF Enzymes LLC hereby notifies the FDA of the determination by BASF Enzymes LLC and an external expert that the phytase 50104 enzyme preparation (marketed as CIBENZA®[1] PHYTAVERSE® L10 Phytase Enzyme and CIBENZA® PHYTAVERSE® G10 Phytase Enzyme), produced from *P. fluorescens* strain BD50104, which expresses a gene encoding the phytase 50104 enzyme, is generally recognized as safe (GRAS), based on scientific procedures, when used as intended in animal food.

B. Name and Address of Notifier

Roxanna Van Dorn
Senior Regulatory Affairs Specialist
BASF Enzymes LLC
3550 John Hopkins Court
San Diego, CA 92121
roxanna.vandorn@basf.com

C. Name of Notified Substance

The notified substance being addressed in this submission is the phytase 50104 enzyme preparation, which is marketed as CIBENZA® PHYTAVERSE® L10 Phytase Enzyme and CIBENZA® PHYTAVERSE® G10 Phytase Enzyme.

The table below is provided to help clarify the different name that are used in this notice.

[1] ®CIBENZA and PHYTAVERSE are trademarks of Novus International, Inc. and are registered in the United States and/or other countries.

Table 1. Naming Convention Used in the Notice

Name	Definition
Phytase 50104 enzyme preparation	The final enzyme preparation. It is either a liquid or a granular formulation and is marketed as CIBENZA® PHYTAVERSE® L10 Phytase Enzyme and CIBENZA® PHYTAVERSE® G10 Phytase Enzyme, respectively.
CIBENZA® PHYTAVERSE® L10 Phytase Enzyme	The liquid formulation of the phytase 50104 enzyme preparation and has a guaranteed minimum phytase activity of 10,000 U/g.
CIBENZA® PHYTAVERSE® G10 Phytase Enzyme	The granular formulation of the phytase 50104 enzyme preparation and has a guaranteed minimum phytase activity of 10,000 U/g.
Phytase 50104 enzyme	The specific phytase enzyme/protein that expressed by *P. fluorescens* strain BD50104.
Phytase 50104 protein	The specific phytase enzyme/protein that expressed by *P. fluorescens* strain BD50104.
Phytase 50104 gene	The specific phytase gene that encodes the phytase 50104 protein.
VR003	The lyophilized test article used to determine the safety of phytase 50104 enzyme in toxicology and genotoxicology studies. It was prepared following a process representative of the manufacturing process (including raw materials) for the commercial enzyme, up to but not including, the final formulation step and was lyophilized.
PHYTAVERSE® L44 Liquid Formulation	The formulated, phytase 50104 enzyme concentrate. It is used to make the liquid and granular formulations of the phytase 50104 enzyme preparation (i.e., CIBENZA® PHYTAVERSE® L10 Phytase Enzyme and CIBENZA® PHYTAVERSE® G10 Phytase Enzyme, respectively).

D. Intended Conditions of Use

CIBENZA® PHYTAVERSE® L10 Phytase Enzyme will be added in a post-pelleting application to complete pelleted feeds. CIBENZA® PHYTAVERSE® G10 Phytase Enzyme will be added to complete mash feeds, complete pelleted feeds, and premixes.

Proposed levels of use: The recommended level of supplementation in a complete feed is 250 to 2000 U/kg of feed.

Animal species intended: CIBENZA® PHYTAVERSE® L10 Phytase Enzyme and CIBENZA® PHYTAVERSE® G10 Phytase Enzyme is intended for use in poultry.

Purpose for which the substance is used in feed: CIBENZA® PHYTAVERSE® L10 Phytase Enzyme and CIBENZA® PHYTAVERSE® G10 Phytase Enzyme will be used to increase the availability of phytin-bound phosphorus in poultry diets.

E. Basis for Conclusion of GRAS Status

The statutory basis for the conclusion of GRAS status for the phytase 50104 enzyme preparation, which is marketed as CIBENZA® PHYTAVERSE® L10 Phytase Enzyme and CIBENZA® PHYTAVERSE® G10 Phytase Enzyme, is based upon scientific procedures, as described in this submission.

F. Premarket Approval Exemption

It is the notifier's view that the notified substance is not subject to the premarket approval requirements of Federal Food, Drug, and Cosmetic Act based on the notifier's conclusion that the phytase 50104 enzyme preparation, which is marketed as CIBENZA® PHYTAVERSE® L10 Phytase Enzyme and CIBENZA® PHYTAVERSE® G10 Phytase Enzyme, is GRAS under the conditions of intended use.

G. Statement of Availability of Data and Information

The data and information that are the basis for BASF Enzymes, LLC conclusion of GRAS status are available for FDA's review. Upon FDA's request, FDA may review and copy the data and information during customary business hours at the address provided below and the notifier will provide FDA with a complete copy of the data and information in either electronic format or

by paper copy. Requests for copies and arrangements for review of materials cited may be directed to:

Roxanna Van Dorn
Senior Regulatory Affairs Specialist
BASF Enzymes LLC
3350 John Hopkins Court
San Diego, CA 92121

H. Statement of Exemption from FOIA Disclosure

The following information is exempt from FOIA Disclosure:

Information	**Reason for Exemption from FOIA Disclosure**
Appendix 1: Phytase 50104 Enzyme Amino Acid Sequence	The native appA protein from *E. coli* K-12 was protein engineered to create the phytase 50104 enzyme. The specific amino acid changes are considered confidential business information; therefore, the amino acid sequence of the phytase 50104 protein is also considered confidential business information.
Appendix 2: Alignment of the Mature Amino Acid Sequences for Phytase 50104 Protein and the Native *E. coli* K-12 and B appA Proteins	The native appA protein from *E. coli* K-12 was protein engineered to create the phytase 50104 enzyme. The specific amino acid changes are considered confidential business information; therefore, the amino acid sequence alignment of the three phytases is also considered confidential business information.
Appendix 3: Phytase 50104 Gene Nucleotide Sequence	The native appA protein from *E. coli* K-12 was protein engineered to create the phytase 50104 enzyme. The specific nucleotide changes are considered confidential business information.

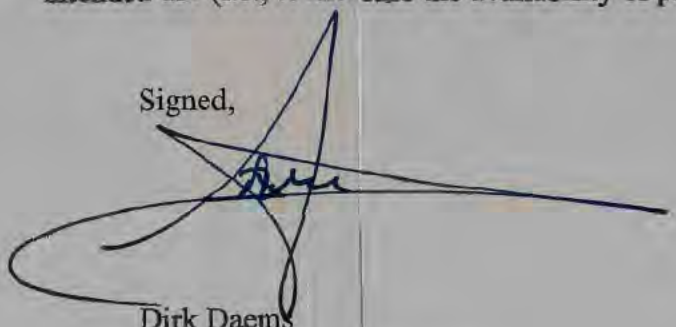
Appendix 4: Alignment of the Mature Amino Acid Sequences for Phytase 50104 Protein and the Native *E. coli* K-12 appA Protein	The native appA protein from *E. coli* K-12 was protein engineered to create the phytase 50104 enzyme. The specific amino acid changes are considered confidential business information; therefore, the amino acid sequence alignment of the two phytases is also considered confidential business information.
Appendix 5: Bioinformatics Analysis of Plasmid pDOW1169_BD50104	This appendix contains confidential information related to our production strain, specifically our expression plasmid.
Appendix 6: Stability of the (b) (4) Gene and the Expression Plasmid (b) (4)_BD50104 in *Pseudomonas fluorescens* BD50104 and Determination of the Phytase 50104 Gene Copy Number in Strain BD50104	This appendix contains confidential information specific to our production organism.
Appendix 7: Plasmid Mobilization Analysis for *Pseudomonas fluorescens* Strain BD50104	This appendix contains confidential information specific to our production organism.
Appendix 8: Characterization of the DNA (b) (4) Expression Cassette) Inserted into the Host Chromosome	This appendix contains confidential information specific to our production organism.
Appendix 10: List of Raw Materials used in the Manufacturing of Phytase 50104 Enzyme Preparation	This appendix contains all raw materials used in the manufacturing of the phytase 50104 enzyme preparation. The raw materials used in fermentation and in recovery are considered confidential business information. The raw materials used to formulate the products of commerce are not confidential and have been disclosed in Part 2 Section C.1.

Appendix 11: Detailed Manufacturing Information: Fermentation, Recovery, and Formulation	This appendix contains detailed information on the manufacturing process of the phytase 50104 enzyme preparations. This information is considered confidential business information.
Appendix 12: Final Product Composition	Although, the raw materials used to formulate the products of commerce are not confidential, the amounts in which they are added are considered confidential business information.

I. Certification

On behalf of BASF Enzymes LLC, I certify to the best of my knowledge, the GRAS Notice is a complete, representative, and balanced submission that includes unfavorable information, known to me, and BASF Enzymes LLC, and pertinent to the evaluation of safety and GRAS status of the phytase 50104 enzyme preparation, which is marketed as CIBENZA® PHYTAVERSE® L10 Phytase Enzyme and CIBENZA® PHYTAVERSE® G10 Phytase Enzyme, under the conditions of intended use (i.e., to increase the availability of phytin-bound phosphorous in poultry diets).

Signed,



Dirk Daems

Director Business Operations

BASF Enzymes LLC

Date: MARCH 31, 2020

PART 2: IDENTITY, METHOD OF MANUFACTURE, SPECIFICATIONS, AND PHYSICAL OR TECHNICAL EFFECT

A. Scientific Data that Identifies the Notified Substance

1. Enzyme identity

a) Identity

The phytase 50104 enzyme is a 6-phytase as defined by the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (IUBMB).

Common Name:	Phytase
Name:	6-phytase
Systematic Name:	Myo-inositol-hexakisphosphate 4-phosphohydrolase
Other names:	4-phytase; phytase; phytate 6-phosphatase; myo-inositol-hexakisphosphate 6-phosphohydrolase (name based on 1L-numbering system and not 1D-numbering)
IUBMB Number:	3.1.3.26
CAS Registry No.:	9001-89-2
Reaction:	*myo*-inositol hexakisphosphate + H_2O = 1D-*myo*-inositol 1,2,3,5,6-pentakisphosphate + phosphate

b) Amino acid sequence

Phytases from the microorganism *E. coli*, including *E. coli* strain K-12 and *E. coli* B, encode for phytase via the (b) (4) gene. The phytase protein, designated as phytase 50104, is encoded by the modified (b) (4) gene derived from *E. coli* strain K-12 and is 411 amino acids in length. The identity of the protein as expressed in the production organism, *P. fluorescens* strain BD50104, has been independently confirmed by amino acid sequence analysis and by amino acid composition analysis. The amino acid sequence for the phytase 50104 protein is provided in Appendix 1.

Since the phytase 50104 enzyme in the CIBENZA® PHYTAVERSE® L10 and G10 Phytase Enzyme products is an *E. coli* based appA phytase, its amino acid sequence is similar to the amino acid sequences of the five *E. coli* based appA phytases listed in the Association of American Feed Control Officials (AAFCO) Official Publication (OP) Table 30.1 (Association of American Feed Control Officals (AAFCO), 2020b) and Section 101 (Association of American

Feed Control Officals (AAFCO), 2020c) and on FDA Center for Veterinary Medicine's (CVM's) Current Animal Food GRAS Notices Inventory (FDA Center for Veterinary Medicine, 2019) (see Appendix 2).

c) Enzyme substrate

The phytase 50104 enzyme in the CIBENZA® PHYTAVERSE® L10 and G10 Phytase Enzyme products is specific for several salt forms of phytic acid, known as phytate or phytin. Like all phytases (including the 12 listed in the 2020 AAFCO OP and on FDA CVM's Current Animal Food GRAS Notices Inventory), it catalyzes the stepwise hydrolysis of phosphate monoesters from the inositol ring of phytate (Association of American Feed Control Officals (AAFCO), 2020b; Association of American Feed Control Officals (AAFCO), 2020c; FDA Center for Veterinary Medicine, 2019; Lei, X.G. and Stahl, C.H., 2001; Wodzinski, R.J. and Ullah, A.H., 1996). The phytase 50104 enzyme is an *E. coli* based appA phytase, and *E. coli* appA phytases exhibit specific activities that are among the highest of all reported phytases (Lim, D. *et al.*, 2000). In addition, it possesses dramatically lower activity on other phosphate-containing substrates such as AMP, ADP, ATP, fructose 1,6-bisphosphate, and glucose 6-phosphate compared to its action on phytate (Greiner, R. *et al.*, 1993; Wyss, M. *et al.*, 1999).

d) Characteristic properties

Catalytic Activity

The phytase 50104 enzyme in CIBENZA® PHYTAVERSE® L10 and G10 Phytase Enzyme products, like other *E. coli* based appA phytases, is a 6-phytase and, therefore, catalyzes initial phosphate ester bond hydrolysis of phytate at position 6 on the inositol ring (Greiner, R. *et al.*, 1993). This initial reaction is extremely rapid and likely represents the major hydrolysis event monitored during initial rate measurements for the phytase 50104 enzyme. In addition, *E. coli* appA phytase catalyzes the removal of additional phosphates from the inositol ring as follows:

$$\text{D-Ins}(1,2,3,4,5)P_5 + H_2O \rightarrow \text{D-Ins}(2,3,4,5)P_4 + P_i$$

$$\text{D-Ins}(2,3,4,5)P_4 + H_2O \rightarrow \text{D-Ins}(2,4,5)P_3 + P_i$$

$$\text{D-Ins}(2,4,5)P_3 + H_2O \rightarrow \text{D-Ins}(2,5)P_2 + P_i$$

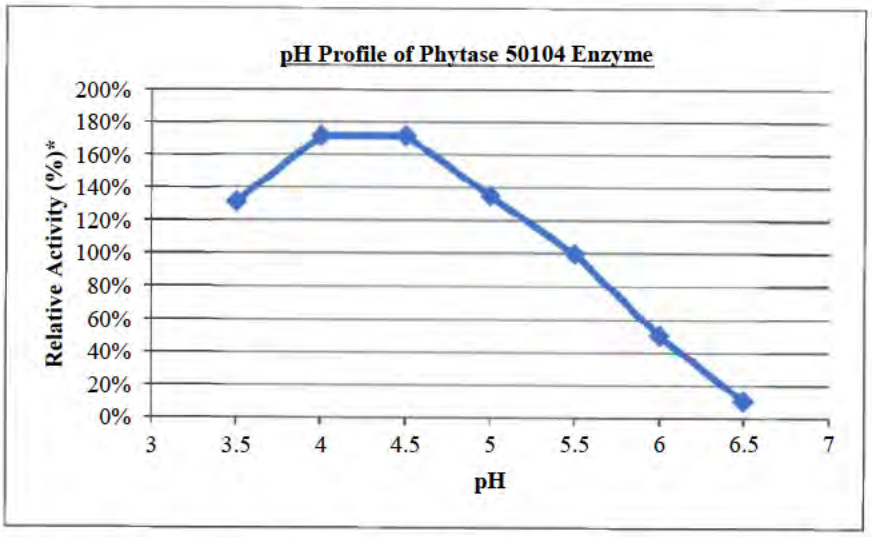
$$\text{D-Ins}(2,5)P_2 + H_2O \rightarrow \text{D-Ins}(2)P + P_i$$

The final reaction, conversion of D-Ins(2,5)P_2 to D-Ins(2)P occurs very slowly (Greiner, R. *et al.*, 1993; Wyss, M. *et al.*, 1999).

pH Performance

The pH performance of the phytase 50104 enzyme was determined, and the pH optimum range was between pH 4 to 4.5 (see Figure 1 below). This is also very similar to the properties reported in the literature for *E. coli* K-12 appA phytase (Golovan, S. *et al.*, 2000; Greiner, R. *et al.*, 1993). In addition, as previously reported for *E. coli* phytase, it does not require calcium or other cofactors for catalytic activity (Greiner, R. *et al.*, 1993).

Figure 1. pH Profile of the Phytase 50104 Enzyme



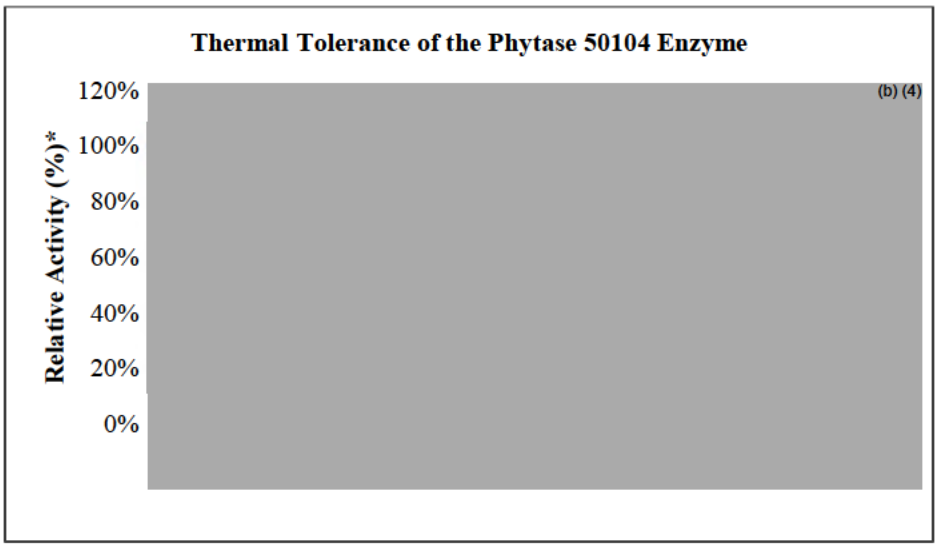
*Relative % activity to the standard conditions of the assay, which is a pH of 5.5.

Thermal Tolerance

[redacted] (b) (4)

. Please see Figure 2 below.

Figure 2. Thermal Tolerance of the Phytase 50104 Enzyme



*Relative % activity to the T=0 (i.e., no incubation at 80°C).

Side Activities

The phytase 50104 enzyme preparation was analyzed for a variety of side activities (i.e., cellulase, xylanase, alpha-amylase, protease, and phosphatase). Enzyme activity was not detected or was below limit of quantitation of the assays when testing for cellulase, xylanase, alpha-amylase, and protease activities. The only significant activity – consistent with a similar published observation made for *E. coli* K-12 app A phytase – is phosphatase (Greiner, R. *et al.*, 1993). The phosphatase activity is expected as phytase belongs to the phosphatase enzyme family, and it is not expected to have any negative effects in poultry diets.

2. Source organism

a) Taxonomic source

The phytase 50104 enzyme in CIBENZA® PHYTAVERSE® L10 and G10 Phytase Enzyme products is produced from *Pseudomonas fluorescens* strain BD50104. The taxonomic designation for strain BD50104 is as follows:

Domain	Bacteria
Phylum BXII	Proteobacteria
Class III	Gammaproteobacteria

Order VII	Pseudomonadales
Family I	Pseudomonadaceae
Genus I	Pseudomonas
Species	fluorescens
Biovar	I

For further information on the construction of the production organism, *P. fluorescens* BD50104 please see Part 2 Section B.1.

b) Part of plant or animal used as source

The source organism is neither a plant nor an animal, but a microorganism. Therefore, there is no part of a plant or an animal to identify.

c) Any known toxicants

Strains of *P. fluorescens* are commonly found on plant surfaces, as well as decaying vegetation, soil, and water (Balows, A., 1992). The ubiquitous nature of *P. fluorescens* on the surface of plants typically grown for human consumption (OECD, 1997) suggests that *P. fluorescens* has been widely consumed by humans for many years. *P. fluorescens* is not reported to be a causative agent of human food poisoning or other disease related to food ingestion (EFSA and ECDC, 2017; FDA, 2018). Derivatives of *P. fluorescens* MB101, i.e., the parental strain of *P. fluorescens* BD50104, have been used safely as production organisms for enzymes used in food production for many years (AFSSA, 2006; FDA Center for Food Safety and Applied Nutrition, 2003a; FDA Center for Food Safety and Applied Nutrition, 2013; FDA Center for Food Safety and Applied Nutrition, 2015).

The Organisation for Economic Co-operation and Development (OECD) and the European Food Safety Agency (EFSA) have conducted literature reviews regarding the safety of *P. fluorescens* (EFSA BIOHAZ Panel *et al.*, 2017; OECD, 1997). Internal literature reviews were also conducted to evaluate the safety of *P. fluorescens*. All literature reviews, OECD and EFSA and internal, found that *P. fluorescens* can be an opportunistic pathogen in immunocompromised individuals.

In addition, published studies have evaluated the pathogenicity and toxigenicity of *P. fluorescens* in mice; no evidence of pathogenicity or toxigenicity was observed under the

conditions of the test (George, S.E. *et al.*, 2000; George, S.E. *et al.*, 1999). Moreover, the pathogenicity and toxigenic potential of orally administered *P. fluorescens* biovar I, strain MB101 was evaluated in Balb/c mice (Landry, T.D. *et al.*, 2003). (Please note that strain MB101 is the parental strain of *P. fluorescens* BD50104.) Under the conditions of the study, there was no evidence of pathogenicity or toxigenicity from *P. fluorescens* strain MB101.

Moreover, published (Pieniazek, J. *et al.*, 2017) and corroborative utility studies conducted with the granular formulation of the phytase 50104 enzyme preparation (i.e., CIBENZA® PHYTAVERSE® G10 Phytase Enzyme) demonstrated that the product is safe for use in poultry. Please see Part 2 Section D for more information on these studies.

Lastly, toxicology and genotoxicity tests conducted using many different enzyme preparations produced by *P. fluorescens* MB101 derivatives have determined that the test materials do not contain toxic or genotoxic substances (FDA Center for Food Safety and Applied Nutrition, 2015; Halich, R. *et al.*, 2012; Landry, T.D. *et al.*, 2003). Toxicology and genotoxicity studies were conducted using test material of the phytase 50104 enzyme produced by *P. fluorescens* BD50104 (i.e., lyophilized phytase 50104 enzyme preparation without formulation ingredients, also known as VR003). These published safety studies also demonstrate that the test material does not contain any toxic or genotoxic substance (Krygier, S. *et al.*, 2014; Krygier, S. *et al.*, 2015).

It can be concluded then that *P. fluorescens*, including those strains derived from MB101, are non-toxigenic and non-pathogenic. Please see Part 6 Section C.2 for more information on the absence of pathogenicity and toxicity.

B. Method of Manufacture

1. Production organism

This section describes the historical activities associated with the construction of *P. fluorescens* BD50104, the origin of the phytase 50104 gene, the construction of the expression plasmid, and the methodology used to introduce the latter into the recipient strain *P. fluorescens* DC454. For a discussion on the safety of the production organism, please see Part 6 Section C.

a) Recipient microorganism

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

Figure 3. Strain lineage

b) Origin of phytase 50104 gene

The phytase 50104 gene was derived from the native *E. coli* K-12 (b) gene encoding the phytase enzyme. The native (b) gene was previously cloned and sequenced (b) (4) To produce the phytase 50104 gene, the native (b) (4) gene from *E. coli* K-12 strain MG1655 (b) (4) was modified for (b) (4) encountered during the production of manufactured feed. The nucleotide sequence for the phytase 50104 gene is provided in Appendix 3.

c) Construction of the expression vector

(b) (4)

(b) (4)

However, (b) (4)

(b) (4)

(Short, J.M., 2001) (b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

Figure 5. Plasmid map of (b) (4)_BD50104

Table 2. Components of (b) (4)_BD50104 expression vector

Name	Start	End	Size (bp)	Source
Plasmid DNA				(b) (4)
(b) (4) terminator				
(b) (4) gene				
(b) (4) promoter				
tac				
RBS				
Phytase 50104 gene				
Nonfunctional DNA				
Terminator region				
Plasmid DNA				
Plasmid DNA				
Plasmid DNA				
repC				
repA				
Plasmid DNA				
(b) (4) (partial)				
repB				

Name	Start	End	Size (bp)	Source
(b) (4)				(b) (4)
(b) (4) (partial)				
Plasmid DNA				
*ori*Tnic				
(b) (4) *C*				
*ori*V				
Plasmid DNA				
Plasmid DNA				

d) Construction of the production organism

(b) (4)

(b) (4)

.

e) Genetic stability and gene copy number

(b) (6)

(b) (6)

(b) (6)

f) Absence of transformable DNA

Among the criteria suggested by the Organization for Economic Co-operation and Development (OECD) is that vectors or plasmids used in modifying a microorganism used for industrial applications should be poorly mobilizable (OECD, 1992). This criterion has been widely adopted and has also been recommended elsewhere (EU Scientific Committee for Food, 1992; NIH, 2019).

(b) (4)

g) Absence of antibiotic resistance

(b) (4)

(see Appendix 5 for further information).

(b) (6)

(please see Appendix 8 for further information).

h) Absence of production organism

2. Manufacturing process

(b) (4)

a) Raw materials

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (6)

b) Master and working cell banks

(b) (4)

c) Fermentation

(b) (4)

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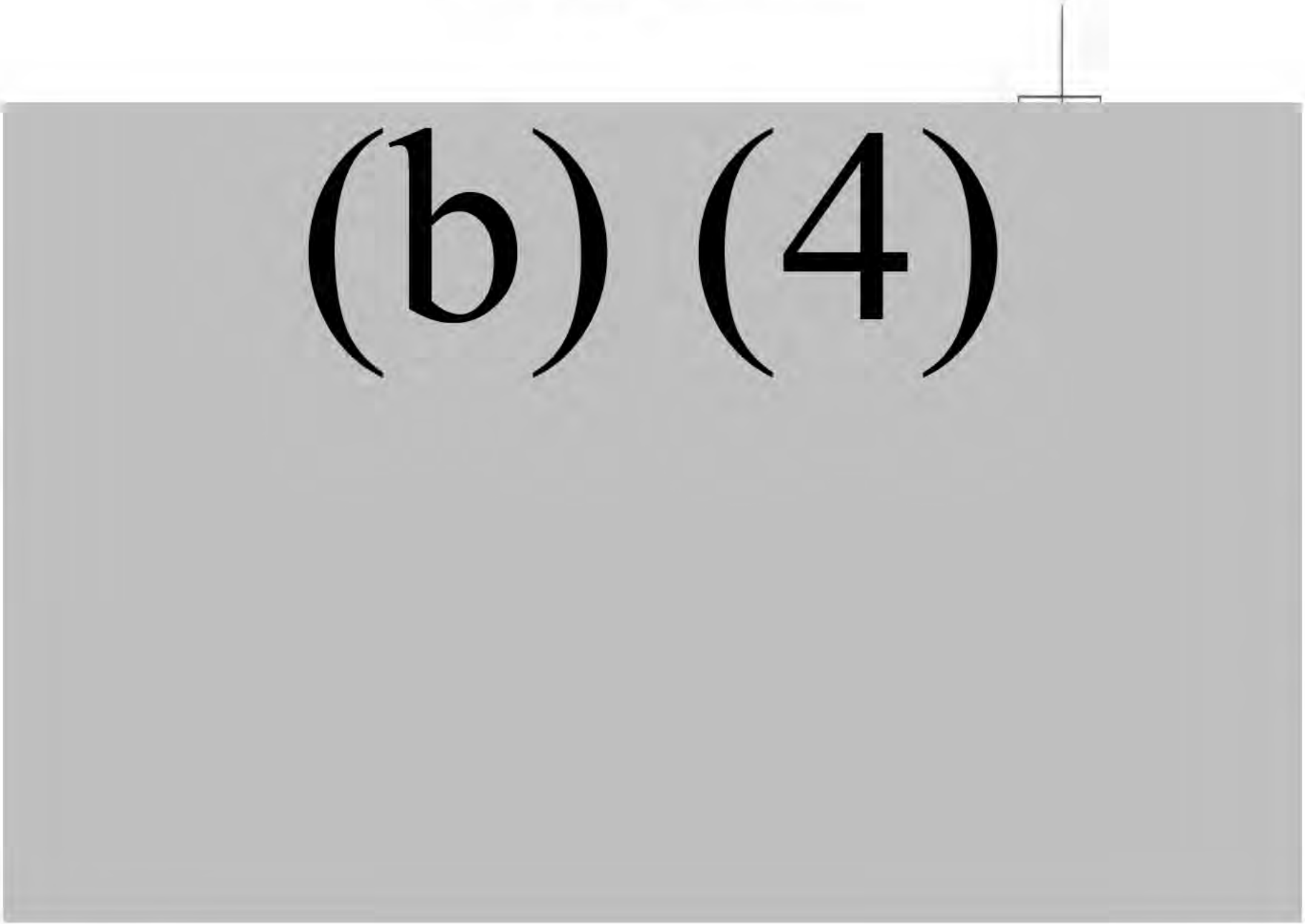
Figure 6: Diagram of the fermentation process



d) Recovery

(b) (4)

(b) (4)

(b) (4)

(b) (4)

[redacted]

e) Formulation

The polished, preformulated concentrate is standardized with food grade ingredients, adjusted to the desired activity, and stabilized with preservatives. This results in a formulated concentrate called PHYTAVERSE® L44 Liquid Formulation.

(b) (4)

[redacted]

Figure 8. (b) (4)

C. Composition and Specifications

1. Finished product composition

The products of commerce are CIBENZA® PHYTAVERSE® L10 Phytase Enzyme (a liquid product) and CIBENZA® PHYTAVERSE® G10 Phytase Enzyme (a granular product). All raw materials used in the final formulation are either approved food additives published in 21 CFR 573, substances that are Generally Recognized as Safe (GRAS) for the intended use, or are otherwise acceptable ingredients for use in animal food, such as those defined in the most recent AAFCO OP (2020), and comply with prescribed limits.

CIBENZA® PHYTAVERSE® L10 Phytase Enzyme is formulated with the following ingredients (in order of predominance): water, liquid *P. fluorescens* fermentation product, sodium chloride, sucrose, sodium citrate, potassium sorbate, sodium benzoate, and sodium propionate. CIBENZA® PHYTAVERSE® L10 Phytase Enzyme is sold with a minimum phytase enzyme guarantee of ≥10,000 U/g.

CIBENZA® PHYTAVERSE® G10 Phytase Enzyme is formulated with the following ingredients (in order of predominance): wheat flour, sucrose, dried *P. fluorescens* fermentation product, sodium citrate, sodium chloride, potassium sorbate, sodium benzoate, and sodium propionate. CIBENZA® PHYTAVERSE® G10 Phytase Enzyme is sold with a minimum phytase enzyme guarantee of ≥10,000 U/g.

The composition for each of the products are provided in Appendix 12. It is possible that other commercial forms of the phytase 50104 enzyme preparation could be developed using other suitable feed grade carriers or preservatives in the future, if there is a market need.

The percent total organic solids (TOS)[2] for the phytase 50104 enzyme preparation is 6 ± 1%. The TOS was calculated for the liquid product (i.e., CIBENZA® PHYTAVERSE® L10 Phytase Enzyme) and is applied to the granulated product (i.e., CIBENZA® PHYTAVERSE® G10 Phytase Enzyme), because they are both made from the same formulated, phytase 50104 enzyme concentrate (i.e., PHYTAVERSE® L44 Liquid Formulation) and formulated to have a guarantee minimum phytase activity of 10, 000 U/g.

2. Finished product specifications

The formulated, phytase 50104 enzyme concentrate (i.e., PHYTAVERSE® L44 Liquid Formulation) has established specifications, which include purity criteria recommended for enzyme preparations as described in the Food Chemical Codex (FCC) (U.S.Pharmacopeial Convention, 2018) and conforms to the general specifications for enzyme preparations used in food processing as proposed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (FAO/WHO, 2006). The formulated, phytase 50104 enzyme concentrate is used to make the products of commerce (i.e., CIBENZA® PHYTAVERSE® L10 Phytase Enzyme and CIBENZA® PHYTAVERSE® G10 Phytase Enzyme). The products of commerce also have established manufacturing and product specifications.

Additionally, the absence of production organism in the final product is a specification even though it is not included in FCC or JECFA specifications. As mentioned in Part 2 Section B.1.h, the phytase 50104 enzyme preparation is tested according to SOP QC0214 for the absence of production organism.

[2] TOS (%) = [100 – (water, % + residue on ignition, % + diluents, % (i.e., formulation ingredients)]

Lastly, the phytase 50104 enzyme preparation is sold with a minimum enzyme activity. Both products, CIBENZA® PHYTAVERSE® L10 Phytase Enzyme and CIBENZA® PHYTAVERSE® G10 Phytase Enzyme, are sold to have a minimum phytase enzyme activity guarantee of 10,000 U/g (according to the method ISO 30024; see Part 2 Section C.3 below for more information on the method).

Provided in Appendix 9 are three Certificates of Analysis for each product of commerce that show testing results for conformance with the purity criteria recommended for enzyme preparations as described in the FCC (U.S.Pharmacopeial Convention, 2018) and with the general specifications for enzyme preparations used in food processing (FAO/WHO, 2006), along with testing results for the absence of production organism, and enzyme activity.

3. Analytical methods

The phytase method, ISO 30024 (Reference number ISO 30024:2009(E); Animal feeding stuffs – determination of phytase activity) is used as the standard method for product release of the phytase 50104 enzyme preparation (i.e., CIBENZA® PHYTAVERSE® L10 Phytase Enzyme and CIBENZA® PHYTAVERSE® G10 Phytase Enzyme).

In summary, phytase releases phosphate from the substrate myo-inositol hexakisphosphate (phytate). In the laboratory, the amount of released inorganic phosphate is determined spectrophotometrically by measuring the formation of a yellow complex with an acidic molybdate/vanadate reagent. The optical density (OD) of the yellow complex is measured at a wavelength of 415 nm, and the inorganic phosphate released is quantified from a phosphate standard calibration curve. One phytase unit (U) is defined as the amount of enzyme that releases 1 µmol of inorganic phosphate from phytate per minute under the reaction conditions specified by the International Standard procedure.

The phytase method ISO 30024 is a validated, analytical method and has been verified by BASF Enzymes LLC and Novus International, Inc. for product release. The verification protocols were based upon the guidelines provided in the VICH text on Validation of Analytical Procedures: Methodology and the United States Pharmacopoeia, USP 37/NF32 (2014) and provide data on the linearity and range, limit of detection, limit of quantitation, precision (repeatability), and intermediate precision. Both companies adopted the ISO method for internal use via standard operating procedures.

A license to the ISO phytase method, ISO 30024 (Reference number ISO 30024:2009(E); Animal feeding stuffs – determination of phytase activity) was purchased for and provided to Dr. Michaela Alewynse (Division on Animal Feeds in the FDA's Center for Veterinary Medicine).

4. Stability

a) Finished product stability

CIBENZA® PHYTAVERSE® L10 Phytase Enzyme

The storage stability of CIBENZA® PHYTAVERSE® L10 Phytase Enzyme was determined using three independent lots of formulated product. The product was stored at elevated temperatures (30 and 40ºC), at ambient temperature (25ºC), and under refrigeration (5ºC) and tested over a 24-month period. To reduce the variability in the data obtained at different time points, the activity results were normalized using samples of the same three lots of CIBENZA® PHYTAVERSE® L10 Phytase Enzyme which were stored frozen at -20ºC. After 24 months of storage, CIBENZA® PHYTAVERSE® L10 Phytase Enzyme retains 97-98% activity when stored under refrigerated conditions (5ºC), retains 64-97% activity at room temperature (25ºC), and retains 59-78% activity at elevated temperature (30ºC).

Based on the results of the study described above, it is concluded that CIBENZA® PHYTAVERSE® L10 Phytase Enzyme will maintain a guaranteed minimum phytase activity of 10,000 U/g when stored for 18 months at 25°C or lower in an unopened container.

CIBENZA® PHYTAVERSE® G10 Phytase Enzyme

The storage stability of CIBENZA® PHYTAVERSE® G10 Phytase Enzyme was determined using three independent lots of granulated product. The product was stored at 25ºC, 60% RH; 30ºC, 70% RH; and 40ºC, 75% RH, and tested over an 18-month period. To reduce the variability in the data obtained at different time points, the activity results were normalized using samples of the same three lots of CIBENZA® PHYTAVERSE® G10 Phytase Enzyme, which were stored frozen at -20ºC. After 18 months of storage, CIBENZA® PHYTAVERSE® G10 Phytase Enzyme retains 84-98% activity when stored at 25ºC, 60% RH, retains 73-80% activity when stored at 30ºC, 70% RH, and retains <50% activity when stored at 40ºC, 75% RH.

Based on the results of the study described above, it is concluded that CIBENZA® PHYTAVERSE® G10 Phytase Enzyme will maintain a guaranteed minimum phytase activity of 10,000U/g for 18 months when stored at room temperature in an unopened container.

b) Stability and homogeneity in premix

CIBENZA® PHYTAVERSE® L10 Phytase Enzyme

CIBENZA® PHYTAVERSE® L10 Phytase Enzyme is applied to complete feed via a post-pellet liquid application. Therefore, stability and homogeneity studies in premix are not applicable.

CIBENZA® PHYTAVERSE® G10 Phytase Enzyme

CIBENZA® PHYTAVERSE® G10 Phytase Enzyme is recommended for use in premix. Therefore, stability and homogeneity studies were conducted with CIBENZA® PHYTAVERSE® G10 Phytase Enzyme in premix. Phytase activity in premixes was determined based on the method ISO 30024 in association with dilution method VDLUFA 27.1.3 (Preparation of Mineral Feed and Premixtures for the Determination of Phytase Activity)."

A premix stability study was conducted using CIBENZA® PHYTAVERSE® G10 Phytase Enzyme. Three batches of CIBENZA® PHYTAVERSE® G10 Phytase Enzyme (lot n^{o}s P23941, P26641, and RO15271001) were used at two inclusion levels in premix to theoretically provide 250 and 500 U/kg in a completed feed. The stability of each of the three batches of the test article at two inclusion levels was determined by monthly measuring of phytase activity in composite samples obtained at mixing and after storage at ambient conditions from 0 to 6 months. According to the results of the stability study in vitamin-mineral premix, CIBENZA® PHYTAVERSE® G10 Phytase Enzyme:

- Was stable over time (up to 6-months storage at ambient conditions) for all three batches at both inclusion levels, as demonstrated by slopes of linear regressions of phytase activity over time not being significantly different from 0 (flat line).
- Presented a good stability (±10% of 0-month value) up to 6-months storage also for all three batches at both inclusion levels. Higher variations at intermediate points were considered to be within the range of expected values considering stability within the batch rather than real activity changes.

The premix stability study report is provided in Appendix 13, and the sources of the vitamins and minerals used in the study are provided in Appendix 14.

A premix homogeneity study was conducted using CIBENZA® PHYTAVERSE® G10 Phytase Enzyme. Three lots of CIBENZA® PHYTAVERSE® G10 Phytase Enzyme (lot nºs P23941, P26641, and RO15271001) were used at two inclusion levels in premix to theoretically provide 250 and 500 U/kg in a completed feed. The homogeneity of each of the three batches of the test article at two inclusion levels was determined by measuring phytase activity in 10 subsamples taken at different location points of the mixer. According to the results of the homogeneity in premix, CIBENZA® PHYTAVERSE® G10 Phytase Enzyme:

- Presented a good mixing homogeneity (CV% 8% to 12%), with actual CVs below or close to the CV of the method itself for all three batches and at both inclusion levels.

The premix homogeneity study report is provided in Appendix 15, and the sources of the vitamins and minerals used in the study are provided in Appendix 14.

c) Stability and homogeneity in feed

CIBENZA® PHYTAVERSE® L10 Phytase Enzyme

A three-month stability study was conducted to evaluate the stability of CIBENZA® PHYTAVERSE® L10 Phytase Enzyme in feed when applied via a post pellet liquid application. Phytase activity in pelleted feed was determined by the method ISO 30024. Three batches of CIBENZA® PHYTAVERSE® L10 Phytase Enzyme (lot nºs CV002C2, 190CV005A3, and PHY-50104-PO030-F4) at two concentrations (250 and 500 U/kg) were added to feed via a post pellet application. For each batch and dose, the stability of the test article was determined by measuring phytase activity in unique feed samples after 0, 1, 2 and 3-months storage at ambient conditions. According to the results, CIBENZA® PHYTAVERSE® L10 Phytase Enzyme:

- Was stable over time (1, 2 and 3-months storage at ambient conditions) for all three batches (A & B & C) at both concentrations tested (250 & 500 U/kg) as demonstrated by the slope of linear regressions of phytase activity over time not being significantly different from 0 (flat line, no significant loss of activity).
- Presented good stability (in general ±10% of 0-month value) up to 3-months in pelleted feeds for all three batches (A & B & C) at both concentrations tested (250 & 500 U/kg).

The stability study report is provided in Appendix 16, and the sources of the vitamins and minerals used in the study are provided in Appendix 17.

Homogeneity of CIBENZA® PHYTAVERSE® L10 Phytase Enzyme in feed when applied via a post pellet liquid application was also evaluated. Three batches of CIBENZA® PHYTAVERSE® L10 Phytase Enzyme (lot n^{o}s CV002C2, 190CV005A3, and PHY-50104-PO030-F4) at the lowest recommended dose (250 U/kg) were added to feed via a post pellet application. For each batch, the homogeneity was determined by measuring phytase activity in 10 subsamples taken at different time points at bagging. Phytase activity in pelleted feed was determined by the method ISO 30024. According to the results of the homogeneity study in feed, CIBENZA® PHYTAVERSE® L10 Phytase Enzyme:

- Presented good mixing homogeneity (CV ~7 to 11%), with actual CVs below or close to the CV of the method itself (10%) for all 3 batches tested in pelleted form (post pellet liquid application).

The homogeneity study report is provided in Appendix 18, and the sources of the vitamins and minerals used in the study are provided in Appendix 17

CIBENZA® PHYTAVERSE® G10 Phytase Enzyme

A three-month stability study was conducted to evaluate the stability of CIBENZA® PHYTAVERSE® G10 Phytase Enzyme in feed. Three batches of CIBENZA® PHYTAVERSE® G10 Phytase Enzyme (lot n^{o}s P23941, P26641, and RO15271001) at two concentrations (250 and 500 U/kg) were added to make mash and pelleted feeds. For each batch, dose, and form, the stability of the test article was determined by measuring phytase activity in unique feed samples after 0, 1, 2 and 3-months storage at ambient conditions. According to the results, CIBENZA® PHYTAVERSE® G10 Phytase Enzyme:

- Was stable over time (1, 2, and 3-months storage at ambient conditions) for all three batches, for both feed forms, and at both concentrations tested as demonstrated by the slope of linear regressions of phytase activity over time not being significantly different from 0 (flat line, no significant loss of activity).

- Presented good stability (in general ±10% of 0-month value) up to three months in feeds for all three batches, for both forms, and at both concentrations tested.

The stability study report is provided in Appendix 19, and the sources of the vitamins and minerals used in the study are provided in Appendix 17.

Homogeneity of CIBENZA® PHYTAVERSE® G10 Phytase Enzyme in feed was also evaluated. Three batches of CIBENZA® PHYTAVERSE® G10 Phytase Enzyme (lot nºs P23941, P26641, and RO15271001) at the lowest recommended dose (250 U/kg) were added to make mash and pelleted feeds. For each batch and form, the homogeneity was determined by measuring phytase activity in 10 subsamples taken at different location points of the mixer (mash) or at bagging (pelleted). Phytase activity in pelleted feed was determined by the method ISO 30024. According to the results of the homogeneity study in feed, CIBENZA® PHYTAVERSE® G10 Phytase Enzyme:

- Presented good mixing homogeneity (CV ~7 to 15%), with actual CVs below or close to the CV of the method itself (10%) for all 3 batches tested in mash and pelleted forms.

The homogeneity study report is provided in Appendix 20 and the sources of the vitamins and minerals used in the study are provided in Appendix 17.

d) Thermostability

CIBENZA® PHYTAVERSE® L10 Phytase Enzyme

CIBENZA® PHYTAVERSE® L10 Phytase Enzyme is applied to complete feed in a post-pelleting application. Therefore, thermostability is not applicable.

CIBENZA® PHYTAVERSE® G10 Phytase Enzyme

CIBENZA® PHYTAVERSE® G10 Phytase Enzyme is recommended for use in pelleted feeds. Therefore, a thermostability study with CIBENZA® PHYTAVERSE® G10 Phytase Enzyme was conducted to determine recommended temperature conditions when pelleting feed.

Three lots of CIBENZA® PHYTAVERSE® G10 Phytase Enzyme (lot nºs P23941, P26641, and RO15271001) at two concentrations (250 and 500 U/kg) were used for the study. The temperatures used to evaluate the temperature conditions when pelleting feed are as follows: 65°C, 75°C, 85°C, 88°C, and 90°C. The conditioning time (also known as the retention time) used in the

study is approximately 60 seconds. The results of the study demonstrate that CIBENZA® PHYTAVERSE® G10 Phytase Enzyme retains greater than 85% of the initial phytase activity when pelleted feed is made using a pelleting temperature of 85°C and a conditioning time of approximately 60 seconds.

The thermostability study report is provided in Appendix 21, and the sources of the vitamins and minerals used in the study are provided in Appendix 22

D. Physical or Technical Effect

The purpose of using phytase as an ingredient in poultry feed is to increase the availability of phytate bound phosphorus in the animal diet and to decrease the phosphorus contribution to manure, which results in the pollution of surface water. The bioavailability of plant phosphorus is limited in common feedstuffs because 1) most of the phosphorus present in plant related feedstuffs is in the form of an organic complex called phytic acid or phytate, and 2) monogastrics such as poultry lack endogenous phytase at the level needed to hydrolyze phytate (Nys, Y. *et al.*, 1996). The chemical name for phytate is myo-inositol 1,2,3,4,5,6-hexakisphosphate, an inositol ring with six phosphate radicals. Phytase liberates phosphorus by cleaving the ortho-phosphate groups from the phytate organic complex.

Like all phytases (including the 12 listed in the 2020 AAFCO OP and on FDA CVM's Current Animal Food GRAS Notices Inventory), the phytase 50104 enzyme, an appA *E. coli* based phytase, catalyzes the stepwise hydrolysis of phosphate monoesters from the inositol ring of phytate (Association of American Feed Control Officals (AAFCO), 2020b; Association of American Feed Control Officals (AAFCO), 2020c; FDA Center for Veterinary Medicine, 2017; FDA Center for Veterinary Medicine, 2019; Lei, X.G. and Stahl, C.H., 2001; Wodzinski, R.J. and Ullah, A.H., 1996). Therefore, the phytase 50104 enzyme will, like other phytase, increase the availability of phytin-bound phosphorus in poultry diets.

Numerous studies have been published demonstrating the effectiveness of *E. coli* based phytases to increase phosphorus availability from phytate in animal feed (Adeola, O. *et al.*, 2004; Jendza, J.A. *et al.*, 2006; Selle, P.H. and Ravindran, V., 2007; Zeng, Z.K. *et al.*, 2014). Within the field of poultry nutrition, experts qualified by scientific training and experience to evaluate safety of feed ingredients generally recognize that the addition of appA *E.coli* based phytases, at appropriate levels to increase digestibility of phytin-bound phosphorus or to increase phosphorus

availability from phytate in poultry diets, is safe. There are several published papers to support the utility of *E. coli* based phytases for use in poultry diets (Onyango, E.M. *et al.*, 2005; Pillai, P.B. *et al.*, 2006; Ribeiro, V. *et al.*, 2016).

Additionally, to demonstrate the utility of the phytase 50104 enzyme preparation to increase the availability of phytin-bound phosphorus in poultry diets, CIBENZA® PHYTAVERSE® G10 Phytase Enzyme was used in three different poultry feeding experiments. The diets used in the studies were representative of U.S. corn and soybean meal diets for poultry. Two of the poultry feeding experiments were published providing pivotal evidence for the utility of phytase 50104 enzyme preparation (Pieniazek, J. *et al.*, 2017). The third experiment is considered corroborative. These three poultry feeding experiments are described below.

Please note that the three poultry feeding experiments described below were conducted with the granulated product form of the phytase 50104 enzyme preparation (i.e., CIBENZA® PHYTAVERSE® G10 Phytase Enzyme). The liquid product (i.e., CIBENZA® PHYTAVERSE® L10 Phytase Enzyme) and the granulated product (i.e., CIBENZA® PHYTAVERSE® G10 Phytase Enzyme) are considered to be sister products, because they are both made from the same formulated, phytase 50104 enzyme concentrate (i.e., PHYTAVERSE® L44 Liquid Formulation) and formulated to have a guaranteed minimum phytase activity of 10,000 U/g.

1. Published utility data

The two poultry feeding experiments (Experiment 1 and Experiment 2) described below are published in Pieniazek, J. *et al.* (2017). The published paper is provided in Appendix 23.

a) Experiment 1

In this experiment, the effects of increasing levels of the commercial, dry product form of phytase 50104 enzyme preparation, i.e., CIBENZA® PHYTAVERSE® G10 Phytase Enzyme, were determined using 576 male Cobb 500 broilers fed diets deficient in available phosphorus (aP) in a 21 day battery study.

1. Experimental Design

Five hundred and seventy-six male Cobb 500 broilers were weighed, wing banded, and allotted to battery cages on day of hatch. Eight broilers were placed per replicate pen, and 12 replicate pens were used per treatment for a total of 72 pens. The negative control (NC) group had

diets formulated with an aP of 0.23% and 0.19% in the starter and grower rations, respectively. There were two positive (PC) control groups. PC1 contained 0.12% more aP compared to the NC for starter and grower rations to give a total of 0.35% aP and 0.31% aP, respectively. PC2 contained 0.22% more aP than the NC in starter and grower rations to give a total of 0.45% aP and 0.41%, respectively. Three levels of phytase were supplemented to the NC diet at 250, 500, and 2,000 U/kg.

Corn-soybean meal diets with supplemental fat were formulated to be both deficient and sufficient in aP. The diets met all other nutrient requirements. Titanium dioxide at 0.4% was added at the expense of corn in the final dietary phase for use as an indigestible marker for the determination of nutrient digestibility. Basal diets were formulated and CIBENZA® PHYTAVERSE® G10 Phytase Enzyme was added over the top to the basal diet during mixing and before pelleting. Diets were mash feed that was steam conditioned for 20 seconds and pelleted at 85ºC. CIBENZA® PHYTAVERSE® G10 Phytase Enzyme was added to deliver 250, 500, and 2,000 U/kg of phytase to the experimental diets.

Broilers were fed a starter diet through day 14 and a grower diet from day 15 to 21. Grower and finisher diets were fed as pellets, while starter diets were crumbled post pelleting. Pelleted samples of all diets and treatment were analyzed for phytase recovery and nutrient content.

Mortalities were collected, recorded and weighed daily. Broilers and feed were weighed weekly on days 7, 14, and 21 for the calculation of body weight (BW) and mortality corrected feed conversion ratio (FCR). On day 20 fecal matter was collected for 24 hours for the determination of total tract AME. On day 21 all remaining birds were euthanized and right tibias were removed and pooled per replicate pen for determination of bone ash. Tibia ash was determined on fat free dry matter basis. Bones were dried at 105ºC for 24 hours then ashed at 600 ºC for 24 hours. Bones were weighed pre- and post ashing. Ileal contents were collected and pooled per replicate pen for the determination of amino acid digestibility. Ileal contents were removed from four centimeters posterior to Meckel's Diverticulum and four centimeters anterior to the ileal-cecal junction. Samples were freeze dried prior to analysis. Sample were then ground for amino acid and titanium concentration determination.

2. Results and Evaluation

The results are describe below and are also provided in Appendix 23.

Birds fed the NC diet had a decreased ($P < 0.05$) bone ash weight and percent compared to birds fed the PC1 and PC2 diets. Supplementing the aP deficient diet with phytase increased ($P < 0.05$) bone ash weight compared to both the NC and PC1. Inclusion of the phytase at 2,000 U/kg increased bone ash weight to levels similar to the PC2 diet. Inclusion of the phytase at 250 and 500 U/kg increased bone ash percent to levels that were similar to the PC1 diet. At 2,000 U/kg inclusion of phytase, bone ash percent increased ($P < 0.05$) to levels similar to the PC2 diet. At phytase inclusion levels of 250, 500, and 2,000 U/kg, bone ash weight and percentage were significantly ($P < 0.05$) higher than NC.

Birds fed the NC diet had lower ($P < 0.05$) body weight (BW) throughout the experiment compared to birds fed PC diets containing 0.35% aP (PC1) and 0.45% aP (PC2). Supplementing the P deficient diet with phytase increased ($P < 0.01$) BW throughout the experiment compared to the NC diet. At the end of the experiment on day 21, supplementing the NC diet with phytase at 250 and 500 U/kg improved ($P < 0.05$) BW to levels comparable to the PC1 diet. Supplementing the NC with 2,000 u/kg increased ($P < 0.05$) BW to levels similar to PC2. Overall, a linear relationship was observed between BW and phytase inclusion. Mortality was highest in the broilers fed NC and decreased ($P < 0.05$) with the inclusion of 250 U/kg phytase or inorganic phosphate in PC1 and PC2 diets.

To determine P equivalency for tibia bone ash weight, tibia bone ash percent, and body weight gain, linear regression analysis was performed. The P equivalency values for tibia ash percent were 0.12%, 0.13%, and 0.21% for phytase inclusion of 250 U/kg, 500 U/kg, and 2,000 U/kg, respectively. Similar trends were seen for tibia ash weight. For body weight gain the P equivalency values for 250 U/kg, 500 U/kg, and 2,000 U/kg were 0.15%, 0.16%, and 0.23%, respectively.

Amino acid digestibility was measured on day 21. Digestibility coefficients of all measured amino acids were reduced ($P < 0.05$) in the NC. Inclusion of phytase at 250 U/kg increased ($P < 0.05$) the digestibility coefficients of all measured amino acids compared to the NC to levels that were similar to the PC1 and PC2. At 500 U/kg inclusion rate, phytase increased ($P < 0.05$) the amino acid digestibility coefficient of aspartic acid, cysteine, glycine, lysine, methionine, phenylalanine, proline, serine, and Total nonessential amino acids (TNEAA) to levels comparable to PC1. Other measured amino acid digestibility coefficients were increased including Total sulfur amino acids (TSAA), Total essential amino acids (TEAA), and Total amino acids (TAA) to levels

comparable to PC1 but they were not statistically different from the NC diet. At phytase inclusion rate of 2,000 U/kg, the digestibility coefficient of cysteine, glycine, lysine, phenylalanine, proline and serine increased ($P < 0.05$) compared to NC levels and were similar to PC1 levels.

3. Conclusion

The addition of phytase 50104 enzyme preparation demonstrated utility to increase the phosphorus availibility from phytate in poultry diets. Parameters of tibia bone ash, tibia bone weight, BW, and amino acid digestibility coefficients showed improvements with the addition of phytase 50104 enzyme preparation to poultry diets deficient in aP. Over a 21 day feeding study, increased ($P < 0.05$) tibia bone ash percentage and tibia bone ash weight were observed for phytase inclusion levels of 250 U/kg, 500 U/kg, and 2,000 U/kg compared to the NC. Inclusion of phytase at 250 and 500 U/kg increased bone ash percent to levels that were similar to the PC1 diet. At 2,000 U/kg inclusion of phytase, bone ash percent increased ($P < 0.05$) to levels similar to the PC2 diet. Supplementing the P deficient diet with phytase increased ($P < 0.01$) BW throughout the experiment compared to the NC diet. At the end of the experiment on day 21, supplementing the NC diet with phytase at 250 and 500 U/kg improved ($P < 0.05$) BW to levels comparable to the PC1 diet. Supplementing the NC with 2,000 u/kg increased ($P < 0.05$) BW to levels similar to PC2. Overall, a linear relationship was observed between BW and phytase inclusion in the diet.

b) Experiment 2

In this experiment, the effects of increasing levels of the commercial, dry product form of phytase 50104 enzyme preparation, i.e., CIBENZA® PHYTAVERSE® G10 Phytase Enzyme, were determined on 1,760 male broilers fed diets deficient in available phosphorus (aP) in a 42 day grow-out experiment.

1. Experimental Design

One thousand, seven hundred and sixty male broilers were weighed, wing banded, and allotted to floor pens and treatment groups based on initial body weights. Forty broilers were placed per replicate pen, with 11 replicate pens per treatment for a total of 44 replicate pens. The positive control (PC) diet contained 0.45%, 0.42%, and 0.38% aP in the starter, grower, and finisher phases, respectively. The aP deficient diet was formulated with a 0.17% reduced aP per dietary phase. Mortalities were collected, recorded and weighed daily. Broilers and feed were

weighed on days 14, 28, and 42. On day 42, six birds per replicate were euthanized and right tibias removed for determination of bone ash using the same method as described above in Experiment 1. Ileal contents were collected similar to Experiment-1 for amino acid digestibility determination.

2. Results and Evaluation

The results are described below and are also provided in Appendix 23.

Tibia bone ash weight and percent were reduced ($P < 0.05$) in the NC group compared to the positive control. The inclusion of phytase 50104 enzyme preparation in the diet at both 500 and 200 U/kg increased ($P < 0.05$) both tibia bone ash weight and percent compared to the NC and these parameters were similar to the PC values. A linear relationship was found between phytase inclusion in the diet and bone mineralization. With increased phytase inclusion, bone mineralization increased ($P < 0.001$).

BW was reduced ($P < 0.05$) throughout the entire experiment for broilers fed the reduced aP diet compared to the PC. Including phytase 50104 enzyme preparation in the diet resulted in increased ($P < 0.05$) BW compared to the NC. On day 14, BW in the 500 U/kg treatment group were comparable to the PC diet. For the 2,000 U/kg phytase treatment group, BW increased ($P < 0.05$) to higher levels than both the NC and PC diets on day 14. On day 42, the inclusion of phytase at 500 U/kg increased ($P < 0.05$) over the NC. Inclusion of phytase at 2,000 U/kg resulted in increased ($P < 0.05$) BW over the NC to levels that were similar to the PC. The experiment found a linear relationship between BW and inclusion of phytase 50104 enzyme preparation in the diet. With increased phytase inclusion, BW increased ($P < 0.001$).

Amino acid digestibility was determined on day 42. The reduced aP diet had reduced ($P < 0.05$) digestibility coefficients of alanine, aspartic acid, cysteine, glycine, histidine, isoleucine, lysine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, TEAA, TNEAA and TAA compared to the PC diet. The inclusion of 500 U/kg did not affect amino acid digestibility coefficients compared to the NC for all measured amino acids in this experiment. The inclusion of 2,000 U/kg resulted in similar amino acid digestibility coefficients as the PC in all measured amino acids. In this experiment, no impact was found on arginine, methionine, or valine digestibility between the PC and NC.

3. Conclusion

The utility of phytase 50104 enzyme preparation to increase phosphorus availability from phytate in poultry diets was demonstrated by the increase in tibia bone ash weight and tibia bone ash percent in broilers fed diets supplemented with phytase at 500 and 2,000 U/kg compared to NC. The addition of phytase 50104 enzyme preparation to poultry diets improved BW over the 42-day study when compared to NC further demonstrating the improved availability of phosphorus in the diet to support growth. Amino acid digestibility coefficients were similar for the 2,000 U/kg group and the PC.

2. Unpublished, corroborative utility data

To demonstrate the utility of phytase 50104 enzyme preparation, a broiler study was conducted at [redacted] (b) (6), (b) (4) The broiler study evaluated the utility of adding CIBENZA® PHYTAVERSE® G10 Phytase Enzyme at two doses (250 and 500 U/kg diet) in diets containing sub-optimal levels of non-phytate phosphorus by assessing tibia ash levels, as an indicator of phosphorus availability. The complete study report is provided in Appendix 24.

a) Experimental Design

A total of 960 Cobb-500 broiler chicks were assigned to 4 treatments with 12 pens/treatment and 20 chicks/pen using a randomized complete block design. The treatment groups consisted of the following:

- Positive control – The diet met or exceeded the NRC 1994 and industry standards.
- Negative control – The diet met or exceeded the NRC 1994 standards with the exception of non-phytate phosphorus (NPP) formulated to 0.3% NPP for starter (days 0 to 14), and 0.26% NPP for grower (days 14 to 28).
- Negative control diet with 250 U CIBENZA® PHYTAVERSE® G10 per kg feed.
- Negative control diet with 500 U CIBENZA® PHYTAVERSE® G10 per kg feed.

One Unit or "U" was defined as the amount of enzyme that catalyzed the release of one micromole phosphate from the phytate per minute at 37°C at pH 5.5 in accordance to the assay.

Starter and grower diets were fed in mash form and were comprised primarily of corn and soybean meal. The starter diet was fed from days 0 to 14, and the grower diet was fed from days

14 to 28. Feed was provided by a feeder tray for each pen for the first four days of the study. Both feed and water were provided *ad libitum* throughout the study.

The test facility, pens, and birds were observed at least twice daily for general flock conditions, lighting, water, feed, ventilation, and unanticipated events. All animals were observed regularly, and any adverse effects were recorded. Birds were weighed by pen at placement (day 0), day 14, and day 28. Feed offered was weighed by pen. Feed removed was weighed by pen on days 14 and 28. Average bird weight gain and average feed intake were calculated for the periods 0-14, 14-28, and 0-28 days. The feed conversion ratio (FCR) (adjusted for mortality and culls) was also calculated.

Percent tibia ash is a direct indicator of broiler (poultry) phosphorus status and the efficacy of CIBENZA® PHYTAVERSE® G10 Phytase Enzyme in the animals fed reduced non-phytate phosphorus. On day 28, at the end of the study, the five (5) surviving birds within each pen with the lowest neck tag numbers were selected for bone-ash measurements. For each pen, the results for all 5 right tibia samples were averaged, so the pen served as the experimental unit.

b) Results and evaluation

The results are described below and are provide in Appendix 24.

Tibia Ash: Results indicate significant treatment effect ($P<0.0001$) for tibia ash %. The percent tibia ash in the positive control (PC) group was significantly higher than the negative control (NC) and 250 U groups (53.50% vs. 44.75% and 51.24%, respectively), but not significantly different from the 500 U group (52.86%). Both the 250 and 500 U groups had significantly higher ash values than the negative control group (51.24% and 52.86% vs. 44. 75%, respectively). Additionally, ash values in the 500 U group were significantly higher than values in the 250 U group (52.86% vs. 51.24%, respectively).

Tibia Ash Minerals: Significant treatment effects ($P<0.0001$) were observed for the percentage of magnesium and phosphorus in tibia ash. For phosphorus and magnesium values, the values in the positive control group were significantly higher than the negative control and 250 U group (17.92%, 0.79% vs. 16.98%, 0.64% and 17.31%, 0.71%, respectively). Phosphorus and magnesium values for the 250 and 500 U groups were significantly higher than the negative control (17.31%, 0.71 % and 17.76%, 0.75% vs. 16.98%, 0.64%, respectively). Calcium values were not affected ($P=0.42$) by treatment. The additional necropsy and bone assessment in the negative control birds at the end of the study resulted in an average hip pop-out score of 1.10 out of 2.00

and an average of 0.82 out of 2.00 for bone softening on gross evaluations. No joint abnormalities were noted on examination of this group.

Body Weight Gain: Significant treatment effects ($P<0.0001$) were observed for average body weight gain for each time period. During Days 0 to 14, body weight gain in the positive control group was not significantly different ($P>0.05$) from the gain observed in the negative control group. Gain in both the 250 and 500 U groups was significantly ($P<0.05$) higher than both the positive and negative control groups (0.304 kg, 0.310 kg and 0.292 kg, 0.282 kg, respectively). During days 14 to 28 and overall (days 0 to 28), gain in the positive control group was significantly higher ($P<0.05$) than the gain observed in the negative control group (0.928 kg vs. 0.751 kg and 1.221 kg vs. 1.033 kg, respectively). Gain in the 250 and 500 U groups was significantly higher ($P<0.05$) than the gain in the negative control group (0. 940 kg and 0. 973 kg, vs. 0. 751 kg, respectively for study days 14 to 28 and 1.244 kg, 1.283 kg vs. 1.033 kg, respectively for 0 to 28 days). Gain in the 500 U dose group was also significantly higher ($P<0.05$) than the gain in positive control group (0.973 kg vs 0.928 kg for study days 14 to 28 and 1.283 kg vs. 1.221 kg for study days 0 to 28).

Pen Daily Feed Intake: Significant treatment effects ($P<0.0001$) were observed for average daily feed intake for days 14 to 28 and 0 to 28. No treatment effects ($P=0.26$) were observed during the first 2 weeks of the treatment period. During days 14 to 28, feed intake in the positive control group was significantly higher ($P<0.05$) than the intake observed in the negative control group (1.96 kg vs. 1.54 kg, respectively). Intake in the 250 and 500 U groups was significantly higher ($P<0.05$) than the intake in the negative control group (1.99 kg and 2.04 kg vs. 1.54 kg, respectively). Intake in the 500 U dose group was also significantly higher ($P<0.05$) than the intake in positive control group and the 250 U group (2.04 kg vs. 1.96 kg and 1.99 kg, respectively). Overall (study days 0 to 28), intake in the positive control group was significantly higher ($P<0.05$) than the intake observed in the negative control group (1.27 kg vs 1.06 kg, respectively). Intake in the 250 and 500 U groups was significantly higher ($P<0.05$) than the intake in the negative control group (1.30 kg and 1.32 kg vs. 1.06 kg, respectively). Intake in the 500 U dose group was also significantly higher ($P<0.05$) than the intake in positive control group (1.32 kg vs. 1.27 kg, respectively).

Average Feed Intake: Significant treatment effects ($P<0.0001$) were observed for average feed intake per bird for days 14 to 28 and 0 to 28. No treatment effects ($P=0.48$) were observed

during the first 2 weeks of the treatment period. During study days 14 to 28, feed intake in the positive control group was significantly higher ($P<0.05$) than the intake observed in the negative control group (1.387 kg vs. 1.213 kg, respectively). Intake in the 250 and 500 U groups was significantly higher ($P<0.05$) than the intake in the negative control group (1.401 kg and 1.449 kg vs. 1.213 kg, respectively). Intake in the 500 U dose group was also significantly higher ($P<0.05$) than the gain in positive control group and the 250 U group (1.449 kg vs. 1.387 kg and 1.401 kg, respectively). Overall (study days 0 to 28), intake in the positive control group was significantly higher ($P<0.05$) than the intake observed in the negative control group (1.798 kg vs. 1.671 kg, respectively). Intake in the 250 and 500 U groups was significantly higher ($P<0.05$) than the intake in the negative control group (1.822 kg and 1.873 kg vs. 1.671 kg, respectively). Intake in the 500 U dose group was also significantly higher ($P<0.05$) than the intake in positive control group (1.873 kg vs. 1.798 kg, respectively).

Feed Conversion Ratio (FCR): Significant treatment effects ($P<0.001$) were observed for feed to gain ratio (FCR, adjusted) for days 0 to 14, 14 to 28, and 0 to 28. During days 0 to 14, 14 to 28 and overall (days 0 to 28), FCR in the positive control group was significantly ($P<0.05$) improved as compared to the FCR observed in the negative control group (1.4038 vs. 1.4572 for days 0 to 14, 1.4939 vs. 1.5744 for study days 14 to 28, and 1.4721 vs. 1.5403 for 0 to 28 days, respectively). FCR in the 250 and 500 U groups was significantly ($P<0.05$) improved versus the FCR in the negative control group (1.3849 and 1.3573 vs. 1.4572, respectively for study days 0 to 14, 1.4902 and 1.4806 vs. 1.5744 for days 14-28, and 1.4643 and 1.4504 vs. 1.5403 for study days 0 to 28, respectively). FCR in the 500 U dose group was also significantly ($P<0.05$) improved as compared to the FCR in positive control group (1.3573 vs. 1.4038 for study days 0 to 14, 1.4806 vs. 1.4939 for days 14-28, and 1.4504 vs. 1.4721 for study days 0 to 28, respectively). During study days 14 to 28, FCR in the positive control group was significantly ($P<0.05$) improved as compared to the FCR observed in the NC group (1.4939 vs. 1.5744, respectively). FCR in the 250 and 500 U groups was significantly ($P<0.05$) improved versus the FCR in the negative control group (1.4902 and 1.4806 vs. 1.5744, respectively). FCR in the 250 U group was not significantly ($P>0.05$) different from the FCR in positive control group, while the FCR in the 500 U group was significantly improved compared to the positive control group.

Mortality: No significant (P=0.55) treatment differences were observed for mortality during the starter phase. During the grower phase, and subsequently overall, mortality rates were significantly higher (P<0.05) in the negative control group as compared to the other 3 groups.

c) Conclusion

In this broiler study, the addition of either 250 or 500 U of CIBENZA® PHYTAVERSE® G10 Phytase Enzyme per kg diet to phosphorus deficient feed resulted in improved growth performance as evidenced by increases in average feed intake, average body weight gain, and a lower average feed conversion ratio in a dose dependent manner, with the higher dose resulting in better performance compared to birds fed a phosphorus deficient diet alone from 0 to 28 days of age. Bone parameters for birds were also improved at both inclusion levels compared to the birds fed a phosphorus deficient diet alone. In addition, the inclusion of CIBENZA® PHYTAVERSE® G10 Phytase Enzyme at the 500 U/kg of phosphorus deficient feed also significantly improved performance parameters compared to a diet supplying a standard level of phosphorus from 0 to 28 days of age.

The results of this study indicate and support the efficacy of phytase50104 phytase enzyme preparation (i.e., CIBENZA® PHYTAVERSE® L10 Phytase Enzyme and CIBENZA® PHYTAVERSE® G10 Phytase Enzyme) in poultry at either 250 or 500 U/kg diet containing sub-optimal levels of non-phytate phosphorus.

Please see Appendix 24 for the complete study report.

3. Dose discussion

The experiments published in Pieniazek, et al. (2017) were conducted by Texas A&M University and are described in Part 2 Section D.1 above. The published paper by Pieniazek et al. (2017) is provided in Appendix 23. A corroborative experiment was conducted at (b) (4) , USA) and is described above in Part 2 Section D.2 with the complete study report provided in Appendix 24. These experiments demonstrate the utility and support the use of the phytase 50104 enzyme preparation (i.e., CIBENZA® PHYTAVERSE® G10 Phytase Enzyme and CIBENZA® PHYTAVERSE® L10 Phytase Enzyme) in poultry diets.

The diets from each experiment were analyzed to confirm the phytase activity in each. The results are provided for each experiment's diet in Tables 3-5 below.

Table 3. Phytase Dose Analysis - Pieniazek et al. (2017) Experiment 1

Diet	Phytase Target Level	Phytase Analyzed Value	% Enzyme Activity of Target Value
Starter phase	250 U/kg	354 U/kg	141.6
Starter phase	500 U/kg	491 U/kg	98.2
Starter phase	2000 U/kg	2059 U/kg	102.95
Grower phase	250 U/kg	270 U/kg	108
Grower phase	500 U/kg	412 U/kg	82.4
Grower phase	2000 U/kg	1738 U/kg	86.9

Table 4. Phytase Dose Analysis - Pieniazek et al. (2017) Experiment 2

Diet	Phytase Target Value	Phytase Analyzed Value	% Enzyme Activity of Target Value
Starter phase	500 U/kg	520 U/kg	104
Starter phase	2000 U/kg	2200 U/kg	110
Grower phase	500 U/kg	430 U/kg	86
Grower phase	2000 U/kg	2000 U/kg	100
Finisher phase	500 U/kg	430 U/kg	86
Finisher phase	2000 U/kg	2100 U/kg	105

Table 5. Phytase Dose Analysis - Corroborative Study at (b) (4)

Diet	Phytase Target Level	Phytase Analyzed Value	% Enzyme Activity of Target Value
Starter phase	250 U/kg	300 U/kg	120
Starter phase	500 U/kg	530 U/kg	106
Grower phase	250 U/kg	298 U/kg	119.2
Grower phase	500 U/kg	539 U/kg	107.8
Finisher phase	250 U/kg	293 U/kg	117.2
Finisher phase	500 U/kg	568 U/kg	113.6

As shown in Tables 3-5, most (~72%) of the target phytase activity levels in the diets were reached for the experiments with the analyzed values being within ±15% of the target value. Fifty percent of the analyzed values were within ±10% of the target values. However, approximately 28% of the analyzed values were outside of the ±15% of the targeted value.

In Pieniazek et al. (2017), the study outcome indicated the use of all inclusion rates (i.e., 250 units (U), 500 U, and 2000 U of targeted dose of phytase per kg diet) are efficacious. The reported analyzed values for phytase activity showed some variation. For instance, in Experiment 1, the starter phase targeted 250 U/kg dose diet had a 354 U/kg analyzed value (+41.6% of target value) for phytase activity. However, in the same experiment, the grower target 250 U/kg dose diet was very close to the target with an analyzed phytase activity value of 270 U/kg (+8% of the targeted value). The analyzed values for 500 U/kg target dose for starter and grower diets in Experiment 1 were 491 U/kg (-1.8% of targeted value) and 412 U/kg (-17.6% of target value), respectively. For the 2000 U/kg target dose in Experiment 1, the analyzed values were 2059 U/kg (+2.95% of target value) and 1738 U/kg (-13.10% of target value) for the starter and grower diets, respectively.

For the corroborative study conducted at (b) (4), we also see variability in the target dose versus the analyzed dose (see Table 5 above). Using the diets from this study, a homogeneity study was conducted and showed that the CIBENZA® PHYTAVERSE® G10 Phytase Enzyme is homogenously mixed into the diets (see Appendix 25). The average phytase activity in the diet dosed with 250 U/kg of CIBENZA® PHYTAVERSE® G10 Phytase Enzyme was 271 U/kg with a CV of 10%. The average activity in the diet dosed with 500 U/kg of CIBENZA® PHYTAVERSE® G10 Phytase Enzyme was 509 U/kg with a CV of 7%. However, three of the ten subsamples for 250 U/kg dose diet were 15% higher than the targeted phytase activity; all other subsamples, for both target doses, were well within ±15% of the targeted dose. With the homogeneity study results in mind, one can conclude that the phytase activity variation seen in the corroborative study at (b) (6), (b) (4) is likely due to sampling variation and/or assay variation. Therefore, it is highly likely that the phytase activity variation seen in Pieniazek et al. (2017) can also be attributed to sampling variation and/or assay variation.

This discrepancy in phytase activity for target dose versus analyzed dose is well documented in the literature and it is widely accepted by highly reputed peer reviewed journals. For example, the study by Walk et al. (2014) has shown high degree of variation in analyzed

phytase values compared to targeted phytase values. The analyzed phytase values were 503, 362, 945, and 1390 U/kg against targeted phytase values of 500, 500, 1000, and 1500 U/kg diet, respectively. The authors mentioned that "these results were expected when sample variation, mixing, and assay errors are considered" (Walk, C.L. *et al.*, 2014).

It is concluded that the variations seen in the utility studies conducted with CIBENZA® PHYTAVERSE® G10 Phytase Enzyme are due to sampling variation and/or assay variation. Therefore, these poultry utility studies still support the use of the phytase 50104 enzyme preparation (i.e., CIBENZA® PHYTAVERSE® L10 Phytase Enzyme and CIBENZA® PHYTAVERSE® G10 Phytase Enzyme) at the inclusion levels between 250 to 2000 U/kg of feed.

4. Recommendation for Use

Product forms of the phytase 50104 enzyme preparation include CIBENZA® PHYTAVERSE® L10 Phytase Enzyme and CIBENZA® PHYTAVERSE® G10 Phytase Enzyme. The products have a guaranteed minimum phytase activity of 10,000 U/g.

The recommended level of supplementation in a complete poultry feed is 250 to 2000 U/kg of feed.

PART 3: TARGET ANIMAL AND HUMAN EXPOSURE

A. Target Animal Exposure

1. Target animal consumption

The phytase 50104 enzyme preparation (i.e., CIBENZA® PHYTAVERSE® L10 Phytase Enzyme and CIBENZA® PHYTAVERSE® G10 Phytase Enzyme) is intended for use in poultry feed. The recommended use rate is 250 to 2000 U/kg feed.

Calculations are provided below in Table 6 for target animal consumption and exposure. For poultry, broiler chickens are considered a worst case due to the ratio of typical feed intake versus body weight. In the calculations below, we are utilizing the typical daily intake (204 g of feed/day) and the typical body weight (2782 g) of 42 day old broiler chicken (Ross, 2019). The safety margin is calculated using the NOAEL from the subchronic (90-day) oral toxicity study (1720 mg TOS/kg – bw/day) and the dietary intake (mg TOS/kg – bw/day).

Table 6. Phytase 50104 enzyme intake estimate and safety margin

Body weight (bw) (kg)	Typical feed intake kg/feed/ day	Phytase 50104 enzyme		Highest expected phytase 50104 enzyme intake		Safety margin (NOAEL/ highest intake)
		U/kg feed	mg TOS/kg feed	U/day	mg TOS/ kg – bw/ day	
2.782	0.204	2000	14	408	1.0266	1675

The safety margin calculations indicate that the worst-case potential animal exposure (poultry) to the phytase 50104 enzyme preparation is well below the NOAEL observed in the subchronic (90-day) oral toxicity study.

2. Amount of other substance that is expected to be formed in or on food because of the use of the notified substance

Like all phytases (including the 12 listed in the 2020 AAFCO OP and on FDA CVM's Current Animal Food GRAS Notices Inventory), the phytase 50104 enzyme catalyzes the stepwise hydrolysis of phosphate monoesters from the inositol ring of phytate (Association of American Feed Control Officals (AAFCO), 2020b; Association of American Feed Control Officals (AAFCO), 2020c; FDA Center for Veterinary Medicine, 2019; Lei, X.G. and Stahl, C.H., 2001;

Wodzinski, R.J. and Ullah, A.H., 1996). The phytase 50104 enzyme will, therefore, liberate phosphorus by cleaving the ortho-phosphate groups from the phytate organic complex.

The use of phytase 50104 enzyme as an ingredient in poultry feed will increase the availability of phytate bound phosphorus in the animal diet (thereby, reducing the need for supplemental phosphorus in the animal diet) and will decrease the phosphorus contribution to manure, which results in the pollution of surface water.

3. Amount of other substance that is present with the notified substance either naturally or due to its manufacture

It is expected that the raw materials used in the fermentation and recovery steps of the manufacturing process for the phytase 50104 enzyme preparation will be consumed during fermentation and/or removed during the various downstream recovery steps in the manufacturing process (see Part 2 Section B.2.d).

In general, the major portion of the raw materials that (b) (4) . A more detailed explanation is provided below.

The first step of the recovery process (b) (4) (see Figure 9). (b) (4) ((b) (4) (b) (4) .

Therefore, [redacted] (b) (4)

Figure 9. [redacted] (b) (4)

* may contain trace amounts of [redacted] (b) (4)

[redacted] (b) (4) Therefore, the maximum residual [redacted] (b) (4) concentration in the animal feed is 15 ng of [redacted] (b) (4) per kg of feed (15 ppt).

To determine the worst-case maximum dietary exposure in poultry to any potential residual [(b) (4)] arising from the use of phytase 50104 enzyme preparation, we are utilizing the typical daily intake (204 g of feed/day) and the typical body weight (2782 g) of 42 day old broiler chicken (Ross, 2019). For poultry, broiler chickens are considered a worst case due to the ratio of typical feed intake versus body weight. Therefore, based on 0.000015 mg [(b) (4)]/kg feed and a diet of 0.204 kg feed/day, the worst-case maximum dietary exposure results in an [(b) (4)] intake of 0.00000306 mg [(b) (4)]/day. In terms of TOS, the dietary intake of [(b) (4)] is 0.000011 mg TOS/kg – bw/day. Please see Table 7.

The safety margin is calculated using the NOAEL from the subchronic (90-day) oral toxicity study (in terms of TOS) and the dietary intake of [(b) (4)] (in terms of TOS); the calculated safety margin is 15,637,386. The safety margin calculation indicates that the worst-case potential animal exposure to potential residues of [(b) (4)] resulting from the use of the phytase 50104 enzyme preparation is well below the NOAEL observed in the oral toxicity studies. Please note that the test article used to determine the safety of phytase 50104 enzyme was prepared following a process representative of the manufacturing process for the commercial enzyme, up to but not including, the final formulation step, and was lyophilized (see Part 6 Section G.1). Therefore, if residues of [(b) (4)] were present in the test article, the residual [(b) (4)] in the test article would be more concentrated than residual [(b) (4)] in the final, formulated product. Additionally, the utility studies conducted in poultry, as described in Part 2 Section D, used phytase 50104 enzyme preparation (i.e., CIBENZA® PHYTAVERSE® G10 Phytase Enzyme) that was manufactured using a process that was representative of the commercial manufacturing process. The animals in those studies did not show any adverse effects. Consequently, there are no safety concerns regarding dietary exposure to any potential residues of [(b) (4)] resulting from use of the phytase 50104 enzyme preparation.

Table 7. Potentia [(b) (4)] intake estimate and safety margin in broilers

Body Weight (kg)	kg/feed/day	[(b) (4)]		Highest expected [(b) (4)] intake		Safety Margin (NOAEL**/highest intake)
		mg/kg feed	mg TOS*/kg feed	mg/day	mg TOS/kg – bw/day	
2.782	0.204	0.000015	0.0015	0.000306	0.000011	15,637,386

* For a worst-case scenario, it is assumed that there is approximately 7.5 ng of [(b) (4)] per 1000 U of phytase activity and that any residues of [(b) (4)] would be in the TOS of the phytase 50104 enzyme preparation. Therefore, [(b) (4)] makes up 0.0001% of the total TOS.
* *The NOAEL for the 90-day oral toxicity study is 1720 mg TOS/kg/day.

B. Human Exposure

1. Potential human exposure to residues in edible animal tissues

a) Residues of the notified substance

Phytase 50104 enzyme is a protein and, like any protein, is expected to be digested into its amino acid constituents in the animal's gastro-intestinal (GI) tract. When the enzyme is digested in the GI tract, it will be broken down into its amino acid constituents making it indistinguishable from other food molecules making the potential for residues in edible animal tissue minimal (Association of American Feed Control Officals (AAFCO), 2020a).

b) Residues of any other substance that is expected to be formed in or on the animal food because of the use of the notified substance

Phosphorus is an essential nutrient to growing animals because it is important for bone formation, bone mineralization, cell metabolism, protein synthesis and is a constituent of cell membranes and intracellular buffers for acid alkaline balance. The phytase 50104 enzyme liberates phosphorus by cleaving the ortho-phosphate groups from the phytate organic complex and frees dietary phosphorus for use. Any liberated phosphorus resulting from the use of the phytase 50104 enzyme preparation is expected to be utilized by the animal.

c) Residues from any other substance that is present with the notified substance whether naturally, due to its manufacture, or produced as a metabolite in edible animal tissues when the notified substance is consumed by a food-producing animal

[redacted] (b) (4) during fermentation to induce the production of phytase 50104 enzyme. It is expected that [redacted] (b) (4)

PART 4: SELF-LIMITING LEVELS OF USE

This part is not applicable. There are no self-limiting levels of use associated with CIBENZA® PHYTAVERSE® L10 Phytase Enzyme and CIBENZA® PHYTAVERSE® G10 Phytase Enzyme that would result in the animal food being unpalatable or technologically impractical.

PART 5: EXPERIENCE BASED ON COMMON USE IN FOOD BEFORE 1958

This part is not applicable. The statutory basis for the notifier's conclusion of GRAS status is based on scientific procedures in accordance with 21 CFR §570.30(a).

PART 6: NARRATIVE

A. Introduction

To assure that the phytase 50104 enzyme preparation (including product forms CIBENZA® PHYTAVERSE® L10 Phytase Enzyme and CIBENZA® PHYTAVERSE® G10 Phytase Enzyme) is safe for its intended use, BASF has had every aspect of the manufacturing process (used to produce the phytase here in question) and the finished phytase products carefully and thoroughly assessed by various appropriately qualified and experienced experts. As the following subsections demonstrate (and discuss in significant detail), BASF's production organism and the phytase 50104 enzyme preparation (i.e., CIBENZA® PHYTAVERSE® L10 Phytase Enzyme and CIBENZA® PHYTAVERSE® G10 Phytase Enzyme) are safe for their intended uses.

B. Safety of Phytase

1. History of safe use

Enzymes have a long history of use in animal foods. As early as the 1920's, researchers showed beneficial effects from poultry feeds supplemented with enzymes (Burnett, G.S., 1962; Fry, R.E. *et al.*, 1958; Hastings, W.H., 1946; Jensen, L.S. *et al.*, 1957; Moran, J.E.T. and McGinnis, J., 1968; Pettersson, D.G., H.; Aman,P., 1990). Phytase was first added to poultry food during a chick study in 1968 (Nelson, T.S. *et al.*, 1968a; Nelson, T.S. *et al.*, 1968b). In the 1980s, Europe's poultry industry saw visible benefits with the use of feed enzymes, specifically xylanases and β-glucanases (Bedford, M.R. and Partridge, G.G., 2010). The 1990's introduced the next major breakthrough in feed enzymes, phytases (Bedford, M.R. and Partridge, G.G., 2010). Today, a wide variety of enzymes are used in animal food and a selection are listed in the 2020 AAFCO OP, specifically in Table 30.1 and in Section 101 (Association of American Feed Control Officals (AAFCO), 2020b; Association of American Feed Control Officals (AAFCO), 2020c). Most recently, FDA CVM has reviewed and issued a No Questions letter for a GRAS Notice on ground grain obtained from a corn variety that expresses an altered *appA* 6-phytase from *E. coli* K-12 (GRAS Notice No. AGRN 27) (FDA Center for Veterinary Medicine, 2019).

Of the enzymes listed in the 2020 AAFCO OP and listed on FDA CVM's Current Animal Food GRAS Notices Inventory, 12 are phytases. Five of these twelve phytases are derived from *E. coli*. More specifically, four of these are *E. coli* K-12 based phytases (all of which are protein

engineered). The first of these was approved by FDA CVM in 2008 through regulatory discretion, and the most recent was reviewed by FDA CVM in 2019 through their GRAS Notification program (GRAS Notice No. AGRN 27).

As is evident, feed enzymes have had a very long history of safe use, and phytases, specifically, have had nearly three decades of safe use in animal food. *E. coli* K-12 based phytases have had a decade of safe use in animal food.

2. Assessment of allergenic potential

The ingestion of food enzymes in general is not considered to be a concern with regard to food allergy (Bindslev-Jensen, C. *et al.*, 2006), and human allergic response to common animal food proteins have not been reported to occur as a result of consuming animal products (Pariza, M.W. and Cook, M., 2010).

Rather, if an allergy were to develop, it would likely result only from inhalation of an enzyme in aerosol or solid form. Therefore, the potential allergenicity of animal food enzymes is limited to occupational settings, i.e., manufacturing and handling (both in producing the enzyme and in adding the enzyme to animal feed) (Pariza, M.W. and Cook, M., 2010). This potential allergenicity has been addressed for the CIBENZA® PHYTAVERSE® L10 and G10 Phytase Enzyme products via their Safety Data Sheets (SDSs).

The allergenic potential of the protein (via the oral route) should be assessed (FAO/WHO, 2001; FAO/WHO, 2009; Ladics, G.S. *et al.*, 2011). A comparison of the amino acid sequence of the modified protein to known protein allergens is one step in a multilevel decision tree to assess allergenic potential (Metcalfe, D.D. *et al.*, 1996).

As recommended by the Joint FAO/WHO Expert Commission, amino acid sequence homology searches comparing the structure of a newly expressed protein and the stepwise, contiguous, identical amino acid segments with all known allergens is an approach for the assessment of allergenic potential (FAO/WHO, 2009). Two such searches were conducted using phytase 50104 protein as the query sequence. A FASTA search to predict overall structural similarities and a search scanning each possible 80 amino acid segment (1-80, 2-81, 3-82, etc.) looking for matches of at least 35% identity were performed against the Food Allergy Research and Resource Program (FARRP) database. The FASTA search results demonstrated that phytase 50104 protein does not have any significant homology to the allergens in the database. The scan of each possible 80 amino acid segment showed that there were no cases where the homology

exceeded 35% identity. This demonstrates that phytase 50104 protein shares no significant amino acid homology with known protein allergens that are present in the current version (2013) of the FARRP database. Based on this analysis, allergenicity (via the oral route) should not occur when using phytase 50104 enzyme.

C. Safety of the Production Organism

As discussed in Pariza and Foster (Pariza, M.W. and Foster, E.M., 1983), Pariza and Johnson (Pariza, M.W. and Johnson, E.A., 2001), and Pariza and Cook (Pariza, M.W. and Cook, M., 2010), the three papers that set forth the gold standard used by the enzyme industry for assessing the safety of enzyme products, the primary consideration in the evaluation of microbial enzyme preparations to be used in human and animal food is the safety of the production organism. This section addresses the safety of the phytase production strain *P. fluorescens* BD50104, whose recipient and parental strains are *P. fluorescens* DC454 and *P. fluorescens* Biovar I, MB101, respectively. Please see Figure 11 in Part 6 Section E for the Pariza and Johnson Decision Tree safety assessment of the phytase 50104 enzyme that is in the products of commerce, CIBENZA® PHYTAVERSE® L10 Phytase Enzyme and CIBENZA® PHYTAVERSE® G10 Phytase Enzyme.

1. History of safe use

P. fluorescens is a common and well-known saprophyte and potential plant pathogen that inhabits plant rhizosphere and phyllosphere environments (OECD, 1997). The microorganism has been used in a variety of industrial applications (Warren, G.J., 1987; Wilson, M. and Lindow, S.E., 1993) to produce biological pesticides (Chew, L. *et al.*, 2005; Herrera, G. *et al.*, 1994) and in the control of diseases in the phyllosphere of plants (Wilson, M. and Lindow, S.E., 1993). The U.S. Environmental Protection Agency (EPA) established an exemption from the requirement of tolerance for residues of *P. fluorescens* in or on the raw agricultural commodity mushrooms (EPA, 1994). More recently, the U.S. EPA issued an exemption from the requirements of tolerance for residues of *P. fluorescens* strain CL145A, which is also a Biovar I strain, in or on all food commodities when applied as a molluscicide (EPA, 2011).

Additionally, three derivatives of *P. fluorescens* Biovar I, strain MB101 have been reviewed by GRAS Panels and/or by the US FDA and found to be safe microorganisms for the production of an alpha-amylase enzyme (GRN 000126), a lipase enzyme (GRN 000462), and a phospholipase C enzyme (GRN 000574) used in food production (FDA Center for Food Safety

and Applied Nutrition, 2003a; FDA Center for Food Safety and Applied Nutrition, 2013; FDA Center for Food Safety and Applied Nutrition, 2015). The alpha-amylase enzyme preparation that was the subject of GRN 000126 was also reviewed by FDA CVM for its use in corn processing applications in which by-products are used in animal feeds. FDA CVM concluded in a regulatory discretion letter (RDL) that animal consumption of feed containing the by-products from food processing applications and ethanol production facilities, that use this alpha-amylase product, did not present an animal safety concern.

Lastly, the French Food Safety Authority (AFSSA) also evaluated the above-mentioned microbial derivative for alpha amylase that was the subject of GRN 000126 and issued an opinion letter (AFSSA, 2006) concluding that the AFSSA "considers that the use of alpha-amylase produced by the genetically-modified strain *Pseudomonas fluorescens* Biovar I presents no health risk for the consumer, under the conditions of use presented by the applicant."

In summary, all of the derivatives of MB101 discussed above contribute to the history of safe use and the safe strain lineage of *P. fluorescens* BD50104 (see Part 6 Section C.3 below for further information).

2. Absence of pathogenicity and toxicity

Strains of *P. fluorescens* are commonly found on plant surfaces, as well as decaying vegetation, soil, and water (Balows, A., 1992). The ubiquitous nature of *P. fluorescens* on the surface of plants typically grown for human consumption (OECD, 1997) suggests that *P. fluorescens* has been widely consumed by humans for many years. *P. fluorescens* has not been reported to be a caustic agent of human food poisoning or other disease related to food ingestion (EFSA and ECDC, 2017; FDA, 2018), and in the specific case of derivatives of *P. fluorescens* strain MB101, i.e., the parental strain of *P. fluorescens* BD50104, have been used safely as production organisms for enzymes used in food production for over the last 10 years (AFSSA, 2006; FDA Center for Food Safety and Applied Nutrition, 2003a; FDA Center for Food Safety and Applied Nutrition, 2013; FDA Center for Food Safety and Applied Nutrition, 2015).

In 1997, OECD evaluated the available literature of *Pseudomonas* used in the assessment of environmental applications involving *Pseudomonas* species. *P. fluorescens* is generally considered to be a saprophyte and potential plant pathogen that inhabits plant rhizosphere and phyllosphere environments. *P. fluorescens* can infect a wide range of animals including horses, chickens, marine turtles, and many fish and invertebrate species. However, because *P. fluorescens*

cannot grow at elevated temperatures like that of the human body, it is unlikely to be more than a rare opportunistic pathogen for warm-blooded animals. *P. fluorescens* can be an opportunistic pathogen in cancer patients and others who are severely immunocompromised but is of little concern for immunocompetent individuals. Fluorescent pseudomonads have not been reported to be potent allergens; however, they do possess a lipopolysaccharide that may cause an allergic response in some individuals (OECD, 1997).

More recently, EFSA evaluated available literature related to the safety of *P. fluorescens* following a recommendation for a Qualified Presumption of Safety[3] (QPS) status (EFSA BIOHAZ Panel *et al.*, 2017). EFSA noted, similar to the references above, that *P. fluorescens* is considered to be an opportunistic pathogen, involved in acute nosocomial infections (Center for Disease Control, 2005; Center for Disease Control, 2006). *P. fluorescens* colonisation was found in immunocompromised individuals (i.e., lung transplant recipients) (Dickson, R.P. *et al.*, 2014). Production of bioactive secondary metabolites, haemolysins, siderophores, type III secretion system, the ability to form biofilms and to adapt to growth at higher temperatures are functional features that have been associated with the ability to cause disease in humans (Mazurier, S. *et al.*, 2015; Scales, B.S. *et al.*, 2014). Moreover, *P. fluorescens* produces pseudomonic acids such as mupirocin, which is used for prevention of methicillin-resistant *Staphylococcus aureus* infections (Sutherland, R. *et al.*, 1985). Based on the evaluation, EFSA declined QPS status to *P. fluorescens* (EFSA BIOHAZ Panel *et al.*, 2017).

Internal literature reviews were also conducted to evaluate the safety of *P. fluorescens*. These evaluations did not reveal any new information than what has already been found by OECD and EFSA.

In addition to the literature reviews described above, *in vivo* studies have been conducted with *P. fluorescens* Biotype A[4]. The U.S. EPA conducted two *in vivo* studies to evaluate the possible health concerns associated with the use of *P. fluorescens* as a microbial pest control agent (George, S.E. *et al.*, 2000; George, S.E. *et al.*, 1999). The results of the study by George and co-workers (George, S.E. *et al.*, 1999) demonstrated that *P. fluorescens* (ATCC® 13525™, a Biotype A strain) was eliminated from the lungs, cecum, small and large intestine by two days post-

[3] The QPS assessment was developed to provide a harmonized, generic pre-assessment to support safety risk assessments performed by EFSA's Scientific Panels. Microorganisms given QPS status have reduced regulatory burden in future submissions made to EFSA.
[4] Under current taxonomic standards, *P. fluorescens* Biotype A is equivalent to *P. fluorescens* Biovar I.

treatment. *P. fluorescens* was detected in the liver and mesenteric lymph node three hours after treatment but had disappeared completely from the tissue within two days of treatment. No mortality in the mice was noted at bacterial concentrations as high as 5.0 x 10^8 CFU/mouse although some mortality was observed at excessively high (~10^9/mouse) bacterial concentrations.

In the second study, male CD-1 mice were treated perorally with an average dose of 1.78 x 10^8 CFU of *P. fluorescens* (ATCC® 13525™, a Biotype A) per mouse (George, S.E. *et al.*, 2000). *P. fluorescens* was recovered in the intestinal tract after three hours but was completely cleared after the first day. *P. fluorescens* was detected in the lungs, intestinal tract (small, large, cecum), mesenteric lymph node (MLN), spleen, and liver three hours after treatment but had completely cleared from all organs and tissues two days after treatment. At the completion of the study, all mice treated with *P. fluorescens* appeared healthy and conventional indicators of morbidity, such as ruffled fur, lethargy, weight loss, conjunctivitis, were not present.

Moreover, the pathogenicity and toxigenic potential of orally administered *P. fluorescens* biovar I, strain MB101 was evaluated in Balb/c mice (Landry, T.D. *et al.*, 2003). (Please note that strain MB101 is the parental strain of *P. fluorescens* BD50104.) Test material was administered by oral gavage in a suspension of bacteria formulated to contain 6 x 10^8 or 1 x 10^8 CFU per mouse. Suitable control groups were included for comparison. Mice were held for up to 21 days, with daily general observations of health. Subgroups of six bacteria-treated mice underwent necropsy on days two, four, and seven; and liver, spleen, MLN, large bowel, small bowel, and cecum were sampled for measuring bacteria. A subgroup of control mice underwent necropsy on day one.

The ability of the test strain, MB101, to infect mice was measured by the recovery of the dosed strain from selected organs and tissues. Oral exposure of *P. fluorescens* resulted in detectable levels of pseudomonads in all mice examined, although significant heterogeneity was noted on day two in the number of CFU recovered on the selective medium within each subgroup of mice. No mortality was observed over a 21-day period following oral administration. Infection with *P. fluorescens* did not result in any clinical signs of morbidity such as ruffled fur or lethargy during the 21-day period. The animals appeared healthy and did not exhibit weight loss, as the body weights of the infected animals were not significantly different from the uninoculated controls. Oral administrations of high doses of *P. fluorescens* biovar I strain MB101 resulted in the translocation of the test strain to the MLN, spleen, and liver of adult male Balb/c mice. The test strain did not appear to be infectious, and the microorganisms were eliminated from these

tissues within four days of exposure. Microorganism capable of growth on Pseudomonas Isolation Agar (PIA) plates were also detected in the bowels and ceca. Elimination of the test strain from the bowels and cecum was difficult to discern, since the normal microbial flora of the uninoculated control mice produced a high level of background CFU on PIA plates.

Bacterial clearance requires an intact and functional immune system that incorporates a cascade of immune responses. In this animal model, bacterial clearance provided an indication of the interaction between the potential pathogenicity of the invading microorganism and the total host immune capability. Similar results were reported by George *et al.* (2000): there was a rapid clearance of *P. fluorescens* from the MLN, spleen, and liver in male CD-1 mice treated orally with high levels (~10^8 CFU/mouse) of this microbial agent. George *et al*. noted some mortality at extremely high levels (~10^9 bacteria/mouse) following *intra nasal* administration.

Additionally, published (Pieniazek, J. *et al.,* 2017) and corroborative utility studies conducted with the granular formulation of the phytase 50104 enzyme preparation (i.e., CIBENZA® PHYTAVERSE® G10 Phytase Enzyme) demonstrated that the product is safe for use in poultry. Please see Part 2 Section D for more information on these studies.

Lastly, toxicology and genotoxicity tests conducted using enzyme preparations produced by *P. fluorescens* MB101 derivatives have determined that the test materials do not contain toxic or genotoxic substances (FDA Center for Food Safety and Applied Nutrition, 2015; Halich, R. *et al.*, 2012; Landry, T.D. *et al.*, 2003). Toxicology and genotoxicity studies were conducted using test material of the phytase 50104 enzyme produced *P. fluorescens* BD50104 (e.g., lyophilized phytase 50104 enzyme preparation without formulation ingredients also known as VR003). These studies also demonstrate that the test material does not contain any toxic or genotoxic substance (Krygier, S. *et al.*, 2014; Krygier, S. *et al.*, 2015).

In summary, the lack of pathogenicity and the lack of toxicity noted above in the published *in vivo* studies demonstrate that *P. fluorescens* Biovar I strains, including those strains derived from *P. fluorescens* Biovar I MB101, are non-toxigenic and non-pathogenic.

3. Safe strain lineage

The production organism used to produce the phytase 50104 enzyme preparation in CIBENZA® PHYTAVERSE® L10 and CIBENZA® PHYTAVERSE® G10 Phytase Enzyme products, i.e., *P. fluorescens* BD50104, is derived from a safe strain lineage originating from *P. fluorescens* MB101.

As described above in Part 6 Sections C.1 and C.2, *P. fluorescens* is non-pathogenic and non-toxigenic. More specifically, *P. fluorescens* MB101 has been found to be non-pathogenic and non-toxigenic (Landry, T.D. *et al.*, 2003). The genotoxicity and oral toxicity studies conducted repeatedly on the enzyme preparations produced using MB101 and its derivatives as the host organisms (including the studies conducted on the phytase 50104 enzyme that is the subject of this AGRN) (FDA Center for Food Safety and Applied Nutrition, 2015; Halich, R. *et al.*, 2012; Krygier, S. *et al.*, 2014; Krygier, S. *et al.*, 2015; Landry, T.D. *et al.*, 2003) confirm that *P. fluorescens* MB101 and its derivatives are non-toxigenic. These enzyme preparations have been assessed by the Pariza and Johnson Decision Tree and were the subject of regulatory submissions (AFSSA, 2006; FDA Center for Food Safety and Applied Nutrition, 2003a; FDA Center for Food Safety and Applied Nutrition, 2013; FDA Center for Food Safety and Applied Nutrition, 2015). MB101 and its derivatives have been used safely as production organisms for food enzymes. These data support and establish the safe strain lineage originating from *P. fluorescens* MB101 as described in Pariza and Cook (2010). (Please see Figure 10 and Table 8.)

Figure 10. Safe strain lineage originating from *P. fluorescens* MB101

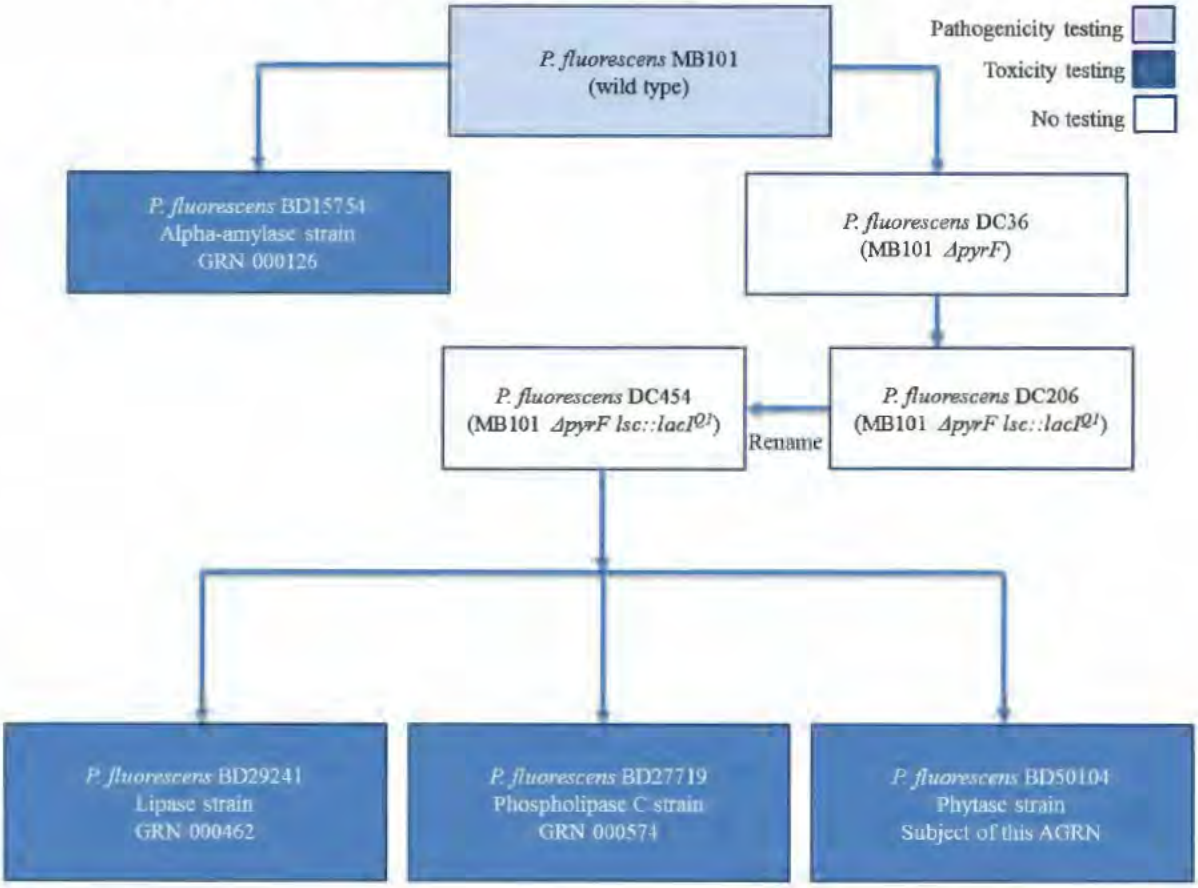


Table 8. Human and animal food enzymes derived from *P. fluorescens* MB101 strain lineage

Enzyme	Production Organism	Recipient Strain	Safety Studies	Published Studies	Current Use
Alpha-amylase	*P. fluorescens* BD15754[a]	*P. fluorescens* MB101	Ames assay; chromosomal aberrations assay, *in vitro*; mouse micronucleus assay, *in vivo*; acute oral toxicity in rats; DRF oral toxicity (14-day) in rats); subchronic (90-day) oral toxicity in rats	Yes (Landry, T.D. *et al.*, 2003)	Human food (GRN 000126)
Lipase	*P. fluorescens* BD29241	*P. fluorescens* DC454	Ames assay; chromosomal aberrations assay, *in vitro*; mouse micronucleus assay, *in vivo*; acute oral toxicity in rats; DRF oral toxicity (14-day) in rats); subchronic (90-day) oral toxicity in rats	Yes (Halich, R. *et al.*, 2012)	Human food (GRN 000462)
Phospholipase C	*P. fluorescens* BD27719	*P. fluorescens* DC454	Ames assay; chromosomal aberrations assay, *in vivo*; subchronic (90-day) oral toxicity in rats	No[b]	Human food (GRN 000574)
Phytase	*P. fluorescens* BD50104	*P. fluorescens* DC454	Ames assay; chromosomal aberrations assay, *in vitro*; mouse micronucleus assay, *in vivo*; acute oral toxicity in rats; subchronic (90-day) oral toxicity in rats	Yes (Krygier, S. *et al.*, 2014; Krygier, S. *et al.*, 2015)	Subject of this AGRN for animal food

[a] The production organism is also known as *P. fluorescens* DC88 or BD5088.
[b] No genotoxicity or oral toxicity effects were noted in any of the studies. Results of the safety studies are summarized in GRN 000574.

D. Safety of the Donor Organism

1. Introduction

This discussion addresses the safety of the bacterium *Escherichia coli* K-12 strain MG1655 (CGSC strain # 6300 /ATCC® 47076™) used as the donor organism of the phytase gene and the (b) (4) gene used to produce the phytase 50104 enzyme preparation here in question. Specifically discussed are the origin and taxonomy of the strain, its pathogenic/toxigenic potential, and a risk

assessment of the intended use of this bacterium as reported in the scientific literature and elsewhere.

2. Taxonomy

Escherichia coli is arguably the most well-studied bacterial species because of its extensive use in studies of physiology, genetics and biochemistry. This species, as well as the family to which it belongs, i.e., Enterobacteriaceae, are found throughout the world in water, soil and, importantly, as normal intestinal flora in humans and other animals (Bettelheim, K.A., 1992).

Enterobacteriaceae are Gram-negative, oxidase-negative, straight, rod-shaped bacteria that do not produce spores. They are chemoorganotrophic and are capable of both respiratory and fermentative metabolism. Growth temperatures range from 22-39^{o}C. Currently, there are 29 recognized genera and over 100 named species (Brenner, D., 1992).

Escherichia coli was first described in 1885 by Theodore Escherich after isolation from the feces of neonates. Since that initial description, *E. coli* has been considered to be a major commensal organism of the large intestine, representing about 1% of the total fecal bacterial population (Muhldorfer, I. *et al.*, 1996). As a result, this microorganism is always likely to be found in sewage and is, thus, an indicator microorganism for assessing the level of fecal contamination found in water for human consumption (American Water Works Association, 2006).

Historically, the classification of strains, until the advent of modern molecular techniques, was largely founded on the basis of serological determinations made using cell surface antigens. In more recent years, the phylogenetic characterization of strains of *E. coli* have been more precisely established by using changes in the primary structure of DNA, RNA, or proteins as indicators of relatedness. In addition, such phylogenetic relationships can also be inferred by the determination of the presence or absence of gene sequences, which reflect the current understanding of the fluid nature of bacterial genomes that occurs as a result of horizontal transmission.

3. Laboratory use of *E. coli* K-12

E. coli strains have been used for the last 60 years in the study of bacterial physiology and genetics. The two most commonly used in the early molecular studies of this organism were two wild-type strains called K-12 and B. Historically, strain K-12 was used in early experiments on

conjugation and recombination while strain B was used for the study of phage biology and genetics (Swartz, J.R., 1996). The use of strain K-12 eventually came to predominate due to its use in the study of recombination and the generation and mapping by conjugation of a large number of mutants in metabolic pathways that aided both the studies of bacterial genetics and physiology. Since *E. coli* K-12 has been widely used extensively in research and in many laboratories throughout the world for decades without inducing any harm, *E. coli* K-12 is generally recognized by experts as safe.

4. Risk assessment of *E. coli* K-12

Although there has been no indication over the sixty years of intensive laboratory study that strain K-12 has the ability to cause disease or have toxigenic potential, it has been only recently that explicit studies in regard to this issue have been carried out.

These studies have focused predominantly on the determination of the presence or absence of known virulence factors, i.e., properties of a microorganism that may contribute to its pathogenic potential, since in recent years it has become apparent that certain *E. coli* strains clearly have the potential to cause disease. Accordingly, the description of the virulence factors of these bacteria has become an area of intense study. Examples of these virulence factors include:

1) capsular polysaccharides which can attenuate or modulate the immune response of the host organism;
2) extended lipopolysaccharide O-antigens (so called smooth strains) which can affect the ability of the complement pathway to promote cell killing and opsonization;
3) fimbriae or pili with the ability to promote specific attachment to epithelial surfaces in mucosal tissue;
4) non-fimbrial cell surface adhesions that promote intimate attachment with cell surfaces through interactions with host proteins;
5) exotoxins that modulate signal transduction pathways or affect cell motility and morphology; and
6) associated protein export pathways that allow for the direct injection of bacterial toxins into the cytoplasm of host cells.

In a study of *E. coli* strains including representatives of the K-12 strain, polymerase chain reaction (PCR) amplification demonstrated the absence of defined virulence genes that are present in known pathogenic isolates of this microorganism (Kuhnert, P. *et al.*, 1997). The authors

concluded that the K-12 strains commonly used in the laboratory are devoid of virulent factors and should be considered nonpathogenic.

A more direct study of the pathogenic potential of K-12 strains was conducted using both a BALB/c mouse and chick gut model. In this study, these two strains were found to be unable to express long-chain lipopolysaccharide (O-antigen) and were serum-sensitive (i.e., susceptible to complement killing). In addition, they were unable to persist or survive in selected mouse tissues or the gut. In the chick model, the two strains were unable to invade the spleen, which is a hallmark of *E. coli* strains able to cause systemic infections. The authors came to the conclusion that the K-12 strains do not possess the recognized pathogenic mechanisms and should be considered nonpathogenic (Chart, H. *et al.*, 2000).

As mentioned above, K-12 became the predominant microorganism of choice for recombinant DNA research because of the great deal of information about recombination and biochemical genetics that was developed using this strain. For this reason, a large body of information was developed that demonstrated that K-12 was safe for recombinant DNA use. Such information resulted in the NIH Guidelines (prepared by the United States National Institute of Health) listing K-12 as safe for recombinant use, as detailed in Appendix C-II-A of the NIH guidelines (NIH, 2019). Such information also resulted in U.S. EPA indicating that K-12 "has a history of safe use" (vis-à-vis recombinant use) (EPA, 1997). Thus, U.S. EPA listed *E. coli* K-12 as safe for use as a recipient microorganism in biotech activities. (40 CFR § 725.420).

5. Summary

In summary, a number of pieces of evidence and expert observations and conclusions demonstrate that the *E. coli* strain K-12 is officially recognized and considered by experts to be a safe organism with no demonstrated pathogenic/toxigenic properties, including:

1) The long-term use of this microorganism in numerous laboratories throughout the world with no reports of illness or disease as a result of its use;
2) The absence of genes encoding defined virulence factors as determined by PCR and other molecular methods;
3) The lack of pathogenic potential in both a mouse and chick animal model; and
4) The inclusion of this strain in the RG1 classification by the NIH Office of Biotechnology Activities and the Recombinant DNA advisory committee.

Finally, it should be noted that this submission refers to only two genes (i.e., the (b) (4) phytase gene and the (b) (4) gene) being used from *E. coli* strain K-12.

E. Safety of the Inserted Genetic Material

Pariza's and Johnson's decision tree for evaluating microbial enzyme safety (Pariza, M.W. and Cook, M., 2010; Pariza, M.W. and Johnson, E.A., 2001) asks several questions relating to the introduced DNA of the genetically modified production microorganism. The first question asks if the expressed enzyme product, which is encoded by the introduced DNA, has a safe history of use. While phytases, including *E. coli* based phytases, themselves do have a long history of safe use in animal food (see Part 6 Section B), the specific phytase of this GRAS Notification does not.

The decision tree then asks whether or not the No-Observed-Adverse-Effect-Level (NOAEL) for the test article in appropriate short-term studies is sufficiently high to ensure safety. The results of the safety studies pertinent to the phytase products can be found in Part 6 Section G, and the worst-case dietary exposure calculations are set forth in Part 3 Section A. The calculations verify that the NOAEL is sufficiently high to ensure safety (Krygier, S. *et al.*, 2014; Krygier, S. *et al.*, 2015).

The next question asks if the test article is free of transferable antibiotic resistance gene DNA. *P. fluorescens* BD50104 does not contain any antibiotic resistance genes, which has been confirmed by bioinformatics analysis and genomic sequencing (see Part 2 Section B.1.g). Additionally, no detectable antimicrobial activity was found in the phytase 50104 enzyme preparation (see Part 2 Section C.2). Furthermore, the expression plasmid (b) (4)_BD50104 is poorly mobilizable (see Part 2 Section B.1.f). For these reasons, the enzyme preparation made from *P. fluorescens* production strain BD50104 is free of transferable antibiotic resistance gene DNA.

The decision tree then asks whether all other introduced DNA is well-characterized and free of attributes that would render it unsafe for constructing microorganisms to be used in producing food-grade products. The sequences of the introduced DNA, expression vector (b) (4)_BD50104 and (b) (4) are known and their gene products are also known. Bioinformatics analysis was conducted on the expression vector. It was not found to contain genes that code for products that are homologous to known toxins or harmful factors. Bioinformatics analysis was also conducted on (b) (4) and its junction region and at its integration location on the

chromosome. None of the putative ORFs generated fortuitously, and none of the ORFs within the (b)(expression cassette encoded any toxins or harmful factors. In addition, the allergenic potential assessment results for the phytase 50104 protein and the toxicity study results using the test article, VR003, demonstrate that the introduced DNA is free of attributes that would render it unsafe for the proposed use.

The final question relevant to genetic modification that the decision tree asks is whether or not the introduced genetic material is randomly integrated into the chromosome. (b) (4) was integrated into the chromosome. The integration was targeted near the levansurcrase locus. The sequence bordering the integration site of the (b) (4)[1] was determined and showed that the (b) (4) was integrated into the chromosome near the *lsc* locus in the recipient strain DC454. The expression vector introduced into strain BD50104 is a self-replicating, extrachromosomal plasmid and thus is not likely to be integrated into the chromosome. Therefore, random integration into the chromosome is highly unlikely.

The answers to the above questions indicate that there are no safety concerns regarding the production strain and introduced DNA here in question; thus, the criteria used in the decision tree for evaluating the safety of a new enzyme (i.e., phytase 50104 enzyme) have been met. This is illustrated in Figure 11.

Figure 11. Pariza and Johnson decision tree

The following analysis is based on the Pariza and Johnson decision tree as adapted for animal feed by Pariza and Cook (Pariza, M.W. *et al.*, 2001; Pariza, M.W. *et al.*, 2010). Decision points that do not pertain are included for completeness but crossed out.
1. Is the production strain genetically modified? **YES** If yes, go to 2. If no, go to 6.
2. Is the production strain modified using rDNA techniques? **YES** If yes, go to 3. If no, go to 3b.
3. Issues relating to the introduced DNA are addressed in 3a-3e. 3a. Do the expressed enzyme product(s) which are encoded by the introduced the DNA have a history of safe use in food or feed? **No, this specific phytase does not have a history of safe use in food or feed. However, other phytases, including those derived from *E. coli*, do have a history of safe use in food or feed.** If yes, go to 3c. If no, go to 3b. 3b. Is the NOAEL for the test article in appropriate short-term oral studies sufficiently high to ensure safety? **YES** If yes, go to 3c. If no, go to 12. 3c. Is the test article free of transferable antibiotic resistance gene DNA? **YES** If yes, go to 3e. If no, go to 3d. ~~3d. Does the resistance gene(s) code for resistance to a drug substance used in treatment of disease agents in man or animal? If yes, go to 12. If no, go to 3e.~~ 3e. Is all other introduced DNA well characterized and free of attributes that would render it unsafe for constructing microorganisms to be used to produce feed-grade products? **YES** If yes, go to 4. If no, go to 12.
4. Is the introduced DNA randomly integrated into the chromosome? **NO** If yes, go to 5. If no go to 6.
~~5. Is the production strain sufficiently well characterized so that one may reasonably conclude that unintended pleiotropic effects which may result in the synthesis of toxins or other unsafe metabolites will not arise due to the genetic modification method that was employed? If yes, go to 6. If no, go to 7.~~
6. Is the production strain derived from a safe strain lineage, as previously demonstrated by repeated assessment via this evaluation? **YES, the production strain is derived from a safe strain lineage, as described in Part 6 Section C.3.** If yes, the test article is ACCEPTED. If no, go to 7. **The test article is ACCEPTED.**
~~7. Is the organism nonpathogenic? If yes, go to 8. In no, go to 12.~~
~~8. Is the test article free of antibiotics? If yes, go to 9. If no, go to 12.~~
~~9. Is the test article free of oral toxins known to be produced by other members of the same species? If yes, go to 11. If no, go to 10.~~
~~10. Are the amounts of such toxins in the test article below levels of concern? If yes, go to 11. If no, go to 12.~~
~~11. Is the NOAEL for the test article in appropriate oral studies sufficiently high to ensure safety? If yes, the test article is ACCEPTED. In no, go to 12.~~
~~12. An undesirable trait or substance may be present and the test article is not acceptable for food use. If the genetic potential for producing the undesirable trait or substance can be permanently inactivated or deleted, the test article may be passed through the decision tree again.~~

F. Safety of the Manufacturing Process

As described in Part 2, the phytase 50104 enzyme preparation, which is marketed as CIBENZA® PHYTAVERSE® L10 Phytase Enzyme and CIBENZA® PHYTAVERSE® G10 Phytase Enzyme products, is made using generally known and accepted methods for the production of microbial enzymes (Aunstrup, K. *et al.*, 1979; Pariza, M.W. and Foster, E.M., 1983). In addition, the CIBENZA® PHYTAVERSE® L10 Phytase Enzyme and CIBENZA® PHYTAVERSE® G10 Phytase Enzyme products are manufactured in accordance with both current Good Manufacturing Practices (cGMP) for animal food and the 1992 Organization for Economic Co-operation and Development's criteria for Good Industrial Large Scale Practice (GILSP) (OECD, 1997). The CIBENZA® PHYTAVERSE® L10 Phytase Enzyme and CIBENZA® PHYTAVERSE® G10 Phytase Enzyme products meet the purity requirements for enzyme preparation of *Food Chemicals Codex* and JECFA. Additionally, the published toxicity studies performed using VR003 (Krygier, S. *et al.*, 2014; Krygier, S. *et al.*, 2015) and the published utility studies (Pieniazek, J. *et al.*, 2017) further show that the manufacturing process, including the raw materials used, is safe for use in the production of an animal food enzyme. Accordingly, the manufacturing process for the CIBENZA® PHYTAVERSE® L10 Phytase Enzyme and CIBENZA® PHYTAVERSE® G10 Phytase Enzyme products should be deemed safe.

G. Safety Studies

As part of the safety assessment, genotoxicity, oral toxicity, and worker safety studies were conducted on the phytase 50104 enzyme test article. The test article production, the studies, and their results are described below in Part 6 Sections G.2, G.3, G.4.a and are published in *Safety evaluation of phytase 50104 enzyme preparation (also known as VR003), expressed in Pseudomonas fluorescens, intended for increasing digestibility of phytase in monogastrics* (Krygier, S. *et al.*, 2014; Krygier, S. *et al.*, 2015) (see Appendix 26).

1. Test article production – VR003 (phytase 50104 enzyme)

The test article used to determine the safety of phytase 50104 enzyme was prepared following a process representative of the manufacturing process (including the raw materials) for the commercial enzyme, up to but not including, the final formulation step. The raw materials were

of the same quality and quantity (relative to scale) for both the test article production process and for the commercial manufacturing process. The test article was produced in a Fermentation and Recovery Pilot Plant in accordance with current good manufacturing practices (cGMP) for animal food. The following SOPs were used to produce the test article: MP0346 for the fermentation protocol, MP0347 for the recovery protocol, MP0348 for the lyophilization protocol, and MP0323 for the milling and blending of phytase 50104 enzyme powder. The test article was analyzed for chemical and microbial composition to ensure conformance to the specifications for enzyme preparations, as outlined in the *Food Chemicals Codex*, 8th Edition (U.S.Pharmacopeial Convention, 2012), and the Joint FAO/WHO Expert Committee on Food Additives (FAO/WHO, 2006). The results from the chemical and microbial composition analysis are provided in Krygier *et al*. (2014 and 2015). The test article for phytase 50104 enzyme was designated as VR003 (and used in the safety studies discussed below in Part 6 Sections G.2, G.3, and G.4.a).

2. Genotoxicity studies

a) Bacterial reverse mutation assay (also referred to as the Ames assay)

The purpose of this study was to evaluate the mutagenic potential of the test article, VR003, by measuring its ability to induce reverse mutations at the histidine loci of several strains of *S. typhimurium* (TA98, TA100, TA1535 and TA1537) and at the tryptophan locus of *E. coli* strain WP2 uvrA in the presence and absence of Aroclor-induced rat liver S9. This study was conducted in compliance with ICH Guideline S2(R1) and OECD Guideline 471.

The assay was performed in two phases, using the plate incorporation method. The first phase, the initial toxicity-mutation assay, was used to establish the dose-range for the confirmatory mutagenicity assay and to provide a preliminary mutagenicity evaluation. The second phase, the confirmatory mutagenicity assay, was used to evaluate and confirm the mutagenic potential of the test article.

In the initial toxicity-mutation assay, the maximum dose tested was 5000 μg per plate; this dose was achieved using a concentration of 50 mg/mL and a 100 μL plating aliquot. The dose levels tested were 1.5, 5.0, 15, 50, 150, 500, 1500 and 5000 μg per plate. No positive mutagenic responses were observed with any of the tester strains in either the presence or absence of S9 activation. Increases in revertant counts (1.6- to 2.5-fold maximum increases) were observed with some test conditions. However, these increases were not considered to be indicative of mutagenic activity because the revertant counts at the peak of the responses were within the historical vehicle

control ranges for each tester strain. Neither precipitate nor toxicity were observed. Based on the findings of the initial toxicity mutation assay, the maximum dose plated in the confirmatory mutagenicity assay was 5000 μg per plate.

In the confirmatory mutagenicity assay, no positive mutagenic responses were observed with any of the tester strains in either the presence or absence of S9 activation. The dose levels tested were 50, 150, 500, 1500 and 5000 μg per plate. Neither precipitate nor toxicity were observed.

Under the conditions of this study, test article VR003 was concluded to be negative in the Bacterial Reverse Mutation Assay.

b) Chromosomal aberrations in cultured human peripheral blood lymphocytes

The purpose of this study was to evaluate the potential of VR003 to induce structural chromosomal aberrations in HPBL in the presence and absence of an exogenous metabolic activation system. This study was conducted using standard procedures (Evans, H.J. and O'Riordan, M.L., 1975; Galloway, S.M. *et al.,* 1994; Preston, R.J. *et al.*, 1981; Swierenga, S.H.H. *et al.,* 1991) and in compliance with OECD Guideline 473.

In the preliminary toxicity assay, the doses tested ranged from 0.5 to 5000 μg/mL. Substantial toxicity (at least 50% reduction in mitotic index relative to the vehicle control) was not observed at any dose level in the non-activated 4 and 20-h exposure groups. Substantial toxicity was observed at dose levels ≥50 μg/mL in the S9 activated 4-h exposure group. Based on these findings, the doses chosen for the chromosome aberration assay ranged from 350 to 5000 μg/mL for the non-activated 4- and 20-h exposure groups, and from 2.5 to 5000 μg/mL for the S9-activated 4-h exposure group.

In the chromosome aberration assay, substantial toxicity was not observed at any dose level in the non-activated 4-h exposure group. Substantial toxicity was observed at dose levels ≥150 μg/mL in the S9 activated 4-h exposure group and at dose levels ≥3500 μg/mL in the non-activated 20-h exposure group. The highest dose analyzed under each treatment condition either produced an approximately 50% reduction in mitotic index or was the highest dose tested in the definitive chromosome aberration assay, which met the dose limit as recommended by the OECD testing guidelines for this assay.

No significant or dose dependent increases in aberrant metaphases, or polyploidy or endoreduplicated cells, were observed in treatment groups with or without S9 ($p > 0.05$; Fisher's Exact and Cochran–Armitage tests). All vehicle control values were within historical ranges, and the positive controls induced significant increases in the percent of aberrant metaphases ($p \leq 0.01$). Thus, all criteria for a valid study were met.

These results indicate VR003 was negative in the *in vitro* chromosome aberration assay in HPBL under the conditions, and according to the criteria of the study protocol.

c) Mouse micronucleus assay

The objective of this study was to evaluate test article VR003 for *in vivo* clastogenic activity and/or disruption of the mitotic apparatus by detecting micronuclei in polychromatic erythrocytes (PCE) cells in mouse bone marrow (Heddle, J.A., 1973; Heddle, J.A. *et al.,* 1983; Schmid, W., 1975). This study was conducted in compliance with ICH Guideline S2(R1) and OECD Guideline 474.

In the dose range finding assay (DRF), the test article was formulated in distilled water with a maximum dose of 2000 mg/kg. The dose levels tested were 500, 1000 and 2000 mg/kg in three animals/sex/group and observed for up to 2 days after dosing for toxic signs and/or mortality. Based upon these results, the high dose for the definitive assay was selected to be 2000 mg/kg, which is the limit dose, based on the ICH and OECD regulatory guidelines.

The definitive assay dose levels tested were the same as the DRF: 500, 1000 and 2000 mg/kg. Since no differences in clinical signs of toxicity were observed between the sexes, only male mice were used for the definitive assay. Groups 1 and 4 consisted of 10 animals designated for either 24 or 48 h bone marrow collections and Groups 2, 3 and 5 consisted of 5 animals designated for 24 h bone marrow collection. Following scheduled euthanasia times, femoral bone marrow was collected; bone marrow slides were prepared and stained with acridine orange. Bone marrow cells [polychromatic erythrocytes (2000 PCEs/animal)] were examined microscopically for the presence of micronuclei (micronucleated PCEs; MPCEs) and statistical analysis of data was performed using the Kastenbaum–Bowman Tables (binomial distribution, $p \leq 0.05$). Scoring was based upon the micronucleated cell, not the micronucleus; thus, occasional cells with more than one micronucleus were counted as one micronucleated PCE (mnPCE), not two (or more) micronuclei. The ratio of polychromatic erythrocytes (PCEs) to total erythrocytes (EC) in the test

article groups relative to the vehicle control groups was also evaluated to reflect the test article's cytotoxicity.

The test article did not induce signs of clinical toxicity in the animals treated at dose levels up to 2000 mg/kg. The test article did not induce statistically significant increases in micronucleated PCEs at any test article dose (500, 1000, 2000 mg/kg). In addition, the test article was not cytotoxic to the bone marrow (i.e., did not produce statistically significant decreases in the PCE:NCE ratio) at any dose of the test article.

Under the conditions of this study, the administration of test article VR003 at doses up to and including a dose of 2000 mg/kg was concluded to be negative in the Micronucleus assay.

3. Oral toxicity studies

a) Acute oral toxicity study in the rat – up-and-down procedure

The purpose of this study was to assess the toxicity of test article VR003 following a single oral dose to the rat. The results of the study are believed to be of value in predicting the likely toxicity of the test article in man by the oral route. The study was conducted in compliance with OECD Guideline 425 and OPPTS Guideline 870.1100.

Initially, one female Sprague Dawley rat was dosed at 2000 mg/kg. No mortality was observed, and dosing continued in four additional females at 2000 mg/kg. A total of five females were dosed. Mortality checks were made once daily. Clinical observations were recorded prior to dosing, as well as at 30 min, 4 h, post-dose, and daily thereafter through Day 15. Body weights were recorded on the day of dosing (Day 1), and on Days 8 and 15. All rats were euthanized by CO_2 asphyxiation and necropsied on Day 15.

For the dose of 2000 mg/kg, no mortality was observed. All animals appeared normal throughout the study at 2000 mg/kg. No biologically relevant effect was observed in the body weights between Days 8 and 15; except one animal lost 7 g of weight between Days 8 and 15 and one animal did not have any change in weight between Days 8 and 15. Terminal necropsy revealed no visible lesions in any of the animals at 2000 mg/kg.

Based on the results of this study, the oral LD_{50} for test article VR003 in rats was estimated to be greater than 2000 mg/kg.

b) 90-day oral toxicity study in rats

The purpose of this study was to evaluate the toxicity of the test article, VR003, when administered orally, via gavage, once daily to Sprague Dawley rats for a minimum of 90 consecutive days (FDA, 2007; Gad, S.C., 1995; Speid, L.H. *et al.*, 1990). This study was conducted in compliance with OECD/OCDE Guideline 408.

The test article, VR003, was supplied by the Sponsor as a light brown lyophilized powder. The test article was then prepared into dosing formulations for oral administration via gavage. One hundred sixty experimentally naïve Sprague Dawley rats (80 males and 80 females), 6–7 weeks old and weighing 136–225 grams for males and females at the outset of the study were assigned to treatment groups.

Animals were dosed at 0, 500, 1000, and 2000 mg/kg once daily for 90 consecutive days. Mortality/morbidity was observed twice daily (a.m. and p.m.) on Day 1 to Day 90 and once prior to euthanasia on Day 91. Body weights were recorded at the time of randomization/selection, prior to dose administration on Days 1, 8, 15, 22, 29, 36, 43, 50, 57, 64, 71, 78, 85, and following the final dose on Day 90. Food consumption was recorded weekly. Ophthalmology examinations were performed before treatment initiation and during the final two weeks of treatment. Blood for evaluation of hematology, coagulation and clinical chemistry was collected prior to terminal sacrifice on Day 91. All surviving animals were sacrificed on Day 91. Selected tissues were harvested at necropsy, selected organs weighed, and selected tissues from the control and high dose groups and all animals that died early evaluated microscopically.

There was no test article-related mortality noted during this study. There were no clinical signs of toxicity noted during the study that were clearly related to the administration of VR003. There were no test article-related changes in group mean body weight or body weight gain for the 500 or 2000 mg/kg males or any of the female dose groups. A statistically significant decrease in group mean body weights was noted for the 1000 mg/kg males from Day 15 to 85. This was most likely due to a statistically significantly decreased group mean body weight gain for this group on Day 15 as well as reduced food consumption values throughout this time frame. The significance of this finding is unknown as a similar trend was not observed in the higher dose group.

The 2000 mg/kg males had statistically significantly decreased group mean food consumption values on Days 57, 64, 85 and 90 while the 2000 mg/kg females had statistically significantly decreased group mean food consumption values on Days 22, 29, and 90, but the group

mean bodyweights for both males and females were not different. The 1000 mg/kg males had statistically significantly reduced group mean food consumption values from Day 15–43 to 57–78 with a concomitant decrease in mean bodyweight. Since this was not a dose dependent trend, the significance is unknown.

There were no test article-related ophthalmological findings noted during the study. There were no test article-related changes in hematology parameters, red blood cell morphology or coagulation parameters. There were no test article-related changes in coagulation parameters.

Cholesterol values were statistically significantly increased for the 2000 mg/kg males. Sodium values were statistically significantly decreased for the 1000 and 2000 mg/kg males. Chloride values were statistically significantly reduced for the 2000 mg/kg males. The limited magnitude of these changes, the fact that they occurred in only one sex, as well as the fact that values were still within historical control values for the laboratory, therefore not considered relevant.

No test article-related macroscopic observations were noted at the terminal sacrifice on Day 91. All gross observations were considered incidental background findings of no toxicologic significance.

There were no definitive test article-related changes in group mean organ weight or organ to body or brain weight ratios.

No toxicologically important test article-related histopathological findings were noted in any tissue.

Based on the findings in this study, the No Observed Adverse Effect Level (NOAEL) following administration of 500, 1000 or 2000 mg/kg test article VR003 once daily by oral gavage for 90 days to Sprague Dawley rats is at least 2000 mg/kg. Findings at 2000 mg/kg were limited to minor changes in food consumption values on a few days during the 90-day dosing period and a few clinical chemistry changes for males that were minor in magnitude and within historical control values for the laboratory, therefore not considered relevant.

4. Worker safety studies

a) Using VR003 (phytase 50104 enzyme)

The test article, VR003, has been evaluated by independent testing laboratories for potential health hazards with respect to dermal exposure and eye exposure. These include a primary eye irritation study, a primary dermal irritation study, a delayed contact hypersensitivity

study. All studies conformed to Good Laboratory Practice Regulations as described in 40 CFR Part 492, OECD Principles of Good Laboratory Practice, and ENV/MC/CHEM(98)17. The results of these studies are summarized in the following table and are published in Krygier *et al*., 2014 and 2015 (see Appendix 26).

Table 9. Results of worker safety studies using VR003

Study	Guidelines for Study Design	Test Object	Concentration of VR003	Result
Primary eye irritation	OPPTS 870.2400 and OECD 405	New Zealand White rabbits (3 female)	10%	**EEC Irritation Rating:** Non-irritating **GHS Classification:** Non-irritating **Kay & Calandra Criteria:** Non-irritating
Primary dermal irritation	OPPTS 870.2500 and OECD 404	New Zealand White rabbits (3 males)	10%	**EEC Irritation Rating:** Non-irritating **GHS Classification:** Non-irritating **Primary Irritation Index:** 0.0
Delayed contact hypersensitivity (Buehler method)	OPPTS 870.2600; OECD 406; and EEC Methods for Skin Sensitization, Method B.6	Guinea pigs (10 male and 10 female)	10%	Did not elicit a delayed contact hypersensitivity response

5. Safety margin calculation

The safety margin calculation for poultry is discussed and provided in Part 3 Section A.1. Briefly, the safety margin calculations for poultry is 1675. The safety margins indicate that the worst-case potential animal exposure (poultry) to the phytase 50104 enzyme preparation is well below the NOAEL observed in the subchronic (90-day) oral toxicity study.

H. Safety of the CIBENZA® PHYTAVERSE® L10 Phytase Enzyme and the CIBENZA® PHYTAVERSE® G10 Phytase Enzyme

1. To animals

Phytase 50104 enzyme is a protein and, like any protein, is expected to be digested into its amino acid constituents in the animal's gastro-intestinal (GI) tract. When the enzyme is digested in the GI tract, it is broken down into its amino acid constituents making it indistinguishable from other food molecules; therefore, the potential for residues in edible animal tissue is minimal. The

primary safety concern is the possible presence of compounds produced or derived from the production organism (Association of American Feed Control Officals (AAFCO), 2020a).

Pariza and Foster (1983), Pariza and Johnson (2001), and Pariza and Cook (2010) are the three papers that set forth the gold standard used by the enzyme industry for assessing the safety of enzyme products. The primary consideration in the safety evaluation of microbial enzyme preparations to be used in human and animal food, in the Pariza decision tree and as noted in the AAFCO OP, is the safety of the production organism. The phytase 50104 enzyme preparation was evaluated according to the Pariza and Johnson decision tree as adapted for animal feed by Pariza and Cook (Pariza, M.W. and Cook, M., 2010; Pariza, M.W. and Johnson, E.A., 2001) (see Figure 11) and as briefly described below:

- The NOAEL for the test article in the oral toxicity studies is sufficiently high enough to ensure safety (see Krygier *et al*. (2014, 2015) and Part 6 Section G.5).
- The test article is free of transferable antibiotic resistance gene DNA (see Part 2 Sections B.1.f and B.1.g).
- All the introduced DNA is well characterized and free of attributes that would render it unsafe for the production organism to be used to produce feed-grade products (see Part 6 Section E).
- The introduced DNA is not randomly integrated in the chromosome (see Part 2 Section B.1).
- The production organism is derived from a safe strain lineage (see Part 6 Section C.3).

Therefore, the phytase 50104 enzyme preparation is found to be acceptable for use in animal food.

Additionally, as noted in Part 6 Section F, the products, CIBENZA® PHYTAVERSE® L10 Phytase Enzyme and CIBENZA® PHYTAVERSE® G10 Phytase Enzyme, are manufactured according to both cGMPs for animal food and the 1992 OECD criteria for GILSP. The products also meet the purity requirements for enzyme preparations as outlined in Food Chemicals Codex and JECFA.

Furthermore, *E. coli* based phytases have been proven to be efficacious for increasing the availability of phytin-bound phosphorus in poultry diets (Adeola, O. *et al.*, 2004; Onyango, E.M. *et al.*, 2005; Pillai, P.B. *et al.*, 2006; Ribeiro, V. *et al.*, 2016), and, therefore the utility of these

enzymes does not pose a safety concern. The phytase 50104 enzyme preparation is no different. As discussed in Part 2 Section D.1, the results of the poultry utility studies indicate and support the addition of CIBENZA® PHYTAVERSE® L10 Phytase Enzyme and CIBENZA® PHYTAVERSE® G10 Phytase Enzyme between 250 to 2000 U/kg of feed containing sub-optimal levels of non-phytate phosphorus.

Therefore, there are no safety concerns for animals (poultry) resulting from the use of the formulated enzyme products, CIBENZA® PHYTAVERSE® L10 Phytase Enzyme and CIBENZA® PHYTAVERSE® G10 Phytase Enzyme.

2. To humans

As shown in Figure 11 and as described briefly below, the phytase 50104 enzyme preparation has passed the safety assessment of Pariza and Johnson (Pariza, M.W. and Cook, M., 2010; Pariza, M.W. and Johnson, E.A., 2001) and is acceptable for use in animal food:

- The NOAEL for the test article in the oral toxicity studies is sufficiently high enough to ensure safety (see Krygier *et al*. (2014, 2015) and Part 6 Section G.5).
- The test article is free of transferable antibiotic resistance gene DNA (see Part 2 Sections B.1.f and B.1.g).
- All the introduced DNA is well characterized and free of attributes that would render it unsafe for the production organism to be used to product feed-grade products (see Part 6 Section E).
- The introduced DNA is not randomly integrated in the chromosome (see Part 2 Section B.1).
- The production organism is derived from a safe strain lineage (see Part 6 Section C.3).

Therefore, the products, CIBENZA® PHYTAVERSE® L10 Phytase Enzyme and CIBENZA® PHYTAVERSE® G10 Phytase Enzyme, do not pose any significant risk of harm to humans who consume edible products from animals that consume the phytase 50104 enzyme preparation.

As demonstrated by the worker safety studies conducted with the phytase 50104 enzyme preparation (see Part 6 Section G.4.a) and as further supported by the allergenic assessment of the phytase 50104 enzyme (see Part 6 Section B.3), the products, CIBENZA® PHYTAVERSE® L10

Phytase Enzyme and CIBENZA® PHYTAVERSE® G10 Phytase Enzyme, do not pose a significant risk of harm to humans who might come into physical contact with the products. However, notwithstanding this conclusion, all enzymes are considered respiratory sensitizers. Therefore, the Safety Data Sheet (SDS) for each product conveys the appropriate hazard communications including information on safe handling and personal protection.

I. Results and Conclusion

The phytase 50104 enzyme preparation, which is marketed as CIBENZA® PHYTAVERSE® L10 Phytase Enzyme and CIBENZA® PHYTAVERSE® G10 Phytase Enzyme, and is the subject of this GRAS Notification, is derived from a genetically modified strain of *P. fluorescens* DC454 that contains an expression vector, (b) (4) _BD50104, which includes the phytase 50104 gene.

BASF Enzymes LLC has determined the phytase 50104 enzyme preparation to be GRAS, through scientific procedures, when used as intended in animal food. The CIBENZA® PHYTAVERSE® L10 Phytase Enzyme product will be added in a post-pelleting application to complete pelleted feeds. The CIBENZA® PHYTAVERSE® G10 Phytase Enzyme product will be added to complete mash feeds, complete pelleted feeds, and premixes. The recommended level of supplementation of each product in a complete, poultry feed is 250 to 2000 U/kg of feed.

The safety of the phytase 50104 enzyme preparation has been evaluated using the safety scheme of Pariza and Johnson as adapted for animal feed by Pariza and Cook (Pariza, M.W. and Cook, M., 2010; Pariza, M.W. and Johnson, E.A., 2001) and others (FAO/WHO, 2006; International Food Biotechnology Council, 1990; OECD, 1997). Published and unpublished information is provided which assesses the safety of the following: recipient strain; introduced genetic material; production microorganism; phytases and their use in animal food; the manufacturing process; and the final, formulated phytase 50104 enzyme preparation.

The safety of the production organism is a prime consideration when assessing the probable degree of safety of an enzyme preparation intended for use in food. If the enzyme production organism is nonpathogenic and nontoxigenic, and the enzyme is made according to current good manufacturing practices (cGMP) for animal food, then one can conclude the food ingredient made from the production microorganism is safe to consume. *P. fluorescens* is well-characterized and complies with the OECD criteria for Good Industrial Large Scale Practice. *P. fluorescens* has been

used in a variety of industrial applications (Chew, L.C. *et al.*, 2005; Herrera, G. *et al.*, 1994; Warren, G.J., 1987; Wilson, M. and Lindow, S.E., 1993). The U.S. EPA established an exemption from the requirement of tolerance for residues of *P. fluorescens* in or on the raw agricultural commodity mushrooms (EPA, 1994). More recently, the U.S. EPA issued an exemption from the requirements of tolerance for residues of *P. fluorescens* strain CL145A, which is a Biovar I strain, in or on all food commodities when applied as a molluscicide (EPA, 2011). Furthermore, the production organism, BD50104, is derived from a safe strain lineage originating from *P. fluorescens* MB101. Derivatives of *P. fluorescens* Biovar I, strain MB101 have been reviewed by a GRAS Panel and/or by the U.S. FDA and were found to be safe microorganisms for the production of enzymes used in food production (FDA Center for Food Safety and Applied Nutrition, 2003a; FDA Center for Food Safety and Applied Nutrition, 2013; FDA Center for Food Safety and Applied Nutrition, 2015). The French Food Safety Authority (AFSSA) also evaluated one of the above-mentioned microbial derivatives and issued an opinion letter (AFSSA, 2006) concluding that the AFSSA "considers that the use of alpha-amylase produced by the genetically-modified strain of *Pseudomonas fluorescens* Biovar I presents no health risk for the consumer, under the conditions of use presented by the applicant."

The introduced DNA is well-characterized and shown to be safe, as further described in Part 2 Section B.1. Additionally, the production organism BD50104 is known to be free of antibiotic resistance markers. The modified phytase gene is derived from *E. coli* K-12. The published utility studies conducted with the granular formulation of the phytase 50104 enzyme preparation (i.e., CIBENZA® PHYTAVERSE® G10 Phytase Enzyme) demonstrated that the product is safe for use in poultry (Pieniazek, J. *et al.*, 2017). The utility studies further support that the introduced DNA is safe. The published toxicity studies performed using VR003 test article further show the introduced DNA is free of attributes that would render it unsafe for use in the production of an animal food enzyme (Krygier, S. *et al.*, 2014; Krygier, S. *et al.*, 2015).

The enzyme phytase has a long history of safe use in animal food. Phytases have been used in animal food for close to 40 years. Many phytase enzyme preparations are commercially available for use in animal food, several of which are protein engineered. The phytase 50104 enzyme preparation from *P. fluorescens* strain BD50104 is similar to other known microbial phytases used in animal food today, including the five other *E. coli* phytase products. Additionally,

like other *E. coli* based phytases the utility of the phytase 50104 enzyme preparation does not pose a safety concern for poultry (Pieniazek, J. *et al.*, 2017).

In assessing the safety of the phytase 50104 enzyme preparation, the following studies were conducted and published (Krygier, S. *et al.*, 2014; Krygier, S. *et al.*, 2015): acute oral toxicity study in rats; 90-day subchronic gavage in rats; chromosomal aberrations test in human lymphocytes, mouse micronucleus assay, and *Salmonella-Escherichia coli/* mammalian-microsome reverse mutation assay. The studies did not find any treatment related toxicity or induction of genetic mutation or chromosomal aberrations in tests using the phytase test preparations derived from the production microorganism. The safety margin calculation indicates the worst-case potential animal exposure to the phytase 50104 enzyme preparation is well below the NOAEL observed in the oral toxicity studies.

The manufacturing process used to make the phytase 50104 enzyme preparation employs a pure culture, submerged fermentation of the *P. fluorescens* production strain, BD50104. Current good manufacturing practice for food is used throughout the process which utilizes generally accepted, published methods for enzyme manufacture and formulation. All raw materials used in the fermentation and recovery processes are of suitable purity and are standard materials used in the enzyme industry. The final phytase 50104 enzyme preparation meet the purity requirements for enzyme preparations as outlined in Food Chemicals Codex and by JECFA. The published toxicity studies performed using VR003 (Krygier, S. *et al.*, 2014; Krygier, S. *et al.*, 2015) and the published utility studies (Pieniazek, J. *et al.*, 2017) further demonstrate that the manufacturing process, including the raw materials, is safe for use in the production of an animal food enzyme.

Based on the information provided in this GRAS Notification, BASF Enzymes LLC concludes that the phytase 50104 enzyme preparation derived from *P. fluorescens*, containing the pDOW1169_BD50104 expression vector that includes the phytase 50104 gene, is GRAS under the intended conditions of use, as specified herein. Additionally, an external expert in the field, Dr. Michael Pariza, also came to the same conclusion (see Appendix 27). Dr. Pariza was given a copy of the GRAS Notification **and** access to all information (including references and appendices) in support of such Notification – i.e., the same aggregate information relied on by BASF Enzymes LLC in reaching its GRAS conclusion. Dr. Pariza reviewed the information, had his questions answered, and then concluded that phytase 50104 enzyme preparation is GRAS, based on scientific procedures, for its intended use.

Please note that BASF Enzymes LLC has reviewed all available data and information and are not aware of any data and information that are, or may appear to be, inconsistent with our conclusion of GRAS status.

PART 7: LIST OF SUPPORTING DATA AND INFORMATION

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Please note, Appendices 1 - 8 and 10 - 12 contain confidential business information.

B. List of References

Please note all references and have been provided with this notice and are generally available.

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Appendix 1: Phytase 50104 Enzyme Amino Acid Sequence

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Appendix 2: Alignment of the Mature Amino Acid Sequences for Phytase 50104 Protein and the Native *E. coli* K12 and B AppA Proteins

Appendix 3: Phytase 50104 Gene Nucleotide Sequence

Appendix 4: Alignment of the Mature Amino Acid Sequences for Phytase 50104 Protein and the Native *E. coli* K12 AppA Protein

Appendix 5: Bioinformatics Analysis of Plasmid [redacted] (b) (4) _BD50104

Bioinformatics Analysis of Plasma [redacted] (b) (4)

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Bioinformatics Analysis of Plasmid [redacted] (b) (4) _BD50104

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Bioinformatics Analysis of Plasmid [redacted] (b) (4) _BD50104

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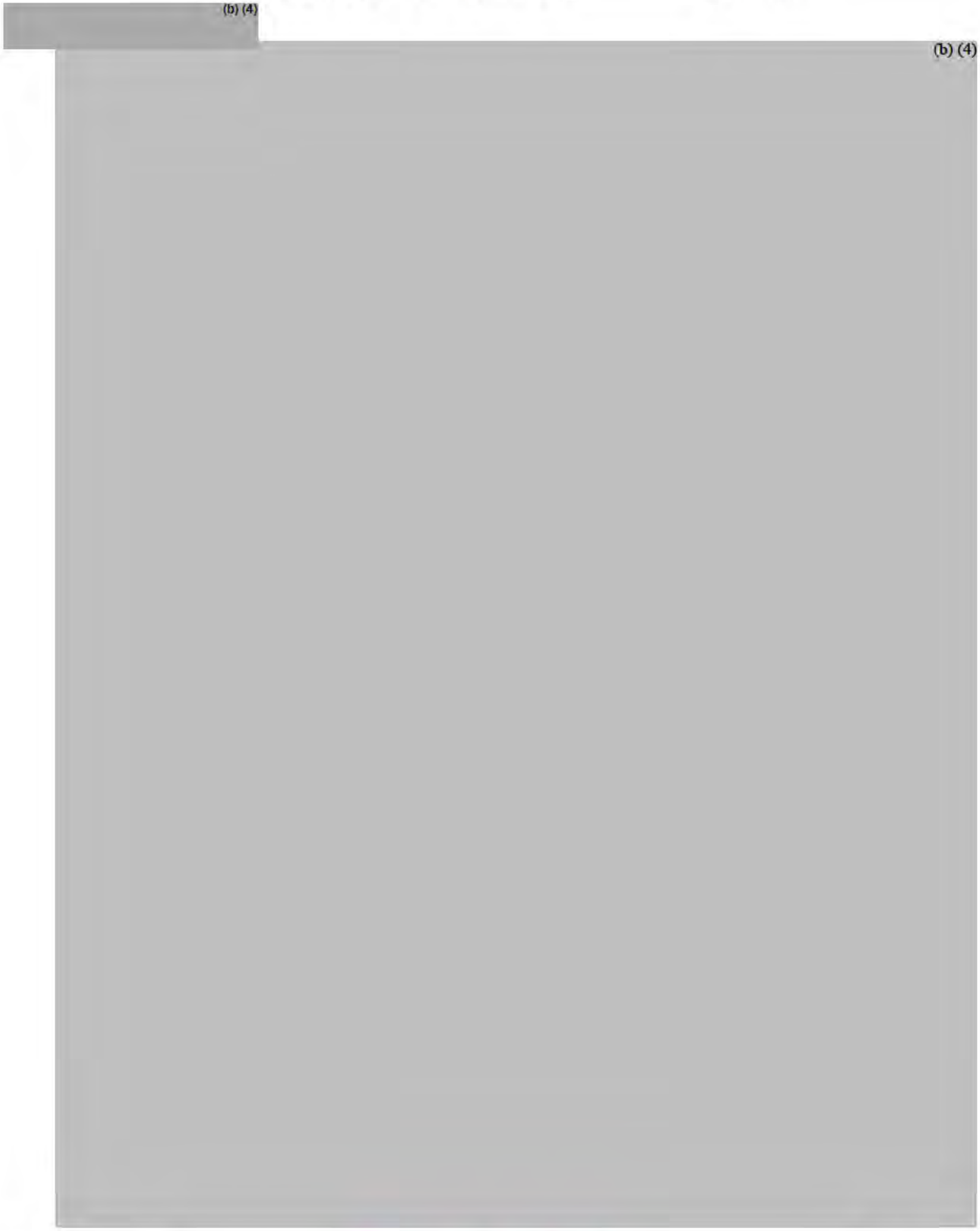
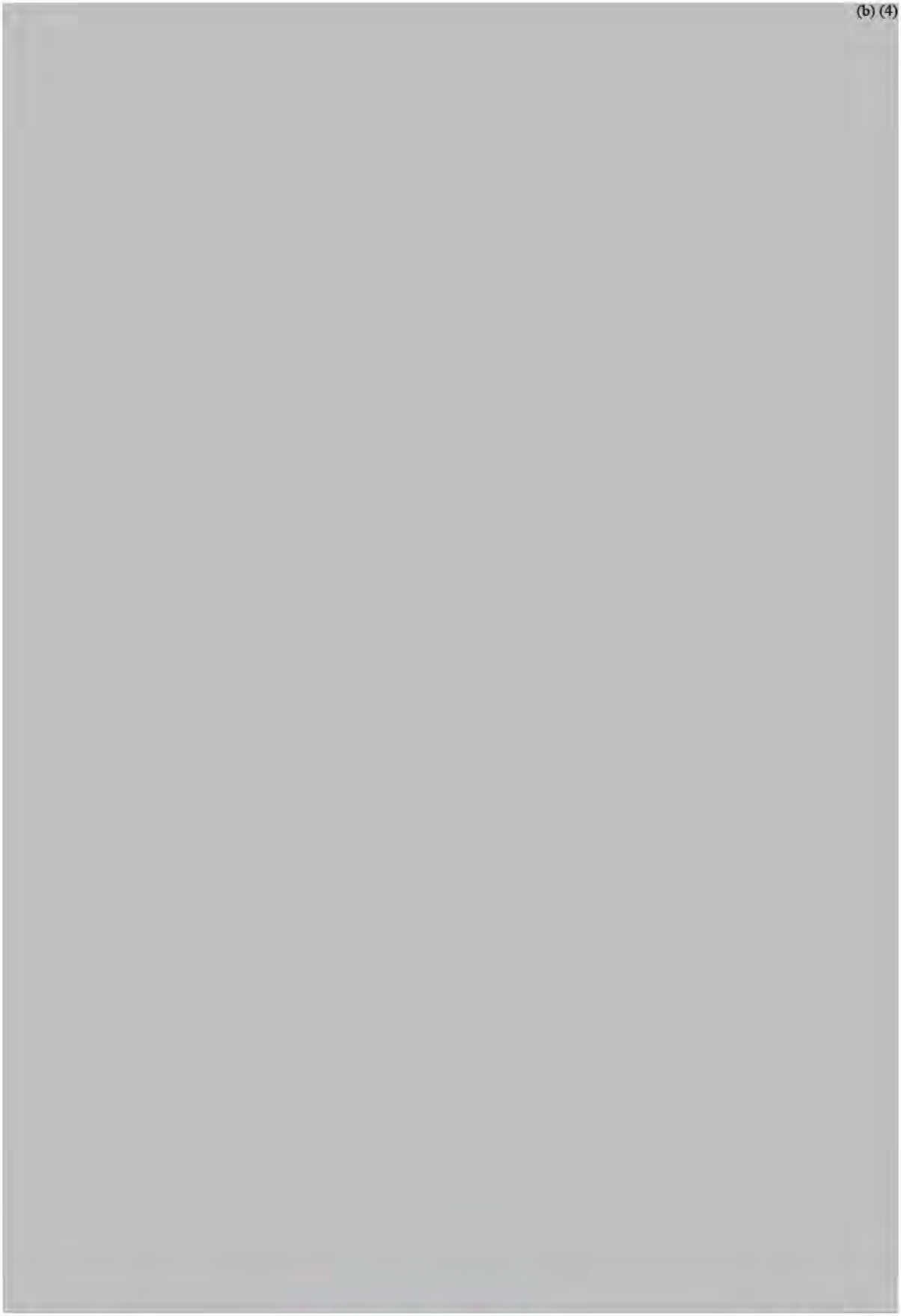
Figure 1. DNA Sequence of (b) (4) _BD50104



(b) (4)



(b) (4)

(b) (4)

Table 1. ORFs and BlastP Summary of Plasmid pDOW1169_BD50104

ORF	length	start	stop	Hit	Putative gene	E-value	Source/Definition	Homologue Accession#	Homologue Description	Homologue Length (AA)	identities AA #	positive AA #
(b) (4)												(b) (4)

Table 1. ORFs and BlastP Summary of Plasmid pDOW1169_BD50104

(Continued)

ORF	length	start	stop	Hit	Putative gene	E-value	Source/Definition	Homologue Accession#	Homologue Description	Homologue Length (AA)	identities AA #	positive AA #
(b) (4)												(b) (4)

Table 1. ORFs and BlastP Summary of Plasmid pDOW1169_BD50104

(Continued)

ORF	length	start	stop	Hit	Putative gene	E-value	Source/Definition	Homologue Accession#	Homologue Description	Homologue Length (AA)	identities AA #	positive AA #
(b) (4)												(b) (4)

Appendix 6: Stability of the [redacted] Gene and the Expression Plasmid [redacted]_BD50104 in *Pseudomonas fluorescens* BD50104 and Determination of the Phytase 50104 Gene Copy Number in Strain BD50104

Stability of the (b) (4) Gene and the Expression Plasmid (b) (4) BD50104 in *Pseudomonas fluorescens* BD50104 and Determination of the Phytase 50104 Gene Copy Number in Strain BD50104

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Stability of the (b) (4) Gene and the Expression Plasmid (b) (4) BD50104 in *Pseudomonas fluorescens* BD50104 and Determination of the Phytase 50104 Gene Copy Number in Strain BD50104

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Date

Stability of the (b) (4)[1] Gene and the Expression Plasmid (b) (4)_BD50104 in *Pseudomonas fluorescens* BD50104 and Determination of the Phytase 50104 Gene Copy Number in Strain BD50104

Table of Contents

Stability of the (b) (4) Gene and the Expression Plasmid (b) (4)_BD50104 in *Pseudomonas fluorescens* BD50104 and Determination of the Phytase 50104 Gene Copy Number in Strain BD50104

Summary

(b) (4)

Introduction

One of the important issues pertinent to recombinant protein production is the stability of the foreign genes and plasmids introduced into a host strain during strain construction. In this case, strain *P. fluorescens* BD50104 is a recombinant production microorganism, which is used to express phytase 50104 protein. This production microorganism has both foreign DNA inserted into its genome and a foreign plasmid, as discussed briefly below.

The platform strain, *P. fluorescens* DC454, was used to generate the antibiotic resistance marker free strain *P. fluroenscens* BD50104, which expresses the phytase 50104 enzyme. The DC454 strain is a derivative of the wild type strain *P. fluorescens* MB101. (b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

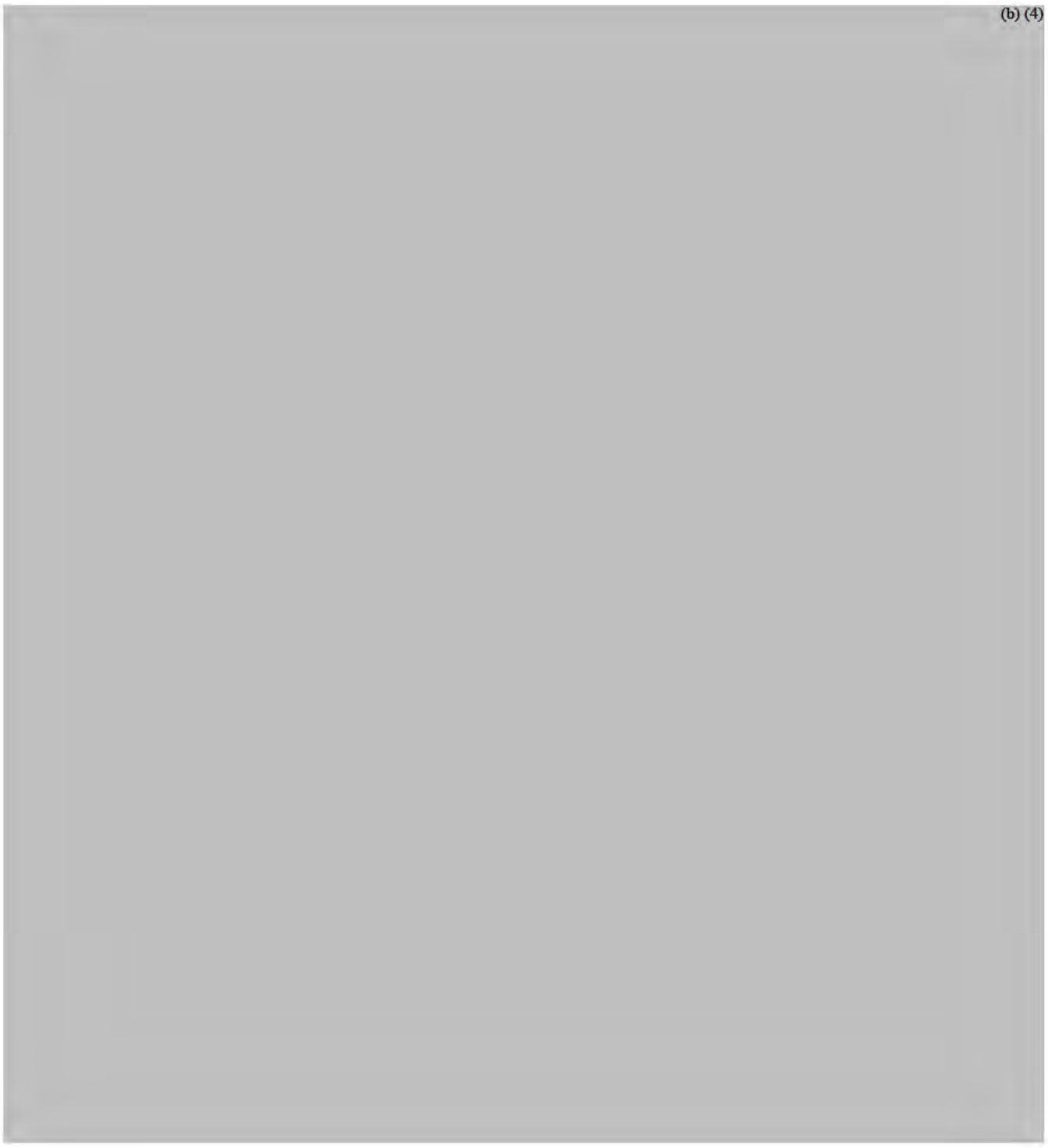
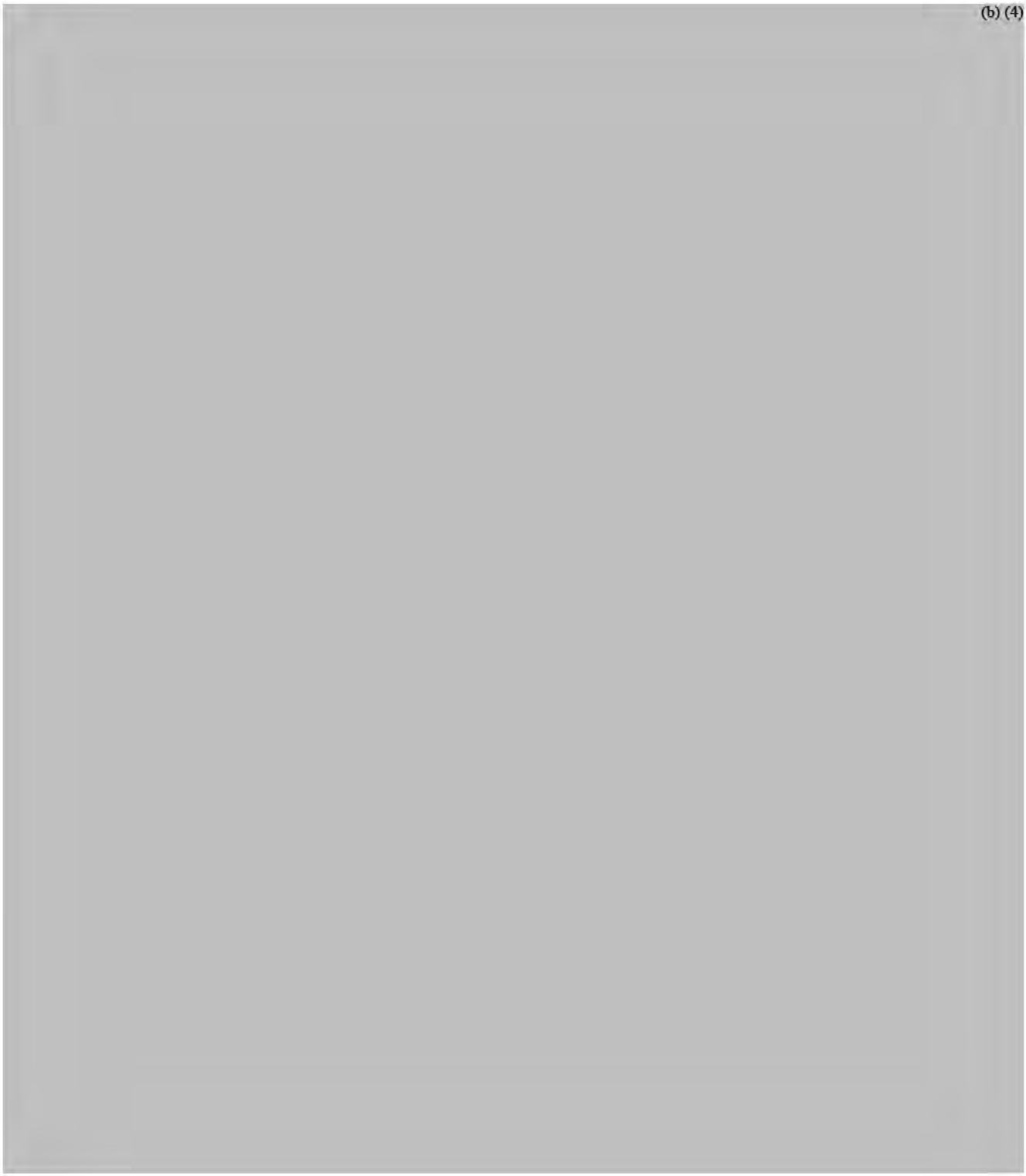


Table 1. PCR reaction conditions.

(b) (4)

(b) (4)

(b) (4)



Conclusion

Based on the results shown above, it has been demonstrated that both the (b) (4) gene, located at the *lsc* locus on the genome, and the expression plasmid are stable during the fermentation process in three independent runs. No loss of the (b) (4) gene was observed. No DNA deletion or rearrangement was observed for the expression plasmid. Furthermore, there was no significant variation in plasmid copy number within the *P. fluorescens* BD50104 production organism through the fermentation process. (b) (4)

Figures

Figure 1. Comparison of undigested plasmid DNA patterns from single colonies.

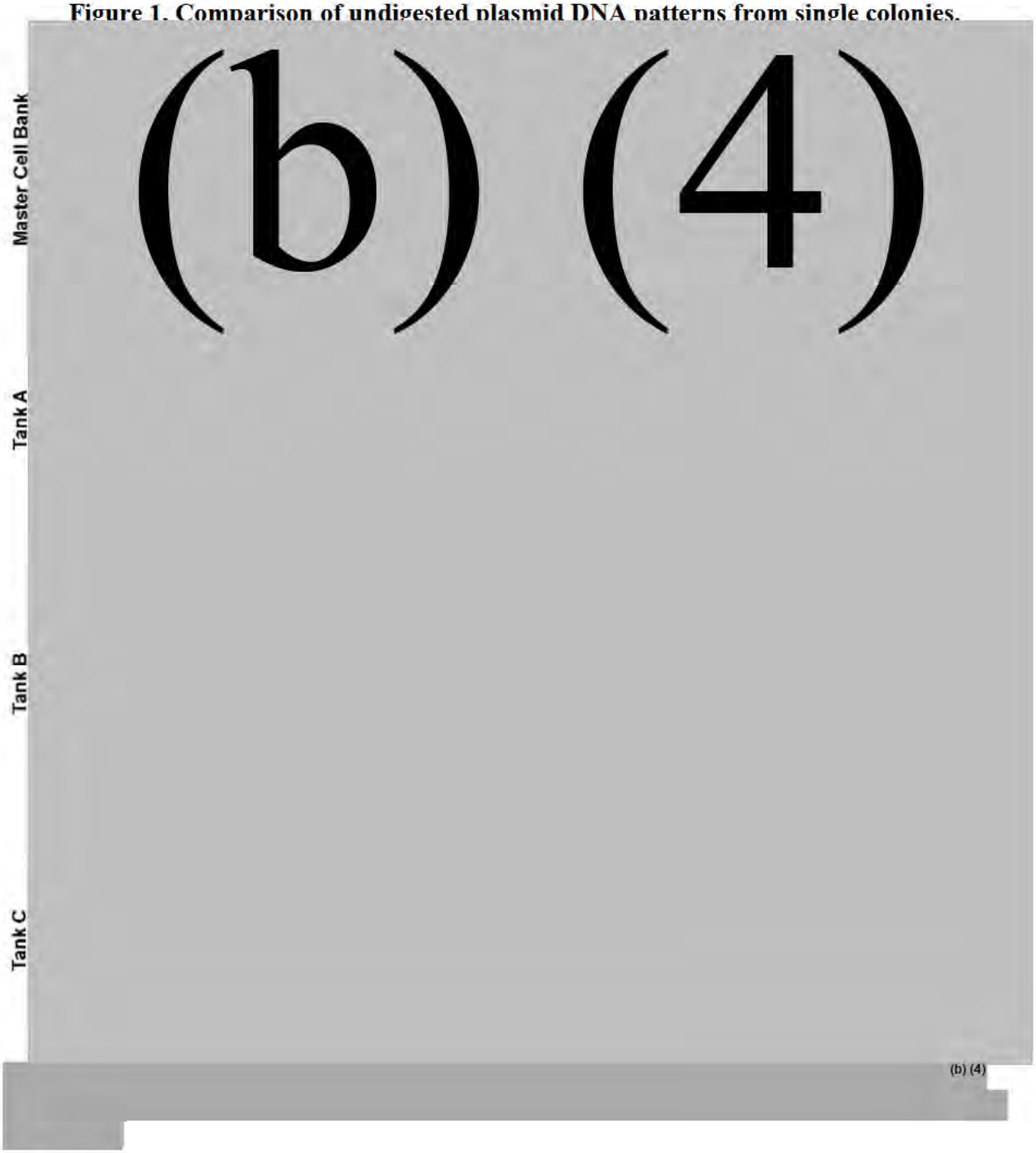


Figure 2. Comparison of XhoI digested plasmid DNA patterns from single colonies.

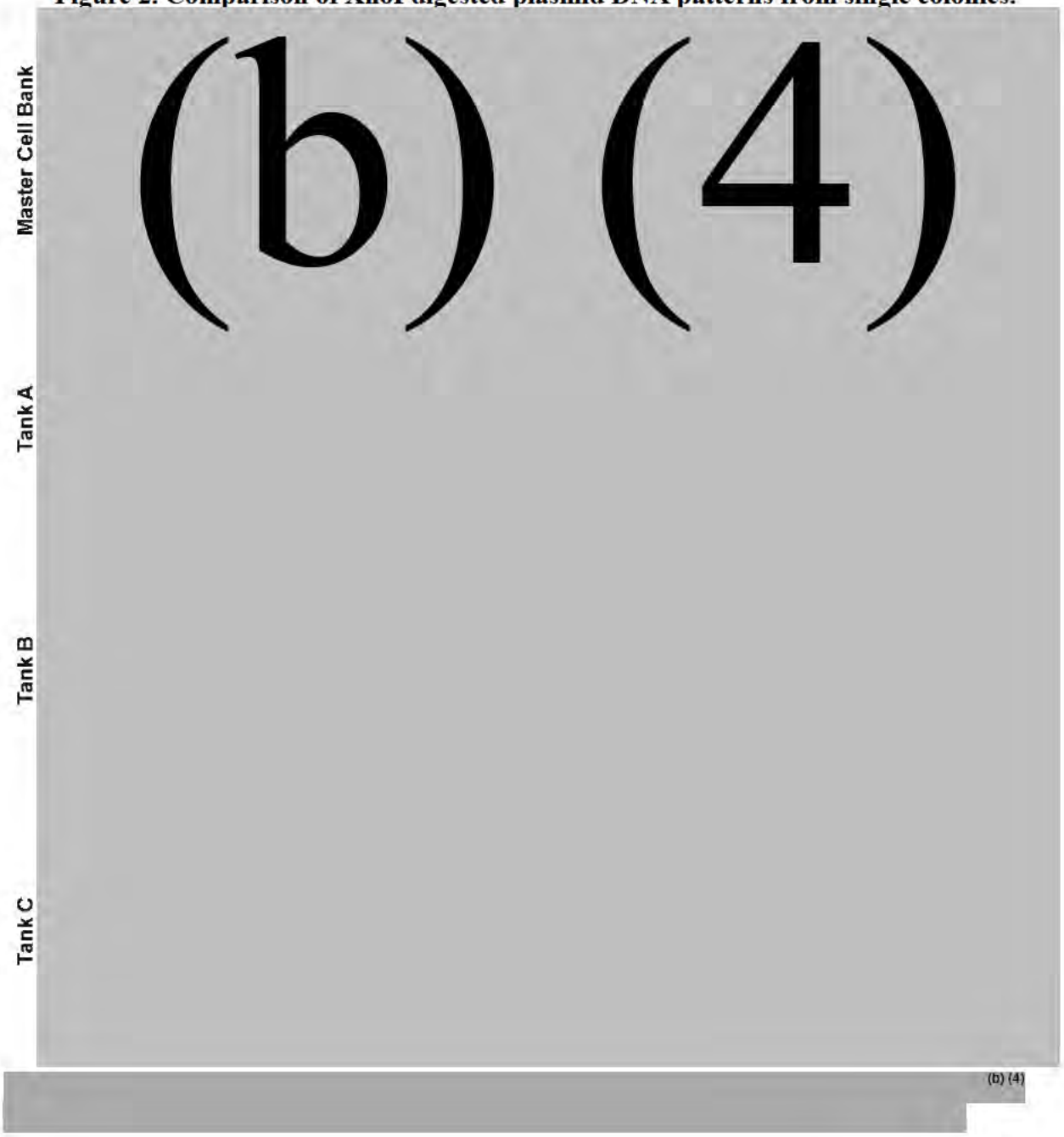
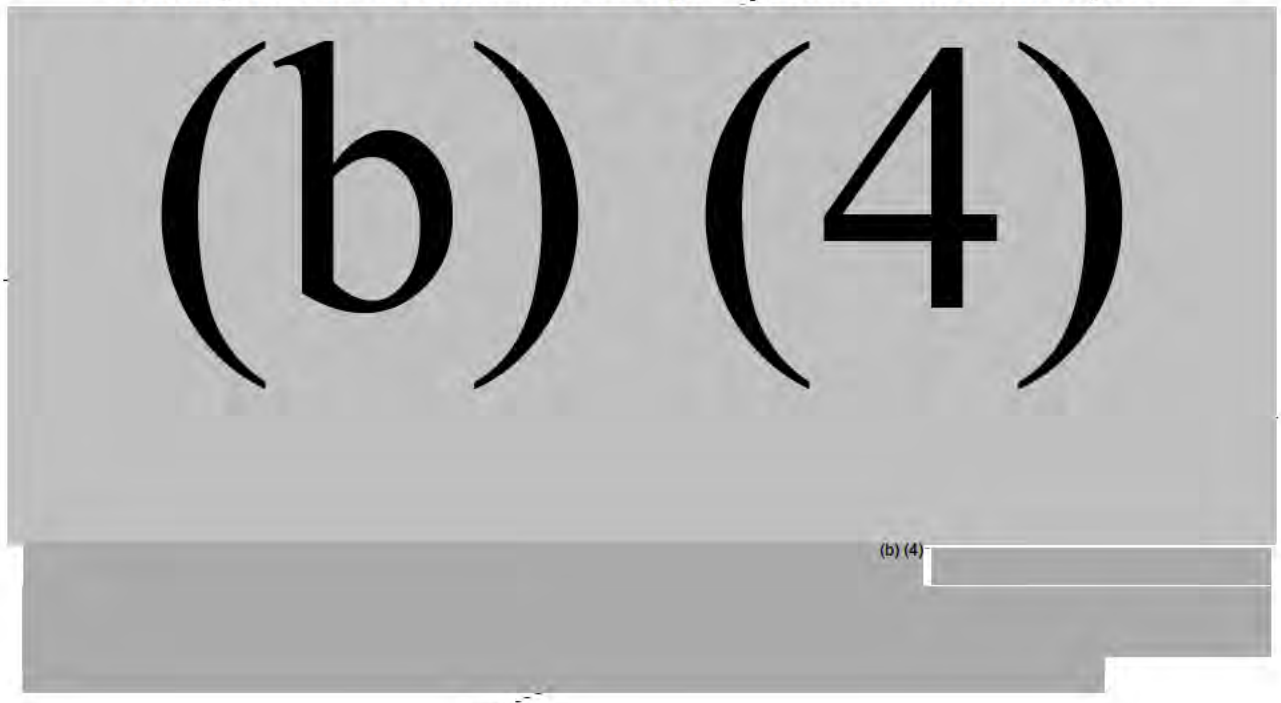


Figure 3. Comparison of undigested and digested plasmid DNA from the master cell bank, working cell bank and fermentation samples collected at different time points.



redact

Figure 4. Standard plot of Cycle number (Ct) vs. the amount of DNA (log) for the Phytaverse and housekeeping (HK) genes.

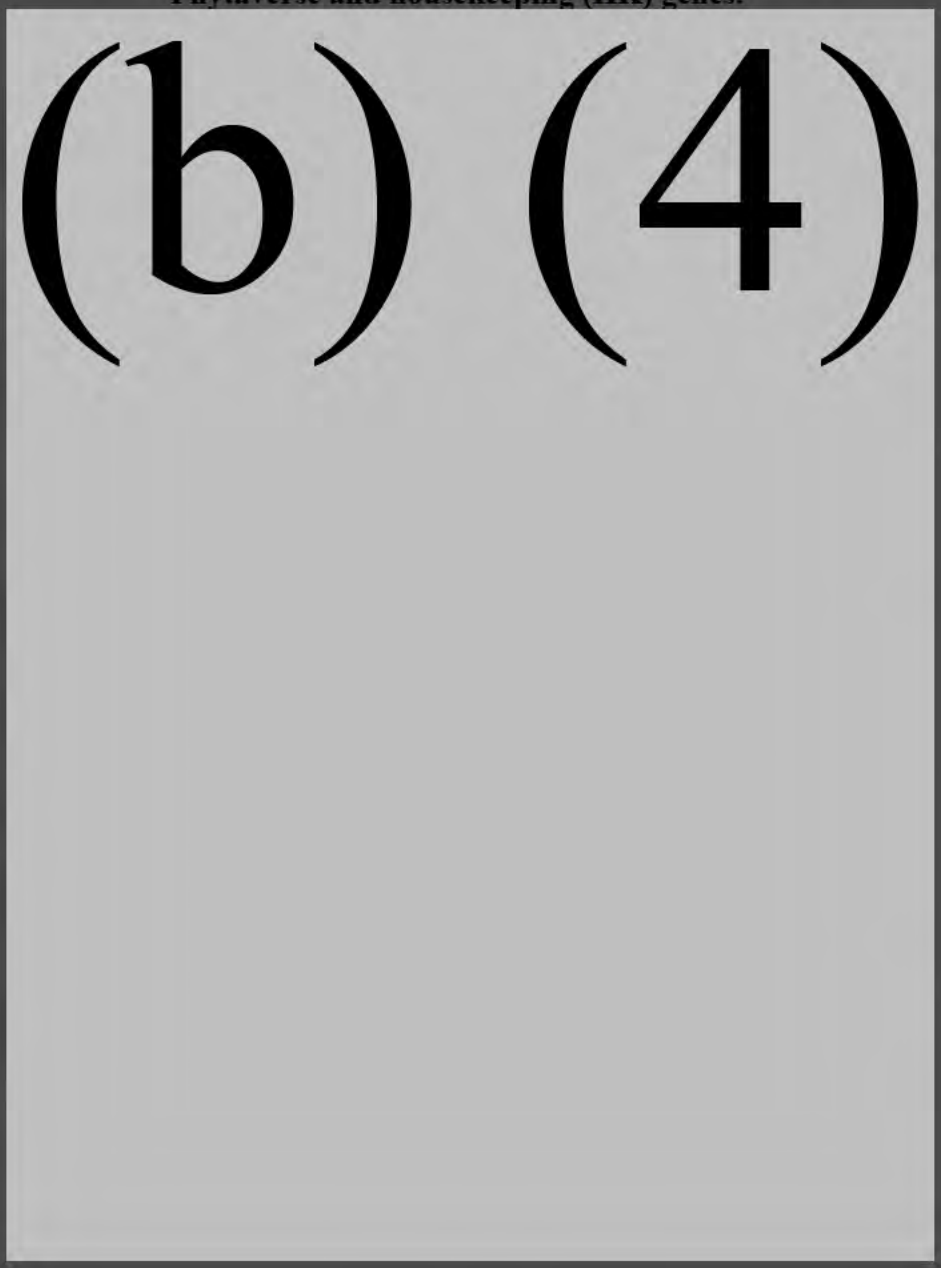
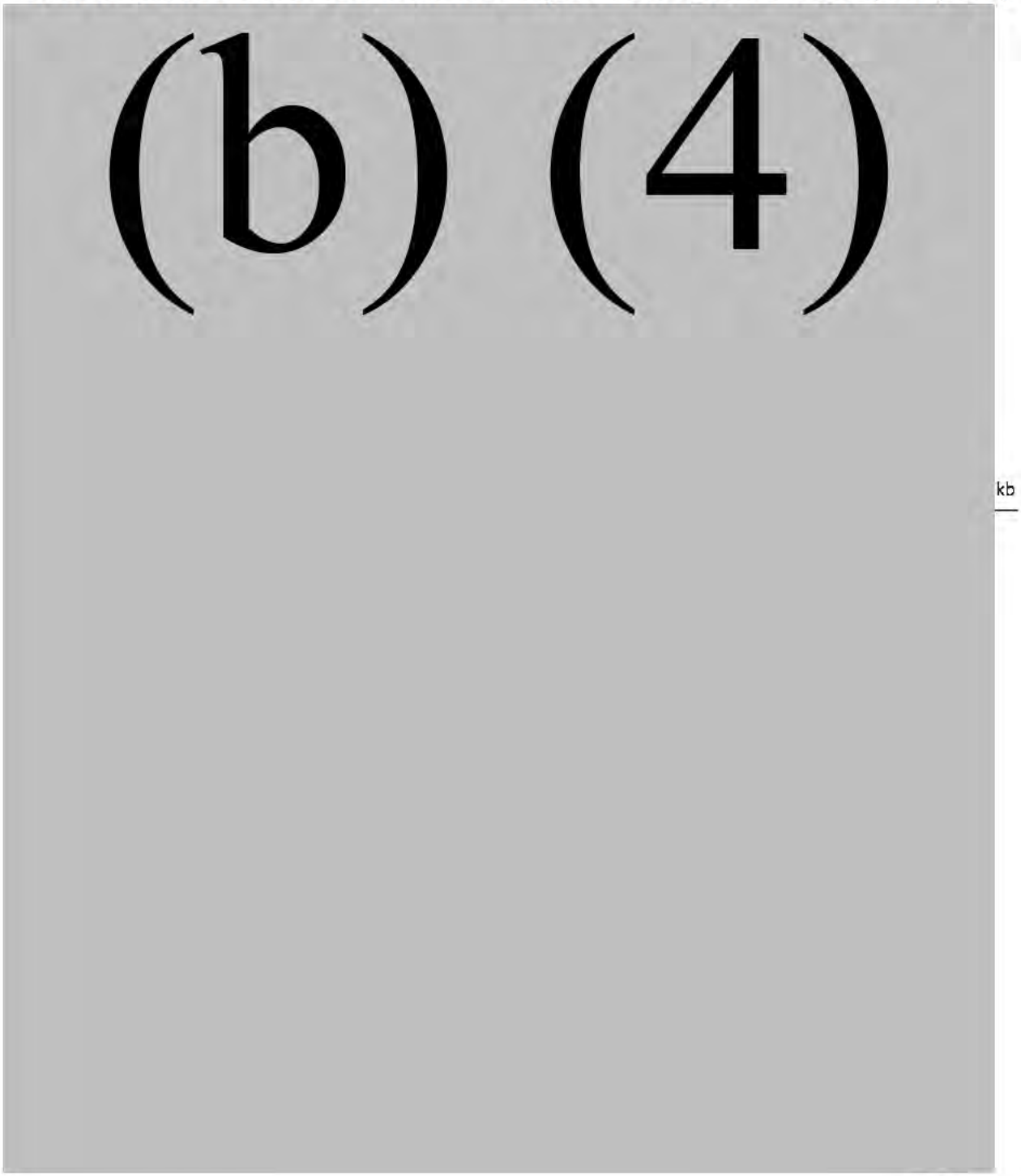


Figure 5. Colony amplification of the (b) (4) gene from single colonies originating either from the master cell bank or fermentation samples collected at the last time point (50 hr).



redact

References

(b) (4)

Appendix 7: Plasmid Mobilization Analysis for *Pseudomonas fluorescens* Strain BD50104

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Plasmid Mobilization Analysis for *Pseudomonas fluorescens* Strain BD50104

Xuqiu Tan

Sr. Manager, Expression /Technology Development

September 15, 2017

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Plasmid Mobilization Analysis for *Pseudomonas fluorescens* Strain BD50104

Author: Xuqiu Tan

9/15/2017

Xuqiu Tan **Date**

Sr. Manager, Expression /Technology Development

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San Diego, CA 92121

Plasmid Mobilization Analysis for *Pseudomonas fluorescens* Strain BD50104

TABLE OF CONTENTS

Plasmid Mobilization Analysis for *Pseudomonas fluorescens* Strain BD50104

INTRODUCTION

Among the criteria suggested by the Organization for Economic Co-operation and Development (OECD) is that vectors or plamids used in modifying a microorganism used by industry should be poorly mobilizable (OECD, Last Accessed 7/28/2017). This criteria has been widely adopted and has also been recommended elsewhere (EU Scientific Committee for Food, 1992; NIH, Last Accessed 8/4/2017).

(b) (4)

Based on these data, the phytase 50104 enzyme preparation is considered to be free of any transformable DNA.

MATERIALS AND METHODS

Bacterial Strains and Plasmids

(b) (4)

(b) (4)

Plasmid Conjugation

Table 2: Setup of Conjugation Experiment

(b) (4)

Table 3: List of Selective Plates for Each Strain

Selective plates	Strain
(b) (4)	

RESULTS

Figure 1: DNA sequence of (b) (4) and (b) (4) _BD50104 around the mutation sites

(b) (4)

Table 4: Mobilization Frequencies of (b) (4) _BD50104

(b) (4)

CONCLUSION

(b) (4)

(b) (4)

(b) (4)

REFERENCE LIST

Appendix 8: Characterization of the DNA (b) (4) Expression Cassette) Inserted into the Host Chromosome

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Characterization of the DNA (b)(4) Expression Cassette) Inserted into the Host Chromosome

Xuqiu Tan
Senior Director, Research

February 21, 2013

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Characterization of the DNA (b) (4) Expression Cassette) Inserted into the Host Chromosome

Author:

Xuqiu Tan
Senior Director, Research
Verenium Corporation

2/24/2013
Date

Characterization of the DNA (b) (4) Expression Cassette) Inserted into the Host Chromosome

Summary

The recipient microorganism used for the CIBENZA® PHYTAVERSE™ Feed Additive enzyme products is *Pseudomonas fluorescens* DC454, which is a derivative of *P. fluorescens* MB101.

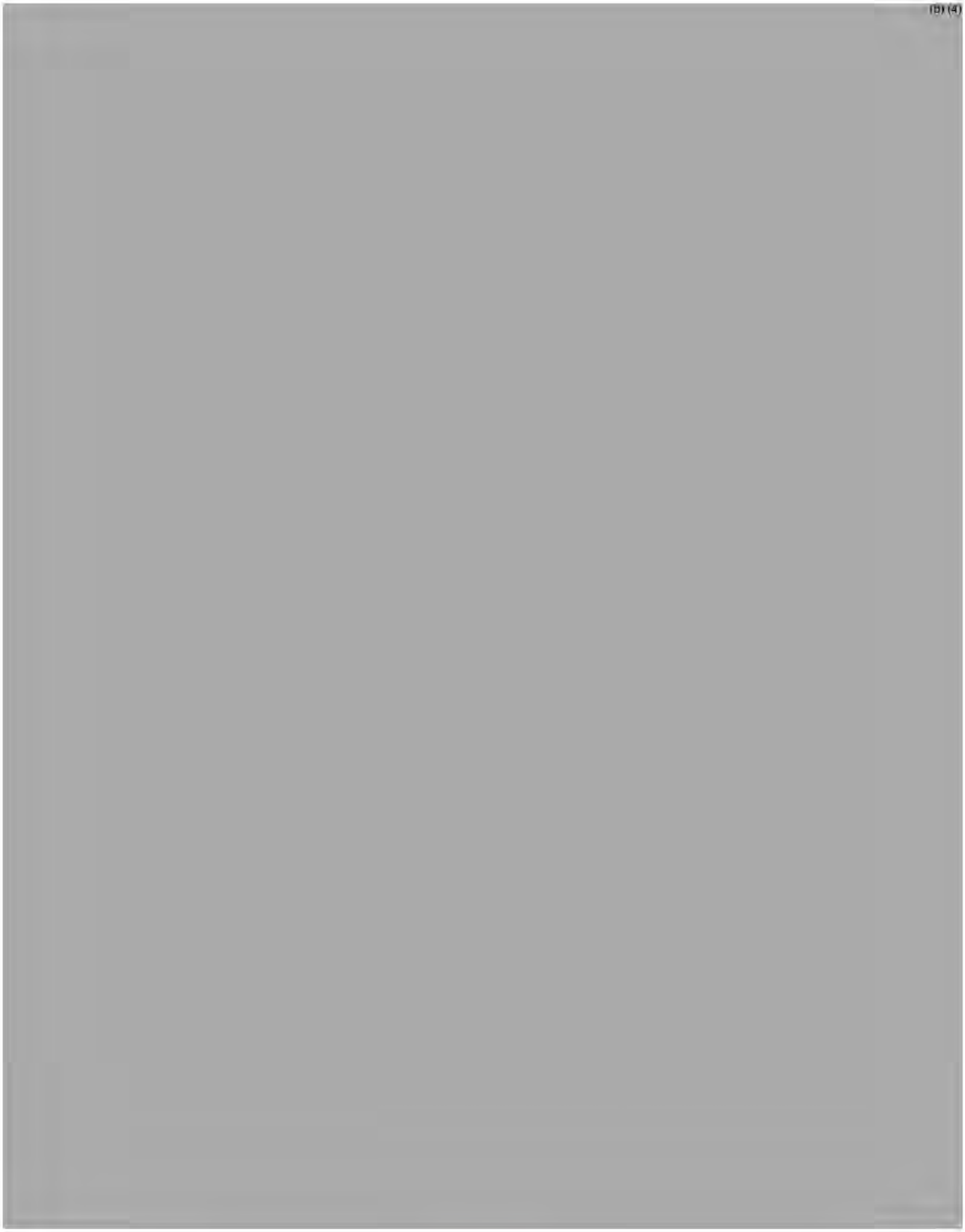
(b) (4)

Detailed Information

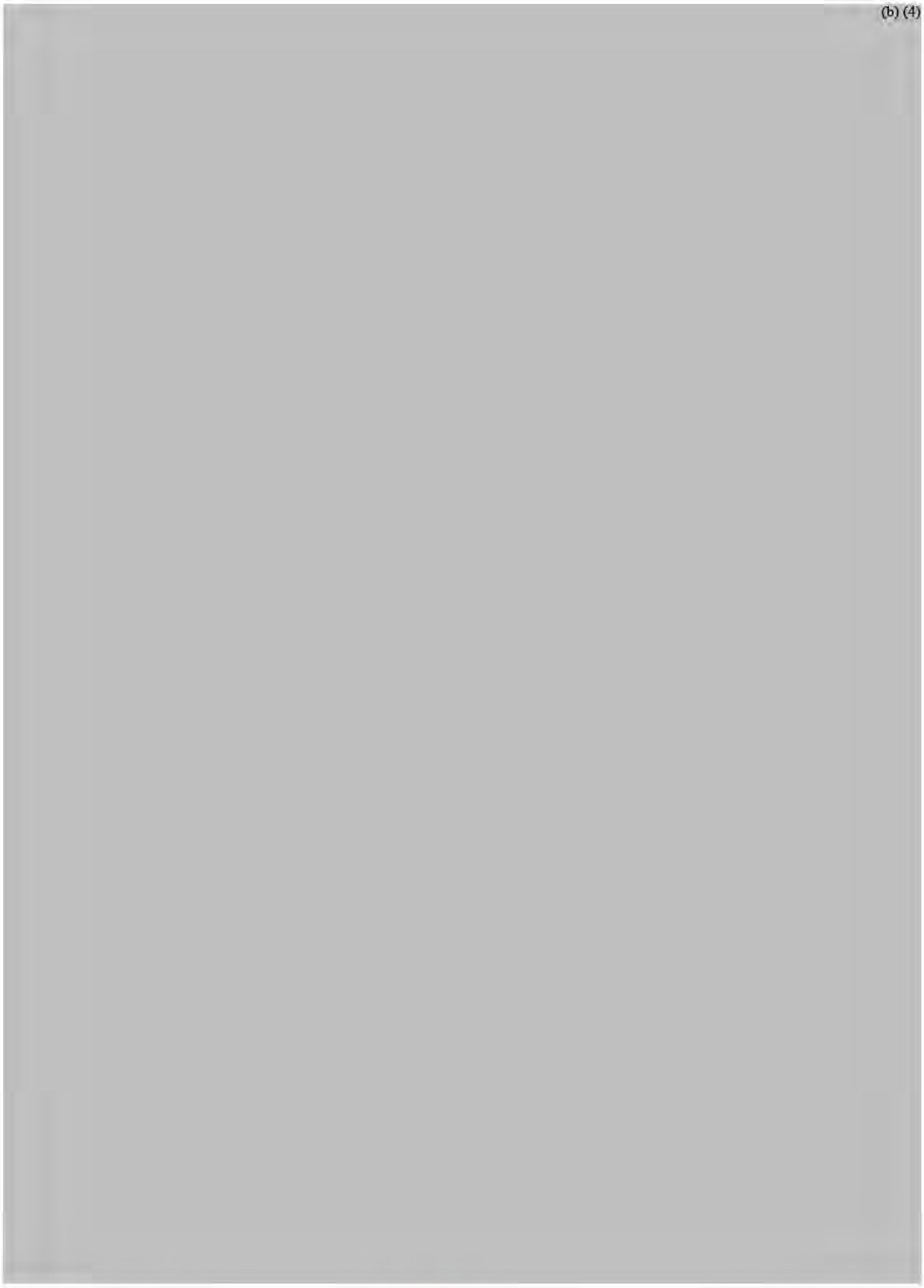
Lineage of DC454

(b) (4)

(b) (4)


(b) (4)

(b) (4)



(b) (4)


(b) (4)

Reference List

Appendix 9: Certificates of Analysis for CIBENZA® PHYTAVERSE® L10 Phytase Enzyme and CIBENZA® PHYTAVERSE® G10 Phytase Enzyme

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Certificate of Analysis

CIBENZA® PHYTAVERSE® L10 Phytase Enzyme (Test Article VR006)

Lot number: CV002C2
Date of Manufacture: August 14, 2014

Specification	Specification Limit	Test Result	Method
Appearance	Amber to brown liquid	(b) (4)	Visual
pH	5.0 - 5.2		Untapped
Specific gravity (g/mL)	1.05 - 1.20		Pycnometer
Sediment (% v/v)	≤ 0.5		QC0232
Activity (U/g)	≥ 10,000		ISO 30024
Lead (mg/kg)	≤ 5		ICP-MS
Arsenic (mg/kg)	< 2		ICP-MS
Cadmium (mg/kg)	< 0.5		ICP-MS
Mercury (mg/kg)	< 0.5		ICP-MS
Total Plate Count (cfu/g)	≤ 50,000		FDA BAM
Total Coliform (MPN/g)	≤ 30		FDA BAM
E. coli (/25g)	Absent		FDA BAM
Salmonella (/25g)	Absent		FDA BAM
Yeast and Mold (CFU/g)	Run and Record		FDA BAM
Staphylococcus aureus (/g)	Absent		FDA BAM
Production Organism (CFU/g)	Absent		QC0214
Antibiotic Activity (Zone of Inhibition)	Absent		JECFA
Mycotoxin			
Aflatoxin B1	NMT 1.0 ppb		HPLC
Aflatoxin B2	NMT 1.0 ppb		HPLC
Aflatoxin G1	NMT 1.0 ppb		HPLC
Aflatoxin G2	NMT 1.0 ppb		HPLC
Fumonisin B1	NMT 0.1 ppm		LCMSMS
Fumonisin B2	NMT 0.1 ppm		LCMSMS
Fumonisin B3	NMT 0.1 ppm		LCMSMS
Ochratoxin A	NMT 2.0 ppb		HPLC
Deoxynivalenol	NMT 0.6 ppm		LCMSMS
Acetyldeoxynivalenol	NMT 0.8 ppm		LCMSMS
Fusarenon X	NMT 0.4 ppm		LCMSMS
Nivalenol	NMT 0.6 ppm		LCMSMS
T-2 Toxin	NMT 0.2 ppm		LCMSMS
HT-2 Toxin	NMT 0.2 ppm		LCMSMS
Neosolaniol	NMT 0.4 ppm		LCMSMS
Diacetoxyscirpenol	NMT 0.4 ppm		LCMSMS
Zearalenone	NMT 43.1 ppb		HPLC
Sterigmatocystin	NMT 200 ppb		TLC

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PCBs	**10,000 pg/g**	(b) (4)	**GC/HRMS**
Dioxins	**1pg/g**		**GC/HRMS**

* Results of retesting performed in May 2017

[1] The limits of detection (LOD) for each of the assays and methods match that of the stated less than (<) values above.

Approved by: _____

Mark Burcin
Sr. Manager, QA/QC

Date: June 7, 2017

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Certificate of Analysis

CIBENZA® PHYTAVERSE® L10 Phytase Enzyme (Test Article VR006)

Lot number: 190CV005A3
Date of Manufacture: August 11, 2014

Specification	Specification Limit	Test Result	Method
Appearance	Amber to brown liquid	(b) (4)	Visual
pH	5.0 - 5.2		Untapped
Specific gravity (g/mL)	1.05 - 1.20		Pycnometer
Sediment (% v/v)	≤ 0.5		QC0232
Activity (U/g)	≥ 10,000		ISO 30024
Lead (mg/kg)	≤ 5		ICP-MS
Arsenic (mg/kg)	< 2		ICP-MS
Cadmium (mg/kg)	< 0.5		ICP-MS
Mercury (mg/kg)	< 0.5		ICP-MS
Total Plate Count (cfu/g)	≤ 50,000		FDA BAM
Total Coliform (cfu/g)	≤ 30		FDA BAM
E. coli (/25g)	Absent		FDA BAM
Salmonella (/25g)	Absent		FDA BAM
Yeast and Mold (MPN/g)	Run and Record		FDA BAM
Staphylococcus aureus (/g)	Absent		FDA BAM
Production Organism (CFU/g)	Absent		QC0214
Antibiotic Activity (Zone of Inhibition)	Absent		JECFA
Mycotoxin			
Aflatoxin B1	NMT 1.0 ppb		HPLC
Aflatoxin B2	NMT 1.0 ppb		HPLC
Aflatoxin G1	NMT 1.0 ppb		HPLC
Aflatoxin G2	NMT 1.0 ppb		HPLC
Fumonisin B1	NMT 0.1 ppm		LCMSMS
Fumonisin B2	NMT 0.1 ppm		LCMSMS
Fumonisin B3	NMT 0.1 ppm		LCMSMS
Ochratoxin A	NMT 2.0 ppb		HPLC
Deoxynivalenol	NMT 0.6 ppm		LCMSMS
Acetyldeoxynivalenol	NMT 0.8 ppm		LCMSMS
Fusarenon X	NMT 0.4 ppm		LCMSMS
Nivalenol	NMT 0.6 ppm		LCMSMS
T-2 Toxin	NMT 0.2 ppm		LCMSMS
HT-2 Toxin	NMT 0.2 ppm		LCMSMS
Neosolaniol	NMT 0.4 ppm		LCMSMS
Diacetoxyscirpenol	NMT 0.4 ppm		LCMSMS
Zearalenone	NMT 43.1 ppb		HPLC
Sterigmatocystin	NMT 200 ppb		TLC

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PCBs	**10,000 pg/g**	(b) (4)	**GC/HRMS**
Dioxins	**1pg/g**		**GC/HRMS**

* Results of retesting performed in May 2017
[1] The limits of detection (LOD) for each of the assays and methods match that of the stated less than (<) values above.

Approved by: _____

Mark Burcin
Sr. Manager, QA/QC

Date: June 7, 2017

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CIBENZA® PHYTAVERSE® L10 Phytase Enzyme (Test Article VR006)

Lot number: PHY-50104-PO030-F4
Date of Manufacture: September 11, 2015

Specification	Specification Limit	Test Result	Method
Appearance	Amber to brown liquid	(b) (4)	Visual
pH	5.0 - 5.2		Untapped
Specific gravity (g/mL)	1.05 - 1.20		Pycnometer
Sediment (% v/v)	≤ 0.5		QC0232
Activity (U/g)	≥ 10,000		ISO 30024
Lead (mg/kg)	≤ 5		ICP-MS
Arsenic (mg/kg)	< 2		ICP-MS
Cadmium (mg/kg)	< 0.5		ICP-MS
Mercury (mg/kg)	< 0.5		ICP-MS
Total Plate Count (cfu/g)	≤ 50,000		FDA BAM
Total Coliform (MPN/g)	≤ 30		FDA BAM
E. coli (/25g)	Absent		FDA BAM
Salmonella (/25g)	Absent		FDA BAM
Yeast and Mold (CFU/g)	Run and Record		FDA BAM
Staphylococcus aureus (/g)	Absent		FDA BAM
Production Organism (CFU/g)	Absent		QC0214
Antibiotic Activity (Zone of Inhibition)	Absent		JECFA
Mycotoxin			
Aflatoxin B1	NMT 1.0 ppb		HPLC
Aflatoxin B2	NMT 1.0 ppb		HPLC
Aflatoxin G1	NMT 1.0 ppb		HPLC
Aflatoxin G2	NMT 1.0 ppb		HPLC
Fumonisin B1	NMT 0.1 ppm		LCMSMS
Fumonisin B2	NMT 0.1 ppm		LCMSMS
Fumonisin B3	NMT 0.1 ppm		LCMSMS
Ochratoxin A	NMT 2.0 ppb		HPLC
Deoxynivalenol	NMT 0.6 ppm		LCMSMS
Acetyldeoxynivalenol	NMT 0.8 ppm		LCMSMS
Fusarenon X	NMT 0.4 ppm		LCMSMS
Nivalenol	NMT 0.6 ppm		LCMSMS
T-2 Toxin	NMT 0.2 ppm		LCMSMS
HT-2 Toxin	NMT 0.2 ppm		LCMSMS
Neosolaniol	NMT 0.4 ppm		LCMSMS
Diacetoxyscirpenol	NMT 0.4 ppm		LCMSMS
Zearalenone	NMT 43.1 ppb		HPLC
Sterigmatocystin	NMT 200 ppb		TLC

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PCBs	**10,000 pg/g**	(b) (4)	**GC/HRMS**
Dioxins	**1pg/g**		**GC/HRMS**

* Results of retesting performed in May 2017
[1] The limits of detection (LOD) for each of the assays and methods match that of the stated less than (<) values above.

Approved by: _____ Date: June 7, 2017

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CIBENZA® PHYTAVERSE® G10 Phytase Enzyme (Test Article VR005)

Lot number: P23941
Date of Manufacture: October 8, 2014

Specification	Specification Limit	Test Result	Method
Appearance	White to Beige granules	(b) (4)	Visual
Bulk Density-untapped (g/cm³)	≥ 0.50		Untapped
Particle size (mesh)	<2% on 20 mesh <10% thru 140 mesh		Sieve Sieve
Activity (U/g)	NLT 10,000		ISO 30024
Loss on Drying (%)	≤ 12		USP 37 <731>
Lead (mg/kg)	≤ 5		ICP-MS
Arsenic (mg/kg)	< 2		ICP-MS
Cadmium (mg/kg)	< 0.5		ICP-MS
Mercury (mg/kg)	< 0.5		ICP-MS
Total Plate Count (cfu/g)	≤ 50,000		FDA BAM
Total Coliform (MPN/g)	≤ 30		FDA BAM
E. coli (/25g)	Absent		FDA BAM
Salmonella (/25g)	Absent		FDA BAM
Yeast and Mold (CFU/g)	Run and Record		FDA BAM
Staphylococcus aureus (/g)	Absent		FDA BAM
Production Organism (CFU/g)	Absent		QC0214
Antibiotic Activity (Zone of Inhibition)	Absent		JECFA
Mycotoxin			
Aflatoxin B1	NMT 1.0 ppb		HPLC
Aflatoxin B2	NMT 1.0 ppb		HPLC
Aflatoxin G1	NMT 1.0 ppb		HPLC
Aflatoxin G2	NMT 1.0 ppb		HPLC
Fumonisin B1	NMT 0.1 ppm		LCMSMS
Fumonisin B2	NMT 0.1 ppm		LCMSMS
Fumonisin B3	NMT 0.1 ppm		LCMSMS
Ochratoxin A	NMT 2.0 ppb		HPLC
Deoxynivalenol	NMT 3.0 ppm		LCMSMS
Acetyldeoxynivalenol	NMT 0.8 ppm		LCMSMS
Fusarenon X	NMT 0.4 ppm		LCMSMS
Nivalenol	NMT 0.6 ppm		LCMSMS
T-2 Toxin	NMT 0.2 ppm		LCMSMS
HT-2 Toxin	NMT 0.2 ppm		LCMSMS
Neosolaniol	NMT 0.4 ppm		LCMSMS
Diacetoxyscirpenol	NMT 0.4 ppm		LCMSMS
Zearalenone	NMT 43.1 ppb		HPLC
Sterigmatocystin	NMT 200 ppb		TLC

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PCBs	**10,000 pg/g**	(b) (4)	**GC/HRMS**
Dioxins	**1 pg/g**		**GC/HRMS**

* Production organism testing was performed on the enzyme concentrate used to produce this dry product.
** Results of retesting performed in March 2017
[1] The limits of detection (LOD) for each of the assays and methods match that of the stated less than (<) values above.

Approved by: _____ Date: May 9, 2017

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CIBENZA® PHYTAVERSE® G10 Phytase Enzyme (Test Article VR005)

Lot number: P26641
Date of Manufacture: October 8, 2014

Specification	Specification Limit	Test Result	Method
Appearance	White to Beige granules	(b) (4)	Visual
Bulk Density-untapped (g/cm^3)	≥ 0.50		Untapped
Particle size (mesh)	<2% on 20 mesh <10% thru 140 mesh		Sieve Sieve
Activity (U/g)	NLT 10,000		ISO 30024
Loss on Drying (%)	≤ 12		USP 37 <731>
Lead (mg/kg)	≤ 5		ICP-MS
Arsenic (mg/kg)	< 2		ICP-MS
Cadmium (mg/kg)	< 0.5		ICP-MS
Mercury (mg/kg)	< 0.5		ICP-MS
Total Plate Count (cfu/g)	≤ 50,000		FDA BAM
Total Coliform (cfu/g)	≤ 30		FDA BAM
E. coli (/25g)	Absent		FDA BAM
Salmonella (/25g)	Absent		FDA BAM
Yeast and Mold (CFU/g)	Run and Record		FDA BAM
Staphylococcus aureus (/g)	Absent		FDA BAM
Production Organism (CFU/g)	Absent		QC0214
Antibiotic Activity (Zone of Inhibition)	Absent		JECFA
Mycotoxin			
Aflatoxin B1	NMT 1.0 ppb		HPLC
Aflatoxin B2	NMT 1.0 ppb		HPLC
Aflatoxin G1	NMT 1.0 ppb		HPLC
Aflatoxin G2	NMT 1.0 ppb		HPLC
Fumonisin B1	NMT 0.1 ppm		LCMSMS
Fumonisin B2	NMT 0.1 ppm		LCMSMS
Fumonisin B3	NMT 0.1 ppm		LCMSMS
Ochratoxin A	NMT 2.0 ppb		HPLC
Deoxynivalenol	NMT 3.0 ppm		LCMSMS
Acetyldeoxynivalenol	NMT 0.8 ppm		LCMSMS
Fusarenon X	NMT 0.4 ppm		LCMSMS
Nivalenol	NMT 0.6 ppm		LCMSMS
T-2 Toxin	NMT 0.2 ppm		LCMSMS
HT-2 Toxin	NMT 0.2 ppm		LCMSMS
Neosolaniol	NMT 0.4 ppm		LCMSMS
Diacetoxyscirpenol	NMT 0.4 ppm		LCMSMS
Zearalenone	NMT 43.1 ppb		HPLC

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Sterigmatocystin	**NMT 200 ppb**	(b) (6), (b) (4)	**TLC**
PCBs	**10,000 pg/g**		**GC/HRMS**
Dioxins	**1 pg/g**		**GC/HRMS**

* Production organism testing was performed on the enzyme concentrate used to produce this dry product.
** Results of retesting performed in March 2017
[1] The limits of detection (LOD) for each of the assays and methods match that of the stated less than (<) values above.

Approved by: _____

Mark Burcin
Sr. Manager, QA/QC

Date: May 9, 2017

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CIBENZA® PHYTAVERSE® G10 Phytase Enzyme (Test Article VR005)

Lot number: RO15271001
Date of Manufacture: September 28, 2015

Specification	Specification Limit	Test Result	Method
Appearance	White to Beige granules	(b) (4)	Visual
Bulk Density-untapped (g/cm^3)	≥ 0.50		Untapped
Particle size (mesh)	<2% on 20 mesh <10% thru 140 mesh		Sieve Sieve
Activity (U/g)	NLT 10,000		ISO 30024
Loss on Drying (%)	≤ 12		USP 37 <731>
Lead (mg/kg)	≤ 5		ICP-MS
Arsenic (mg/kg)	< 2		ICP-MS
Cadmium (mg/kg)	< 0.5		ICP-MS
Mercury (mg/kg)	< 0.5		ICP-MS
Total Plate Count (cfu/g)	≤ 50,000		FDA BAM
Total Coliform (cfu/g)	≤ 30		FDA BAM
E. coli (/25g)	Absent		FDA BAM
Salmonella (/25g)	Absent		FDA BAM
Yeast and Mold (CFU/g)	Run and Record		FDA BAM
Staphylococcus aureus (/g)	Absent		FDA BAM
Production Organism (CFU/g)	Absent		QC0214
Antibiotic Activity (Zone of Inhibition)	Absent		JECFA
Mycotoxin			
Aflatoxin B1	NMT 1.0 ppb		HPLC
Aflatoxin B2	NMT 1.0 ppb		HPLC
Aflatoxin G1	NMT 1.0 ppb		HPLC
Aflatoxin G2	NMT 1.0 ppb		HPLC
Fumonisin B1	NMT 0.1 ppm		LCMSMS
Fumonisin B2	NMT 0.1 ppm		LCMSMS
Fumonisin B3	NMT 0.1 ppm		LCMSMS
Ochratoxin A	NMT 2.0 ppb		HPLC
Deoxynivalenol	NMT 3.0 ppm		LCMSMS
Acetyldeoxynivalenol	NMT 0.8 ppm		LCMSMS
Fusarenon X	NMT 0.4 ppm		LCMSMS
Nivalenol	NMT 0.6 ppm		LCMSMS
T-2 Toxin	NMT 0.2 ppm		LCMSMS
HT-2 Toxin	NMT 0.2 ppm		LCMSMS
Neosolaniol	NMT 0.4 ppm		LCMSMS
Diacetoxyscirpenol	NMT 0.4 ppm		LCMSMS
Zearalenone	NMT 43.1 ppb		HPLC

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Certificate of Analysis

Sterigmatocystin	**NMT 200 ppb**	(b) (4)	**TLC**
PCBs	**10,000 pg/g**		**GC/HRMS**
Dioxins	**1 pg/g**		**GC/HRMS**

* Production organism testing was performed on the enzyme concentrate used to produce this dry product.
** Results of retesting performed in March 2017
[1] The limits of detection (LOD) for each of the assays and methods match that of the stated less than (<) values above.

Approved by: _____ Date: May 9, 2017

Mark Burcin
Sr. Manager, QA/QC

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San Diego, CA 92121
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Appendix 10: List of Raw Materials Used in the Manufacturing of Phytase 50104 Enzyme Preparation

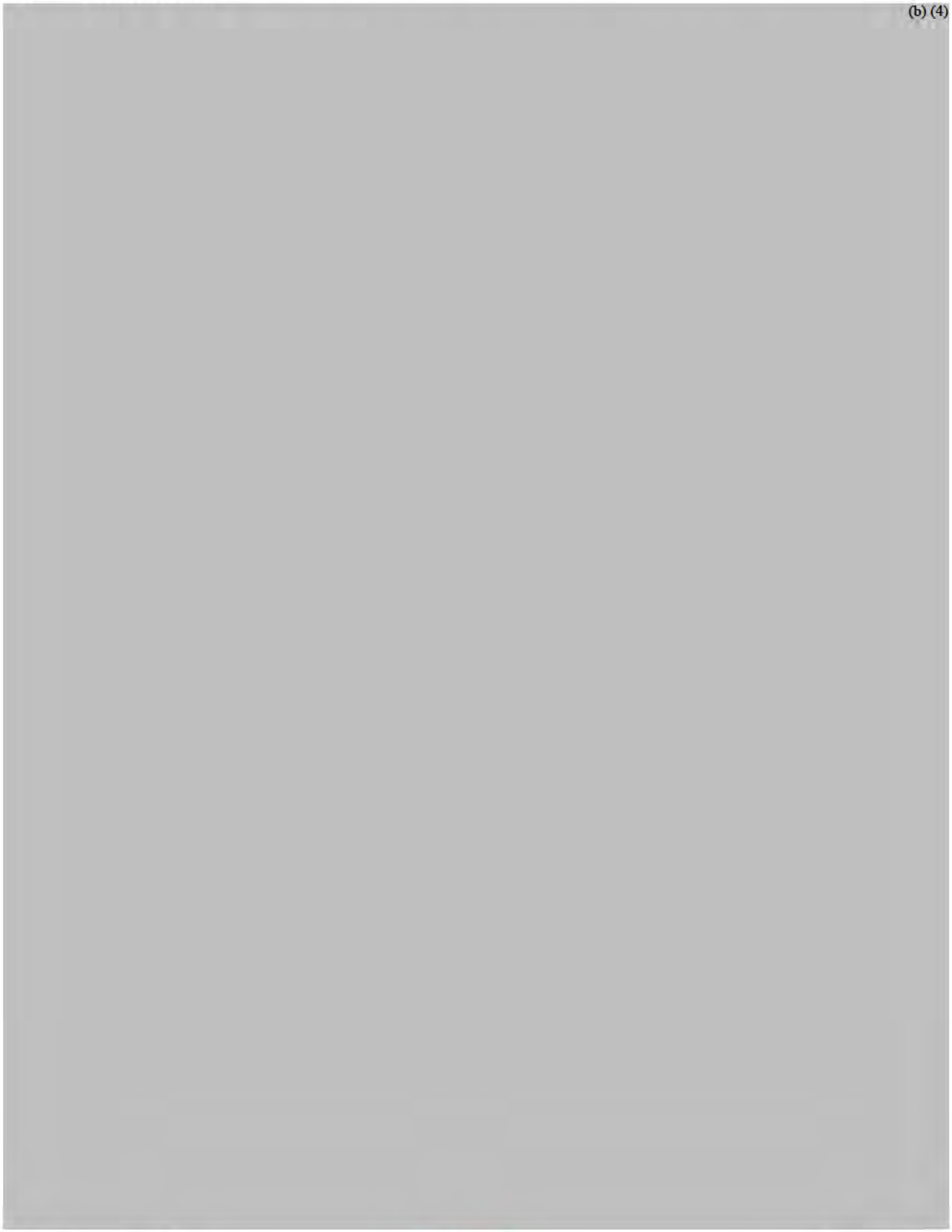
INGREDIENT	FORMULA
Fermentation	
(b) (4)	
Recovery Raw Materials	
(b) (4)	
Low microbial wheat flour (for granulated product only)	N/A
Sucrose	$C_{12}H_{22}O_{11}$
Potassium sorbate	$KC_6H_7O_2$
Sodium benzoate	$NaC_7H_5O_2$
Sodium propionate	$NaC_3H_5O_2$
Citric acid, anhydrous	$C_6H_8O_7$
Trisodium citrate, dihydrate	$Na_3C_6H_5O_7 . 2H_2O$
Sodium chloride	NaCl

Appendix 11: Detailed Manufacturing Information: Fermentation, Recovery, and Formulation

Detailed Manufacturing Information: Fermentation, Recovery, and Formulation

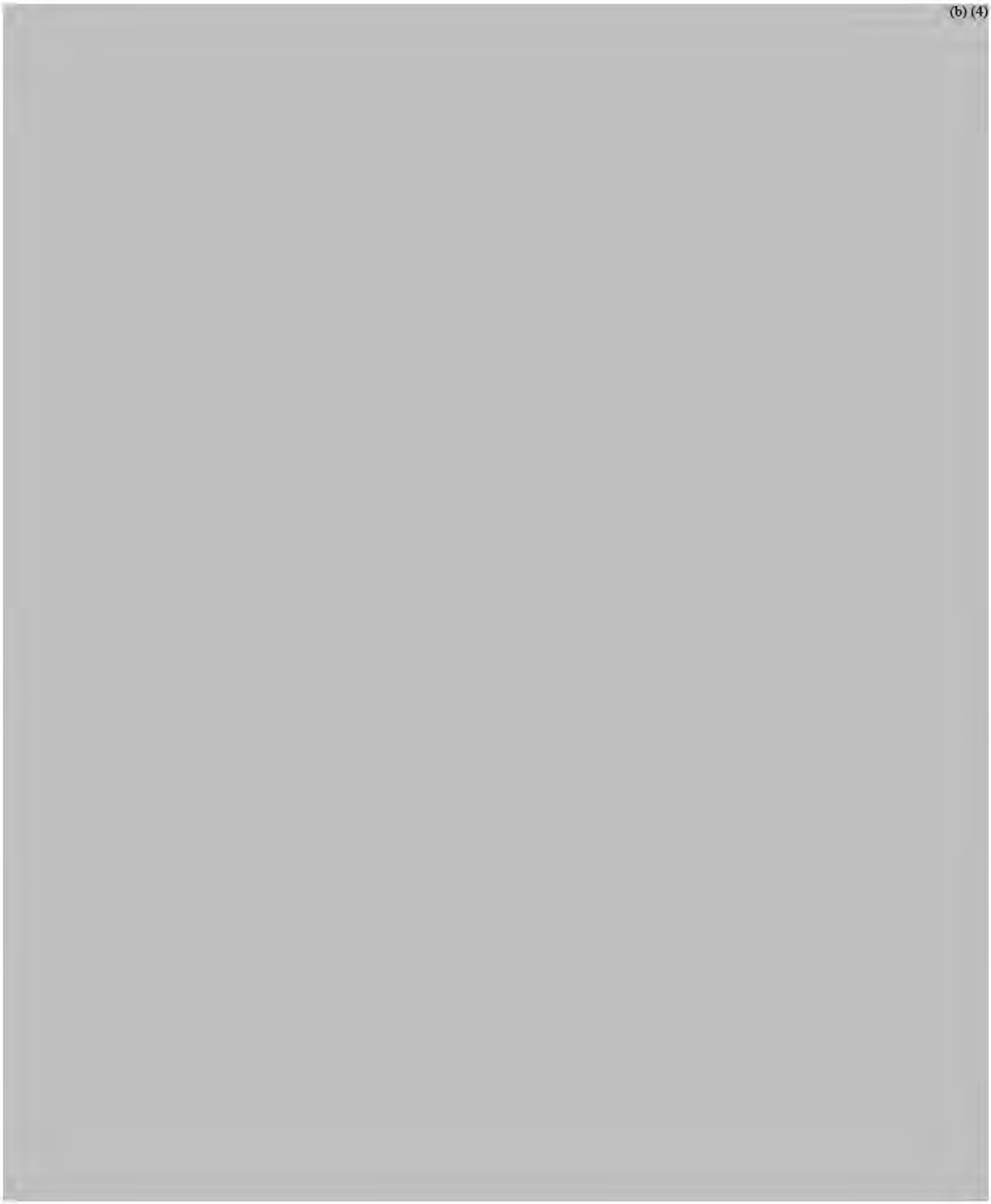
A. Fermentation

(b) (4)



(b) (4)

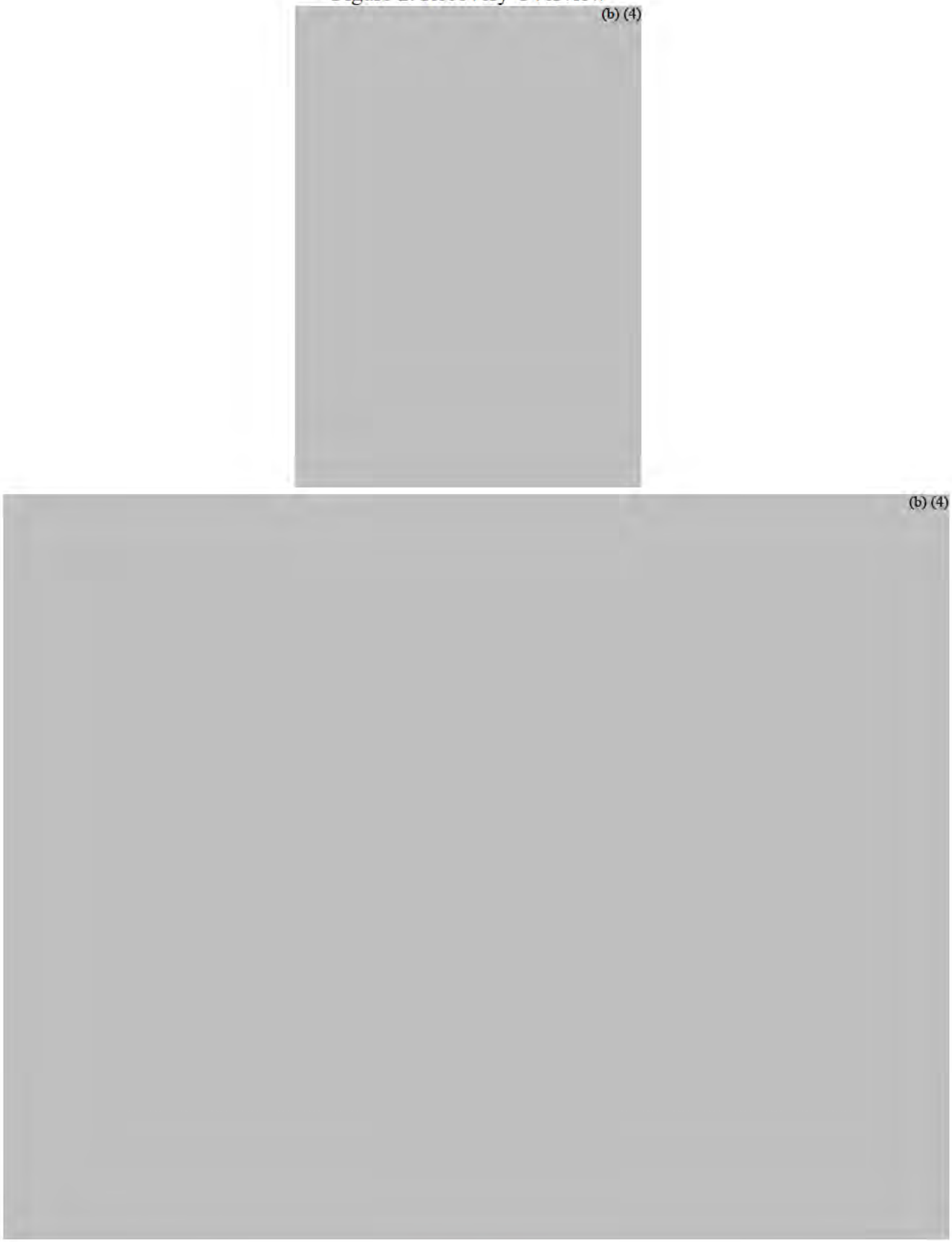
(b) (4)



(b) (4)

(b) (4)

Figure 2. Recovery Overview



(b) (4)

(b) (4)

(b) (4)

Appendix 12: Final Product Composition

CIBENZA® PHYTAVERSE® L10 Phytase Enzyme Composition

Chemical Name	Amount w/w% (b) (6), (b) (4)
Water	
Liquid *P. fluorescens* Fermentation Product	
Sodium Chloride	
Sucrose	
Sodium Citrate	
Potassium Sorbate	
Sodium Benzoate	
Sodium Propionate	

CIBENZA® PHYTAVERSE® G10 Phytase Enzyme Composition

Chemical Name	Amount w/w%
Wheat Flour	(b) (4)
Sucrose	
Dried *P. fluorescens* Fermentation Product	
Sodium Citrate	
Sodium Chloride	
Potassium Sorbate	
Sodium Benzoate	
Sodium Propionate	

Appendix 13: Stability Evaluation of CIBENZA® PHYTAVERSE® G10 Phytase Enzyme in Premix

(b) (4)

Stability evaluation of
CIBENZA® PHYTAVERSE® G10 phytase enzyme in premix

Unique Study Code: F584

FINAL REPORT
Date: March 8, 2018

Study sponsor: Novus Europe S.A./N.V. and BASF Enzymes LLC.

Signed by Study Director, Study Sponsors and Study Monitor:

(b) (4), (b) (6)			
	2018.03.09	8 March 2018	March 9, 2018
Study Director	Study Sponsors		Study Monitor
(b) (4)	Elkin Amaya Senior Regulatory Affairs Manager, Novus Europe S.A./N.V. Novus- Edifici CEPID, Tecnoparc Reus, Avda. Cambra del Comerç 42 ES-43204, Reus, Spain	Roxanna Van Dorn Senior Regulatory Affairs Specialist BASF Enzymes LLC 3550 John Hopkins Court, San Diego, CA 92121, United States of America	Drew Lichtenstein Research Manager, Specialty Products Novus International 20 Research Park Dr., St. Charles, MO 63304, United States of America

Table of contents

1 Summary

The objective of this study was to evaluate the Stability of CIBENZA® PHYTAVERSE® G10 phytase enzyme in vitamin-mineral premix.

The stability of each of the three batches of the test article at two inclusion levels was determined by monthly measuring phytase activity in composite samples obtained at mixing, and after storage at ambient conditions from 0 to 6 months.

Results are presented next in Summary Table 1.

Summary Table 1. Stability of CIBENZA® PHYTAVERSE® G10 phytase enzyme in premix

		N	Phytase U/kg as is	DM %	Phytase U/kg 97% DM	Phytase activity % 0 month as is	Phytase activity % 0 month 97%DM
Tr	month						
A250	0	2	23636	98.8	23215	100.0	100.0
	1	1	(b) (4)				
	2	1					
	3	1					
	4	1					
	5	1					
	6	1					
	Phytase U/kg 97% DM =		(b) (4) × month		P of error refusing slope being 0 = 0.222		
A500	0	2	53321	98.7	52388	100.0	100.0
	1		(b) (4)				
	2						
	3						
	4						
	5						
	6						
	Phytase U/kg 97% DM =		(b) (6) × month		P of error refusing slope being 0 = 0.119		
B250	0	2	27710	98.9	27191	100.0	100.0
	1	1	(b) (4)				
	2	1					
	3	1					
	4	1					
	5	1					
	6	1					
	Phytase U/kg 97% DM =		(b) (4) × month		P of error refusing slope being 0 = 0.114		
B500	0	2	49697	98.9	48740	100.0	100.0
	1	1	(b) (4)				
	2	1					
	3	1					
	4	1					
	5	1					
	6	1					
	Phytase U/kg 97% DM =		(b) (4) × month		P of error refusing slope being 0 = 0.163		
C250	0	2	27836	98.8	27328	100.0	100.0
	1	1	(b) (4)				
	2	1					
	3	1					
	4	1					
	5	1					
	6	3					
	Phytase U/kg 97% DM =		(b) (4) × month		P of error refusing slope being 0 = 0.082		

C500	0	2	51278	98.8	50332	100.0	100.0
	1	1					
	2	1					
	3	1					
	4	1					
	5	1					
	6	1					

(b) (4)

Phytase U/kg 97% DM = (b) (4) × month P of error refusing slope being 0 = 0.200

least squares regressions of Phytase U/kg 88% DM over time, with the upper and lower 95% confidence limits

A250 | Fit Plot for U_kg_as_is | C250

(b) (6)

According the results of the present stability study in vitamin-mineral premix, CIBENZA® PHYTAVERSE® G10 phytase enzyme:

- Was stable over time (up to 6-months storage at ambient conditions) for all three batches (A & B & C) at both 250 and 500 U/kg, as demonstrated by slopes of linear regressions of phytase activity over time not being significantly different from 0 (flat line).
- Presented a good stability (±10% of 0-month value) up to 6-months storage also for all three batches at both 250 and 500 U/kg. Higher variations at intermediate points were considered to be within the range of expected values considering stability within the batch rather than real activity changes.

2 Quality statement

The study, Stability evaluation of CIBENZA® PHYTAVERSE® G10 phytase enzyme in premix (Unique Study Code: F584), was conducted in compliance with current quality standards and regulatory requirements as applicable for EU and US feed additive applications.

Procedures, documentation, equipment and records were examined in order to assure that the study was performed in accordance with the regulations specified herein and with the protocol and relevant Standard Operating Procedures.

Signed and dated:

(b) (4), (b) (6)			
March 8, 2018			
Study Director	Study Sponsors		Study Monitor
(b) (4)	Elkin Amaya Senior Regulatory Affairs Manager, Novus Europe S.A./N.V. Novus- Edifici CEPID, Tecnoparc Reus, Avda. Cambra del Comerç 42 ES-43204, Reus, Spain	Roxanna Van Dorn Senior Regulatory Affairs Specialist BASF Enzymes LLC 3550 John Hopkins Court, San Diego, CA 92121, United States of America	Drew Lichtenstein Research Manager, Specialty Products Novus International 20 Research Park Dr., St. Charles, MO 63304, United States of America

3 Study title and unique study code

Stability evaluation of CIBENZA® PHYTAVERSE® G10 phytase enzyme in vitamin-mineral premix.

Unique study code: F584

4 Study objective

To evaluate the stability of three batches of CIBENZA® PHYTAVERSE® G10 phytase enzyme at two doses each in vitamin-mineral premix.

5 Study location

(b) (6), (b) (4)

6 Important dates & duration of the study

Date of feed manufacture: 24th July 2017
Duration of study: 1 day mixing, 6-months storage for stability

7 Test products

Table 1. Details of test product

Code	Product	Provider	Lot nº Manufacture Date	Active substance	Activity (U/g)†	
					Guaranteed	Analysed
A	CIBENZA® PHYTAVERSE® G10 Phytase Enzyme	Novus International, Inc.	Lot: P23941 Made: 08 October 2014	6-phytase	10,000	13,951
B	CIBENZA® PHYTAVERSE® G10 Phytase Enzyme	Novus International, Inc.	Lot: P26641 Made: 08 October 2014	6-phytase	10,000	13,742
C	CIBENZA® PHYTAVERSE® G10 Phytase Enzyme	Novus International, Inc.	Lot: RO15271001 Made: 28 September 2015	6-phytase	10,000	13,522

† One phytase unit is the amount of enzyme that releases 1 µmol of inorganic phosphate from phytate per minute under the conditions of the assay (Phytate concentration: 5.0 mM, pH: 5.5, Temperature: 37ºC, Incubation time: 30 min) (ISO 30024:2009). Normal analytical variation for the method is 10%.

8 Key study personnel

Study Director: (b) (6), (b) (4)

External Study Monitor: Drew Lichtenstein, Ph.D. Research Manager, Specialty Products, Novus International, Inc., 20 Research Park Dr., St. Charles, MO 63304, United States of America, Tel: +1 314 453-7793, E-mail: drew.lichtenstein@novusint.com

Study Sponsors: 1) Elkin Amaya, Senior Regulatory Affairs Manager, EMEA, Novus Europe S.A./N.V. Novus- Edifici CEPID, Tecnoparc Reus, Av. Cambra del Comerç, 42 ES-43204, Reus, Spain Tel: +34 676 004 728, E-mail: elkin.amaya@novusint.com

2) Roxanna Van Dorn, Sr. Regulatory Affairs Specialist, Phone: +1 858 431-8590, Mobile: +1-858-349-7339, Fax: +1-973-307-2549, E-mail: roxanna.vandorn@basf.com. Postal Address: BASF Enzymes LLC, 3550 John Hopkins Court, San Diego, CA 92121 United States

Feed mill & supervision of diet manufacture: (b) (6), (b) (4)

Premix analysis (DM and CIBENZA® PHYTAVERSE® G10 phytase enzyme): (b) (6), (b) (4)

Optional/back-up facility for premix analysis (DM and CIBENZA® PHYTAVERSE® G10 phytase enzyme): Drew Lichtenstein, Novus International, Inc., 20 Research Park Drive, Saint Charles, MO, 63304; United States of America.

9 Material and methods

9.1 Experimental treatments

Number of treated and control groups: Commercial vitamin-mineral premix (inclusion level 10 kg/ton feed) was used as matrix for stability purposes.

CIBENZA® PHYTAVERSE® G10 phytase enzyme from each batch was added to the vitamin-mineral premix to theoretically provide 250 and 500 U/kg feed as detailed in Table 2.

Table 2. Experimental Treatments

Treatment	Product	CIBENZA® PHYTAVERSE® G10 phytase enzyme		
		U/kg feed	mg in 10 g premix (equivalent to mg/kg feed)†	g to add to 10 kg premix†
A2	CIBENZA® PHYTAVERSE® G10 phytase enzyme batch P23941	250	(b) (4)	
A5		500		
B2	CIBENZA® PHYTAVERSE® G10 phytase enzyme batch P26641	250		
B5		500		
C2	CIBENZA® PHYTAVERSE® G10 phytase enzyme batch RO15271001	250		
C5		500		

† inclusion based on actual activity of each batch

9.2 Treatment application

CIBENZA® PHYTAVERSE® G10 phytase enzyme was mixed with the vitamin-mineral premix in serial mixing steps (details provided under Section 9.3 & 9.6).

9.3 Detailed study design

Figure 1. Basic study design
For each batch and dose of enzyme: The stability of the test article in the vitamin-mineral premix was determined by measuring phytase activity of composite samples obtained at mixing, and after storage at ambient conditions for the following periods and for each batch of enzyme: • 0 months (samples used only for this time point) • 1 months (samples used only for this time point) • 2 months (samples used only for this time point) • 3 months (samples used only for this time point) • 4 months (samples used only for this time point) • 5 months (samples used only for this time point) • 6 months (samples used only for this time point) Premix was produced as follows: 10 kg of Vitamin and Mineral premix was mixed with the corresponding amount of CIBENZA® PHYTAVERSE® phytase enzyme depending on actual activity of each batch as detailed in Table 2

9.4 Premix composition

A standard commercial vitamin-mineral premix was used. The composition of the vitamin-mineral premix is presented next:

Table 3. Composition of vitamin-mineral premix

	Units	per kg of vitamin-mineral premix	when premix added at 10 kg/ton feed, results in the following values per kg of feed
Vitamins, provitamins and similar			
(b) (4)	IU	1 000 000	(b) (4)
	IU	350 000	
	mg	3 000	
	mg	210	
	mg	855	
	mg	470	
	mg	5	
	mg	300	
	mg	2 000	
	mg	1 520	
	mg	6 710	
	mg	150	
	mg	25	
	mg	6 500	
	mg	150	
	mg	1 500	
	mg	8 000	
	mg	8 500	
	mg	20	

Table 3. Composition of vitamin-mineral premix			
	Units	per kg of vitamin-mineral premix	when premix added at 10 kg/ton feed, results in the following values per kg of feed
Amino acids			
(b) (4)	g	50	(b) (4)
	g	150	
	mg	5 000	
	g	10	
	g	146	
	g	100	
		up to 1 kg	

9.5 Premix analyses

Phytase activity in premixes was determined based on "ISO 30024:2009. Animal feeding stuffs – Determination of phytase activity in association with dilution method VDLUFA 27.1.3 (dilution of mineral feeds and premixtures with maize meal (blank feed) before applying the EN ISO 30024 analytical method)."

Dry Matter was determined according AOAC method 934.01: Moisture in Animal Feed.

Premix with no addition of CIBENZA® PHYTAVERSE® G10 phytase enzyme was previously analyzed to confirm the absence of phytase activity before mixing.

9.6 Premixture manufacture

The calculated amount of product for each CIBENZA® PHYTAVERSE® G10 phytase enzyme batch and dose (Table 2) was first manually premixed with (b) (4) .

9.6.1 Short description of the process

Under general and corporative (b) (4) ; (b) (4) (b) (4) .

9.7 Premix samples at manufacture

For each CIBENZA® PHYTAVERSE® G10 phytase enzyme batch and dose:

- After mixing of the product with the vitamin mineral premix, 10 grab samples (~550 g each) were taken from several points of the mixer. From these 10 grab premix samples:
  - Triplicate (NOVUS, (b) (4)) (b) (4) at each time point one sample was sent to NOVUS, a second one analyzed for phytase activity at (b) (4) lab while the third sample was retained at (b) (4) -20°C as a backup sample).

Each sample was labelled with the unique study code (F584), treatment code (A2 / A5 / B2 / B5 / C2 / C5), sample number (i.e. NOVUS samples 1.11 to 17; (b) (4) samples 2.11 to 2.17; backup samples 3.11 to 3.17), the date of manufacture and the analysis required (DM, phytase activity).

9.8 Feed sampling plan

Table 4. Sampling plan

CIBENZA® PHYTAVERSE® G10 phytase enzyme	dose intended for XXX U/kg feed	Months storage	Final Samples		
			NOVUS	(b) (4)	
A	250	10 × ~550 g grab samples homogenized and split: 21 × 250g			
		0	1 × 250g	1 × 250g	1 × 250g
		1	(b) (4)		
		2			
		3			
		4			
		5			
		6			
A	500	10 × ~550 g grab samples homogenized and split: 21 × 250g			
		0	1 × 250g	1 × 250g	1 × 250g
		1	(b) (4)		
		2			
		3			
		4			
		5			
		6			
B	250	10 × ~550 g grab samples homogenized and split: 21 × 250g			
		0	1 × 250g	1 × 250g	1 × 250g
		1	(b) (4)		
		2			
		3			
		4			
		5			
		6			
B	500	10 × ~550 g grab samples homogenized and split: 21 × 250g			
		0	1 × 250g	1 × 250g	1 × 250g
		1	(b) (4)		
		2			
		3			
		4			
		5			
		6			
C	250	10 × ~550 g grab samples homogenized and split: 21 × 250g			
		0	1 × 250g	1 × 250g	1 × 250g
		1	(b) (4)		
		2			
		3			
		4			
		5			
		6			

Table 4. Sampling plan					
CIBENZA® PHYTAVERSE® G10 phytase enzyme	dose intended for XXX U/kg feed	Months storage	Final Samples		
			NOVUS	(b) (4)	
C	500	10 × ~550 g grab samples homogenized and split: 21 × 250g			
		0	1 × 250g	1 × 250g	1 × 250g
		1	(b) (4)		
		2			
		3			
		4			
		5			
		6			

For stability analysis, all samples were kept together at (b) (4)s feed mill in a cardboard box protected from light and at room temperature. Samples were dispatched to NOVUS Reus and (b) (4) lab for analysis or (b) (4) storage after the corresponding time (0, 1, 2, 3, 4, 5 or 6 months) (except for 5-months NOVUS & backup samples that were stored 6-months at ambient conditions at the feed mill by error; backup samples used for analysis were: All 0-month, A250 4-months, A500 6-months, and C250 5-months {actually 6-months stored} & 6-months).

9.9 Statistics

The CIBENZA® PHYTAVERSE® G10 phytase enzyme activity was assessed in the premix after the maximum storage period (6-months). The data was fitted to a least squares regression, with the upper and lower 95% confidence limits shown. The regression line of CIBENZA® PHYTAVERSE® G10 phytase enzyme activity vs. time was calculated and the slope tested to determine if it was significantly different from 0.

10 Results

The results are summarized in Table 5.

Table 5. Stability of CIBENZA® PHYTAVERSE® G10 phytase enzyme in premix							
Tr	month	N	Phytase U/kg as is	DM %	Phytase U/kg 97% DM	Phytase activity % 0 month as is	Phytase activity % 0 month 97%DM
A250	0	2	23636	98.8	23215	100.0	100.0
	1	1	(b) (4)				
	2	1					
	3	1					
	4	1					
	5	1					
	6	1					
	Phytase U/kg 97% DM = (b) (6) × month P of error refusing slope being 0 = 0.222						
A500	0	2	53321	98.7	52388	100.0	100.0
	1	1	(b) (6)				
	2	1					
	3	1					
	4	1					
	5	1					
	6	2					
	Phytase U/kg 97% DM = (b) (6) × month P of error refusing slope being 0 = 0.119						

Table 5. Stability of CIBENZA® PHYTAVERSE® G10 phytase enzyme in premix

Tr	month	N	Phytase U/kg as is	DM %	Phytase U/kg 97% DM	Phytase activity % 0 month as is	Phytase activity % 0 month 97%DM
B250	0	2	27710	98.9	27191	100.0	100.0
	1	1	(b) (4)				
	2	1					
	3	1					
	4	1					
	5	1					
	6	1					
	Phytase U/kg 97% DM = (b) (4) × month P of error refusing slope being 0 = 0.114						
B500	0	2	49697	98.9	48740	100.0	100.0
	1	1	(b) (4)				
	2	1					
	3	1					
	4	1					
	5	1					
	6	1					
	Phytase U/kg 97% DM = (b) (4) × month P of error refusing slope being 0 = 0.163						
C250	0	2	27836	98.8	27328	100.0	100.0
	1	1	(b) (4)				
	2	1					
	3	1					
	4	1					
	5	1					
	6	3					
	Phytase U/kg 97% DM = (b) (4) × month P of error refusing slope being 0 = 0.082						
C500	0	2	51278	98.8	50332	100.0	100.0
	1	1	(b) (4)				
	2	1					
	3	1					
	4	1					
	5	1					
	6	1					
	Phytase U/kg 97% DM = (b) (4) × month P of error refusing slope being 0 = 0.200						

† One phytase unit is the amount of enzyme that releases 1 µmol of inorganic phosphate from phytate per minute under the conditions of the assay (Phytate concentration: 5.0 mM, pH: 5.5, Temperature: 37°C, Incubation time: 30 min) (ISO 30024:2009). Normal analytical variation for the method is 10%.

Figure 2. Least squares regressions of Phytase U/kg 97% DM over time, with the upper and lower 95% confidence limits

Figure 2. Least squares regressions of Phytase U/kg 97% DM over time, with the upper and lower 95% confidence limits

† One phytase unit is the amount of enzyme that releases 1 µmol of inorganic phosphate from phytate per minute under the conditions of the assay (Phytate concentration: 5.0 mM, pH: 5.5, Temperature: 37°C, Incubation time: 30 min) (ISO 30024:2009). Normal analytical variation for the method is 10%.

11 Discussion

CIBENZA® PHYTAVERSE® G10 phytase enzyme activity results were also standardized considering a common Dry Matter content of 97%. This value was close to the average DM values from previous studies, but lower than the actual DM content in the present study (99.0%; DM range: 98.7-99.97%). DM did not greatly vary with time.

The backup samples were also analyzed at 0-month for all treatments, at 4-months for A250, at 6-months for A500, and for C250 at 5-months {actually 6-months stored at ambient conditions at the feed mill} & 6-months. The average value between the original and backup sample was taken into account for all except A250 4-months and C250 5-months: A250→ The original 4-months sample was probably spoiled and discarded by the lab technician, using the A250 backup instead; C250→ The original intent was to have it tested as a 5-month backup for C250 5-month original sample, but due to the error in storage, it could not be used as such. Therefore, the original C250 5-month sample was reported as is, and no backup sample was available for this time point.

The regression lines of CIBENZA® PHYTAVERSE® G10 phytase enzyme activities *vs.* time was calculated for the three batches and both concentrations per batch, and the slopes were not significantly ($P>0.05$) different from 0, meaning that no significant loss of activity was detected in any case. Final phytase activity (6-months stability) standardized at 97% DM content was generally within ±10% of that of 0-month value except for A250 (113% activity from 0-month); however, this >100% value for the A250 treatment is considered to be related to the analytical variation at each time point (% of activity were 100%, 101%, 110%, 101%, 103%, 102% and 113% for 0, 1, 2, 3, 4, 5 and 6-months respectively).

12 Conclusions

According the results of the present stability in vitamin-mineral premix, CIBENZA® PHYTAVERSE® G10 phytase enzyme:

- Was stable over time (up to 6-months storage at ambient conditions) for all three batches (A & B & C) at both 250 and 500 U/kg, as demonstrated by slopes of linear regressions of phytase activity over time not being significantly different from 0 (flat line).
- Presented a good stability (±10% of 0-month value) up to 6-months storage also for all three batches at both 250 and 500 U/kg. Higher variations at punctual points were considered to be within the range of expected values considering stability within the batch rather than real activity loss.

13 References

ISO 30024:2009. Animal feeding stuffs – Determination of phytase activity

VLLUFA 27.1.3. Preparation of Mineral Feed and Premixtures for the Determination of Phytase Activity

Regulation (EC) Nº 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition.http://eur-lex.europa.eu/en/index.htm

SAS Institute Inc. 2011. Base SAS® 9.3 Guide to Information Maps. Cary, NC: SAS Institute Inc.

Statutory Instrument 1999 No. 1663. The Feeding Stuffs (Sampling and Analysis) Regulation 1999.

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Appendix 1- *Curricula vitae* of Study Director & Study Monitor

Study Director:
Name: (b) (6)
Qualifications: (b) (6)

Present Position: (b) (6)
Experience: (b) (6)

Study Monitor:
Name: Drew Lichtenstein
Qualifications: B.S. Biochemistry (Michigan State University 1982), PhD Biochemistry (University of Wisconsin-Madison 1990)
Present Position: Research Manager, Specialty Products, Novus International
Experience: Over 35 years research experience in biochemistry and cell biology; more than 8 years of experience in animal feed enzymes.

Appendix 2 - Certificate of analysis of CIBENZA® PHYTAVERSE® G10 phytase enzyme used (3 batches)

NOVUS

NOVUS INTERNATIONAL INC.
20 RESEARCH PARK DRIVE
ST. CHARLES, MO 63304

DATE:	09 May 2017
PRODUCT:	20002453
PRODUCT DESCRIPTION:	CIBENZA PHYTAVERSE G10 20 KG BAG
LOT NUMBER:	P23941
DATE OF MANUFACTURE:	8 October 2014
DATE OF ANALYSIS:	21 March 2017
DATE OF PACKAGING:	8 October 2014

CERTIFICATE OF ANALYSIS

CHARACTERISTIC	SPECIFICATION	RESULTS
Appearance	White to Beige Granules	(b) (4)
Phytase Activity, U/g	>=10000	

Approved by:

SABINA DIAZ
REGULATORY AFRAIRS MANAGER, EMEA

The value and properties stated above are based upon test and analysis of samples of material. The exclusive commitment of Novus with respect to such values and properties is as set forth in the sales contract between your company and Novus for such material or the acknowledgment of Novus for the above described shipment of material, whichever is applicable.

NOVUS

NOVUS INTERNATIONAL INC.
20 RESEARCH PARK DRIVE
ST. CHARLES, MO 63304

DATE:	09 May 2017
PRODUCT:	20002453
PRODUCT DESCRIPTION:	CIBENZA PHYTAVERSE G10 20 KG BAG
LOT NUMBER:	P26641
DATE OF MANUFACTURE:	8 October 2014
DATE OF ANALYSIS:	21 March 2017
DATE OF PACKAGING:	8 October 2014

CERTIFICATE OF ANALYSIS

CHARACTERISTIC	SPECIFICATION	RESULTS
Appearance	White to Beige Granules	(b) (4)
Phytase Activity, U/g	>=10000	

Approved by:

SABINA DIAZ
REGULATORY AFRAIRS MANAGER, EMEA

The value and properties stated above are based upon test and analysis of samples of material. The exclusive commitment of Novus with respect to such values and properties is as set forth in the sales contract between your company and Novus for such material or the acknowledgment of Novus for the above described shipment of material, whichever is applicable.

NOVUS

NOVUS INTERNATIONAL INC.
20 RESEARCH PARK DRIVE
ST. CHARLES, MO 63304

DATE:	09 May 2017
PRODUCT:	20002453
PRODUCT DESCRIPTION:	CIBENZA PHYTAVERSE G10 20 KG BAG
LOT NUMBER:	RO15271001
DATE OF MANUFACTURE:	28 September 2015
DATE OF ANALYSIS:	21 March 2017
DATE OF PACKAGING:	28 September 2015

CERTIFICATE OF ANALYSIS

CHARACTERISTIC	SPECIFICATION	RESULTS
Appearance	White to Beige Granules	(b) (4)
Phytase Activity, U/g	>=10000	(b) (4)

Approved by:

SABINA DIAZ
REGULATORY AFRAIRS MANAGER, EMEA

The value and properties stated above are based upon test and analysis of samples of material. The exclusive commitment of Novus with respect to such values and properties is as set forth in the sales contract between your company and Novus for such material or the acknowledgment of Novus for the above described shipment of material, whichever is applicable.

Appendix 3- Relevant laboratory reports

CERTIFICATE OF ANALYSIS

Company:	Novus Europe S.A./N.V.		
Type of sample:	F584 vitamin-mineral premix		
Laboratory ref. :	0-month & backup	171310-5	171589-94
	1-months	171456-61	
	2-months	171595-600	
	3-months	171793-8	
	4-months & backup	171792-7	172040
	5-months	172270-5	
	6-months & backup	180307-12	181394-6
Reception date:	25th July 2017		
Analysis starting date:	25th July 2017		
Analysis finishing date:	23rd February 2018		

Sample description:
See Results section

Analysis performed:

- AOAC, 2000:
  - Moisture -dry matter- by oven drying –method 2 (SOP 0602-L-10001)
- Other
  - Phytase (SOP 0602-L-10143; ISO 30024:2009. Animal feeding stuffs – Determination of phytase activity.)

Results:

LAB. REF.	SAMPLE DESCRIPTION	PHYTASE (FTU/Kg)	DRY MATTER (%)
171310	TA-2 24/07/2017	(b) (4)	(b) (4)
171311	TA-5 24/07/2017		
171312	TB-2 24/07/2017		
171313	TB-5 24/07/2017		
171314	TC-2 24/07/2017		
171315	TC-5 24/07/2017		
171589	TA-2 24/07/2017 muestra backup 0 meses		
171590	TA-5 24/07/2017 muestra backup 0 meses		
171591	TB-2 24/07/2017 muestra backup 0 meses		
171592	TB-5 24/07/2017 muestra backup 0 meses		
171593	TC-2 24/07/2017 muestra backup 0 meses		
171594	TC-5 24/07/2017 muestra backup 0 meses		
171456	TA-2 Premix + G10 1 month		
171459	TA-5 Premix + G10 1 month		
171457	TB-2 Premix + G10 1 month		
171460	TB-5 Premix + G10 1 month		
171458	TC-2 Premix + G10 1 month		
171461	TC-5 Premix + G10 1 month		
171595	TA-2 Premix + G10 2 month		
171596	TA-5 Premix + G10 2 month		
171597	TB-2 Premix + G10 2 month		
171598	TB-5 Premix + G10 2 month		
171599	TC-2 Premix + G10 2 month		
171600	TC-5 Premix + G10 2 month		
171793	TA-2 Premix + G10 3 month		
171794	TA-5 Premix + G10 3 month		
171795	TB-2 Premix + G10 3 month		
171796	TB-5 Premix + G10 3 month		

171797	TC-2 Premix + G10 3 month		(b) (4)
171798	TC-5 Premix + G10 3 month		
171792	TA-2 Premix + G10 4 month		
172040	TA-2 Premix + G10 4 month BACKUP		
171793	TA-5 Premix + G10 4 month		
171794	TB-2 Premix + G10 4 month		
171795	TB-5 Premix + G10 4 month		
171796	TC-2 Premix + G10 4 month		
171797	TC-5 Premix + G10 4 month		
172270	TA-2 Premix + G10 5 month		
172271	TA-5 Premix + G10 5 month		
172272	TB-2 Premix + G10 5 month		
172273	TB-5 Premix + G10 5 month		
172274	TC-2 Premix + G10 5 month		
172275	TC-5 Premix + G10 5 month		
180307	TA-2 Premix + G10 6 month		
180310	TA-5 Premix + G10 6 month		
180308	TB-2 Premix + G10 6 month		
180311	TB-5 Premix + G10 6 month		
180309	TC-2 Premix + G10 6 month		
180312	TC-5 Premix + G10 6 month		
181394	TC-2 Premix 5 month Back up (6 month @ambient conditions)		
181395	TA-5 Premix 6 month Back up		
181396	TC-2 Premix 6 month Back up		

(b) (4), (b) (6)

Signature: (b) (6), (b) (4)

Date: 8TH MARCH 2018

Appendix 4- Raw data

Obs	enzyme	Tr	lab_ref	dose	U_kg_as_is	DM_p	month
1	(b) (4)		171310	250		(b) (4)	0
2			171311	500			0
3			171312	250			0
4			171313	500			0
5			171314	250			0
6			171315	500			0
7			171589	250			0
8			171590	500			0
9			171591	250			0
10			171592	500			0
11			171593	250			0
12			171594	500			0
13			171456	250			1
14			171459	500			1
15			171457	250			1
16			171460	500			1
17			171458	250			1
18			171461	500			1
19			171595	250			2
20			171596	500			2
21			171597	250			2
22			171598	500			2
23			171599	250			2
24			171600	500			2
25			171793	250			3
26			171794	500			3
27			171795	250			3
28			171796	500			3
29			171797	250			3
30			171798	500			3
31			172040	250			4
32			171793	500			4
33			171794	250			4
34			171795	500			4
35			171796	250			4
36			171797	500			4
37			172270	250			5
38			172271	500			5
39			172272	250			5
40			172273	500			5
41			172274	250			5
42			172275	500			5
43			170307	250			6
44			170310	500			6
45			170308	250			6
46			170311	500			6
47			170309	250			6
48			170312	500			6
49			181394	250			6
50			181395	500			6
51			181396	250			6

Appendix 5 - Statistical printouts

Obs	enzyme	Tr	lab_ref	dose	U_kg_as_is	DM_p	month
1	(b) (4)		171310	250		(b) (4)	0
2			171311	500			0
3			171312	250			0
4			171313	500			0
5			171314	250			0
6			171315	500			0
7			171589	250			0
8			171590	500			0
9			171591	250			0
10			171592	500			0
11			171593	250			0
12			171594	500			0
13			171456	250			1
14			171459	500			1
15			171457	250			1
16			171460	500			1
17			171458	250			1
18			171461	500			1
19			171595	250			2
20			171596	500			2
21			171597	250			2
22			171598	500			2
23			171599	250			2
24			171600	500			2
25			171793	250			3
26			171794	500			3
27			171795	250			3
28			171796	500			3
29			171797	250			3
30			171798	500			3
31			172040	250			4
32			171793	500			4
33			171794	250			4
34			171795	500			4
35			171796	250			4
36			171797	500			4
37			172270	250			5
38			172271	500			5
39			172272	250			5
40			172273	500			5
41			172274	250			5
42			172275	500			5
43			170307	250			6
44			170310	500			6
45			170308	250			6
46			170311	500			6
47			170309	250			6
48			170312	500			6
49			181394	250			6
50			181395	500			6
51			181396	250			6

Obs	enzyme	dose	month	Tr	_FREQ_	U_kg_as_is	DM_p	U_kg_97_pc_DM
1	(b) (4)		0	A250	2	(b) (4)		
2			1	A250	1			
3			2	A250	1			
4			3	A250	1			
5			4	A250	1			
6			5	A250	1			
7			6	A250	1			
8			0	A500	2			
9			1	A500	1			
10			2	A500	1			
11			3	A500	1			
12			4	A500	1			
13			5	A500	1			
14			6	A500	2			
15			0	B250	2			
16			1	B250	1			
17			2	B250	1			
18			3	B250	1			
19			4	B250	1			
20			5	B250	1			
21			6	B250	1			
22			0	B500	2			
23			1	B500	1			
24			2	B500	1			
25			3	B500	1			
26			4	B500	1			
27			5	B500	1			
28			6	B500	1			
29			0	C250	2			
30			1	C250	1			
31			2	C250	1			
32			3	C250	1			
33			4	C250	1			
34			5	C250	1			
35			6	C250	3			
36			0	C500	2			
37			1	C500	1			
38			2	C500	1			
39			3	C500	1			
40			4	C500	1			
41			5	C500	1			
42			6	C500	1			

Obs	enzyme	Tr	lab_ref	dose	U_kg_as_is	DM_p	month	U_kg_97_pc_DM	pc_Om_as_is	pc_Om_97_pc_DM	pc_Om_DM
1	(b) (4)		171310	250	(b) (4)		0	(b) (4)			
2			171311	500			0				
3			171312	250			0				
4			171313	500			0				
5			171314	250			0				
6			171315	500			0				
7			171589	250			0				
8			171590	500			0				
9			171591	250			0				
10			171592	500			0				
11			171593	250			0				
12			171594	500			0				
13			171456	250			1				
14			171459	500			1				
15			171457	250			1				
16			171460	500			1				
17			171458	250			1				
18			171461	500			1				
19			171595	250			2				
20			171596	500			2				
21			171597	250			2				
22			171598	500			2				
23			171599	250			2				
24			171600	500			2				
25			171793	250			3				
26			171794	500			3				
27			171795	250			3				
28			171796	500			3				
29			171797	250			3				
30			171798	500			3				
31			172040	250			4				
32			171793	500			4				
33			171794	250			4				
34			171795	500			4				
35			171796	250			4				
36			171797	500			4				
37			172270	250			5				
38			172271	500			5				
39			172272	250			5				
40			172273	500			5				
41			172274	250			5				

42	(b) (4)	172275	500	(b) (4)	5
43		170307	250		6
44		170310	500		6
45		170308	250		6
46		170311	500		6
47		170309	250		6
48		170312	500		6
49		181394	250		6
50		181395	500		6
51		181396	250		6

		N	U_kg_-as_is	DM_p	U_kg_-97_pc-_DM	pc_Om-_as_is	pc_Om-_97_p-c_DM	pc_Om-_DM
		N	Mean	Mean	Mean	Mean	Mean	Mean
Tr	month							
A250	0	2	23636	98.8	23215	100.0	100.0	100.0
	1	1	(b) (4)					
	2	1						
	3	1						
	4	1						
	5	1						
	6	1						
A500	0	2	53321	98.7	52388	100.0	100.0	100.0
	1	1	(b) (4)					
	2	1						
	3	1						
	4	1						
	5	1						
	6	2						
B250	0	2	27710	98.9	27191	100.0	100.0	100.0
	1	1	(b) (4)					
	2	1						
	3	1						
	4	1						

	5	1	(b) (4)				
	6	1					
B500	0	2	49697	98.9	48740	100.0	100.0	100.0
	1	1	(b) (4)				
	2	1					
	3	1					
	4	1					
	5	1					
	6	1					
C250	0	2	27836	98.8	27328	100.0	100.0	100.0
	1	1	(b) (4)				
	2	1					
	3	1					
	4	1					
	5	1					
	6	3					
C500	0	2	51278	98.8	50332	100.0	100.0	100.0
	1	1	(b) (4)				
	2	1					
	3	1					
	4	1					
	5	1					
	6	1					

Obs	enzyme	dose	month	Tr	_FREQ_	U_kg_ as_is	DM_p	U_kg_97_ pc_DM	pc_Om_ as_is	pc_Om_ 97_pc_DM	pc_Om_DM
1	(b) (4)		0	A250	2	(b) (4)					
2			1	A250	1						
3			2	A250	1						
4			3	A250	1						
5			4	A250	1						
6			5	A250	1						
7			6	A250	1						
8			0	A500	2						
9			1	A500	1						
10			2	A500	1						
11			3	A500	1						
12			4	A500	1						
13			5	A500	1						
14			6	A500	2						
15			0	B250	2						
16			1	B250	1						
17			2	B250	1						
18			3	B250	1						
19			4	B250	1						
20			5	B250	1						
21			6	B250	1						
22			0	B500	2						
23			1	B500	1						
24			2	B500	1						
25			3	B500	1						
26			4	B500	1						
27			5	B500	1						
28			6	B500	1						
29			0	C250	2						
30			1	C250	1						
31			2	C250	1						
32			3	C250	1						
33			4	C250	1						
34			5	C250	1						
35			6	C250	3						
36			0	C500	2						
37			1	C500	1						
38			2	C500	1						
39			3	C500	1						
40			4	C500	1						
41			5	C500	1						
42			6	C500	1						

---------- Tr=A250 ----------

The GLM Procedure

Number of Observations Read 7
Number of Observations Used 7

---------- Tr=A250 ----------

The GLM Procedure
Dependent Variable: U_kg_as_is

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	2518500.223	2518500.223	2.11	0.2061
Error	5	5968822.134	1193764.427		
Corrected Total	6	8487322.357			

R-Square	Coeff Var	Root MSE	U_kg_as_is Mean
0.296737	4.425907	1092.595	24686.36

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	2518500.223	2518500.223	2.11	0.2061

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	2518500.223	2518500.223	2.11	0.2061

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	23786.62500	744.4781861	31.95	<.0001
month	299.91071	206.4810980	1.45	0.2061

---------- Tr=A250 ----------

The GLM Procedure
Dependent Variable: U_kg_97_pc_DM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	2288002.269	2288002.269	1.94	0.2222
Error	5	5889253.594	1177850.719		
Corrected Total	6	8177255.864			

R-Square	Coeff Var	Root MSE	U_kg_97_pc_DM Mean
0.279801	4.483302	1085.288	24207.34

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	2288002.269	2288002.269	1.94	0.2222

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	2288002.269	2288002.269	1.94	0.2222

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	23349.77110	739.4993322	31.58	<.0001
month	285.85725	205.1002123	1.39	0.2222

---------- Tr=A250 ----------

The GLM Procedure
Dependent Variable: pc_Om_as_is

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	45.0829582	45.0829582	2.11	0.2061
Error	5	106.8461922	21.3692384		
Corrected Total	6	151.9291504			

R-Square	Coeff Var	Root MSE	pc_Om_as_is Mean
0.296737	4.425907	4.622687	104.4461

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	45.08295822	45.08295822	2.11	0.2061

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	45.08295822	45.08295822	2.11	0.2061

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	100.6393984	3.14983049	31.95	<.0001
month	1.2688994	0.87360580	1.45	0.2061

---------- Tr=A250 ----------

The GLM Procedure
Dependent Variable: pc_Om_97_pc_DM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	42.4548130	42.4548130	1.94	0.2222
Error	5	109.2774965	21.8554993		
Corrected Total	6	151.7323095			

R-Square	Coeff Var	Root MSE	pc_Om_97_pc_DM Mean
0.279801	4.483302	4.674987	104.2755

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	42.45481298	42.45481298	1.94	0.2222

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	42.45481298	42.45481298	1.94	0.2222

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	100.5814444	3.18546639	31.58	<.0001
month	1.2313583	0.88348941	1.39	0.2222

---------- Tr=A500 ----------

The GLM Procedure

Number of Observations Read 7
Number of Observations Used 7

---------- Tr=A500 ----------

The GLM Procedure

Dependent Variable: U_kg_as_is

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	38506073.58	38506073.58	3.39	0.1251
Error	5	56839175.92	11367835.18		
Corrected Total	6	95345249.50			

R-Square	Coeff Var	Root MSE	U_kg_as_is Mean
0.403859	6.684355	3371.622	50440.50

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	38506073.58	38506073.58	3.39	0.1251

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	38506073.58	38506073.58	3.39	0.1251

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	53958.58929	2297.373169	23.49	<.0001
month	-1172.69643	637.176674	-1.84	0.1251

---------- Tr=A500 ----------

The GLM Procedure

Dependent Variable: U_kg_97_pc_DM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	39249816.49	39249816.49	3.53	0.1189
Error	5	55530999.47	11106199.89		
Corrected Total	6	94780815.97			

R-Square	Coeff Var	Root MSE	U_kg_97_pc_DM Mean
0.414111	6.736402	3332.597	49471.46

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	39249816.49	39249816.49	3.53	0.1189

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	39249816.49	39249816.49	3.53	0.1189

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	53023.36467	2270.781793	23.35	<.0001
month	-1183.96755	629.801553	-1.88	0.1189

---------- Tr=A500 ----------

The GLM Procedure
Dependent Variable: pc_Om_as_is

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	135.4380867	135.4380867	3.39	0.1251
Error	5	199.9214285	39.9842857		
Corrected Total	6	335.3595151			

R-Square	Coeff Var	Root MSE	pc_Om_as_is Mean
0.403859	6.684355	6.323313	94.59870

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	135.4380867	135.4380867	3.39	0.1251

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	135.4380867	135.4380867	3.39	0.1251

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	101.1967054	4.30861145	23.49	<.0001
month	-2.1993350	1.19499381	-1.84	0.1251

---------- Tr=A500 ----------

The GLM Procedure
Dependent Variable: pc_Om_97_pc_DM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	143.0113516	143.0113516	3.53	0.1189
Error	5	202.3337686	40.4667537		
Corrected Total	6	345.3451202			

R-Square	Coeff Var	Root MSE	pc_Om_97_pc_DM Mean
0.414111	6.736402	6.361348	94.43243

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	143.0113516	143.0113516	3.53	0.1189

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	143.0113516	143.0113516	3.53	0.1189

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	101.2124000	4.33452831	23.35	<.0001
month	-2.2599886	1.20218185	-1.88	0.1189

---------- Tr=B250 ----------

The GLM Procedure

Number of Observations Read 7
Number of Observations Used 7

---------- Tr=B250 ----------

The GLM Procedure
Dependent Variable: U_kg_as_is

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	12476910.04	12476910.04	3.54	0.1187
Error	5	17622962.82	3524592.56		
Corrected Total	6	30099872.86			

R-Square	Coeff Var	Root MSE	U_kg_as_is Mean
0.414517	7.286347	1877.390	25765.86

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	12476910.04	12476910.04	3.54	0.1187

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	12476910.04	12476910.04	3.54	0.1187

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	27768.46429	1279.225538	21.71	<.0001
month	-667.53571	354.793328	-1.88	0.1187

---------- Tr=B250 ----------

The GLM Procedure
Dependent Variable: U_kg_97_pc_DM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	12489187.41	12489187.41	3.66	0.1141
Error	5	17082956.17	3416591.23		
Corrected Total	6	29572143.58			

R-Square	Coeff Var	Root MSE	U_kg_97_pc_DM Mean
0.422329	7.319296	1848.402	25253.83

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	12489187.41	12489187.41	3.66	0.1141

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	12489187.41	12489187.41	3.66	0.1141

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	27257.41765	1259.473899	21.64	<.0001
month	-667.86406	349.315209	-1.91	0.1141

---------- Tr=B250 ----------

The GLM Procedure
Dependent Variable: pc_Om_as_is

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	162.4927513	162.4927513	3.54	0.1187
Error	5	229.5122515	45.9024503		
Corrected Total	6	392.0050028			

R-Square	Coeff Var	Root MSE	pc_Om_as_is Mean
0.414517	7.286347	6.775135	92.98397

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	162.4927513	162.4927513	3.54	0.1187

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	162.4927513	162.4927513	3.54	0.1187

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	100.2109862	4.61647614	21.71	<.0001
month	-2.4090065	1.28038011	-1.88	0.1187

---------- Tr=B250 ----------

The GLM Procedure
Dependent Variable: pc_Om_97_pc_DM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	168.9164726	168.9164726	3.66	0.1141
Error	5	231.0472734	46.2094547		
Corrected Total	6	399.9637459			

R-Square	Coeff Var	Root MSE	pc_Om_97_pc_DM Mean
0.422329	7.319296	6.797754	92.87441

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	168.9164726	168.9164726	3.66	0.1141

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	168.9164726	168.9164726	3.66	0.1141

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	100.2428993	4.63188835	21.64	<.0001
month	-2.4561619	1.28465469	-1.91	0.1141

---------- Tr=B500 ----------

The GLM Procedure

Number of Observations Read 7
Number of Observations Used 7

---------- Tr=B500 ----------

The GLM Procedure
Dependent Variable: U_kg_as_is

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	40677187.6	40677187.6	2.61	0.1670
Error	5	77882964.8	15576593.0		
Corrected Total	6	118560152.4			

R-Square	Coeff Var	Root MSE	U_kg_as_is Mean
0.343093	7.687170	3946.719	51341.64

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	40677187.58	40677187.58	2.61	0.1670

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	40677187.58	40677187.58	2.61	0.1670

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	54957.55357	2689.235874	20.44	<.0001
month	-1205.30357	745.859834	-1.62	0.1670

---------- Tr=B500 ----------

The GLM Procedure
Dependent Variable: U_kg_97_pc_DM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	40668387.7	40668387.7	2.67	0.1631
Error	5	76124095.0	15224819.0		
Corrected Total	6	116792482.7			

R-Square	Coeff Var	Root MSE	U_kg_97_pc_DM Mean
0.348211	7.757353	3901.899	50299.37

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	40668387.66	40668387.66	2.67	0.1631

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	40668387.66	40668387.66	2.67	0.1631

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	53914.88647	2658.696290	20.28	<.0001
month	-1205.17319	737.389677	-1.63	0.1631

---------- Tr=B500 ----------

The GLM Procedure
Dependent Variable: pc_Om_as_is

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	164.7021662	164.7021662	2.61	0.1670
Error	5	315.3485718	63.0697144		
Corrected Total	6	480.0507380			

R-Square	Coeff Var	Root MSE	pc_Om_as_is Mean
0.343093	7.687170	7.941644	103.3104

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	164.7021662	164.7021662	2.61	0.1670

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	164.7021662	164.7021662	2.61	0.1670

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	110.5863664	5.41131845	20.44	<.0001
month	-2.4253289	1.50082970	-1.62	0.1670

---------- Tr=B500 ----------

The GLM Procedure
Dependent Variable: pc_Om_97_pc_DM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	171.1902219	171.1902219	2.67	0.1631
Error	5	320.4380962	64.0876192		
Corrected Total	6	491.6283181			

R-Square	Coeff Var	Root MSE	pc_Om_97_pc_DM Mean
0.348211	7.757353	8.005474	103.1985

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	171.1902219	171.1902219	2.67	0.1631

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	171.1902219	171.1902219	2.67	0.1631

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	110.6164445	5.45481128	20.28	<.0001
month	-2.4726376	1.51289244	-1.63	0.1631

---------- Tr=C250 ----------

The GLM Procedure

Number of Observations Read 7
Number of Observations Used 7

---------- Tr=C250 ----------

The GLM Procedure
Dependent Variable: U_kg_as_is

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	10099809.72	10099809.72	4.70	0.0824
Error	5	10753002.63	2150600.53		
Corrected Total	6	20852812.36			

R-Square	Coeff Var	Root MSE	U_kg_as_is Mean
0.484338	5.626080	1466.493	26065.98

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	10099809.72	10099809.72	4.70	0.0824

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	10099809.72	10099809.72	4.70	0.0824

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	27867.74405	999.2462668	27.89	<.0001
month	-600.58929	277.1410501	-2.17	0.0824

---------- Tr=C250 ----------

The GLM Procedure
Dependent Variable: U_kg_97_pc_DM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	10110859.49	10110859.49	4.73	0.0817
Error	5	10691719.03	2138343.81		
Corrected Total	6	20802578.52			

R-Square	Coeff Var	Root MSE	U_kg_97_pc_DM Mean
0.486039	5.720129	1462.308	25564.24

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	10110859.49	10110859.49	4.73	0.0817

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	10110859.49	10110859.49	4.73	0.0817

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	27366.99712	996.3947420	27.47	<.0001
month	-600.91774	276.3501794	-2.17	0.0817

---------- Tr=C250 ----------

The GLM Procedure
Dependent Variable: pc_Om_as_is

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	130.3512314	130.3512314	4.70	0.0824
Error	5	138.7815388	27.7563078		
Corrected Total	6	269.1327702			

R-Square	Coeff Var	Root MSE	pc_Om_as_is Mean
0.484338	5.626080	5.268426	93.64292

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	130.3512314	130.3512314	4.70	0.0824

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	130.3512314	130.3512314	4.70	0.0824

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	100.1158379	3.58982690	27.89	<.0001
month	-2.1576379	0.99563884	-2.17	0.0824

---------- Tr=C250 ----------

The GLM Procedure
Dependent Variable: pc_Om_97_pc_DM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	135.3817057	135.3817057	4.73	0.0817
Error	5	143.1592597	28.6318519		
Corrected Total	6	278.5409653			

R-Square	Coeff Var	Root MSE	pc_Om_97_pc_DM Mean
0.486039	5.720129	5.350874	93.54464

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	135.3817057	135.3817057	4.73	0.0817

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	135.3817057	135.3817057	4.73	0.0817

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	100.1412711	3.64600601	27.47	<.0001
month	-2.1988772	1.01122012	-2.17	0.0817

---------- Tr=C500 ----------

The GLM Procedure

Number of Observations Read 7
Number of Observations Used 7

---------- Tr=C500 ----------

The GLM Procedure
Dependent Variable: U_kg_as_is

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	24992416.51	24992416.51	1.84	0.2328
Error	5	67840833.42	13568166.68		
Corrected Total	6	92833249.93			

R-Square	Coeff Var	Root MSE	U_kg_as_is Mean
0.269218	7.416808	3683.499	49664.21

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	24992416.51	24992416.51	1.84	0.2328

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	24992416.51	24992416.51	1.84	0.2328

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	52498.51786	2509.881663	20.92	<.0001
month	-944.76786	696.115925	-1.36	0.2328

---------- Tr=C500 ----------

The GLM Procedure
Dependent Variable: U_kg_97_pc_DM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	27975065.59	27975065.59	2.18	0.2000
Error	5	64216585.30	12843317.06		
Corrected Total	6	92191650.89			

R-Square	Coeff Var	Root MSE	U_kg_97_pc_DM Mean
0.303445	7.371371	3583.757	48617.25

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	27975065.59	27975065.59	2.18	0.2000

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	27975065.59	27975065.59	2.18	0.2000

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	51615.90898	2441.919048	21.14	<.0001
month	-999.55464	677.266487	-1.48	0.2000

---------- Tr=C500 ----------

The GLM Procedure
Dependent Variable: pc_Om_as_is

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	95.0505345	95.0505345	1.84	0.2328
Error	5	258.0105639	51.6021128		
Corrected Total	6	353.0610984			

R-Square	Coeff Var	Root MSE	pc_Om_as_is Mean
0.269218	7.416808	7.183461	96.85381

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	95.05053450	95.05053450	1.84	0.2328

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	95.05053450	95.05053450	1.84	0.2328

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	102.3811962	4.89470365	20.92	<.0001
month	-1.8424608	1.35754654	-1.36	0.2328

---------- Tr=C500 ----------

The GLM Procedure
Dependent Variable: pc_Om_97_pc_DM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	110.4268794	110.4268794	2.18	0.2000
Error	5	253.4841999	50.6968400		
Corrected Total	6	363.9110793			

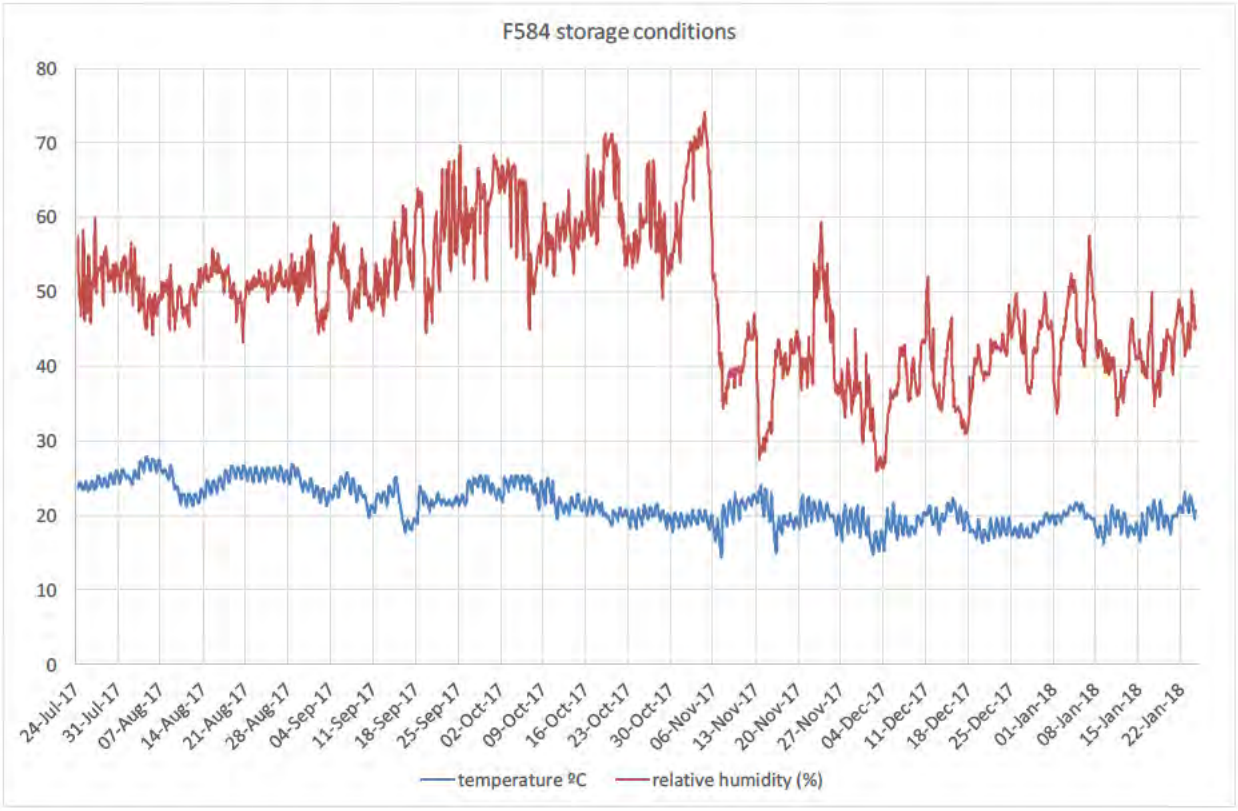
R-Square	Coeff Var	Root MSE	pc_Om_97_pc_DM Mean
0.303445	7.371371	7.120171	96.59223

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	110.4268794	110.4268794	2.18	0.2000

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	110.4268794	110.4268794	2.18	0.2000

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	102.5499429	4.85157898	21.14	<.0001
month	-1.9859046	1.34558591	-1.48	0.2000

Appendix 6 – Temperature and relative humidity during storage of stability samples



Appendix 14: Sources of Vitamins and Minerals in Premix

Date **27th March 2018**
Product: **CIBENZA® PHYTAVERSE® G10 Phytase Enzyme**

TO WHOM IT MAY CONCERN:

The table below provides source and regulatory status for the ingredients in the vitamin-mineral premix used in "Stability evaluation of CIBENZA® PHYTAVERSE® G10 phytase enzyme in premix" (Unique Study Code: F584) conducted at (b) (4)

#	Vitamin/Mineral	Source	Regulatory status to support ingredient use in US
1	(b) (4)		
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			

#	Vitamin/Mineral	Source	Regulatory status to support ingredient use in US
19	(b) (4)		
20			
21			
22			
23			
24			
25			
26			

Sincerely,

(b) (4), (b) (6)

(b) (4), (b) (6)

(b) (4)

Date **27th March 2018**
Product: **CIBENZA® PHYTAVERSE® G10 Phytase Enzyme**

TO WHOM IT MAY CONCERN:

The table below provides source and regulatory status for the ingredients in the vitamin-mineral premix used in "Homogeneity evaluation of CIBENZA® PHYTAVERSE® G10 phytase enzyme in premix" (Unique Study Code: F562) conducted at (b) (4).

#	Vitamin/Mineral	Source	Regulatory status to support ingredient use in US
1	(b) (4)		
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			

#	Vitamin/Mineral	Source	Regulatory status to support ingredient use in US
19	(b) (4)		(b) (4)
20			
21			
22			
23			
24			
25			
26			
27			

Sincerely,

(b) (4), (b) (6)

(b) (4)

(b) (4)

Appendix 15: Homogeneity Evaluation of CIBENZA® PHYTAVERSE® G10 Phytase Enzyme in Premix

(b) (4)

Homogeneity evaluation of CIBENZA® PHYTAVERSE® G10 phytase enzyme in premix

Unique Study Code: F562

FINAL REPORT
Date: 28th December 2017

Study sponsor: Novus Europe S.A./N.V. and BASF Enzymes LLC.

Signed by Study Director, Study Sponsors and Study Monitor:

(b) (4), (b) (6) 28th December 2017	2018.01.11	18 Jan 2018	Jan. 4, 2018
Study Director	Study Sponsors		Study Monitor
(b) (4)	Elkin Amaya Senior Regulatory Affairs Manager, Novus Europe S.A./N.V. Novus- Edifici CEPID, Tecnoparc Reus, Avda. Cambra del Comerç 42 ES-43204, Reus, Spain	Roxanna Van Dorn Senior Regulatory Affairs Specialist BASF Enzymes LLC 3550 John Hopkins Court, San Diego, CA 92121, United States of America	Drew Lichtenstein Research Manager, Specialty Products Novus International 20 Research Park Dr., St. Charles, MO 63304, United States of America

Table of contents

1 Summary

The objective of this study was to evaluate the Homogeneity of CIBENZA® PHYTAVERSE® G10 phytase enzyme in vitamin-mineral premix.

The homogeneity of each of the three batches of the test article at two inclusion levels was determined by measuring phytase activity in 10 subsamples taken at different location points of the mixer.

Results are presented next in Summary Table 1.

Summary Table 1. Homogeneity of CIBENZA® PHYTAVERSE® G10 phytase enzyme in premix

	Phytase U/kg (as is)						Phytase U/kg (97% DM)					
	N	Mean	CV	StdDev	Max	Min	N	Mean	CV	StdDev	Max	Min
Tr			(b) (4)									(b) (4)
A250	10	24075					10	24007				
A500	10	49890					10	49591				
B250	10	25761					10	25848				
B500	10	48614					10	48778				
C250	10	26167					10	26889				
C500	10	50800					10	51432				

† One phytase unit is the amount of enzyme that releases 1 µmol of inorganic phosphate from phytate per minute under the conditions of the assay (Phytate concentration: 5.0 mM, pH: 5.5, Temperature: 37°C, Incubation time: 30 min) (ISO 30024:2009). Normal analytical variation for the method is 10%.

According the results of the present homogeneity in vitamin-mineral premix, CIBENZA® PHYTAVERSE® G10 phytase enzyme:

- Presented a good mixing homogeneity (CV 8% to 12%), actual CVs below or close to ×1 the CV of the method itself for all the three batches tested and at both inclusion levels.

2 Quality statement

The study, Homogeneity evaluation of CIBENZA® PHYTAVERSE® G10 phytase enzyme in premix (Unique Study Code: F562), was conducted in compliance with current quality standards and regulatory requirements as applicable for EU and US feed additive applications.

Procedures, documentation, equipment and records were examined in order to assure that the study was performed in accordance with the regulations specified herein and with the protocol and relevant Standard Operating Procedures.

Signed and dated:

(b) (4), (b) (6) 28th December 2017	2018.01.11	18 Jan 2018	Jan. 4, 2018
Study Director	Study Sponsors		Study Monitor
(b) (4)	Elkin Amaya Senior Regulatory Affairs Manager, Novus Europe S.A./N.V. Novus- Edifici CEPID, Tecnoparc Reus, Avda. Cambra del Comerç 42 ES-43204, Reus, Spain	Roxanna Van Dorn Senior Regulatory Affairs Specialist BASF Enzymes LLC 3550 John Hopkins Court, San Diego, CA 92121, United States of America	Drew Lichtenstein Research Manager, Specialty Products Novus International 20 Research Park Dr., St. Charles, MO 63304, United States of America

3 Study title and unique study code

Homogeneity evaluation of CIBENZA® PHYTAVERSE® G10 phytase enzyme in vitamin-mineral premix.

Unique study code: F562

4 Study objective

To evaluate the homogeneity of three batches of CIBENZA® PHYTAVERSE® G10 phytase enzyme at two doses each in vitamin-mineral premix.

5 Study location

(b) (4)

6 Important dates & duration of the study

Date of feed manufacture: 29th May 2017
Duration of study: 1 day mixing, 10 days analysis

7 Test products

Table 1. Details of test product

Code	Product	Provider	Lot n° Manufacture Date	Active substance	Activity (U/g)†	
					Guaranteed	Analysed
A	CIBENZA® PHYTAVERSE® G10 Phytase Enzyme	Novus International, Inc.	Lot: P23941 Made: 08 October 2014	6-phytase	10,000	13,951
B	CIBENZA® PHYTAVERSE® G10 Phytase Enzyme	Novus International, Inc.	Lot: P26641 Made: 08 October 2014	6-phytase	10,000	13,742
C	CIBENZA® PHYTAVERSE® G10 Phytase Enzyme	Novus International, Inc.	Lot: RO15271001 Made: 28 September 2015	6-phytase	10,000	13,522

† One phytase unit is the amount of enzyme that releases 1 µmol of inorganic phosphate from phytate per minute under the conditions of the assay (Phytate concentration: 5.0 mM, pH: 5.5, Temperature: 37°C, Incubation time: 30 min) (ISO 30024:2009). Normal analytical variation for the method is 10%.

8 Key study personnel

Study Director: (b) (4)

External Study Monitor: Drew Lichtenstein, Ph.D. Research Manager, Specialty Products, Novus International, Inc., 20 Research Park Dr., St. Charles, MO 63304, United States of America, Tel: +1 314 453-7793, E-mail: drew.lichtenstein@novusint.com

Study Sponsors: 1) Elkin Amaya, Senior Regulatory Affairs Manager, EMEA, Novus Europe S.A./N.V. Novus- Edifici CEPID, Tecnoparc Reus, Av. Cambra del Comerç, 42 ES-43204, Reus, Spain Tel: +34 676 004 728, E-mail: elkin.amaya@novusint.com

2) Roxanna Van Dorn, Sr. Regulatory Affairs Specialist, Phone: +1 858 431-8590, Mobile: +1-858-349-7339, Fax: +1-973-307-2549, E-mail: roxanna.vandorn@basf.com. Postal Address: BASF Enzymes LLC, 3550 John Hopkins Court, San Diego, CA 92121 United States

Feed mill & supervision of diet manufacture: (b) (4)

Feed analysis (DM and CIBENZA® PHYTAVERSE® G10 phytase enzyme): (b) (4)

Optional/back-up facility for feed analysis (DM and CIBENZA® PHYTAVERSE® G10 phytase enzyme): Drew Lichtenstein, Novus International, Inc., 20 Research Park Drive, Saint Charles, MO, 63304; United States of America.

9 Material and methods

9.1 Experimental treatments

Number of treated and control groups: Commercial vitamin-mineral premix (inclusion level 10 kg/ton feed) was used as matrix for homogeneity purposes.

CIBENZA® PHYTAVERSE® G10 phytase enzyme from each batch was added to the vitamin-mineral premix to theoretically provide 250 and 500 U/kg feed as detailed in Table 2.

Table 2. Experimental Treatments

Treatment	Product	CIBENZA® PHYTAVERSE® G10 phytase enzyme		
		U/kg feed	mg in 10 g premix (equivalent to mg/kg feed)†	g to add to 10 kg premix†
A2	CIBENZA® PHYTAVERSE® G10 phytase enzyme batch P23941	250	(b) (4)	
A5		500		
B2	CIBENZA® PHYTAVERSE® G10 phytase enzyme batch P26641	250		
B5		500		
C2	CIBENZA® PHYTAVERSE® G10 phytase enzyme batch RO15271001	250		
C5		500		

† inclusion based on actual activity of each batch

9.2 Treatment application

CIBENZA® PHYTAVERSE® G10 phytase enzyme was mixed with the vitamin-mineral premix in serial mixing steps (details provided under Section 9.3 & 9.6).

9.3 Detailed study design

Figure 1. Basic study design
For each batch and dose of enzyme: The homogeneity of the test article in the vitamin-mineral premix was determined by measuring phytase activity in: • 10 subsamples taken at different places of the mixer Premix was produced as follows: 10 kg of Vitamin and Mineral premix was mixed with the corresponding amount of CIBENZA® PHYTAVERSE® phytase enzyme depending on actual activity of each batch as detailed in Table 2

9.4 Premix composition

A standard commercial vitamin-mineral premix was used. The composition of the vitamin-mineral premix is presented next:

Table 3. Composition of vitamin-mineral premix

	Units	per kg of vitamin-mineral premix	when premix added at 10 kg/ton feed, results in the following values per kg of feed
Vitamins, provitamins and similar			
(b) (4)	IU	1 000 000	(b) (4)
	IU	350 000	
	mg	3 000	
	mg	210	
	mg	855	
	mg	470	
	mg	5	
	mg	300	
	mg	2 000	
	mg	1 520	
	mg	6 710	
	mg	150	
	mg	25	
	mg	70 000	
	mg	6 500	
	mg	150	
	mg	1 500	
	mg	8 000	
	mg	8 500	
	mg	20	
	g	50	
	g	150	
	mg	5 000	
		up to 1 kg	

9.5 Premix analyses

Phytase activity in premixes was determined based on "ISO 30024:2009. Animal feeding stuffs – Determination of phytase activity in association with dilution method VDLUFA 27.1.3 (dilution of mineral feeds and premixtures with maize meal (blank feed) before applying the EN ISO 30024 analytical method)."

Dry Matter was determined according AOAC method 934.01: Moisture in Animal Feed.

Premix with no addition of CIBENZA® PHYTAVERSE® G10 phytase enzyme was previously analyzed to confirm the absence of phytase activity before mixing.

9.6 Premixture manufacture

The calculated amount of product for each CIBENZA® PHYTAVERSE® G10 phytase enzyme batch and dose (Table 2) was first manually premixed with [redacted] (b) (4)

9.6.1 Short description of the process

Under general and corporative [redacted] (b) (4)

9.7 Premix samples at manufacture

For each CIBENZA® PHYTAVERSE® G10 phytase enzyme batch and dose:

- After mixing of the product with the vitamin mineral premix, 10 grab samples (~550 g each) were taken from several points of the mixer. From these 10 grab premix samples:
  - Triplicate (NOVUS, [redacted] (b) (4) backup) [redacted] (b) (4))

Each sample was placed in single-ply kraft 80 g paper bags. Bags were ply folded to simulate commercial bags and labelled with the unique study code (F562), treatment code (A2 / A5 / B2 / B5 / C2 / C5), sample number (i.e. NOVUS samples 1.1 to 10; [redacted] (b) (4) samples 2.1 to 2.10; backup samples 3.1 to 3.10), the date of manufacture and the analysis required (DM, phytase activity).

9.8 Feed sampling plan

Table 4. Sampling plan			
Treatment		n at sampling	Final Samples
			NOVUS [redacted]
A2	(premix dose intended for 250 U/kg feed)	10 × ~550 g	[redacted]
A5	(premix dose intended for 500 U/kg feed)	10 × ~550 g	
B2	(premix dose intended for 250 U/kg feed)	10 × ~550 g	
B5	(premix dose intended for 500 U/kg feed)	10 × ~550 g	
C2	(premix dose intended for 250 U/kg feed)	10 × ~550 g	
C5	(premix dose intended for 500 U/kg feed)	10 × ~550 g	

For homogeneity analysis, samples were analysed in IRTA's lab within 10 working days after production of the premix containing CIBENZA® PHYTAVERSE® phytase enzyme, keeping samples refrigerated (-4°C) before analysis. Samples were dispatched to NOVUS ([redacted], Novus Reus) and [redacted] for analysis or [redacted] backup storage.

9.9 Statistics

Key parameters:

- Homogeneity: Mean CIBENZA® PHYTAVERSE® G10 phytase enzyme activity (arithmetic mean) and variation (standard deviation) was used to express the result as a unique value described as the coefficient of variation.
- Stability: The CIBENZA® PHYTAVERSE® G10 phytase enzyme activity will be assessed in the feeds after the maximum storage period (3 month).

Calculations:

$$\%CV = \frac{s}{\bar{y}} \times 100$$

$$\bar{y} = \frac{\sum y_i}{n}$$

$$s = \sqrt{s^2}$$

$$s^2 = \frac{\sum y_i^2 - n\bar{y}^2}{n-1}$$

where:

%CV= coefficient of variation

Σ= summation

s= standard deviation

y_i= individual result from each sample

s^2= variance

n= total number of samples

$\bar{y}$ = mean

10 Results

The results are summarized in Table 5.

Table 5. Homogeneity of CIBENZA® PHYTAVERSE® G10 phytase enzyme in premix												
	Phytase U/kg (as is)						Phytase U/kg (97% DM)					
	N	Mean	CV	StdDev	Max	Min	N	Mean	CV	StdDev	Max	Min
Tr												
A2	10	24075	[redacted]				10	24007	[redacted]			
A5	10	49890					10	49591				
B2	10	25761					10	25848				
B5	10	48614					10	48778				
C2	10	26167					10	26889				
C5	10	50800					10	51432				

† One phytase unit is the amount of enzyme that releases 1 µmol of inorganic phosphate from phytate per minute under the conditions of the assay (Phytate concentration: 5.0 mM, pH: 5.5, Temperature: 37°C, Incubation time: 30 min) (ISO 30024:2009). Normal analytical variation for the method is 10%.

11 Discussion

CIBENZA® PHYTAVERSE® G10 phytase enzyme activity results were also standardized considering a common Dry Matter content of 97%. This value was close to the average DM values (96.4%; DM range: 93.7-97.8%).

The homogeneity of mixing for the three CIBENZA® PHYTAVERSE® G10 phytase enzyme batches tested and at both inclusion levels, expressed as Coefficients of Variation ranged from 7.7% to 12.4% when standardized at 97% DM content. These CVs of the homogeneity were well below ×2 the CV of the normal analytical variation of the method itself (normal analytical CV is 10%), and therefore the CVs of the homogeneity could be considered good.

12 Conclusions

According the results of the present homogeneity in vitamin-mineral premix, CIBENZA® PHYTAVERSE® G10 phytase enzyme:

- Presented a good mixing homogeneity (CV 8% to 12%), actual CVs below or close to ×1 the CV of the method itself for all 3 batches tested and at both inclusion levels.

13 References

ISO 30024:2009. Animal feeding stuffs – Determination of phytase activity

VLLUFA 27.1.3. Preparation of Mineral Feed and Premixtures for the Determination of Phytase Activity

Regulation (EC) Nº 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition.http://eur-lex.europa.eu/en/index.htm

SAS Institute Inc. 2011. Base SAS® 9.3 Guide to Information Maps. Cary, NC: SAS Institute Inc.

Statutory Instrument 1999 No. 1663. The Feeding Stuffs (Sampling and Analysis) Regulation 1999.

14 List of Appendices

Appendix 1 - Curricula vitae of Study Director & Study Monitor
Appendix 2 - Certificate of analysis of CIBENZA® PHYTAVERSE® G10 phytase enzyme used (3 batches)
Appendix 3 - Relevant laboratory reports
Appendix 4 - Raw data
Appendix 5 - Statistical printouts

Appendix 1- *Curricula vitae* of Study Director & Study Monitor

Study Director:
Name: (b) (6)
Qualifications: (b) (6)
(b) (6)
Present Position: (b) (4)
Experience: (b) (4), (b) (6)

Study Monitor:
Name: Drew Lichtenstein
Qualifications: B.S. Biochemistry (Michigan State University 1982), PhD Biochemistry (University of Wisconsin-Madison 1990)
Present Position: Research Manager, Specialty Products, Novus International
Experience: Over 35 years research experience in biochemistry and cell biology; more than 8 years of experience in animal feed enzymes.

Appendix 2 - Certificate of analysis of CIBENZA® PHYTAVERSE® G10 phytase enzyme used (3 batches)

NOVUS

NOVUS INTERNATIONAL INC.
20 RESEARCH PARK DRIVE
ST. CHARLES, MO 63304

DATE:	09 May 2017
PRODUCT:	20002453
PRODUCT DESCRIPTION:	CIBENZA PHYTAVERSE G10 20 KG BAG
LOT NUMBER:	P23941
DATE OF MANUFACTURE:	8 October 2014
DATE OF ANALYSIS:	21 March 2017
DATE OF PACKAGING:	8 October 2014

CERTIFICATE OF ANALYSIS

CHARACTERISTIC	SPECIFICATION	RESULTS
Appearance	White to Beige Granules	(b) (4)
Phytase Activity, U/g	>=10000	(b) (4)

Approved by:

SABINA DIAZ
REGULATORY AFRAIRS MANAGER, EMEA

The value and properties stated above are based upon test and analysis of samples of material. The exclusive commitment of Novus with respect to such values and properties is as set forth in the sales contract between your company and Novus for such material or the acknowledgment of Novus for the above described shipment of material, whichever is applicable.

NOVUS

NOVUS INTERNATIONAL INC.
20 RESEARCH PARK DRIVE
ST. CHARLES, MO 63304

DATE:	09 May 2017
PRODUCT:	20002453
PRODUCT DESCRIPTION:	CIBENZA PHYTAVERSE G10 20 KG BAG
LOT NUMBER:	P26641
DATE OF MANUFACTURE:	8 October 2014
DATE OF ANALYSIS:	21 March 2017
DATE OF PACKAGING:	8 October 2014

CERTIFICATE OF ANALYSIS

CHARACTERISTIC	SPECIFICATION	RESULTS
Appearance	White to Beige Granules	(b) (4)
Phytase Activity, U/g	>=10000	(b) (4)

Approved by:

SABINA DIAZ
REGULATORY AFRAIRS MANAGER, EMEA

The value and properties stated above are based upon test and analysis of samples of material. The exclusive commitment of Novus with respect to such values and properties is as set forth in the sales contract between your company and Novus for such material or the acknowledgment of Novus for the above described shipment of material, whichever is applicable.

NOVUS

NOVUS INTERNATIONAL INC.
20 RESEARCH PARK DRIVE
ST. CHARLES, MO 63304

DATE:	09 May 2017
PRODUCT:	20002453
PRODUCT DESCRIPTION:	CIBENZA PHYTAVERSE G10 20 KG BAG
LOT NUMBER:	RO15271001
DATE OF MANUFACTURE:	28 September 2015
DATE OF ANALYSIS:	21 March 2017
DATE OF PACKAGING:	28 September 2015

CERTIFICATE OF ANALYSIS

CHARACTERISTIC	SPECIFICATION	RESULTS
Appearance	White to Beige Granules	(b) (4)
Phytase Activity, U/g	>=10000	(b) (4)

Approved by:

SABINA DIAZ
REGULATORY AFRAIRS MANAGER, EMEA

The value and properties stated above are based upon test and analysis of samples of material. The exclusive commitment of Novus with respect to such values and properties is as set forth in the sales contract between your company and Novus for such material or the acknowledgment of Novus for the above described shipment of material, whichever is applicable.

Appendix 2- Relevant laboratory reports

CERTIFICATE OF ANALYSIS

Company:	Novus Europe S.A./N.V.
Type of sample:	F562bis Vitamin-Mineral Premix + CIBENZA® PHYTAVERSE® G10
Laboratory ref. :	170814 170815 170816 170817 170818 170819 170820 170821 170822 170823 170824 170825 170826 170827 170828 170829 170830 170831 170832 170833 170834 170835 170836 170837 170838 170839 170840 170841 170842 170843 170844 170845 170846 170847 170848 170849 170850 170851 170852 170853 170854 170855 170856 170857 170858 170859 170860 170861 170862 170863 170864 170865 170866 170867 170868 170869 170870 170871 170872 170873
Reception date:	30 May 2017
Analysis starting date:	31 May 2017
Analysis finishing date:	05 June 2017

Sample description:
See Results section

Analysis performed:
Dry Matter; phytase activity

Results:

LAB_REF	Tr	location	U/kg	DM_p
170814	A250	1	(b) (4)	(b) (4)
170815	A250	2		
170816	A250	3		
170817	A250	4		
170818	A250	5		
170819	A250	6		
170820	A250	7		
170821	A250	8		
170822	A250	9		
170823	A250	10		
170824	A500	1		
170825	A500	2		
170826	A500	3		
170827	A500	4		
170828	A500	5		
170829	A500	6		
170830	A500	7		
170831	A500	8		
170832	A500	9		
170833	A500	10		

LAB_REF	Tr	location	U/kg	DM_p
170834	B250	1	(b) (4)	(b) (4)
170835	B250	2		
170836	B250	3		
170837	B250	4		
170838	B250	5		
170839	B250	6		
170840	B250	7		
170841	B250	8		
170842	B250	9		
170843	B250	10		
170844	B500	1		
170845	B500	2		
170846	B500	3		
170847	B500	4		
170848	B500	5		
170849	B500	6		
170850	B500	7		
170851	B500	8		
170852	B500	9		
170853	B500	10		

LAB_REF	Tr	location	U/kg	DM_p
170854	C250	1	(b) (4)	(b) (4)
170855	C250	2		
170856	C250	3		
170857	C250	4		
170858	C250	5		
170859	C250	6		
170860	C250	7		
170861	C250	8		
170862	C250	9		
170863	C250	10		
170864	C500	1		
170865	C500	2		
170866	C500	3		
170867	C500	4		
170868	C500	5		
170869	C500	6		
170870	C500	7		
170871	C500	8		
170872	C500	9		
170873	C500	10		

(b) (4), (b) (6)

Signature: (b) (4), (b) (6)

Date: 20TH NOVEMBER 2017

Appendix 3- Raw data

Obs	lab_ref	Tr	location	U_kg_as_is	DM_p	U_kg_97_p_DM
1	170814	A250	1	23480	(b) (4)	(b) (4)
2	170815	A250	2	23710		
3	170816	A250	3	26391		
4	170817	A250	4	25979		
5	170818	A250	5	21762		
6	170819	A250	6	24451		
7	170820	A250	7	23254		
8	170821	A250	8	23570		
9	170822	A250	9	20301		
10	170823	A250	10	27856		
11	170824	A500	1	48107		
12	170825	A500	2	43318		
13	170826	A500	3	54024		
14	170827	A500	4	59318		
15	170828	A500	5	40714		
16	170829	A500	6	55704		
17	170830	A500	7	46425		
18	170831	A500	8	56875		
19	170832	A500	9	46856		
20	170833	A500	10	47557		
21	170834	B250	1	23803		
22	170835	B250	2	21031		
23	170836	B250	3	23413		
24	170837	B250	4	26716		
25	170838	B250	5	28245		
26	170839	B250	6	24278		
27	170840	B250	7	27670		
28	170841	B250	8	27890		
29	170842	B250	9	27746		
30	170843	B250	10	26822		
31	170844	B500	1	43705		
32	170845	B500	2	42037		
33	170846	B500	3	51321		
34	170847	B500	4	51209		
35	170848	B500	5	53256		
36	170849	B500	6	42694		
37	170850	B500	7	55863		
38	170851	B500	8	51815		
39	170852	B500	9	50028		
40	170853	B500	10	44216		
41	170854	C250	1	26263		
42	170855	C250	2	26317		
43	170856	C250	3	26705		
44	170857	C250	4	23484		
45	170858	C250	5	26348		
46	170859	C250	6	26671		
47	170860	C250	7	28148		
48	170861	C250	8	27886		
49	170862	C250	9	28080		
50	170863	C250	10	21765		
51	170864	C500	1	52588		
52	170865	C500	2	52275		
53	170866	C500	3	44347		
54	170867	C500	4	41319		
55	170868	C500	5	54739		
56	170869	C500	6	53611		
57	170870	C500	7	54993		
58	170871	C500	8	46648		
59	170872	C500	9	52398		
60	170873	C500	10	55082		

Appendix 4 - Statistical printouts

(b) (4) Trial F562 09:25 Friday, November 17, 2017 1

Obs	lab_ref	Tr	location	U_kg_as_is	DM p (b) (4)	U_kg_97_p_DM (b) (4)
1	170814	A250	1	23480		
2	170815	A250	2	23710		
3	170816	A250	3	26391		
4	170817	A250	4	25979		
5	170818	A250	5	21762		
6	170819	A250	6	24451		
7	170820	A250	7	23254		
8	170821	A250	8	23570		
9	170822	A250	9	20301		
10	170823	A250	10	27856		
11	170824	A500	1	48107		
12	170825	A500	2	43318		
13	170826	A500	3	54024		
14	170827	A500	4	59318		
15	170828	A500	5	40714		
16	170829	A500	6	55704		
17	170830	A500	7	46425		
18	170831	A500	8	56875		
19	170832	A500	9	46856		
20	170833	A500	10	47557		
21	170834	B250	1	23803		
22	170835	B250	2	21031		
23	170836	B250	3	23413		
24	170837	B250	4	26716		
25	170838	B250	5	28245		
26	170839	B250	6	24278		
27	170840	B250	7	27670		
28	170841	B250	8	27890		
29	170842	B250	9	27746		
30	170843	B250	10	26822		
31	170844	B500	1	43705		
32	170845	B500	2	42037		
33	170846	B500	3	51321		
34	170847	B500	4	51209		
35	170848	B500	5	53256		
36	170849	B500	6	42694		
37	170850	B500	7	55863		
38	170851	B500	8	51815		
39	170852	B500	9	50028		
40	170853	B500	10	44216		
41	170854	C250	1	26263		
42	170855	C250	2	26317		
43	170856	C250	3	26705		
44	170857	C250	4	23484		
45	170858	C250	5	26348		
46	170859	C250	6	26671		
47	170860	C250	7	28148		
48	170861	C250	8	27886		
49	170862	C250	9	28080		
50	170863	C250	10	21765		
51	170864	C500	1	52588		
52	170865	C500	2	52275		
53	170866	C500	3	44347		
54	170867	C500	4	41319		
55	170868	C500	5	54739		
56	170869	C500	6	53611		
57	170870	C500	7	54993		
58	170871	C500	8	46648		
59	170872	C500	9	52398		
60	170873	C500	10	55082		

	U_kg_as_is						U_kg_97_p_DM					
	N	Mean	CV	StdDev	Max	Min	N	Mean	CV	StdDev	Max	Min
Tr			(b) (4)						(b) (4)			
A250	10	24075			(b) (4)		10	24007			(b) (4)	
A500	10	49890			(b) (4)		10	49591			(b) (4)	
B250	10	25761			(b) (4)		10	25848			(b) (4)	
B500	10	48614			(b) (4)		10	48778			(b) (4)	
C250	10	26167			(b) (4)		10	26889			(b) (4)	
C500	10	50800			(b) (4)		10	51432			(b) (4)	

Appendix 16: Stability Evaluation of CIBENZA® PHYTAVERSE® L10 Phytase Enzyme in Feed

(b) (4)

Stability evaluation of
CIBENZA® PHYTAVERSE® L10 phytase enzyme in feed

Unique Study Code: F600

FINAL REPORT
Date: 9th May 2018

Study sponsor: Novus International Inc. and BASF Enzymes LLC.

Signed by Study Director, Study Sponsor and Study Monitor:

(b) (4), (b) (5)			
9th May 2018	MAY 14, 2018	10 May 2018	10, May 2018
Study Director	Study Sponsors		Study Monitor
(b) (4)	Gavin Bowman Director, Global Regulatory Affairs, Novus International 20 Research Park Dr., St. Charles, MO 63304, United States of America	Roxanna Van Dorn Senior Regulatory Affairs Specialist BASF Enzymes LLC 3550 John Hopkins Court, San Diego, CA 92121, United States of America	Drew Lichtenstein Research Manager, Specialty Products Novus International 20 Research Park Dr., St. Charles, MO 63304, United States of America

Table of contents

1 Summary

The objective of this study was to evaluate the Stability of CIBENZA® PHYTAVERSE® L10 phytase enzyme in feeds (post pellet liquid application).

For each batch and dose, the stability of the test article was determined by measuring phytase activity in unique feed samples after 0, 1, 2 and 3-months storage at ambient conditions.

Results are presented next in Summary Table 1.

Summary Table 1. Stability of CIBENZA® PHYTAVERSE® L10 phytase enzyme in feeds

		N	Phytase U/kg as is	DM %	Phytase U/kg 88% DM	Phytase % 0 month as is	Phytase % 0 month 88%DM
Tr form	month						
A250pellet	0	2	262	87.1	265	100.0	100.0
	1	1	(b) (4)				
	2	1					
	3	1					
A500pellet	0	2	555	87.3	559	100.0	100.0
	1	1	(b) (4)				
	2	1					
	3	1					
B250pellet	0	2	297	87.3	299	100.0	100.0
	1	1	(b) (4)				
	2	1					
	3	1					
B500pellet	0	1	535	87.4	539	100.0	100.0
	1	1	(b) (4)				
	2	1					
	3	1					
C250pellet	0	1					
	1	1	(b) (4)				
	2	1					
	3	1					
C500pellet	0	1	541	87.5	544	100.0	100.0
	1	1	(b) (4)				
	2	1					
	3	1					

(b) (4)

† One phytase unit is the amount of enzyme that releases 1 µmol of inorganic phosphate from phytate per minute under the conditions of the assay (Phytate concentration: 5.0 mM, pH: 5.5, Temperature: 37ºC, Incubation time: 30 min) (ISO 30024:2009). Normal analytical variation for the method is 10%.

According to the results of the present stability study in feeds, CIBENZA® PHYTAVERSE® L10 phytase enzyme:

- Was stable over time (1, 2 and 3-months storage at ambient conditions) for all three batches (A & B & C) at both concentrations tested (250 & 500 U/kg) as demonstrated by the slope of linear regressions of phytase activity over time not being significantly different from 0 (flat line, no significant loss of activity).
- Presented good stability (in general ±10% of 0-month value) up to 3-months in pelleted feeds for all three batches (A & B & C) at both concentrations tested (250 & 500 U/kg). Exceptions were: A500 (82%) and B250 (87%). These lower activities at 3 months for A500 and B250 were considered to be within the range of expected values, especially considering the other dose for the same batches of enzyme (i.e. A250 and B500) did not differ from their respective T=0 activity by more than 10% (A250 (93%) as reference for A500 and B500 (102%) as reference for B250).

2 Quality statement

The study, Stability evaluation of CIBENZA® PHYTAVERSE® L10 phytase enzyme in feed (Unique Study Code: F600), was conducted in compliance with current quality standards and regulatory requirements as applicable for US animal food requirements.

Procedures, documentation, equipment and records were examined in order to assure that the study was performed in accordance with the regulations specified herein and with the protocol and relevant Standard Operating Procedures.

Signed and dated:

(b) (4), (b) (6) 9th May 2018	MAY 14, 2018	10 May 2018	10, May 2018
Study Director	Study Sponsors		Study Monitor
(b) (4)	Gavin Bowman Director, Global Regulatory Affairs Novus International 20 Research Park Dr., St. Charles, MO 63304, United States of America	Roxanna Van Dorn Senior Regulatory Affairs Specialist BASF Enzymes LLC 3550 John Hopkins Court, San Diego, CA 92121, United States of America	Drew Lichtenstein Research Manager, Specialty Products Novus International 20 Research Park Dr., St. Charles, MO 63304, United States of America

3 Study title and unique study code

Stability evaluation of CIBENZA® PHYTAVERSE® L10 phytase enzyme in pelleted feed.

Unique study code: F600

4 Study objective

To evaluate the stability of three batches of CIBENZA® PHYTAVERSE® L10 phytase enzyme in pelleted feeds (post pellet liquid application).

5 Study location

(b) (4)

6 Important dates & duration of the study

Date of feeds manufacture: 27th November 2017
Duration of study: 1 day at feed mill, 3-months storage for stability 14th March 2018 end of analysis

7 Test products

Table 1. Details of test product

Code	Product	Provider	Lot nº Manufacture Date	Active substance	Activity (U/g)†	
					Guaranteed	Analysed
A	CIBENZA® PHYTAVERSE® L10 Phytase Enzyme	Novus International, Inc.	CV002C2	6-phytase	10,000	12,247
B	CIBENZA® PHYTAVERSE® L10 Phytase Enzyme	Novus International, Inc.	190CV005A3	6-phytase	10,000	11,860
C	CIBENZA® PHYTAVERSE® L10 Phytase Enzyme	Novus International, Inc.	PHY-50104-PO030-F4	6-phytase	10,000	12,247

† One phytase unit is the amount of enzyme that releases 1 µmol of inorganic phosphate from phytate per minute under the conditions of the assay (Phytate concentration: 5.0 mM, pH: 5.5, Temperature: 37ºC, Incubation time: 30 min) (ISO 30024:2009). Normal analytical variation for the method is 10%.

8 Key study personnel

Study Director: (b) (4), (b) (6)
cat

External Study Monitor: Drew Lichtenstein, Ph.D. Research Manager, Specialty Products, Novus International, Inc., 20 Research Park Dr., St. Charles, MO 63304, United States of America, Tel: +1 314 453-7793, E-mail: drew.lichtenstein@novusint.com

Study Sponsors: 1) Gavin Bowman, Director, Global Regulatory Affairs, Novus International, 20 Research Park Dr., St. Charles, MO 63304, United States of America Tel: +1 636 926 7402, E-mail: gavin.bowman@novusint.com

2) Roxanna Van Dorn, Sr. Regulatory Affairs Specialist, Phone: +1 858 431-8590, Mobile: +1-858-349-7339, Fax: +1-973-307-2549, E-mail: roxanna.vandorn@basf.com. Postal Address: BASF Enzymes LLC, 3550 John Hopkins Court, San Diego, CA 92121 United States

Feed mill & supervision of diet manufacture: [redacted] (b) (4), (b) (6)

Feed analysis (DM and CIBENZA® PHYTAVERSE® L10 phytase enzyme): [redacted] (b) (4), (b) (6)

Optional/back-up facility for feed analysis (DM and CIBENZA® PHYTAVERSE® L10 phytase enzyme): Drew Lichtenstein, Novus International, Inc., 20 Research Park Drive, Saint Charles, MO, 63304; United States of America.

9 Material and methods

9.1 Experimental treatments

Number of treated and control groups: Corn/soya based diet was used for stability purposes.

CIBENZA® PHYTAVERSE® L10 phytase liquid enzyme from each batch was added post pelleting to the feed to provide 250 and 500 U/kg feed as detailed in Table 2.

Table 2. Experimental Treatments

Treatment	Product	CIBENZA® PHYTAVERSE® L10 phytase enzyme			
		U/kg feed	mg/kg feed†	g to add to 300 kg feed†	g for 2.4 kg dilution‡
A250	CIBENZA® PHYTAVERSE® L10 phytase enzyme batch CV002C2	250	[redacted] (b) (4)		
A500		500			
B250	CIBENZA® PHYTAVERSE® L10 phytase enzyme batch 190CV005A3	250			
B500		500			
C250	CIBENZA® PHYTAVERSE® L10 phytase enzyme batch PHY-50104-PO030-F4	250			
C500		500			

† inclusion based on actual activity of each batch

‡ diluted product applied at 6 kg/ton; 0.6 kg of diluted product is needed to fill the pipeline for post pellet application

9.2 Treatment application

CIBENZA® PHYTAVERSE® L10 phytase enzyme was applied post pelleting.

9.3 Detailed study design

Figure 1. Basic study design

For each batch and dose of enzyme:

The stability of the test article in pelleted feeds was determined by measuring phytase activity of composite samples obtained at the time of feed manufacturing and after storage at ambient conditions for the following periods and for each batch of enzyme:

- 0 months
- 1 months
- 2 months
- 3 months

The amount of endogenous phytase in blank feed has been determined in other studies being values below the level of quantitation.

Feeds were produced as follows:

- Firstly, a 300 kg batch of mash feed was produced.
- Secondly, mash feed was pelleted.
- Thirdly, the corresponding amount of CIBENZA® PHYTAVERSE® L10 phytase enzyme as detailed in Table 2 was applied post pelleting, and the feed was later bagged.

9.4 Feed composition

Feeds did not contain any antibiotics or any other growth promoters. The ingredients, premix and the calculated analyses of the diets are presented in Table 3 to Table 5.

Table 3. Composition (g/kg) of the basal diet

Corn	577
Soybean meal 48%	373
Fat blend	13.69
Dicalcium phosphate	6.81
Calcium carbonate	12.12
Methionine Hydroxy Analogue	1.75
Premix Min-Vit	10.00
Sodium chloride	1.94
L-lysine HCL	2.91
L-threonine	0.65

Table 4. Composition of vitamin-mineral premix			
	Units	per kg of vitamin-mineral premix	when premix added at 10 kg/ton feed, results in the following values per kg of feed
Vitamins, provitamins and similar			
(b) (4)	IU	1 000 000	(b) (4)
	IU	350 000	
	mg	3 000	
	mg	210	
	mg	855	
	mg	470	
	mg	5	
	mg	300	
	mg	2 000	
	mg	1 520	
	mg	6 710	
	mg	150	
	mg	25	
	mg	70 000	
	mg	6 500	
	mg	150	
	mg	1 500	
	mg	8 000	
	mg	8 500	
	mg	20	
	g	50	
	g	150	
	mg	5 000	
		up to 1 kg	

Table 5. Calculated analyses of the basal diet (g/kg)	
Metabolizable Energy kcal/kg	2864
Dry Matter	868
Ash	58
Crude Fiber	27
Ether Extract	41
Crude Protein	227
Ca	9.6
P	5.0
Dig lysine	14.1
Dig SAA	9.4
Dig threonine	8.4

9.5 Feed analyses

Phytase activity in feeds was determined based on ISO 30024:2009. Animal feeding stuffs – Determination of phytase activity.

Dry Matter was determined according AOAC method 934.01: Loss on Drying (Moisture) at 95°-100°C for Feeds.

Premix was firstly analyzed to confirm the absence of phytase activity.

9.6 Feeds manufacture

All the process is automated and controlled by a computer provided with software from (b) (4) so that the incorporation of ingredients and the functioning of the equipment is regulated and recorded by the software. The addition of manual ingredients (vitamins, amino acids and oligo minerals, as well as test products) is made by means of a bar code system.

Feed ingredients were ground through a 40HP hammer mill (Rosal VRE-40) with a horizontal axis and a 3 mm sieve, provided with an automatic feeder.

The feed mixer was a 1000 L Rosal mixer with a double horizontal ribbon, which is sufficient for 300 to 500 kg of feed. The amount of feed prepared was 300 kg per treatment. Fat was added by means of a dosing device provided by three nozzles (b) (4) Mineral-vitamin premix and amino acids were manually added to the mixer. The mixing time was 6 min.

Mash feed was then pelleted in a pelleting press (MABRIK PVR-40) provided with a die of 280 mm of internal diameter with holes of 3×36 mm. The compression group consists of 2 rollers. The feeder is of stainless steel of progressive opening and is moved by a reducing engine. The conditioner is of stainless steel with adjustable blades, prepared for the reception of water and steam. The steam generator has a manometer to reduce the pressure to 2.5-3 kg/cm^2 and a flux regulator valve. Pelleting is automatically regulated by the software of the system which adjusts the temperature of the mash feed at the end of the conditioner (approximately 30 to 38 seconds of conditioning time). The pelletization temperature was adjusted to a mean temperature of 65ºC being the actual maximum temperature 64.8ºC. Temperatures were recorded at fixed intervals (i.e. 5 seconds) in the outlet of the conditioner and outlet of die. The vertical cooler (MABRIK, S.A) works by air aspiration provided by a 7.5 HP turbine.

(b) (4)

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9.6.1 Short description of the process

(b) (4)

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9.7 Feeds samples at manufacture

For each CIBENZA® PHYTAVERSE® L10 phytase enzyme batch and dose:

- 10 grab samples of pelleted feed (~1.1 kg each) were taken at fixed interval times before bagging.
- A portion of these grab pelleted feed samples was combined and homogenized and then:
    - Triplicate (NOVUS, (b) (4) backup) (b) (4)

[redacted]; at each time point one sample was sent to NOVUS, a second one analyzed for phytase activity at [redacted] lab, while the third sample was retained at [redacted] at -20°C as a backup sample; 0-month samples were subjected to proximate analysis.

Stability samples were labelled with the unique study code (F600), treatment code (A250 / A500 / B250 / B500 / C250 / C500), date of manufacture and the analysis required (DM, phytase activity, proximate).

9.8 Feed sampling plan

Table 6. Sampling plan

Treatment	Feed form	Month storage	Analysis	Final Samples		
				NOVUS	[redacted]	[redacted]
A250	PELLET	10x1.1kg samples: A portion of each of 10 samples combined and homogenized then split for stability: 3x4x250 g				
		0	stability & proximate	1 × 250g	1 × 250g	1 × 250g
		1	stability	[redacted]		
		2	stability			
		3	stability			
A500	PELLET	10x1.1kg samples: A portion of each of 10 samples combined and homogenized then split for stability: 3x4x250 g				
		0	stability & proximate	1 × 250g	1 × 250g	1 × 250g
		1	stability	[redacted]		
		2	stability			
		3	stability			
B250	PELLET	10x1.1kg samples: A portion of each of 10 samples combined and homogenized then split for stability: 3x4x250 g				
		0	stability & proximate	1 × 250g	1 × 250g	1 × 250g
		1	stability	[redacted]		
		2	stability			
		3	stability			
B500	PELLET	10x1.1kg samples: A portion of each of 10 samples combined and homogenized then split for stability: 3x4x250 g				
		0	stability & proximate	1 × 250g	1 × 250g	1 × 250g
		1	stability	[redacted]		
		2	stability			
		3	stability			
C250	PELLET	10x1.1kg samples: A portion of each of 10 samples combined and homogenized then split for stability: 3x4x250 g				
		0	stability & proximate	1 × 250g	1 × 250g	1 × 250g
		1	stability	[redacted]		
		2	stability			
		3	stability			
C500	PELLET	10x1.1kg samples: A portion of each of 10 samples combined and homogenized then split for stability samples				
		0	stability & proximate	1 × 250g	1 × 250g	1 × 250g
		1	stability	[redacted]		
		2	stability			
		3	stability			

For stability analysis, A250, B250, C250, A500, B500 and C500 0-month stability samples were analyzed in [redacted] lab within 10 working days after production of the feeds containing CIBENZA® PHYTAVERSE® L10 phytase enzyme. The initial samples to be tested at time zero were refrigerated (4°C) to make sure they reflected the activity values at time zero. All other samples were kept together at [redacted] in a cardboard box protected from light and at room temperature. Samples were dispatched to NOVUS Reus, and [redacted] lab for analysis or [redacted] storage as backup samples after the corresponding time (1, 2 or 3-months).

9.9 Statistics

For each CIBENZA® PHYTAVERSE® L10 phytase enzyme batch and dose:

- The CIBENZA® PHYTAVERSE® L10 phytase enzyme activity was assessed in the feeds after the maximum storage period (3-months). The data was fitted to a least squares regression, with the upper and lower 95% confidence limits shown. The regression line of CIBENZA® PHYTAVERSE® L10 phytase enzyme activity vs. time was calculated and the slope tested to be significantly different from 0.

10 Results

The results are summarized in Table 7 and Table 8. Values from proximate analysis were within expected ranges.

Table 7. Analyzed values of experimental diets

Sample	Dry matter (%)	Crude protein (%)	Ether extract (%)	Ash (%)
A250 pellet	87.0	22.9	3.9	5.5
A500 pellet	87.3	23.0	3.8	5.5
B250 pellet	87.3	23.0	3.8	5.5
B500 pellet	87.4	22.9	3.8	5.5
C250 pellet	87.2	23.3	3.9	5.4
C500 pellet	87.5	23.2	3.8	5.5

Table 8. Stability of CIBENZA® PHYTAVERSE® L10 phytase enzyme in feeds (actual & relative values)

		N	Phytase U/kg as is	DM %	Phytase U/kg 88% DM	Phytase % 0 month as is	Phytase % 0 month 88%DM
Tr_form	month						
A250pellet	0	2	262	87.1	265	100.0	100.0
	1	1	[redacted]				
	2	1					
	3	1					
A500pellet	0	2	555	87.3	559	100.0	100.0
	1	1	[redacted]				
	2	1					
	3	1					
B250pellet	0	2	297	87.3	299	100.0	100.0
	1	1	[redacted]				
	2	1					
	3	1					
B500pellet	0	1	535	87.4	539	100.0	100.0
	1	1	[redacted]				
	2	1					
	3	1					
C250pellet	0	1	323	87.2	326	100.0	100.0
	1	1	[redacted]				
	2	1					
	3	1					

C500pellet	0	1	541	87.5	544	100.0	100.0
	1	1	(b) (4)				
	2	1					
	3	1					

† One phytase unit is the amount of enzyme that releases 1 µmol of inorganic phosphate from phytate per minute under the conditions of the assay (Phytate concentration: 5.0 mM, pH: 5.5, Temperature: 37°C, Incubation time: 30 min) (ISO 30024:2009). Normal analytical variation for the method is 10%.

Table 9. Stability of CIBENZA® PHYTAVERSE® L10 phytase enzyme in feeds (regressions)

(b) (4)

† One phytase unit is the amount of enzyme that releases 1 µmol of inorganic phosphate from phytate per minute under the conditions of the assay (Phytate concentration: 5.0 mM, pH: 5.5, Temperature: 37°C, Incubation time: 30 min) (ISO 30024:2009). Normal analytical variation for the method is 10%.

11 Discussion

Dry matter was quite similar among samples (87.3%±0.7) and the correction for constant DM (88%) did not greatly change the results; DM did not vary over storage time.

All samples were analyzed in duplicate, and when phytase analysis results presented unexpected values, the back-up samples were also analyzed in duplicate. The duplicate analyses were below the expected range of variation of the method (~10%) for all 27 analyses. The back-up samples analyzed were: A250, A500 and B250 all from 0-months; for these samples, average values of original and back-up samples were taken into account. Including the A250 and A500 back-up samples in the analysis resulted in lower phytase activity than the original samples alone, while the opposite was true for B250.

Phytase results for A250 and A500 slightly decreased over time, with the phytase activity at the end of the 3-months storage period 93% and 82%, respectively, of the initial activity. The slope of regression lines of phytase activity over time of storage were not significantly different from 0 (P=0.663 and P=0.116 respectively). In the case of A500 relative values for 1-, 2- and 3-months were 87%, 81% and 82% respectively, that might indicate slight loss of activity (not significant by regression). However, the decrease in A250 was smaller and no differences should be expected from different dosages of the same batch; these variations could be considered analytical artifacts more than real loss of activity.

For B batch, results for B250 were 87%, 89% and 87% at 1-, 2- and 3-months storage, but loss of activity was not significant according to the slope of the regression line (P=0.234). Moreover, for B500, 97%, 92% and 102% of the T=0 activity was retained at 1-, 2- and 3-months storage, respectively, and the regression line could not be distinguished from a flat line (P=0.994 for B500). As with batch A, no differences should be expected from different dosages of the same batch; the variations in B250 could be considered analytical artifacts more than real loss of activity

Finally, for C batch, both C250 and C500 presented fairly constant values through storage: 97%, 99% and 95% at 1-, 2- and 3-months storage for C250; 107%, 100% and 93% at 1-, 2- and 3-months storage for C500; slopes of the regression lines were not significantly different from 0 in both cases (P=0.191 and P=0.338).

12 Conclusions

According to the results of the present stability study in feeds, CIBENZA® PHYTAVERSE® L10 phytase enzyme:

- Was stable over time (1, 2 and 3-months storage at ambient conditions) for all three batches (A & B & C) at both concentrations tested (250 & 500 U/kg) as demonstrated by the slope of linear regressions of phytase activity over time not being significantly different from 0 (flat line, no significant loss of activity).
- Presented good stability (in general ±10% of 0-month value) up to 3-months in pelleted feeds for all three batches (A & B & C) at both concentrations tested (250 & 500 U/kg). Exceptions were: A500 (82%) and B250 (87%). These lower activities at 3 months for A500 and B250 were considered to be within the range of expected values, especially considering the other dose for the same batches of enzyme (i.e. A250 and B500) did not differ from their respective T=0 activity by more than 10% (A250 (93%) as reference for A500 and B500 (102%) as reference for B250).

13 References

ISO 30024:2009. Animal feeding stuffs – Determination of phytase activity

SAS Institute Inc. 2012. Base SAS® 9.4 Guide to Information Maps. Cary, NC: SAS Institute Inc.

Statutory Instrument 1999 No. 1663. The Feeding Stuffs (Sampling and Analysis) Regulation 1999.

14 List of Appendices

Appendix 1 - Curricula vitae of Study Director & Study Monitor
Appendix 2 - Certificate of analysis of CIBENZA® PHYTAVERSE® L10 phytase enzyme used (3 batches)
Appendix 3 - Relevant laboratory reports
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Appendix 5 - Statistical printouts
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Appendix 7 – Temperature and relative humidity during storage of stability samples

Appendix 1- *Curricula vitae* of Study Director & Study Monitor

Study Director:
Name: (b) (6)

Study Monitor:
Name: Drew Lichtenstein
Qualifications: B.S. Biochemistry (Michigan State University 1982), PhD Biochemistry (University of Wisconsin-Madison 1990)
Present Position: Research Manager, Specialty Products, Novus International
Experience: Over 35 years research experience in biochemistry and cell biology; more than 8 years of experience in animal feed enzymes.

Appendix 2 - Certificate of analysis of CIBENZA® PHYTAVERSE® L10 phytase enzyme used (3 batches)

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Certificate of Analysis

CIBENZA® PHYTAVERSE® L10 Phytase Enzyme (Test Article VR006)

Lot number: CV002C2
Date of Manufacture: August 14, 2014

Specification	Specification Limit	Test Result (b) (4)	Method
Appearance	Amber to brown liquid		Visual
pH	5.0 - 5.2		Untapped
Specific gravity (g/mL)	1.05 - 1.20		Pycnometer
Sediment (% v/v)	≤ 0.5		QC0232
Activity (U/g)	≥ 10,000		ISO 30024
Lead (mg/kg)	≤ 5		ICP-MS
Arsenic (mg/kg)	< 2		ICP-MS
Cadmium (mg/kg)	< 0.5		ICP-MS
Mercury (mg/kg)	< 0.5		ICP-MS
Total Plate Count (cfu/g)	≤ 50,000		FDA BAM
Total Coliform (MPN/g)	≤ 30		FDA BAM
E. coli (/25g)	Absent		FDA BAM
Salmonella (/25g)	Absent		FDA BAM
Yeast and Mold (CFU/g)	Run and Record		FDA BAM
Staphylococcus aureus (/g)	Absent		FDA BAM
Production Organism (CFU/g)	Absent		QC0214
Antibiotic Activity (Zone of Inhibition)	Absent		JECFA
Mycotoxin			
Aflatoxin B1	NMT 1.0 ppb		HPLC
Aflatoxin B2	NMT 1.0 ppb		HPLC
Aflatoxin G1	NMT 1.0 ppb		HPLC
Aflatoxin G2	NMT 1.0 ppb		HPLC
Fumonisin B1	NMT 0.1 ppm		LCMSMS
Fumonisin B2	NMT 0.1 ppm		LCMSMS
Fumonisin B3	NMT 0.1 ppm		LCMSMS
Ochratoxin A	NMT 2.0 ppb		HPLC
Deoxynivalenol	NMT 0.6 ppm		LCMSMS
Acetyldeoxynivalenol	NMT 0.8 ppm		LCMSMS
Fusarenon X	NMT 0.4 ppm		LCMSMS
Nivalenol	NMT 0.6 ppm		LCMSMS
T-2 Toxin	NMT 0.2 ppm		LCMSMS
HT-2 Toxin	NMT 0.2 ppm		LCMSMS
Neosolaniol	NMT 0.4 ppm		LCMSMS
Diacetoxyscirpenol	NMT 0.4 ppm		LCMSMS
Zearalenone	NMT 43.1 ppb		HPLC
Sterigmatocystin	NMT 200 ppb		TLC

BASF Enzymes LLC
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www.basf.us

Certificate of Analysis

PCBs	**10,000 pg/g**	(b) (4)	**GC/HRMS**
Dioxins	**1pg/g**		**GC/HRMS**

* Results of retesting performed in May 2017
[1] The limits of detection (LOD) for each of the assays and methods match that of the stated less than (<) values above.

Approved by: _____ Date: June 7, 2017

Mark Burcin
Sr. Manager, QA/QC

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Certificate of Analysis

CIBENZA® PHYTAVERSE® L10 Phytase Enzyme (Test Article VR006)

Lot number: 190CV005A3
Date of Manufacture: August 11, 2014

Specification	Specification Limit	Test Result (b) (4)	Method
Appearance	Amber to brown liquid		Visual
pH	5.0 - 5.2		Untapped
Specific gravity (g/mL)	1.05 - 1.20		Pycnometer
Sediment (% v/v)	≤ 0.5		QC0232
Activity (U/g)	≥ 10,000		ISO 30024
Lead (mg/kg)	≤ 5		ICP-MS
Arsenic (mg/kg)	< 2		ICP-MS
Cadmium (mg/kg)	< 0.5		ICP-MS
Mercury (mg/kg)	< 0.5		ICP-MS
Total Plate Count (cfu/g)	≤ 50,000		FDA BAM
Total Coliform (cfu/g)	≤ 30		FDA BAM
E. coli (/25g)	Absent		FDA BAM
Salmonella (/25g)	Absent		FDA BAM
Yeast and Mold (MPN/g)	Run and Record		FDA BAM
Staphylococcus aureus (/g)	Absent		FDA BAM
Production Organism (CFU/g)	Absent		QC0214
Antibiotic Activity (Zone of Inhibition)	Absent		JECFA
Mycotoxin			
Aflatoxin B1	NMT 1.0 ppb		HPLC
Aflatoxin B2	NMT 1.0 ppb		HPLC
Aflatoxin G1	NMT 1.0 ppb		HPLC
Aflatoxin G2	NMT 1.0 ppb		HPLC
Fumonisin B1	NMT 0.1 ppm		LCMSMS
Fumonisin B2	NMT 0.1 ppm		LCMSMS
Fumonisin B3	NMT 0.1 ppm		LCMSMS
Ochratoxin A	NMT 2.0 ppb		HPLC
Deoxynivalenol	NMT 0.6 ppm		LCMSMS
Acetyldeoxynivalenol	NMT 0.8 ppm		LCMSMS
Fusarenon X	NMT 0.4 ppm		LCMSMS
Nivalenol	NMT 0.6 ppm		LCMSMS
T-2 Toxin	NMT 0.2 ppm		LCMSMS
HT-2 Toxin	NMT 0.2 ppm		LCMSMS
Neosolaniol	NMT 0.4 ppm		LCMSMS
Diacetoxyscirpenol	NMT 0.4 ppm		LCMSMS
Zearalenone	NMT 43.1 ppb		HPLC
Sterigmatocystin	NMT 200 ppb		TLC

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Certificate of Analysis

PCBs	**10,000 pg/g**	(b) (4)	**GC/HRMS**
Dioxins	**1pg/g**		**GC/HRMS**

* Results of retesting performed in May 2017
[1] The limits of detection (LOD) for each of the assays and methods match that of the stated less than (<) values above.

Approved by: _____ Date: June 7, 2017

Mark Burcin
Sr. Manager, QA/QC

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Certificate of Analysis

CIBENZA® PHYTAVERSE® L10 Phytase Enzyme (Test Article VR006)

Lot number: PHY-50104-PO030-F4
Date of Manufacture: September 11, 2015

Specification	Specification Limit	Test Result	Method
Appearance	Amber to brown liquid	(b) (4)	Visual
pH	5.0 - 5.2		Untapped
Specific gravity (g/mL)	1.05 - 1.20		Pycnometer
Sediment (% v/v)	≤ 0.5		QC0232
Activity (U/g)	≥ 10,000		ISO 30024
Lead (mg/kg)	≤ 5		ICP-MS
Arsenic (mg/kg)	< 2		ICP-MS
Cadmium (mg/kg)	< 0.5		ICP-MS
Mercury (mg/kg)	< 0.5		ICP-MS
Total Plate Count (cfu/g)	≤ 50,000		FDA BAM
Total Coliform (MPN/g)	≤ 30		FDA BAM
E. coli (/25g)	Absent		FDA BAM
Salmonella (/25g)	Absent		FDA BAM
Yeast and Mold (CFU/g)	Run and Record		FDA BAM
Staphylococcus aureus (/g)	Absent		FDA BAM
Production Organism (CFU/g)	Absent		QC0214
Antibiotic Activity (Zone of Inhibition)	Absent		JECFA
Mycotoxin			
Aflatoxin B1	NMT 1.0 ppb		HPLC
Aflatoxin B2	NMT 1.0 ppb		HPLC
Aflatoxin G1	NMT 1.0 ppb		HPLC
Aflatoxin G2	NMT 1.0 ppb		HPLC
Fumonisin B1	NMT 0.1 ppm		LCMSMS
Fumonisin B2	NMT 0.1 ppm		LCMSMS
Fumonisin B3	NMT 0.1 ppm		LCMSMS
Ochratoxin A	NMT 2.0 ppb		HPLC
Deoxynivalenol	NMT 0.6 ppm		LCMSMS
Acetyldeoxynivalenol	NMT 0.8 ppm		LCMSMS
Fusarenon X	NMT 0.4 ppm		LCMSMS
Nivalenol	NMT 0.6 ppm		LCMSMS
T-2 Toxin	NMT 0.2 ppm		LCMSMS
HT-2 Toxin	NMT 0.2 ppm		LCMSMS
Neosolaniol	NMT 0.4 ppm		LCMSMS
Diacetoxyscirpenol	NMT 0.4 ppm		LCMSMS
Zearalenone	NMT 43.1 ppb		HPLC
Sterigmatocystin	NMT 200 ppb		TLC

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PCBs	**10,000 pg/g**	(b) (4)	**GC/HRMS**
Dioxins	**1pg/g**		**GC/HRMS**

* Results of retesting performed in May 2017
[1] The limits of detection (LOD) for each of the assays and methods match that of the stated less than (<) values above.

Approved by: _____ Date: June 7, 2017

Mark Burcin
Sr. Manager, QA/QC

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Appendix 3 - Relevant laboratory reports

CERTIFICATE OF ANALYSIS

Company:	Novus International Ltd and BASF Enzymes LLC
Type of sample:	F600 feeds
Laboratory ref. :	172041 to 172046 180016 to 180021 180129 to 180134 181560 to 181565 181801 to 181803
Reception date:	28th November 2017
Analysis starting date:	7th December 2017
Analysis finishing date:	14th March 2018

Sample description:
See Results section

Analysis performed:

- Moisture -dry matter- by oven drying –method 2 (SOP 0602-L-10001) (AOAC, 2000)
- Nitrogen -crude protein- by combustion -Dumas method (SOP 0602-L-10118) (AOAC, 2000)
- Ether extract on a Soxtec system -method 3B (SOP 0602-L-10003) (AOAC, 2000)
- Ash after muffle furnace incineration -method 12 (SOP 0602-L-10002) (AOAC, 2000)
- Phytase (SOP 0602-L-10143; ISO 30024:2009. Animal feeding stuffs – Determination of phytase activity.)

Results:

LAB. REF.	SAMPLE DESCRIPTION	CRUDE PROTEIN (%)	ETHER EXTRACT (%)	ASH (%)
172041	A250 pellet	(b) (4)		
172042	A500 pellet			
172043	B250 pellet			
172044	B500 pellet			
172045	C250 pellet			
172046	C500 pellet			

LAB. REF.	SAMPLE DESCRIPTION	PHYTASE (U/kg)	DRY MATTER (%)
172041	A250 pellet stab 0 mes	(b) (4)	
172042	A500 pellet stab 0 mes		
172043	B250 pellet stab 0 mes		
172044	B500 pellet stab 0 mes		
172045	C250 pellet stab 0 mes		
172046	C500 pellet stab 0 mes		

LAB. REF.	SAMPLE DESCRIPTION	PHYTASE (U/kg)	DRY MATTER (%)
180016	A250 pellet stab 1 mes	(b) (4)	
180017	A500 pellet stab 1 mes		
180018	B250 pellet stab 1 mes		
180019	B500 pellet stab 1 mes		
180020	C250 pellet stab 1 mes		
180021	C500 pellet stab 1 mes		

LAB. REF.	SAMPLE DESCRIPTION	PHYTASE (U/kg)	DRY MATTER (%)
180129	A250 pellet STAB 2 meses	(b) (4)	
180130	B250 pellet STAB 2 meses		
180131	C250 pellet STAB 2 meses		
180132	A500 pellet STAB 2 meses		
180133	B500 pellet STAB 2 meses		
180134	C500 pellet STAB 2 meses		

LAB. REF.	SAMPLE DESCRIPTION	PHYTASE (U/kg)	DRY MATTER (%)
181560	A250 pellet STAB 3 meses	(b) (4)	
181562	B250 pellet STAB 3 meses		
181564	C250 pellet STAB 3 meses		
181561	A500 pellet STAB 3 meses		
181563	B500 pellet STAB 3 meses		
181565	C500 pellet STAB 3 meses		

LAB. REF.	SAMPLE DESCRIPTION	PHYTASE (U/kg)	DRY MATTER (%)
181801	A250 Pellet BACKUP 0 meses	(b) (4)	
181802	A500 Pellet BACKUP 0 meses		
181803	B250 Pellet BACKUP 0 meses		

(b) (4), (b) (6)

Signature: (b) (4), (b) (6)

Date: 21 MARCH 2018

Appendix 4 - Raw data

Obs	enzyme	form	Tr	Tr_form	lab_ref	dose	U_kg_as_is	DM_p	month	U_kg_88_pc_DM
1	A	pellet	A250	A250pellet	172041	250		(b) (4)	0	(b) (4)
2	A	pellet	A500	A500pellet	172042	500			0	
3	B	pellet	B250	B250pellet	172043	250			0	
4	B	pellet	B500	B500pellet	172044	500			0	
5	C	pellet	C250	C250pellet	172045	250			0	
6	C	pellet	C500	C500pellet	172046	500			0	
7	A	pellet	A250	A250pellet	180016	250			1	
8	A	pellet	A500	A500pellet	180017	500			1	
9	B	pellet	B250	B250pellet	180018	250			1	
10	B	pellet	B500	B500pellet	180019	500			1	
11	C	pellet	C250	C250pellet	180020	250			1	
12	C	pellet	C500	C500pellet	180021	500			1	
13	A	pellet	A250	A250pellet	180129	250			2	
14	B	pellet	B250	B250pellet	180130	250			2	
15	C	pellet	C250	C250pellet	180131	250			2	
16	A	pellet	A500	A500pellet	180132	500			2	
17	B	pellet	B500	B500pellet	180133	500			2	
18	C	pellet	C500	C500pellet	180134	500			2	
19	A	pellet	A250	A250pellet	181560	250			3	
20	A	pellet	A500	A500pellet	181561	500			3	
21	B	pellet	B250	B250pellet	181562	250			3	
22	B	pellet	B500	B500pellet	181563	500			3	
23	C	pellet	C250	C250pellet	181564	250			3	
24	C	pellet	C500	C500pellet	181565	500			3	
25	A	pellet	A250	A250pellet	181801	250			0	
26	A	pellet	A500	A500pellet	181802	500			0	
27	B	pellet	B250	B250pellet	181803	250			0	

Appendix 5 - Statistical printouts

Obs	enzyme	form	Tr	Tr_form	lab_ref	dose	U_kg_as_is	DM_p	month	U_kg_88_pc_DM
1	A	pellet	A250	A250pellet	172041	250		(b) (4)	0	(b) (4)
2	A	pellet	A500	A500pellet	172042	500			0	
3	B	pellet	B250	B250pellet	172043	250			0	
4	B	pellet	B500	B500pellet	172044	500			0	
5	C	pellet	C250	C250pellet	172045	250			0	
6	C	pellet	C500	C500pellet	172046	500			0	
7	A	pellet	A250	A250pellet	180016	250			1	
8	A	pellet	A500	A500pellet	180017	500			1	
9	B	pellet	B250	B250pellet	180018	250			1	
10	B	pellet	B500	B500pellet	180019	500			1	
11	C	pellet	C250	C250pellet	180020	250			1	
12	C	pellet	C500	C500pellet	180021	500			1	
13	A	pellet	A250	A250pellet	180129	250			2	
14	B	pellet	B250	B250pellet	180130	250			2	
15	C	pellet	C250	C250pellet	180131	250			2	
16	A	pellet	A500	A500pellet	180132	500			2	
17	B	pellet	B500	B500pellet	180133	500			2	
18	C	pellet	C500	C500pellet	180134	500			2	
19	A	pellet	A250	A250pellet	181560	250			3	
20	A	pellet	A500	A500pellet	181561	500			3	
21	B	pellet	B250	B250pellet	181562	250			3	
22	B	pellet	B500	B500pellet	181563	500			3	
23	C	pellet	C250	C250pellet	181564	250			3	
24	C	pellet	C500	C500pellet	181565	500			3	
25	A	pellet	A250	A250pellet	181801	250			0	
26	A	pellet	A500	A500pellet	181802	500			0	
27	B	pellet	B250	B250pellet	181803	250			0	

		N	U_kg_-as_is	DM_p	U_kg_-88_pc-_DM	pc_Om-_as_is	pc_Om-_88_p-c_DM	pc_Om-_DM
			Mean	Mean	Mean	Mean	Mean	Mean
Tr_form	month							
A250pellet	0	2	262	87.1	265	100.0	100.0	100.0
	1	1	(b) (4)					
	2	1						
	3	1						
A500pellet	0	2	555	87.3	559	100.0	100.0	100.0
	1	1	(b) (4)					
	2	1						
	3	1						
B250pellet	0	2	297	87.3	299	100.0	100.0	100.0
	1	1	(b) (4)					
	2	1						
	3	1						
B500pellet	0	1	535	87.4	539	100.0	100.0	100.0
	1	1	(b) (4)					
	2	1						
	3	1						
C250pellet	0	1	323	87.2	326	100.0	100.0	100.0
	1	1	(b) (4)					
	2	1						
	3	1						
C500pellet	0	1	541	87.5	544	100.0	100.0	100.0
	1	1	(b) (4)					
	2	1						
	3	1						

Obs	enzyme	dose	month	Tr	Tr_form	_FREQ_	U_kg_ as_is	DM_p	U_kg_88_ pc_DM	pc_Om_ as_is	pc_Om_ 88_pc_DM	pc_Om_DM
1	A	250	0	A250	A250pellet	2	262	87.065	(b) (4)			
2	A	250	1	A250	A250pellet	1	216	86.820				
3	A	250	2	A250	A250pellet	1	214	87.180				
4	A	250	3	A250	A250pellet	1	243	87.070				
5	A	500	0	A500	A500pellet	2	555	87.325				
6	A	500	1	A500	A500pellet	1	483	87.190				
7	A	500	2	A500	A500pellet	1	451	87.310				
8	A	500	3	A500	A500pellet	1	457	87.310				
9	B	250	0	B250	B250pellet	2	297	87.315				
10	B	250	1	B250	B250pellet	1	257	87.150				
11	B	250	2	B250	B250pellet	1	264	87.370				
12	B	250	3	B250	B250pellet	1	257	87.180				
13	B	500	0	B500	B500pellet	1	535	87.360				
14	B	500	1	B500	B500pellet	1	521	87.530				
15	B	500	2	B500	B500pellet	1	491	87.440				
16	B	500	3	B500	B500pellet	1	546	87.490				
17	C	250	0	C250	C250pellet	1	323	87.240				
18	C	250	1	C250	C250pellet	1	313	87.410				
19	C	250	2	C250	C250pellet	1	319	87.610				
20	C	250	3	C250	C250pellet	1	307	87.430				
21	C	500	0	C500	C500pellet	1	541	87.500				
22	C	500	1	C500	C500pellet	1	578	87.580				
23	C	500	2	C500	C500pellet	1	541	87.860				
24	C	500	3	C500	C500pellet	1	502	87.950				

---------- Tr_form=A250pellet ----------

The GLM Procedure

Number of Observations Read 4
Number of Observations Used 4

---------- Tr_form=A250pellet ----------

The GLM Procedure

Dependent Variable: U_kg_as_is

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	174.050000	174.050000	0.25	0.6690
Error	2	1414.700000	707.350000		
Corrected Total	3	1588.750000			

R-Square	Coeff Var	Root MSE	U_kg_as_is Mean
0.109552	11.37799	26.59605	233.7500

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	174.0500000	174.0500000	0.25	0.6690

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	174.0500000	174.0500000	0.25	0.6690

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	242.6000000	22.25185386	10.90	0.0083
month	-5.9000000	11.89411619	-0.50	0.6690

---------- Tr_form=A250pellet ----------

The GLM Procedure

Dependent Variable: U_kg_88_pc_DM

Sum of

Source	DF	Squares	Mean Square	F Value	Pr > F
Model	1	183.767041	183.767041	0.26	0.6625
Error	2	1429.644878	714.822439		
Corrected Total	3	1613.411919			

R-Square	Coeff Var	Root MSE	U_kg_88_pc_DM Mean
0.113900	11.31242	26.73616	236.3434

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	183.7670414	183.7670414	0.26	0.6625

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	183.7670414	183.7670414	0.26	0.6625

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	245.4370570	22.36907926	10.97	0.0082
month	-6.0624589	11.95677581	-0.51	0.6625

---------- Tr_form=A250pellet ----------

The GLM Procedure

Dependent Variable: pc_Om_as_is

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	25.3554571	25.3554571	0.25	0.6690
Error	2	206.0923023	103.0461512		
Corrected Total	3	231.4477595			

R-Square	Coeff Var	Root MSE	pc_Om_as_is Mean
0.109552	11.37799	10.15117	89.21756

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	25.35545714	25.35545714	0.25	0.6690

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	25.35545714	25.35545714	0.25	0.6690

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	92.59541985	8.49307399	10.90	0.0083
month	-2.25190840	4.53973900	-0.50	0.6690

---------- Tr_form=A250pellet ----------

The GLM Procedure

Dependent Variable: pc_Om_88_pc_DM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	26.2021267	26.2021267	0.26	0.6625
Error	2	203.8436050	101.9218025		
Corrected Total	3	230.0457317			

R-Square	Coeff Var	Root MSE	pc_Om_88_pc_DM Mean
0.113900	11.31242	10.09563	89.24376

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	26.20212669	26.20212669	0.26	0.6625

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	26.20212669	26.20212669	0.26	0.6625

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	92.67756060	8.44661244	10.97	0.0082

```
month          -2.28919753      4.51490426      -0.51      0.6625
```



```
---------------------------------------- Tr_form=A500pellet ----------------------------------------
                                The GLM Procedure
                          Number of Observations Read           4
                          Number of Observations Used           4
```



```
---------------------------------------- Tr_form=A500pellet ----------------------------------------
                                The GLM Procedure
                       Dependent Variable: U_kg_as_is
                                              Sum of
Source                         DF            Squares     Mean Square    F Value    Pr > F
Model                           1       5313.800000     5313.800000       6.99    0.1183
Error                           2       1521.200000      760.600000
Corrected Total                 3       6835.000000

                  R-Square     Coeff Var      Root MSE    U_kg_as_is Mean
                  0.777440      5.668855      27.57898           486.5000

Source                         DF          Type I SS     Mean Square    F Value    Pr > F
month                           1       5313.800000     5313.800000       6.99    0.1183

Source                         DF       Type III SS     Mean Square    F Value    Pr > F
month                           1       5313.800000     5313.800000       6.99    0.1183

                                            Standard
               Parameter         Estimate           Error    t Value    Pr > |t|
               Intercept      535.4000000     23.07422805      23.20      0.0019
               month          -32.6000000     12.33369369      -2.64      0.1183
```



```
---------------------------------------- Tr_form=A500pellet ----------------------------------------
                                The GLM Procedure
                       Dependent Variable: U_kg_88_pc_DM
                                              Sum of
Source                         DF            Squares     Mean Square    F Value    Pr > F
Model                           1       5411.883148     5411.883148       7.14    0.1161
Error                           2       1515.646808      757.823404
Corrected Total                 3       6927.529956

                  R-Square     Coeff Var      Root MSE    U_kg_88_pc_DM Mean
                  0.781214      5.612453      27.52859              490.4913

Source                         DF          Type I SS     Mean Square    F Value    Pr > F
month                           1       5411.883148     5411.883148       7.14    0.1161

Source                         DF       Type III SS     Mean Square    F Value    Pr > F
month                           1       5411.883148     5411.883148       7.14    0.1161

                                            Standard
               Parameter         Estimate           Error    t Value    Pr > |t|
               Intercept      539.8405386     23.03207291      23.44      0.0018
               month          -32.8994928     12.31116082      -2.67      0.1161
```



```
---------------------------------------- Tr_form=A500pellet ----------------------------------------
                                The GLM Procedure
                        Dependent Variable: pc_Om_as_is
                                              Sum of
Source                         DF            Squares     Mean Square    F Value    Pr > F
Model                           1       172.5119714     172.5119714       6.99    0.1183
Error                           2        49.3856018      24.6928009
```

```
Corrected Total              3     221.8975732

                R-Square     Coeff Var      Root MSE    pc_Om_as_is Mean
                0.777440      5.668855      4.969185            87.65766

Source                       DF       Type I SS     Mean Square    F Value    Pr > F
month                         1     172.5119714     172.5119714       6.99    0.1183

Source                       DF     Type III SS     Mean Square    F Value    Pr > F
month                         1     172.5119714     172.5119714       6.99    0.1183

                                          Standard
           Parameter         Estimate           Error    t Value    Pr > |t|
           Intercept      96.46846847      4.15751857      23.20      0.0019
           month          -5.87387387      2.22228715      -2.64      0.1183
```



```
------------------------------------- Tr_form=A500pellet -------------------------------------
                                The GLM Procedure
                          Dependent Variable: pc_Om_88_pc_DM
                                       Sum of
Source                       DF         Squares     Mean Square    F Value    Pr > F
Model                         1     173.0038095     173.0038095       7.14    0.1161
Error                         2      48.4512811      24.2256405
Corrected Total               3     221.4550905

            R-Square     Coeff Var      Root MSE    pc_Om_88_pc_DM Mean
            0.781214      5.612453      4.921955              87.69704

Source                       DF       Type I SS     Mean Square    F Value    Pr > F
month                         1     173.0038095     173.0038095       7.14    0.1161

Source                       DF     Type III SS     Mean Square    F Value    Pr > F
month                         1     173.0038095     173.0038095       7.14    0.1161

                                          Standard
           Parameter         Estimate           Error    t Value    Pr > |t|
           Intercept      96.52040196      4.11800296      23.44      0.0018
           month          -5.88224123      2.20116517      -2.67      0.1161
```



```
------------------------------------- Tr_form=B250pellet -------------------------------------
                                The GLM Procedure
                        Number of Observations Read           4
                        Number of Observations Used           4
```



```
------------------------------------- Tr_form=B250pellet -------------------------------------
                                The GLM Procedure
                          Dependent Variable: U_kg_as_is
                                       Sum of
Source                       DF         Squares     Mean Square    F Value    Pr > F
Model                         1      638.450000      638.450000       2.79    0.2370
Error                         2      458.300000      229.150000
Corrected Total               3     1096.750000

                R-Square     Coeff Var      Root MSE    U_kg_as_is Mean
                0.582129      5.632633      15.13770           268.7500

Source                       DF       Type I SS     Mean Square    F Value    Pr > F
month                         1     638.4500000     638.4500000       2.79    0.2370

Source                       DF     Type III SS     Mean Square    F Value    Pr > F
month                         1     638.4500000     638.4500000       2.79    0.2370
```

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	285.7000000	12.66510955	22.56	0.0020
month	-11.3000000	6.76978582	-1.67	0.2370

(b) (4) Trial F600, stability pellet feeds 170
07:52 Saturday, March 17, 2018

Tr_form=B250pellet

The GLM Procedure

Dependent Variable: U_kg_88_pc_DM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	642.239504	642.239504	2.84	0.2340
Error	2	452.456203	226.228101		
Corrected Total	3	1094.695707			

R-Square	Coeff Var	Root MSE	U_kg_88_pc_DM Mean
0.586683	5.549345	15.04088	271.0389

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	642.2395043	642.2395043	2.84	0.2340

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	642.2395043	642.2395043	2.84	0.2340

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	288.0390914	12.58410390	22.89	0.0019
month	-11.3334858	6.72648647	-1.68	0.2340

(b) (4) Trial F600, stability pellet feeds 171
07:52 Saturday, March 17, 2018

Tr_form=B250pellet

The GLM Procedure

Dependent Variable: pc_Om_as_is

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	72.3792357	72.3792357	2.79	0.2370
Error	2	51.9561496	25.9780748		
Corrected Total	3	124.3353853			

R-Square	Coeff Var	Root MSE	pc_Om_as_is Mean
0.582129	5.632633	5.096869	90.48822

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	72.37923568	72.37923568	2.79	0.2370

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	72.37923568	72.37923568	2.79	0.2370

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	96.19528620	4.26434665	22.56	0.0020
month	-3.80471380	2.27938916	-1.67	0.2370

(b) (4) Trial F600, stability pellet feeds 172
07:52 Saturday, March 17, 2018

Tr_form=B250pellet

The GLM Procedure

Dependent Variable: pc_Om_88_pc_DM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	71.6807149	71.6807149	2.84	0.2340
Error	2	50.4988931	25.2494465		
Corrected Total	3	122.1796079			

R-Square	Coeff Var	Root MSE	pc_Om_88_pc_DM Mean
0.586683	5.549345	5.024883	90.54912

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	71.68071486	71.68071486	2.84	0.2340

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	71.68071486	71.68071486	2.84	0.2340

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	96.22858249	4.20411853	22.89	0.0019
month	-3.78630994	2.24719588	-1.68	0.2340

---------- Tr_form=B500pellet ----------

The GLM Procedure

Number of Observations Read	4
Number of Observations Used	4

---------- Tr_form=B500pellet ----------

The GLM Procedure

Dependent Variable: U_kg_as_is

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.450000	0.450000	0.00	0.9837
Error	2	1700.300000	850.150000		
Corrected Total	3	1700.750000			

R-Square	Coeff Var	Root MSE	U_kg_as_is Mean
0.000265	5.572352	29.15733	523.2500

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	0.45000000	0.45000000	0.00	0.9837

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	0.45000000	0.45000000	0.00	0.9837

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	522.8000000	24.39477403	21.43	0.0022
month	0.3000000	13.03955521	0.02	0.9837

---------- Tr_form=B500pellet ----------

The GLM Procedure

Dependent Variable: U_kg_88_pc_DM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.064644	0.064644	0.00	0.9939
Error	2	1722.862426	861.431213		
Corrected Total	3	1722.927070			

R-Square	Coeff Var	Root MSE	U_kg_88_pc_DM Mean
0.000038	5.574460	29.35015	526.5111

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	0.06464421	0.06464421	0.00	0.9939

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	0.06464421	0.06464421	0.00	0.9939

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	526.3405147	24.55609597	21.43	0.0022
month	0.1137051	13.12578541	0.01	0.9939

---------- Tr_form=B500pellet ----------

The GLM Procedure

Dependent Variable: pc_Om_as_is

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.01572190	0.01572190	0.00	0.9837
Error	2	59.40431479	29.70215739		
Corrected Total	3	59.42003668			

R-Square	Coeff Var	Root MSE	pc_Om_as_is Mean
0.000265	5.572352	5.449969	97.80374

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	0.01572190	0.01572190	0.00	0.9837

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	0.01572190	0.01572190	0.00	0.9837

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	97.71962617	4.55977085	21.43	0.0022
month	0.05607477	2.43730004	0.02	0.9837

---------- Tr_form=B500pellet ----------

The GLM Procedure

Dependent Variable: pc_Om_88_pc_DM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.00222578	0.00222578	0.00	0.9939
Error	2	59.32033693	29.66016846		
Corrected Total	3	59.32256271			

R-Square	Coeff Var	Root MSE	pc_Om_88_pc_DM Mean
0.000038	5.574460	5.446115	97.69763

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	0.00222578	0.00222578	0.00	0.9939

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	0.00222578	0.00222578	0.00	0.9939

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	97.66597850	4.55654671	21.43	0.0022
month	0.02109873	2.43557667	0.01	0.9939

---------- Tr_form=C250pellet ----------

The GLM Procedure

Number of Observations Read	4
Number of Observations Used	4

---------- Tr_form=C250pellet ----------

The GLM Procedure

Dependent Variable: U_kg_as_is

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	88.2000000	88.2000000	3.00	0.2254
Error	2	58.8000000	29.4000000		
Corrected Total	3	147.0000000			

R-Square	Coeff Var	Root MSE	U_kg_as_is Mean
0.600000	1.718598	5.422177	315.5000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	88.20000000	88.20000000	3.00	0.2254

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	88.20000000	88.20000000	3.00	0.2254

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	321.8000000	4.53651849	70.94	0.0002
month	-4.2000000	2.42487113	-1.73	0.2254

Tr_form=C250pellet

The GLM Procedure

Dependent Variable: U_kg_88_pc_DM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	101.8345986	101.8345986	3.79	0.1909
Error	2	53.7058037	26.8529018		
Corrected Total	3	155.5404023			

R-Square	Coeff Var	Root MSE	U_kg_88_pc_DM Mean
0.654715	1.631672	5.181979	317.5870

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	101.8345986	101.8345986	3.79	0.1909

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	101.8345986	101.8345986	3.79	0.1909

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	324.3564747	4.33555432	74.81	0.0002
month	-4.5129724	2.31745126	-1.95	0.1909

Tr_form=C250pellet

The GLM Procedure

Dependent Variable: pc_Om_as_is

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	8.45402525	8.45402525	3.00	0.2254
Error	2	5.63601683	2.81800842		
Corrected Total	3	14.09004208			

R-Square	Coeff Var	Root MSE	pc_Om_as_is Mean
0.600000	1.718598	1.678692	97.67802

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	8.45402525	8.45402525	3.00	0.2254

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	8.45402525	8.45402525	3.00	0.2254

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	99.62848297	1.40449489	70.94	0.0002
month	-1.30030960	0.75073410	-1.73	0.2254

Tr_form=C250pellet

The GLM Procedure
Dependent Variable: pc_Om_88_pc_DM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	9.59303155	9.59303155	3.79	0.1909
Error	2	5.05919870	2.52959935		
Corrected Total	3	14.65223024			

R-Square	Coeff Var	Root MSE	pc_Om_88_pc_DM Mean
0.654715	1.631672	1.590471	97.47494

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	9.59303155	9.59303155	3.79	0.1909

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	9.59303155	9.59303155	3.79	0.1909

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	99.55265111	1.33068386	74.81	0.0002
month	-1.38513765	0.71128044	-1.95	0.1909

---------- Tr_form=C500pellet ----------

The GLM Procedure

Number of Observations Read	4
Number of Observations Used	4

---------- Tr_form=C500pellet ----------

The GLM Procedure
Dependent Variable: U_kg_as_is

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	1185.800000	1185.800000	1.39	0.3593
Error	2	1703.200000	851.600000		
Corrected Total	3	2889.000000			

R-Square	Coeff Var	Root MSE	U_kg_as_is Mean
0.410453	5.399109	29.18219	540.5000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	1185.800000	1185.800000	1.39	0.3593

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	1185.800000	1185.800000	1.39	0.3593

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	563.6000000	24.41556880	23.08	0.0019
month	-15.4000000	13.05067048	-1.18	0.3593

---------- Tr_form=C500pellet ----------

The GLM Procedure
Dependent Variable: U_kg_88_pc_DM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	1350.183427	1350.183427	1.56	0.3384
Error	2	1734.462143	867.231071		
Corrected Total	3	3084.645570			

R-Square	Coeff Var	Root MSE	U_kg_88_pc_DM Mean
0.437711	5.430824	29.44879	542.2527

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	1350.183427	1350.183427	1.56	0.3384

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	1350.183427	1350.183427	1.56	0.3384

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	566.9018737	24.63862314	23.01	0.0019
month	-16.4327930	13.16989804	-1.25	0.3384

---------- Tr_form=C500pellet ----------

The GLM Procedure

Dependent Variable: pc_Om_as_is

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	40.51510006	40.51510006	1.39	0.3593
Error	2	58.19304977	29.09652489		
Corrected Total	3	98.70814983			

R-Square	Coeff Var	Root MSE	pc_Om_as_is Mean
0.410453	5.399109	5.394119	99.90758

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	40.51510006	40.51510006	1.39	0.3593

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	40.51510006	40.51510006	1.39	0.3593

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	104.1774492	4.51304414	23.08	0.0019
month	-2.8465804	2.41232356	-1.18	0.3593

---------- Tr_form=C500pellet ----------

The GLM Procedure

Dependent Variable: pc_Om_88_pc_DM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	45.6089095	45.6089095	1.56	0.3384
Error	2	58.5897630	29.2948815		
Corrected Total	3	104.1986725			

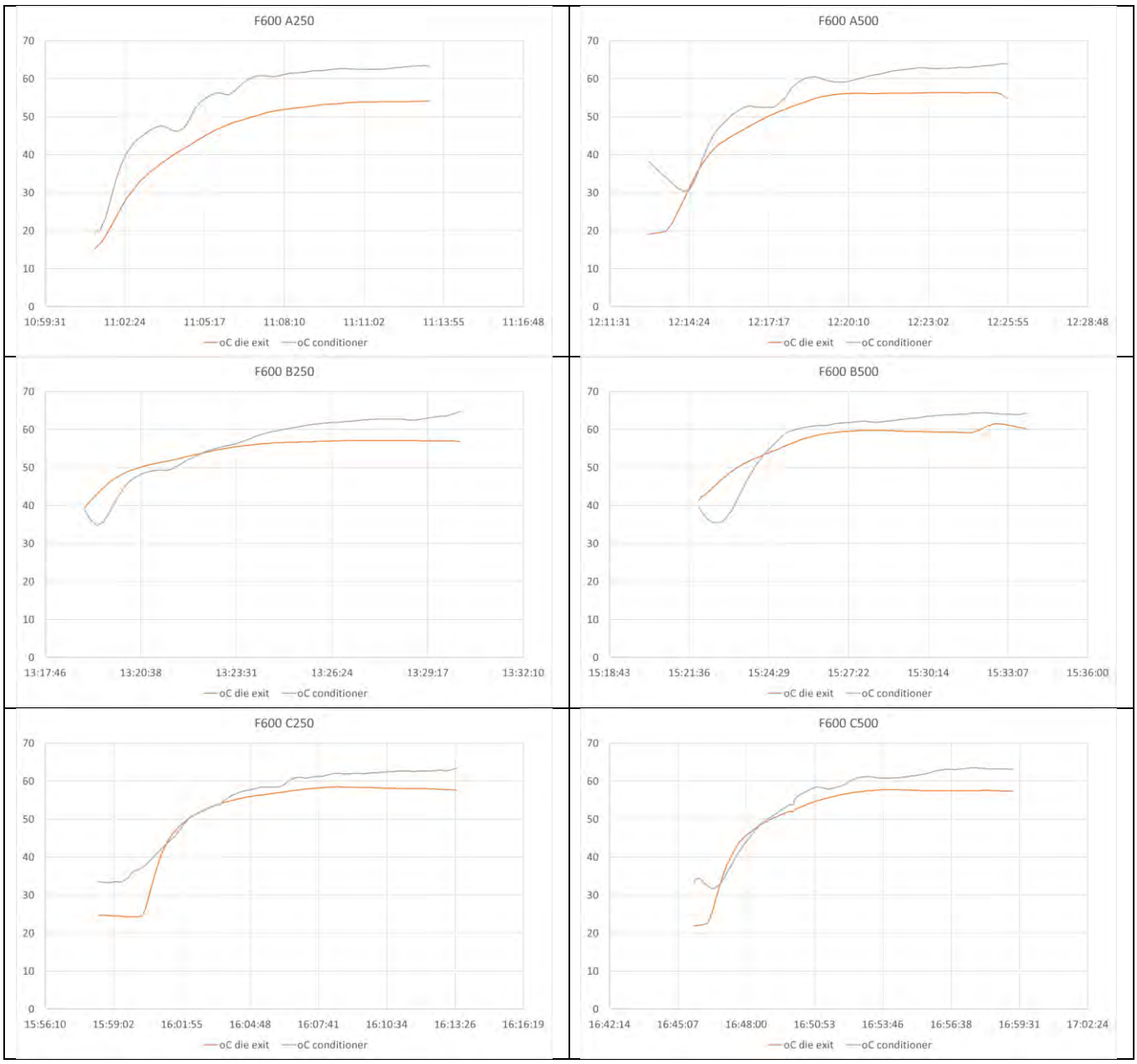
R-Square	Coeff Var	Root MSE	pc_Om_88_pc_DM Mean
0.437711	5.430824	5.412475	99.66213

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	45.60890952	45.60890952	1.56	0.3384

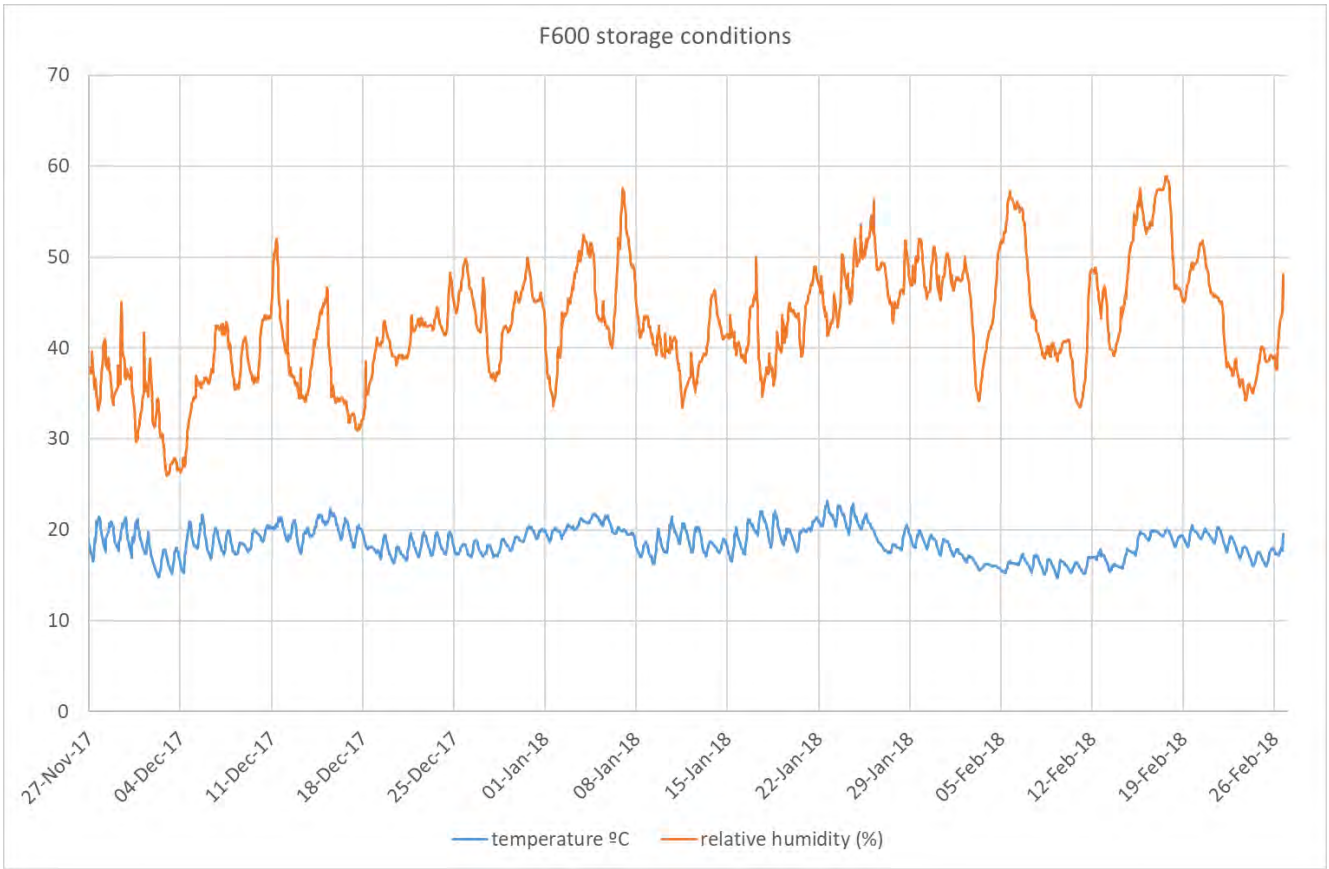
Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	45.60890952	45.60890952	1.56	0.3384

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	104.1924740	4.52840116	23.01	0.0019
month	-3.0202288	2.42053223	-1.25	0.3384

Appendix 6 – Temperature profile in the conditioner during pelleting



Appendix 7 – Temperature and relative humidity during storage of stability samples



Appendix 17: Sources of Vitamins and Minerals Used in the In-Feed Stability Studies

Date **27th March 2018**
Products: **CIBENZA® PHYTAVERSE® L10 Phytase Enzyme and CIBENZA® PHYTAVERSE® G10 Phytase Enzyme**

TO WHOM IT MAY CONCERN:

The table below provides source and regulatory status for the ingredients in the vitamin-mineral premix used in the following studies conducted at (b) (4):

1) Homogeneity evaluation of CIBENZA PHYTAVERSE G10 phytase enzyme in feed (Unique Study Code: F598),
2) Stability evaluation of CIBENZA PHYTAVERSE G10 phytase enzyme in feed (Unique Study Code: F597),
3) Homogeneity evaluation of CIBENZ PHYTAVERSE L10 phytase enzyme in feed (Unique Study Code: F599), and
4) Stability evaluation of CIBENZA PHYTAVERSE L10 phytase enzyme in feed (Unique Study Code: F600).

#	Vitamin/Mineral	Source	Regulatory status to support ingredient use in US
1	(b) (4)		
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			

#	Vitamin/Mineral	Source	Regulatory status to support ingredient use in US
15	(b) (4)		
16			
17			
18			
19			
20			
21			
22			
23			
24			
25			
26			
27			

Sincerely,

(b) (4)

(b) (6), (b) (4)

(b) (4)

Appendix 18: Homogeneity Evaluation of CIBENZA® PHYTAVERSE® L10 Phytase Enzyme in Feed

(b) (4)

(b) (4)

(b) (4), (b) (6)

(b) (4), (b) (6)

(b) (4)

(b) (4)

Table of contents

1 Summary

The objective of this study was to evaluate the Homogeneity of CIBENZA® PHYTAVERSE® L10 phytase enzyme in feeds (post pellet liquid application).

For each batch, the homogeneity of the test article was determined by measuring phytase activity in 10 subsamples taken at different time points at bagging.

Results are presented next in Summary Table 1.

Summary Table 1. Homogeneity of CIBENZA® PHYTAVERSE® L10 phytase enzyme in feeds

		Phytase U/kg as is						Phytase U/kg 88% DM					
		N	Mean	CV	StdDev	Max	Min	N	Mean	CV	StdDev	Max	Min
Tr	form			(b) (4)		(b) (4)				(b) (4)		(b) (4)	
A250	pellet	10	264					10	268				
B250	pellet	10	277					10	280				
C250	pellet	10	284					10	286				

† One phytase unit is the amount of enzyme that releases 1 µmol of inorganic phosphate from phytate per minute under the conditions of the assay (Phytate concentration: 5.0 mM, pH: 5.5, Temperature: 37°C, Incubation time: 30 min) (ISO 30024:2009). Normal analytical variation for the method is 10%.

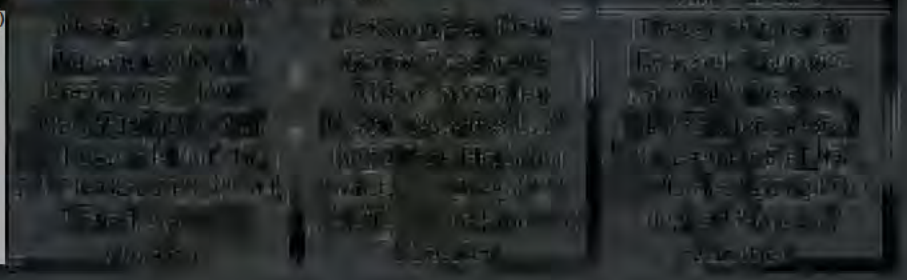
According the results of the present homogeneity study in feeds, CIBENZA® PHYTAVERSE® L10 phytase enzyme:

- Presented good mixing homogeneity (CV ~7 to 11%), actual CVs below to 2× the CV of the method itself (10%) for all 3 batches tested in pelleted form (post pellet application).

(b) (4)

(b) (4), (b) (6)

(b) (4), (b) (6)



3 Study title and unique study code

Homogeneity evaluation of CIBENZA® PHYTAVERSE® L10 phytase enzyme in feed.

Unique study code: F599

4 Study objective

To evaluate the homogeneity of three batches of CIBENZA® PHYTAVERSE® L10 phytase enzyme in pelleted feeds (post pellet liquid application of CIBENZA® PHYTAVERSE® L10 phytase enzyme).

5 Study location

(b) (4)

6 Important dates & duration of the study

Date of feeds manufacture: 27th November 2017
Duration of study: 1 day at feed mill, 12th December 2017 end of analysis

7 Test products

Table 1. Details of test product

Code	Product	Provider	Lot n° Manufacture Date	Active substance	Activity (U/g)†	
					Guaranteed	Analysed
A	CIBENZA® PHYTAVERSE® L10 Phytase Enzyme	Novus International, Inc.	CV002C2	6-phytase	10,000	12,247
B	CIBENZA® PHYTAVERSE® L10 Phytase Enzyme	Novus International, Inc.	190CV005A3	6-phytase	10,000	11,860
C	CIBENZA® PHYTAVERSE® L10 Phytase Enzyme	Novus International, Inc.	PHY-50104-PO030-F4	6-phytase	10,000	12,247

† One phytase unit is the amount of enzyme that releases 1 µmol of inorganic phosphate from phytate per minute under the conditions of the assay (Phytate concentration: 5.0 mM, pH: 5.5, Temperature: 37°C, Incubation time: 30 min) (ISO 30024:2009). Normal analytical variation for the method is 10%.

8 Key study personnel

Study Director: (b) (4), (b) (6)

External Study Monitor: Drew Lichtenstein, Ph.D. Research Manager, Specialty Products, Novus International, Inc., 20 Research Park Dr., St. Charles, MO 63304, United States of America, Tel: +1 314 453-7793, E-mail: drew.lichtenstein@novusint.com

Study Sponsors: 1) Gavin Bowman, Director, Global Regulatory Affairs, Novus International, 20 Research Park Dr., St. Charles, MO 63304, United States of America Tel: +1 636 926 7402, E-mail: gavin.bowman@novusint.com

2) Roxanna Van Dorn, Sr. Regulatory Affairs Specialist, Phone: +1 858 431-8590, Mobile: +1-858-349-7339, Fax: +1-973-307-2549, E-mail: roxanna.vandorn@basf.com. Postal Address: BASF Enzymes LLC, 3550 John Hopkins Court, San Diego, CA 92121 United States

Feed mill & supervision of diet manufacture: (b) (4), (b) (6)

Feed analysis (DM and CIBENZA® PHYTAVERSE® L10 phytase enzyme): (b) (4), (b) (6)

Optional/back-up facility for feed analysis (DM and CIBENZA® PHYTAVERSE® L10 phytase enzyme): Drew Lichtenstein, Novus International, Inc., 20 Research Park Drive, Saint Charles, MO, 63304; United States of America.

9 Material and methods

9.1 Experimental treatments

Number of treated and control groups: Corn/soya based diet was used for homogeneity purposes.

CIBENZA® PHYTAVERSE® L10 phytase liquid enzyme from each batch was added post pelleting to the feed to provide 250 and 500 U/kg feed as detailed in Table 2.

Table 2. Experimental Treatments

Treatment	Product	CIBENZA® PHYTAVERSE® L10 phytase enzyme			
		U/kg feed	mg/kg feed†	g to add to 300 kg feed†	g for 2.4 kg dilution‡
A250	CIBENZA® PHYTAVERSE® L10 phytase enzyme batch CV002C2	250	20.41	(b) (4)	
A500		500	40.83		
B250	CIBENZA® PHYTAVERSE® L10 phytase enzyme batch 190CV005A3	250	21.08		
B500		500	42.16		
C250	CIBENZA® PHYTAVERSE® L10 phytase enzyme batch PHY-50104-PO030-F4	250	20.41		
C500		500	40.83		

† inclusion based on actual activity of each batch

‡ diluted product applied at 6 kg/ton; 0.6 kg of diluted product is needed to fill the pipeline for post pellet application

9.2 Treatment application

CIBENZA® PHYTAVERSE® L10 phytase enzyme was applied post pelleting.

9.3 Detailed study design

Figure 1. Basic study design
For each batch and dose of enzyme: The homogeneity of the test article in pelleted feeds was determined by measuring phytase activity in: • 10 subsamples taken at fixed intervals at bagging The amount of endogenous phytase in blank feed has been determined in other studies being values below the level of quantitation. Feeds were produced as follows: • Firstly, a 300 kg batch of mash feed was produced. • Secondly, mash feed was pelleted. • Thirdly, the corresponding amount of CIBENZA® PHYTAVERSE® L10 phytase enzyme as detailed in Table 2 was applied post pelleting, and later bagged

9.4 Feed composition

Feeds did not contain any antibiotics or any other growth promoters. The ingredients, premix and the calculated analyses of the diets are presented in Table 3 to Table 5.

Table 3. Composition (g/kg) of the basal diet	
Corn	577
Soybean meal 48%	373
Fat blend	13.69
Dicalcium phosphate	6.81
Calcium carbonate	12.12
Methionine Hydroxy Analogue	1.75
Premix Min-Vit	10.00
Sodium chloride	1.94
L-lysine HCL	2.91
L-threonine	0.65

Table 4. Composition of vitamin-mineral premix			
	Units	per kg of vitamin-mineral premix	when premix added at 10 kg/ton feed, results in the following values per kg of feed
Vitamins, provitamins and similar			
(b) (4)	IU	1 000 000	(b) (4)
	IU	350 000	
	mg	3 000	
	mg	210	
	mg	855	
	mg	470	
	mg	5	
	mg	300	
	mg	2 000	
	mg	1 520	
	mg	6 710	
	mg	150	
	mg	25	
	mg	70 000	
	mg	6 500	
	mg	150	
	mg	1 500	
	mg	8 000	
	mg	8 500	
	mg	20	
	g	50	
	g	150	
	mg	5 000	
		up to 1 kg	u

Table 5. Calculated analyses of the basal diet (g/kg)	
Metabolizable Energy kcal/kg	2864
Dry Matter	868
Ash	58
Crude Fiber	27
Ether Extract	41
Crude Protein	227
Ca	9.6
P	5.0
Dig lysine	14.1
Dig SAA	9.4
Dig threonine	8.4

9.5 Feed analyses

Phytase activity in feeds was determined based on ISO 30024:2009. Animal feeding stuffs – Determination of phytase activity.

Dry Matter was determined according AOAC method 934.01: Loss on Drying (Moisture) at 95°-100°C for Feeds.

Premix was firstly analyzed to confirm the absence of phytase activity.

9.6 Feeds manufacture

All the process is automated and controlled by a computer provided with software from (b) (4) so that the incorporation of ingredients and the functioning of the equipment is regulated and recorded by the software. The addition of manual ingredients (vitamins, amino acids and oligo minerals, as well as test products) is made by means of a bar code system.

Feed ingredients were ground through a 40HP hammer mill (Rosal VRE-40) with a horizontal axis and a 3 mm sieve, provided with an automatic feeder.

The feed mixer was a 1000 L Rosal mixer with a double horizontal ribbon, which is sufficient for 300 to 500 kg of feed. The amount of feed prepared was 300 kg per treatment. Fat was added by means of a dosing device provided by three nozzles ((b) (4)). Mineral-vitamin premix and amino acids were manually added to the mixer. The mixing time was 6 min.

Mash feed was then pelleted in a pelleting press (MABRIK PVR-40) provided with a die of 280 mm of internal diameter with holes of 3×36 mm. The compression group consists of 2 rollers. The feeder is of stainless steel of progressive opening and is moved by a reducing engine. The conditioner is of stainless steel with adjustable blades, prepared for the reception of water and steam. The steam generator has a manometer to reduce the pressure to 2.5-3 kg/cm^2 and a flux regulator valve. Pelleting is automatically regulated by the software of the system which adjusts the temperature of the mash feed at the end of the conditioner (approximately 30 to 38 seconds of conditioning time). The pelletization temperature was adjusted to a mean temperature of approximately 65ºC being the actual maximum temperature 64.8ºC. Temperatures were recorded at fixed intervals (i.e. 5 seconds) in the outlet of the conditioner and outlet of die. The vertical cooler (MABRIK, S.A) works by air aspiration provided by a 7.5 HP turbine.

(b) (4)

9.6.1 Short description of the process

(b) (4)

9.7 Feeds samples at manufacture

For each CIBENZA® PHYTAVERSE® L10 phytase enzyme batch and dose:

- 10 grab samples of pelleted feed (~1.1 kg each) were taken at fixed interval times before bagging. From these 10 grab pelleted feed samples:
  - Triplicate (NOVUS, (b) (4)) (b) (4)

Homogeneity samples were placed in zip-lock plastic bags labelled with the unique study code (F599), treatment code (A250 / A500 / B250 / B500 / C250 / C500), date of manufacture and the analysis required (DM, phytase activity).

9.8 Feed sampling plan

Table 6. Sampling plan

Treatment	Feed form	n at sampling	Final Samples		
			NOVUS	(b) (4)	(b) (4)
A250	pellet	10 × ~1.1 kg			
A500	pellet	10 × ~1.1 kg			
B250	pellet	10 × ~1.1 kg			
B500	pellet	10 × ~1.1 kg			
C250	pellet	10 × ~1.1 kg			
C500	pellet	10 × ~1.1 kg			

For homogeneity analysis, A250, B250 and C250 samples were analyzed in (b) (4) lab within 10 working days after production of the feeds containing CIBENZA® PHYTAVERSE® L10 phytase enzyme; the A500, B500 and C500 homogeneity samples were kept frozen serving as back up samples. The 250 U/kg samples were refrigerated (4°C) until tested to make sure they reflected accurate activity values at the time the feed was manufactured. One set of samples was dispatched to NOVUS (Reus, Spain) as backup samples. A second set of samples was sent to (b) (4) lab for analysis. A third set of samples was sent (b) (4) lab for storage as backup samples.

9.9 Statistics

For each CIBENZA® PHYTAVERSE® L10 phytase enzyme batch:

- Homogeneity: Mean CIBENZA® PHYTAVERSE® L10 phytase enzyme activity (arithmetic mean) and variation (standard deviation) was used to express the result as a unique value described as the coefficient of variation.

Calculations:

$$\%CV = \frac{s}{\bar{y}} \times 100$$

$$\bar{y} = \frac{\Sigma y_i}{n}$$

$$s = \sqrt{s^2}$$

$$s^2 = \frac{\Sigma (y_i - \bar{y})^2}{n-1}$$

where:

%CV= coefficient of variation

Σ= summation

s= standard deviation

y_i= individual result from each sample

s^2= variance

n= total number of samples

$\bar{y}$ = mean

10 Results

The results are summarized in Table 7 and Table 8. Values from proximate analysis were within expected ranges.

Table 7. Analyzed values of experimental diets

Sample	Dry matter (%)	Crude protein (%)	Ether extract (%)	Ash (%)
A250 pellet	87.0	22.9	3.9	5.5
A500 pellet	87.3	23.0	3.8	5.5
B250 pellet	87.3	23.0	3.8	5.5
B500 pellet	87.4	22.9	3.8	5.5
C250 pellet	87.2	23.3	3.9	5.4
C500 pellet	87.5	23.2	3.8	5.5

Table 8. Homogeneity of CIBENZA® PHYTAVERSE® L10 phytase enzyme in feeds

		Phytase U/kg as is						Phytase U/kg 88% DM					
		N	Mean	CV	StdDev	Max	Min	N	Mean	CV	StdDev	Max	Min
Tr	form						(b) (4)						
A250	pellet	10	264					10	268				(b) (4)
B250	pellet	10	277					10	280				
C250	pellet	10	284					10	286				

† One phytase unit is the amount of enzyme that releases 1 µmol of inorganic phosphate from phytate per minute under the conditions of the assay (Phytate concentration: 5.0 mM, pH: 5.5, Temperature: 37°C, Incubation time: 30 min) (ISO 30024:2009). Normal analytical variation for the method is 10%.

11 Discussion

Dry matter was quite similar among samples (87.1%±0.4) and the correction for constant DM (88%) did not change the results of the coefficients of variation for homogeneity. Phytase activity ranged from 223 to 320 U/kg as is (225 to 323 U/kg at 88% DM). Considering each enzyme batch, the average activities were: 264 U/kg as is for A250, 277 U/kg as is for B250, and 284 U/kg as is for C250.

The homogeneity of mixing for the three CIBENZA® PHYTAVERSE® L10 phytase enzyme batches tested expressed as Coefficients of Variation were 7%, 7% and 11% when standardized at 88% DM content for A250, B250 and C250 respectively. These small variations among batches are considered within the expected fluctuations due to the method variability itself.

All these CVs of the homogeneity were close to 1× or even below the CV of the normal analytical variation of the method itself (normal analytical CV is 10%), and therefore the CVs of the homogeneity were considered good ($CV<2\times_{analytical}CV$).

Per the protocol, back up samples of A500, B500, and C500 were not tested, because the lowest inclusion rate of 250 U/kg demonstrated good homogeneity.

12 Conclusions

According the results of the present homogeneity study in feeds, CIBENZA® PHYTAVERSE® L10 phytase enzyme:

- Presented good mixing homogeneity (CV ~7 to 11%), actual CVs below to 2× the CV of the method itself (10%) for all 3 batches tested.

13 References

ISO 30024:2009. Animal feeding stuffs – Determination of phytase activity

SAS Institute Inc. 2012. Base SAS® 9.4 Guide to Information Maps. Cary, NC: SAS Institute Inc.

Statutory Instrument 1999 No. 1663. The Feeding Stuffs (Sampling and Analysis) Regulation 1999.

14 List of Appendices

Appendix 1 - Curricula vitae of Study Director & Study Monitor
Appendix 2 - Certificate of analysis of CIBENZA® PHYTAVERSE® L10 phytase enzyme used (3 batches)
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Appendix 1- *Curricula vitae* of Study Director & Study Monitor

Study Director:
Name: (b) (4), (b) (6)

(b) (4), (b) (6)

Study Monitor:
Name: Drew Lichtenstein
Qualifications: B.S. Biochemistry (Michigan State University 1982), PhD Biochemistry (University of Wisconsin-Madison 1990)
Present Position: Research Manager, Specialty Products, Novus International
Experience: Over 35 years research experience in biochemistry and cell biology; more than 8 years of experience in animal feed enzymes.

Appendix 2 - Certificate of analysis of CIBENZA® PHYTAVERSE® L10 phytase enzyme used (3 batches)

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Certificate of Analysis

CIBENZA® PHYTAVERSE® L10 Phytase Enzyme (Test Article VR006)

Lot number: CV002C2
Date of Manufacture: August 14, 2014

Specification	Specification Limit	Test Result	Method
Appearance	Amber to brown liquid	Pass	Visual
pH	5.0 - 5.2	(b) (4)	Untapped
Specific gravity (g/mL)	1.05 - 1.20		Pycnometer
Sediment (% v/v)	≤ 0.5		QC0232
Activity (U/g)	≥ 10,000		ISO 30024
Lead (mg/kg)	≤ 5		ICP-MS
Arsenic (mg/kg)	< 2		ICP-MS
Cadmium (mg/kg)	< 0.5		ICP-MS
Mercury (mg/kg)	< 0.5		ICP-MS
Total Plate Count (cfu/g)	≤ 50,000		FDA BAM
Total Coliform (MPN/g)	≤ 30		FDA BAM
E. coli (/25g)	Absent		FDA BAM
Salmonella (/25g)	Absent		FDA BAM
Yeast and Mold (CFU/g)	Run and Record		FDA BAM
Staphylococcus aureus (/g)	Absent		FDA BAM
Production Organism (CFU/g)	Absent		QC0214
Antibiotic Activity (Zone of Inhibition)	Absent		JECFA
Mycotoxin			
Aflatoxin B1	NMT 1.0 ppb		HPLC
Aflatoxin B2	NMT 1.0 ppb		HPLC
Aflatoxin G1	NMT 1.0 ppb		HPLC
Aflatoxin G2	NMT 1.0 ppb		HPLC
Fumonisin B1	NMT 0.1 ppm		LCMSMS
Fumonisin B2	NMT 0.1 ppm		LCMSMS
Fumonisin B3	NMT 0.1 ppm		LCMSMS
Ochratoxin A	NMT 2.0 ppb		HPLC
Deoxynivalenol	NMT 0.6 ppm		LCMSMS
Acetyldeoxynivalenol	NMT 0.8 ppm		LCMSMS
Fusarenon X	NMT 0.4 ppm		LCMSMS
Nivalenol	NMT 0.6 ppm		LCMSMS
T-2 Toxin	NMT 0.2 ppm		LCMSMS
HT-2 Toxin	NMT 0.2 ppm		LCMSMS
Neosolaniol	NMT 0.4 ppm		LCMSMS
Diacetoxyscirpenol	NMT 0.4 ppm		LCMSMS
Zearalenone	NMT 43.1 ppb		HPLC
Sterigmatocystin	NMT 200 ppb		TLC

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(b) (4)

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Certificate of Analysis

CIBENZA® PHYTAVERSE® L10 Phytase Enzyme (Test Article VR006)

Lot number: 190CV005A3
Date of Manufacture: August 11, 2014

Specification	Specification Limit	Test Result	Method
Appearance	Amber to brown liquid	(b) (4)	Visual
pH	5.0 - 5.2		Untapped
Specific gravity (g/mL)	1.05 - 1.20		Pycnometer
Sediment (% v/v)	≤ 0.5		QC0232
Activity (U/g)	≥ 10,000		ISO 30024
Lead (mg/kg)	≤ 5		ICP-MS
Arsenic (mg/kg)	< 2		ICP-MS
Cadmium (mg/kg)	< 0.5		ICP-MS
Mercury (mg/kg)	< 0.5		ICP-MS
Total Plate Count (cfu/g)	≤ 50,000		FDA BAM
Total Coliform (cfu/g)	≤ 30		FDA BAM
E. coli (/25g)	Absent		FDA BAM
Salmonella (/25g)	Absent		FDA BAM
Yeast and Mold (MPN/g)	Run and Record		FDA BAM
Staphylococcus aureus (/g)	Absent		FDA BAM
Production Organism (CFU/g)	Absent		QC0214
Antibiotic Activity (Zone of Inhibition)	Absent		JECFA
Mycotoxin			
Aflatoxin B1	NMT 1.0 ppb		HPLC
Aflatoxin B2	NMT 1.0 ppb		HPLC
Aflatoxin G1	NMT 1.0 ppb		HPLC
Aflatoxin G2	NMT 1.0 ppb		HPLC
Fumonisin B1	NMT 0.1 ppm		LCMSMS
Fumonisin B2	NMT 0.1 ppm		LCMSMS
Fumonisin B3	NMT 0.1 ppm		LCMSMS
Ochratoxin A	NMT 2.0 ppb		HPLC
Deoxynivalenol	NMT 0.6 ppm		LCMSMS
Acetyldeoxynivalenol	NMT 0.8 ppm		LCMSMS
Fusarenon X	NMT 0.4 ppm		LCMSMS
Nivalenol	NMT 0.6 ppm		LCMSMS
T-2 Toxin	NMT 0.2 ppm		LCMSMS
HT-2 Toxin	NMT 0.2 ppm		LCMSMS
Neosolaniol	NMT 0.4 ppm		LCMSMS
Diacetoxyscirpenol	NMT 0.4 ppm		LCMSMS
Zearalenone	NMT 43.1 ppb		HPLC
Sterigmatocystin	NMT 200 ppb		TLC

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Certificate of Analysis

CIBENZA® PHYTAVERSE® L10 Phytase Enzyme (Test Article VR006)

Lot number: PHY-50104-PO030-F4
Date of Manufacture: September 11, 2015

Specification	Specification Limit	Test Result (b) (4)	Method
Appearance	Amber to brown liquid		Visual
pH	5.0 - 5.2		Untapped
Specific gravity (g/mL)	1.05 - 1.20		Pycnometer
Sediment (% v/v)	≤ 0.5		QC0232
Activity (U/g)	≥ 10,000		ISO 30024
Lead (mg/kg)	≤ 5		ICP-MS
Arsenic (mg/kg)	< 2		ICP-MS
Cadmium (mg/kg)	< 0.5		ICP-MS
Mercury (mg/kg)	< 0.5		ICP-MS
Total Plate Count (cfu/g)	≤ 50,000		FDA BAM
Total Coliform (MPN/g)	≤ 30		FDA BAM
E. coli (/25g)	Absent		FDA BAM
Salmonella (/25g)	Absent		FDA BAM
Yeast and Mold (CFU/g)	Run and Record		FDA BAM
Staphylococcus aureus (/g)	Absent		FDA BAM
Production Organism (CFU/g)	Absent		QC0214
Antibiotic Activity (Zone of Inhibition)	Absent		JECFA
Mycotoxin			
Aflatoxin B1	NMT 1.0 ppb		HPLC
Aflatoxin B2	NMT 1.0 ppb		HPLC
Aflatoxin G1	NMT 1.0 ppb		HPLC
Aflatoxin G2	NMT 1.0 ppb		HPLC
Fumonisin B1	NMT 0.1 ppm		LCMSMS
Fumonisin B2	NMT 0.1 ppm		LCMSMS
Fumonisin B3	NMT 0.1 ppm		LCMSMS
Ochratoxin A	NMT 2.0 ppb		HPLC
Deoxynivalenol	NMT 0.6 ppm		LCMSMS
Acetyldeoxynivalenol	NMT 0.8 ppm		LCMSMS
Fusarenon X	NMT 0.4 ppm		LCMSMS
Nivalenol	NMT 0.6 ppm		LCMSMS
T-2 Toxin	NMT 0.2 ppm		LCMSMS
HT-2 Toxin	NMT 0.2 ppm		LCMSMS
Neosolaniol	NMT 0.4 ppm		LCMSMS
Diacetoxyscirpenol	NMT 0.4 ppm		LCMSMS
Zearalenone	NMT 43.1 ppb		HPLC
Sterigmatocystin	NMT 200 ppb		TLC

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Certificate of Analysis

PCBs	**10,000 pg/g**	(b) (4)	**GC/HRMS**
Dioxins	**1pg/g**		**GC/HRMS**

* Results of retesting performed in May 2017
[1] The limits of detection (LOD) for each of the assays and methods match that of the stated less than (<) values above.

Approved by: _____ Date: June 7, 2017

Mark Burcin
Sr. Manager, QA/QC

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Appendix 3 - Relevant laboratory reports

CERTIFICATE OF ANALYSIS

Company:	Novus International Ltd and BASF Enzymes LLC
Type of sample:	F599 feeds
Laboratory ref. :	172041 to 172046 172126 to 172135 172140 to 172149 172157 to 172166
Reception date:	28th November 2017
Analysis starting date:	7th December 2017
Analysis finishing date:	12th December 2017

Sample description:
See Results section

Analysis performed:

- Moisture -dry matter- by oven drying –method 2 (SOP 0602-L-10001) (AOAC, 2000)
- Nitrogen -crude protein- by combustion -Dumas method (SOP 0602-L-10118) (AOAC, 2000)
- Ether extract on a Soxtec system -method 3B (SOP 0602-L-10003) (AOAC, 2000)
- Ash after muffle furnace incineration -method 12 (SOP 0602-L-10002) (AOAC, 2000)
- Phytase (SOP 0602-L-10143; ISO 30024:2009. Animal feeding stuffs – Determination of phytase activity.)

Results:

LAB. REF.	SAMPLE DESCRIPTION	CRUDE PROTEIN (%)	ETHER EXTRACT (%)	ASH (%)
172041	A250 pellet	(b) (4)		
172042	A500 pellet			
172043	B250 pellet			
172044	B500 pellet			
172045	C250 pellet			
172046	C500 pellet			

LAB. REF.	DESCRIPTION	PHYTASE U/kg	DM %
172126	A250 PELLET HOM 1	(b) (4)	
172127	A250 PELLET HOM 2		
172128	A250 PELLET HOM 3		
172129	A250 PELLET HOM 4		
172130	A250 PELLET HOM 5		
172131	A250 PELLET HOM 6		
172132	A250 PELLET HOM 7		
172133	A250 PELLET HOM 8		
172134	A250 PELLET HOM 9		
172135	A250 PELLET HOM 10		

LAB. REF.	DESCRIPTION	PHYTASE U/kg	DM %
172140	B250 PELLET HOM 1	(b) (4)	
172141	B250 PELLET HOM 2		
172142	B250 PELLET HOM 3		
172143	B250 PELLET HOM 4		
172144	B250 PELLET HOM 5		
172145	B250 PELLET HOM 6		
172146	B250 PELLET HOM 7		
172147	B250 PELLET HOM 8		
172148	B250 PELLET HOM 9		
172149	B250 PELLET HOM 10		

LAB. REF.	DESCRIPTION	PHYTASE U/kg	DM %
172157	C250 PELLET HOM 1	(b) (4)	
172158	C250 PELLET HOM 2		
172159	C250 PELLET HOM 3		
172160	C250 PELLET HOM 4		
172161	C250 PELLET HOM 5		
172162	C250 PELLET HOM 6		
172163	C250 PELLET HOM 7		
172164	C250 PELLET HOM 8		
172165	C250 PELLET HOM 9		
172166	C250 PELLET HOM 10		

(b) (4), (b) (6)

(b) (4), (b) (6)

Signature:

Date: 21 MARCH 2018

Appendix 4 - Raw data

Obs	enzyme	form	homogeneity	Trt	lab_ref	dose	Tr	location	U_kg_as_is	DM_p	U_kg_88_p_DM
1	A	pellet	yes	A250pellet	172126	250	A250	1	259	(b) (4)	
2	A	pellet	yes	A250pellet	172127	250	A250	2	245		
3	A	pellet	yes	A250pellet	172128	250	A250	3	279		
4	A	pellet	yes	A250pellet	172129	250	A250	4	267		
5	A	pellet	yes	A250pellet	172130	250	A250	5	271		
6	A	pellet	yes	A250pellet	172131	250	A250	6	265		
7	A	pellet	yes	A250pellet	172132	250	A250	7	299		
8	A	pellet	yes	A250pellet	172133	250	A250	8	238		
9	A	pellet	yes	A250pellet	172134	250	A250	9	253		
10	A	pellet	yes	A250pellet	172135	250	A250	10	265		
11	B	pellet	yes	B250pellet	172140	250	B250	1	282		
12	B	pellet	yes	B250pellet	172141	250	B250	2	252		
13	B	pellet	yes	B250pellet	172142	250	B250	3	267		
14	B	pellet	yes	B250pellet	172143	250	B250	4	291		
15	B	pellet	yes	B250pellet	172144	250	B250	5	301		
16	B	pellet	yes	B250pellet	172145	250	B250	6	267		
17	B	pellet	yes	B250pellet	172146	250	B250	7	301		
18	B	pellet	yes	B250pellet	172147	250	B250	8	290		
19	B	pellet	yes	B250pellet	172148	250	B250	9	253		
20	B	pellet	yes	B250pellet	172149	250	B250	10	270		
21	C	pellet	yes	C250pellet	172157	250	C250	1	223		
22	C	pellet	yes	C250pellet	172158	250	C250	2	268		
23	C	pellet	yes	C250pellet	172159	250	C250	3	304		
24	C	pellet	yes	C250pellet	172160	250	C250	4	272		
25	C	pellet	yes	C250pellet	172161	250	C250	5	317		
26	C	pellet	yes	C250pellet	172162	250	C250	6	254		
27	C	pellet	yes	C250pellet	172163	250	C250	7	320		
28	C	pellet	yes	C250pellet	172164	250	C250	8	307		
29	C	pellet	yes	C250pellet	172165	250	C250	9	287		
30	C	pellet	yes	C250pellet	172166	250	C250	10	288		

Appendix 5 - Statistical printouts

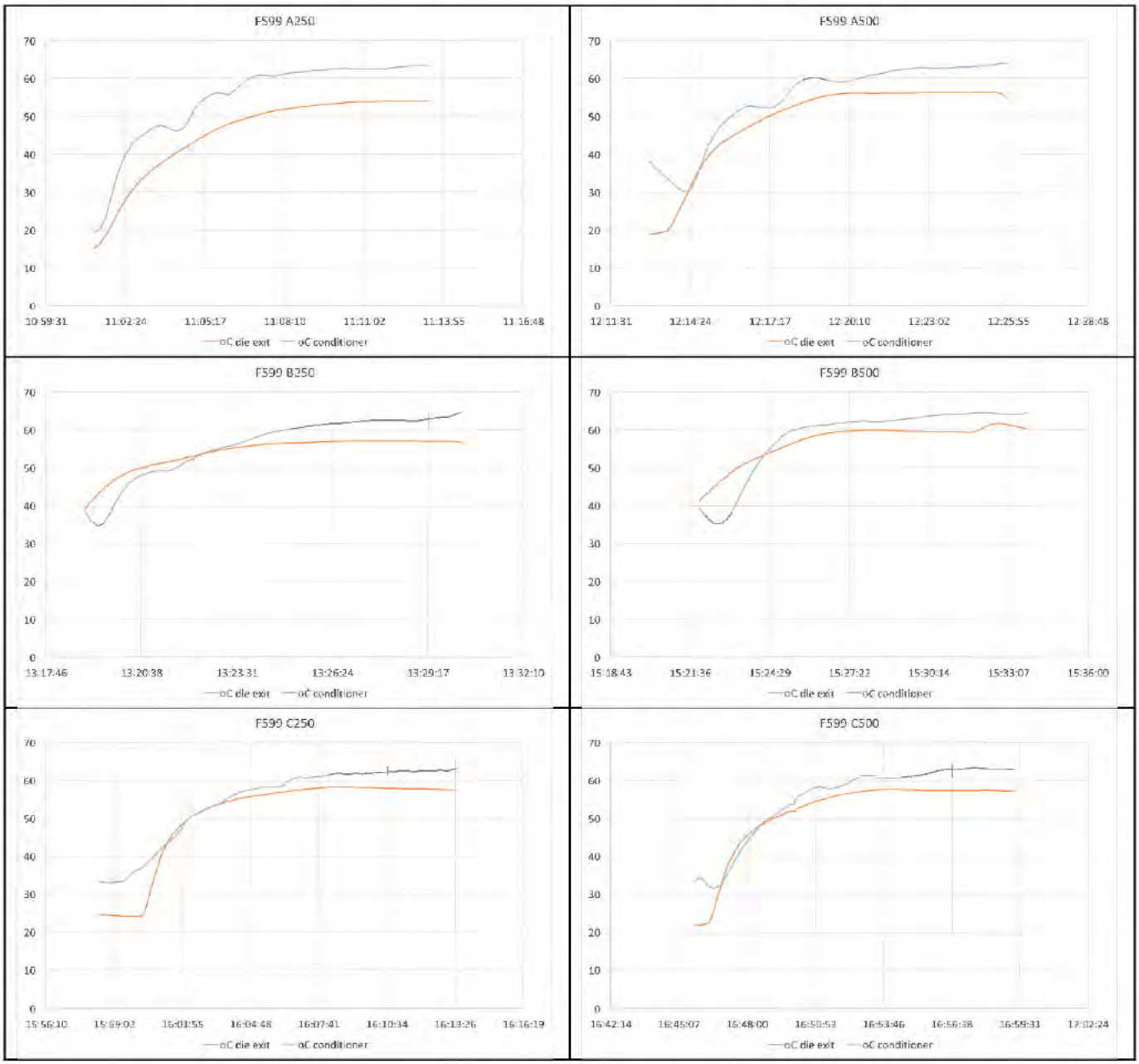
(b) (4) Trial F599 15:34 Friday, January 12, 2018 3

Obs	enzyme	form	homogeneity	Trt	lab_ref	dose	Tr	location	U_kg_as_is	DM_p	U_kg_88_p_DM
1	A	pellet	yes	A250pellet	172126	250	A250	1	259	(b) (4)	(b) (4)
2	A	pellet	yes	A250pellet	172127	250	A250	2	245		
3	A	pellet	yes	A250pellet	172128	250	A250	3	279		
4	A	pellet	yes	A250pellet	172129	250	A250	4	267		
5	A	pellet	yes	A250pellet	172130	250	A250	5	271		
6	A	pellet	yes	A250pellet	172131	250	A250	6	265		
7	A	pellet	yes	A250pellet	172132	250	A250	7	299		
8	A	pellet	yes	A250pellet	172133	250	A250	8	238		
9	A	pellet	yes	A250pellet	172134	250	A250	9	253		
10	A	pellet	yes	A250pellet	172135	250	A250	10	265		
11	B	pellet	yes	B250pellet	172140	250	B250	1	282		
12	B	pellet	yes	B250pellet	172141	250	B250	2	252		
13	B	pellet	yes	B250pellet	172142	250	B250	3	267		
14	B	pellet	yes	B250pellet	172143	250	B250	4	291		
15	B	pellet	yes	B250pellet	172144	250	B250	5	301		
16	B	pellet	yes	B250pellet	172145	250	B250	6	267		
17	B	pellet	yes	B250pellet	172146	250	B250	7	301		
18	B	pellet	yes	B250pellet	172147	250	B250	8	290		
19	B	pellet	yes	B250pellet	172148	250	B250	9	253		
20	B	pellet	yes	B250pellet	172149	250	B250	10	270		
21	C	pellet	yes	C250pellet	172157	250	C250	1	223		
22	C	pellet	yes	C250pellet	172158	250	C250	2	268		
23	C	pellet	yes	C250pellet	172159	250	C250	3	304		
24	C	pellet	yes	C250pellet	172160	250	C250	4	272		
25	C	pellet	yes	C250pellet	172161	250	C250	5	317		
26	C	pellet	yes	C250pellet	172162	250	C250	6	254		
27	C	pellet	yes	C250pellet	172163	250	C250	7	320		
28	C	pellet	yes	C250pellet	172164	250	C250	8	307		
29	C	pellet	yes	C250pellet	172165	250	C250	9	287		
30	C	pellet	yes	C250pellet	172166	250	C250	10	288		

		U_kg_as_is						U_kg_88_p_DM					
		N	Mean	CV	StdDev	Max	Min	N	Mean	CV	StdDev	Max	Min
Tr	form			(b)(4)						(b)(4)			
A250	pellet	10	264			299	238	10	268			303	241
B250	pellet	10	277			301	252	10	280			305	254
C250	pellet	10	284			320	223	10	286			323	225

		U_kg_as_is						DM_p					
		N	Mean	CV	StdDev	Max	Min	N	Mean	CV	StdDev	Max	Min
Tr	form			(b)(4)						(b)(4)			
A250	pellet	10	264			299	238	10	86.8			86.9	86.7
B250	pellet	10	277			301	252	10	87.2			87.5	86.8
C250	pellet	10	284			320	223	10	87.2			87.4	87.0
All		30	275			320	223	30	87.1			87.5	86.7

Appendix 6 – Temperature profile in the conditioner during pelleting



Appendix 19: Stability Evaluation of CIBENZA® PHYTAVERSE® G10 Phytase Enzyme in Feed

(b) (4)

Stability evaluation of CIBENZA® PHYTAVERSE® G10 phytase enzyme in feed

Unique Study Code: F597

FINAL REPORT
Date: 2nd June 2018

Study sponsor: Novus International Inc. and BASF Enzymes LLC.

Signed by Study Director, Study Sponsor and Study Monitor:

(b) (4)			
	June 11/2018	11 June 2018	11, June 2018
Study Director	Study Sponsors		Study Monitor
(b) (4)	Gavin Bowman Director, Global Regulatory Affairs Novus International 20 Research Park Dr., St. Charles, MO 63304, United States of America	Roxanna Van Dorn Senior Regulatory Affairs Specialist BASF Enzymes LLC 3550 John Hopkins Court, San Diego, CA 92121, United States of America	Drew Lichtenstein Research Manager, Specialty Products Novus International 20 Research Park Dr., St. Charles, MO 63304, United States of America

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1 Summary

The objective of this study was to evaluate the stability of CIBENZA® PHYTAVERSE® G10 phytase enzyme in feeds.

For each batch, dose and form, the stability of the test article was determined by measuring phytase activity in unique feed samples after 0, 1, 2 and 3-months storage at ambient conditions.

Results are presented next in Summary Table 1.

Summary Table 1. Stability of CIBENZA® PHYTAVERSE® G10 phytase enzyme in feeds

		N	Phytase U/kg as is	DM %	Phytase U/kg 88% DM	Phytase % 0 month as is	Phytase % 0 month 88%DM
Tr form	month						
A250mash	0	1	319	87.3	322	100.0	100.0
	1	1	340	87.2	(b) (4)		
	2	1	343	87.7			
	3	1	312	86.6			
A250pellet	0	2	299	87.2	302	100.0	100.0
	1	1	271	87.3	(b) (4)		
	2	2	223	87.7			
	3	2	250	87.7			
A500mash	0	1	624	87.5	628	100.0	100.0
	1	1	597	87.4	(b) (4)		
	2	1	637	87.8			
	3	1	657	87.5			
A500pellet	0	1	491	87.2	496	100.0	100.0
	1	1	491	87.2	(b) (4)		
	2	1	438	87.5			
	3	1	532	87.5			
B250mash	0	2	329	87.3	331	100.0	100.0
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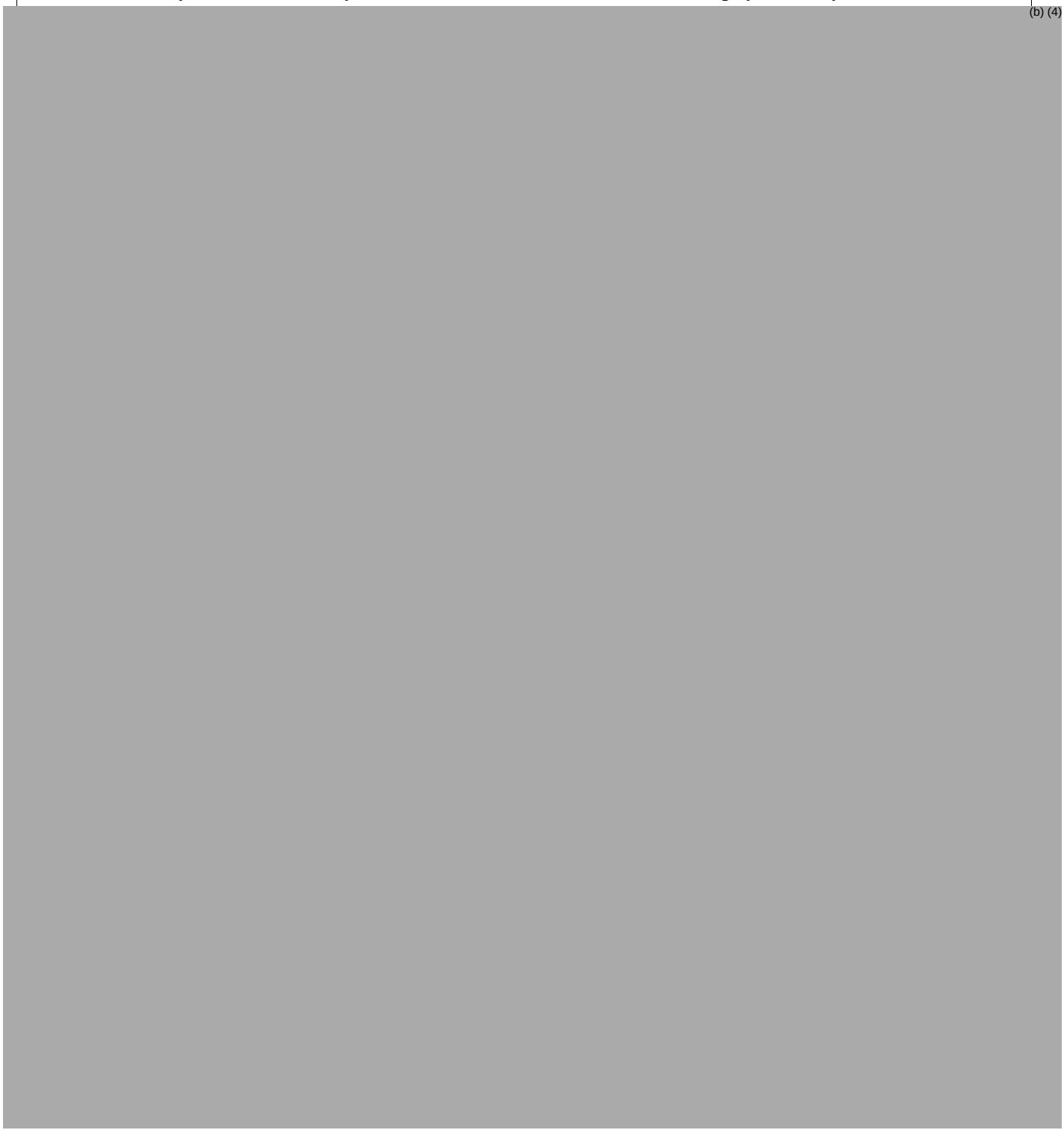
	1	1	224	87.3	(b) (4)		
	2	2	219	87.4			
	3	1	269	87.3			
B500mash	0	1	638	87.3	643	100.0	100.0
	1	1	554	87.3	(b) (4)		
	2	1	566	87.6			
	3	1	624	87.5			
B500pellet	0	1	489	87.1	494	100.0	100.0
	1	1	409	87.1	(b) (4)		
	2	1	444	87.5			
	3	1	469	87.3			
C250mash	0	1	308	87.7	309	100.0	100.0
	1	1	319	87.3	(b) (4)		
	2	1	305	87.6			
	3	1	340	87.7			
C250pellet	0	1	232	86.7	235	100.0	100.0
	1	1	294	86.8	(b) (4)		
	2	1	193	87.0			
	3	1	280	87.0			
C500mash	0	1	541	87.5	544	100.0	100.0
	1	1	556	87.7	(b) (4)		
	2	1	536	87.8			
	3	1	444	87.7			
C500pellet	0	1	455	86.9	461	100.0	100.0
	1	1	465	87.0	(b) (4)		
	2	1	377	87.2			
	3	1	490	87.1			

Summary Table 1. Stability of CIBENZA® PHYTAVERSE® G10 phytase enzyme in feeds

(b) (4)



According to the results of the present stability study in feeds, CIBENZA® PHYTAVERSE® G10 phytase enzyme:

- Was stable over time (1, 2 and 3-months storage at ambient conditions) for all three batches (A & B & C), for both feed forms (mash & pellet) and at both concentrations tested (250 & 500 U/kg) as demonstrated by the slope of linear regressions of phytase activity over time not being significantly different from 0 (flat line, no significant loss of activity).
- Presented good stability (in general ±10% of 0-month value) up to 3-months in pelleted feeds for all three batches (A & B & C), for both feed forms (mash & pellet) and at both concentrations tested (250 & 500 U/kg). Exceptions at three months were: A250 pellet (84%), B250 mash (78%) and C500 mash (82%) on the lower side, and B250 pellet (114%) and C250 pellet (120%) on the upper side. The variation in activity at 3-months was considered to be within the range of expected values, especially considering the other dose/form for the same batches of enzyme did not differ from their respective T=0 activity by more than 14% (A500 pellet [108%] and A250 mash [98%] as references for A250 pellet, B500 mash [98%] and B250 pellet [114%] as references for B250 mash, and C250 mash [110%] and C500 pellet [108%] as references for C500 mash).

2 Quality statement

The study, Stability evaluation of CIBENZA® PHYTAVERSE® G10 phytase enzyme in feed (Unique Study Code: F597), was conducted in compliance with current quality standards and regulatory requirements as applicable for US animal food requirements.

Procedures, documentation, equipment and records were examined in order to assure that the study was performed in accordance with the regulations specified herein and with the protocol and relevant Standard Operating Procedures.

Signed and dated:

(b) (4), (b) (6)			
2nd June 2018	June 11/2018	11 June 2018	11 June 2018
Study Director	Study Sponsors		Study Monitor
(b) (4)	Gavin Bowman Director, Global Regulatory Affairs Novus International 20 Research Park Dr., St. Charles, MO 63304, United States of America	Roxanna Van Dorn Senior Regulatory Affairs Specialist BASF Enzymes LLC 3550 John Hopkins Court, San Diego, CA 92121, United States of America	Drew Lichtenstein Research Manager, Specialty Products Novus International 20 Research Park Dr., St. Charles, MO 63304, United States of America

3 Study title and unique study code

Stability evaluation of CIBENZA® PHYTAVERSE® G10 phytase enzyme in feed.

Unique study code: F597

4 Study objective

To evaluate the Stability of three batches of CIBENZA® PHYTAVERSE® G10 phytase enzyme in mash and pelleted feeds.

5 Study location

(b) (4)

6 Important dates & duration of the study

Date of feeds manufacture: 23rd and 24th November 2017
Duration of study: 2 days at feed mill, 14th March 2018 end of analysis

7 Test products

Table 1. Details of test product

Code	Product	Provider	Lot nº Manufacture Date	Active substance	Activity (U/g)†	
					Guaranteed	Analysed
A	CIBENZA® PHYTAVERSE® G10 Phytase Enzyme	Novus International, Inc.	Lot: P23941 Made: 08 October 2014	6-phytase	10,000	13,951
B	CIBENZA® PHYTAVERSE® G10 Phytase Enzyme	Novus International, Inc.	Lot: P26641 Made: 08 October 2014	6-phytase	10,000	13,742
C	CIBENZA® PHYTAVERSE® G10 Phytase Enzyme	Novus International, Inc.	Lot: RO15271001 Made: 28 September 2015	6-phytase	10,000	13,522

† One phytase unit is the amount of enzyme that releases 1 µmol of inorganic phosphate from phytate per minute under the conditions of the assay (Phytate concentration: 5.0 mM, pH: 5.5, Temperature: 37°C, Incubation time: 30 min) (ISO 30024:2009). Normal analytical variation for the method is 10%.

8 Key study personnel

Study Director: (b) (4), (b) (6)
(b) (4)

External Study Monitor: Drew Lichtenstein, Ph.D. Research Manager, Specialty Products, Novus International, Inc., 20 Research Park Dr., St. Charles, MO 63304, United States of America, Tel: +1 314 453-7793, E-mail: drew.lichtenstein@novusint.com

Study Sponsors: 1) Gavin Bowman, Director, Global Regulatory Affairs, Novus International, 20 Research Park Dr., St. Charles, MO 63304, United States of America Tel: +1 636 926 7402, E-mail: gavin.bowman@novusint.com

2) Roxanna Van Dorn, Sr. Regulatory Affairs Specialist, Phone: +1 858 431-8590, Mobile: +1-858-349-7339, Fax: +1-973-307-2549, E-mail: roxanna.vandorn@basf.com. Postal Address: BASF Enzymes LLC, 3550 John Hopkins Court, San Diego, CA 92121 United States

Feed mill & supervision of diet manufacture: (b) (4), (b) (6)

Feed analysis (DM and CIBENZA® PHYTAVERSE® G10 phytase enzyme): (b) (4), (b) (6)

Optional/back-up facility for feed analysis (DM and CIBENZA® PHYTAVERSE® G10 phytase enzyme): Drew Lichtenstein, Novus International, Inc., 20 Research Park Drive, Saint Charles, MO, 63304; United States of America.

9 Material and methods

9.1 Experimental treatments

Number of treated and control groups: Corn/soya based diet was used for stability purposes.

CIBENZA® PHYTAVERSE® G10 phytase enzyme from each batch was added in serial mixing steps to the mash feed to provide 250 and 500 U/kg feed as detailed in Table 2, that was later pelleted.

Table 2. Experimental Treatments

Treatment	Product	CIBENZA® PHYTAVERSE® G10 phytase enzyme		
		U/kg feed	mg/kg feed†	g to add to 200 kg feed†
A250	CIBENZA® PHYTAVERSE® G10 phytase enzyme batch P23941	250	(b) (4)	
A500		500		
B250	CIBENZA® PHYTAVERSE® G10 phytase enzyme batch P26641	250		
B500		500		
C250	CIBENZA® PHYTAVERSE® G10 phytase enzyme batch RO15271001	250		
C500		500		

† inclusion based on actual activity of each batch

9.2 Treatment application

CIBENZA® PHYTAVERSE® G10 phytase enzyme was mixed with a fraction of 10 kg soya in serial mixing steps, mash feed was then produced and later pelleted.

9.3 Detailed study design

Figure 1. Basic study design
For each batch and dose of enzyme: The stability of the test article in mash and pelleted feeds was determined by measuring phytase activity of composite samples obtained at the time of feed manufacturing and after storage at ambient conditions for the following periods and for each batch of enzyme: • 0 months • 1 months • 2 months • 3 months The amount of endogenous phytase in blank feed has been determined in previous studies being values below the level of quantitation and therefore was not determined in this study. Feeds were produced as follows: • Firstly, a fraction of 10 kg soya from the feed was mixed in serial mixing steps with the corresponding amount of CIBENZA® PHYTAVERSE® G10 phytase enzyme depending on actual activity of each batch as detailed in Table 2. • Secondly, a 200 kg batch of mash feed was produced by including the 10 kg soya containing CIBENZA® PHYTAVERSE® G10 phytase enzyme prepared as described above. • Mash feed was then pelleted and bagged.

9.4 Feed composition

Feeds did not contain any enzymes, antibiotics or any other growth promoters. Feed for fattening turkeys during the Grower phase was used as a matrix. The ingredients, premix and the calculated and actual analyses of the diets are presented in Table 3 to Table 5.

Table 3. Composition (g/kg) of the basal diet		
Corn		(b) (4)
Soybean meal 48%		
Fat blend		
Dicalcium phosphate		
Calcium carbonate		
Methionine Hydroxy Analogue		
Premix Min-Vit		
Sodium chloride		
L-lysine HCL		
L-threonine		

Table 4. Composition of vitamin-mineral premix			
	Units	per kg of vitamin-mineral premix	when premix added at 10 kg/ton feed, results in the following values per kg of feed
Vitamins, provitamins and similar			
vitamin A (3a672)	IU	1 000 000	(b) (4)
vitamin D_3 (E-671)	IU	350 000	
vitamin E (alfa-tocopherol, 3a700)	mg	3 000	
vitamin B_1 (thiamine mononitrate 3a821)	mg	210	
vitamin B_2 3,000 mg	mg	855	
vitamin B_6 (3a831)	mg	470	
vitamin B_{12} 13 mg	mg	5	
vitamin K_3 (menadione sodium bisulphate, 3a710)	mg	300	
vitamin C	mg	2 000	
calcium pantothenate (3a841)	mg	1 520	
nicotinic acid (3a314)	mg	6 710	
folic acid (3a316)	mg	150	
biotin (3a880)	mg	25	
choline chloride	mg	70 000	
Oligoelements			
Fe (E-1) (from $FeSO_4 \cdot H_2O$)	mg	6 500	
I (3b202) (from $Ca(IO3)_2$)	mg	150	
Cu (E-4) (from $CuSO_4 \cdot 5H_2O$)	mg	1 500	
Mn (E-5) (from MnO)	mg	8 000	
Zn (3b603) (from ZnO)	mg	8 500	
Se (E-8) (from Na_2SeO_3)	mg	20	
Amino acids			
L-lysine monochlorhydrate	g	50	
DL-methionine	g	150	
Other			
ethoxyquin (E324)	mg	5 000	
Mg oxide, Na bicarbonate, Na chloride, Ca carbonate		up to 1 kg	

Table 5. Calculated analyses of the basal diet (g/kg)	
Metabolizable Energy kcal/kg	2864
Dry Matter	868
Ash	58
Crude Fiber	27
Ether Extract	41
Crude Protein	227
Ca	9.6
P	5.0
Dig lysine	14.1
Dig SAA	9.4
Dig threonine	8.4

9.5 Feeds manufacture

All the process is automated and controlled by a computer provided with software from (b) (4) so that the incorporation of ingredients and the functioning of the equipment is regulated and recorded by the software. The addition of manual ingredients (vitamins, amino acids and oligo minerals, as well as test products) is made by means of a bar code system.

Feed ingredients were ground through a 40HP hammer mill (Rosal VRE-40) with a horizontal axis and a 3 mm sieve, provided with an automatic feeder.

The feed mixer was a 500 L Rosal mixer with a double horizontal ribbon, which is sufficient for 200 to 250 kg of feed. The amount of feed prepared was 200 kg per treatment. Fat was added by means of a dosing device provided by three nozzles (b) (4) The mixing time was 6 min. The calculated amount of product for each CIBENZA® PHYTAVERSE® G10 phytase enzyme batch and dose (Table 2) was manually premixed with the corresponding vitamin-mineral premix and amino acids and then added to 10 kg of soya in a commercial mixer and mixed for 6 minutes. Then this premix was incorporated into the final mixture and mixed for 6 minutes.

Mash feed was then pelleted in a pelleting press (MABRIK PVR-40) provided with a die of 280 mm of internal diameter with holes of 3×36 mm. The compression group consists of 2 rollers. The feeder is of stainless steel of progressive opening and is moved by a reducing engine. The conditioner is of stainless steel with adjustable blades, prepared for the reception of water and steam. The steam generator has a manometer to reduce the pressure to 2.5-3 kg/cm^2 and a flux regulator valve. Pelleting is automatically regulated by the software of the system which adjusts the temperature of the mash feed at the end of the conditioner (approximately 30 to 40 seconds of conditioning time). The pelletization temperature was adjusted to a mean temperature of 65ºC and a maximum temperature of up to 66.2ºC. Temperatures were recorded at fixed intervals (i.e. 10 seconds) in the outlet of the conditioner and outlet of die. The vertical cooler (MABRIK, S.A) works by air aspiration provided by a 7.5 HP turbine.

9.5.1 Short description of the process

Under general and corporative (b) (4) (b) (4) .

9.6 Feeds samples at manufacture

For each CIBENZA® PHYTAVERSE® G10 phytase enzyme batch and dose:

- 10 grab samples of mash feed (~1.1 kg each) were taken from several points of the mixer.
- A portion of these grab mash feed samples was combined and homogenized and then:
  - Triplicate (NOVUS, (b) (4) backup) (b) (4) at each time point one sample was sent to NOVUS, a second one analyzed for phytase activity at (b) (4) lab, while the third sample was retained at (b) (4) at -20°C as a backup sample).
- 10 grab samples of pelleted feed (~1.1 kg each) were taken at bagging.
- A portion of these grab pelleted feed samples was combined and homogenized and then:
  - Triplicate (NOVUS, (b) (4) backup) (b) (4) at each time point one sample was sent to NOVUS, a second one analyzed for phytase activity at (b) (4)'s lab, while the third sample was retained at (b) (4) at -20°C as a backup sample; all 0 month (A250, B250, C250, A500, B500, & C500) pelleted feeds were subjected to proximate analysis).

Stability samples were labelled with the unique study code (F597), treatment code (A250 / A500 / B250 / B500 / C250 / C500), feed form (mash / pellet), date of manufacture and the analysis required (DM, phytase activity, proximate).

9.7 Feed sampling plan

Table 6. Sampling plan

Treatment	Feed form	Month storage	Analysis	Final Samples		
				NOVUS	(b) (4) lab	(b) (4) backup
A250	MASH	10x1.1kg samples: A portion of each of 10 samples combined and homogenized then split for stability: 3x4x250 g				
		0	stability	1 × 250g	1 × 250g	1 × 250g
		1	stability	(b) (4)		
		2	stability			
		3	stability			
	PELLET	10x1.1kg samples: A portion of each of 10 samples combined and homogenized then split for stability: 3x4x250 g				
		0	stability & proximate	1 × 250g	1 × 250g	1 × 250g
		1	stability	(b) (4)		
		2	stability			
		3	stability			
A500	MASH	10x1.1kg samples: A portion of each of 10 samples combined and homogenized then split for stability: 3x4x250 g				
		0	stability	1 × 250g	1 × 250g	1 × 250g
		1	stability	(b) (4)		
		2	stability			
		3	stability			
	PELLET	10x1.1kg samples: A portion of each of 10 samples combined and homogenized then split for stability: 3x4x250 g				
		0	stability & proximate	1 × 250g	1 × 250g	1 × 250g
		1	stability	(b) (4)		
		2	stability			
		3	stability			
B250	MASH	10x1.1kg samples: A portion of each of 10 samples combined and homogenized then split for stability: 3x4x250 g				
		0	stability	1 × 250g	1 × 250g	1 × 250g
		1	stability	(b) (4)		
		2	stability			
		3	stability			
	PELLET	10x1.1kg samples: A portion of each of 10 samples combined and homogenized then split for stability: 3x4x250 g				
		0	stability & proximate	1 × 250g	1 × 250g	1 × 250g
		1	stability	(b) (4)		
		2	stability			
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Table 6. Sampling plan						
Treatment	Feed form	Month storage	Analysis	Final Samples		
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B500	MASH	10x1.1kg samples: A portion of each of 10 samples combined and homogenized then split for stability: 3x4x250 g				
		0	stability	1 × 250g	1 × 250g	1 × 250g
		1	stability	(b) (4)		
		2	stability			
		3	stability			
	PELLET	10x1.1kg samples: A portion of each of 10 samples combined and homogenized then split for stability: 3x4x250 g				
		0	stability & proximate	1 × 250g	1 × 250g	1 × 250g
		1	stability	(b) (4)		
		2	stability			
		3	stability			
C250	MASH	10x1.1kg samples: A portion of each of 10 samples combined and homogenized then split for stability: 3x4x250 g				
		0	stability	1 × 250g	1 × 250g	1 × 250g
		1	stability	(b) (4)		
		2	stability			
		3	stability			
	PELLET	10x1.1kg samples: A portion of each of 10 samples combined and homogenized then split for stability: 3x4x250 g				
		0	Stability & proximate	1 × 250g	1 × 250g	1 × 250g
		1	stability	(b) (4)		
		2	stability			
		3	stability			
C500	MASH	10x1.1kg samples: A portion of each of 10 samples combined and homogenized then split for stability samples				
		0	stability	1 × 250g	1 × 250g	1 × 250g
		1	stability	(b) (4)		
		2	stability			
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	PELLET	10x1.1kg samples: A portion of each of 10 samples combined and homogenized then split for stability samples				
		0	stability & proximate	1 × 250g	1 × 250g	1 × 250g
		1	stability	(b) (4)		
		2	stability			
		3	stability			

For stability analysis, A250, B250, C250, A500, B500 and C500 0-month stability samples were analysed in (b) (4) lab within 10 working days after production of the feeds containing CIBENZA® PHYTAVERSE® G10 phytase enzyme. The initial samples to be tested at time zero were refrigerated (4°C) until analysed to make sure they reflected the activity values at time zero. All other samples were kept together at (b) (4) in a cardboard box (temperature and humidity monitored) protected from light and at room temperature. Samples were dispatched to NOVUS-Reus for backup, and (b) (4) lab for analysis or (b) (4) storage after the corresponding time (1, 2 or 3-months). When phytase analysis results presented unexpected values, the back-up samples were also analysed; this was the case for the following samples: A250 pellet & B250 mash both at 0, 2 & 3-months, and B250 pellet at 2 months (average values of original and back-up samples were taken into account).

9.8 Statistics

For each CIBENZA® PHYTAVERSE® G10 phytase enzyme batch, dose and feed form:

- The data was fitted to least squares regression, with the upper and lower 95% confidence limits shown. The regression line of CIBENZA® PHYTAVERSE® G10 phytase enzyme activity *vs.* time was calculated and the slope tested to be significantly different from 0.

10 Results

The results are summarized in Table 7 to Table 9. Values from proximate analysis were within expected ranges.

Table 7. Analyzed values of experimental diets

Sample	Dry matter (%)	Crude protein (%)	Ether extract (%)	Ash (%)
A250 pellet	87.2	22.8	4.1	5.5
A500 pellet	87.2	22.9	4.0	5.5
B250 pellet	87.2	23.0	4.0	5.5
B500 pellet	87.1	23.0	3.9	5.4
C250 pellet	86.7	23.2	3.8	5.4
C500 pellet	86.9	23.0	3.6	5.5

Table 8. Stability of CIBENZA® PHYTAVERSE® G10 phytase enzyme in feeds (actual & relative values)

		N	Phytase U/kg as is	DM %	Phytase U/kg 88% DM	Phytase % 0 month as is	Phytase % 0 month 88%DM
Tr_form	month						
A250mash	0	1	319	87.3	322	100.0	100.0
	1	1	340	87.2	(b) (4)		
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Table 8. Stability of CIBENZA® PHYTAVERSE® G10 phytase enzyme in feeds (actual & relative values)

		N	Phytase U/kg as is	DM %	Phytase U/kg 88% DM	Phytase % 0 month as is	Phytase % 0 month 88%DM
B500pellet	0	1	489	87.1	494	100.0	100.0
	1	1	(b) (4)				
	2	1					
	3	1					
C250mash	0	1	308	87.7	309	100.0	100.0
	1	1	(b) (4)				
	2	1					
	3	1					
C250pellet	0	1	232	86.7	235	100.0	100.0
	1	1	(b) (4)				
	2	1					
	3	1					
C500mash	0	1	541	87.5	544	100.0	100.0
	1	1	(b) (4)				
	2	1					
	3	1					
C500pellet	0	1	455	86.9	461	100.0	100.0
	1	1	(b) (4)				
	2	1					
	3	1					

† One phytase unit is the amount of enzyme that releases 1 µmol of inorganic phosphate from phytate per minute under the conditions of the assay (Phytate concentration: 5.0 mM, pH: 5.5, Temperature: 37ºC, Incubation time: 30 min) (ISO 30024:2009). Normal analytical variation for the method is 10%.

Table 9. Stability of CIBENZA® PHYTAVERSE® G10 phytase enzyme in feeds (regressions)

(b) (4)

Table 9. Stability of CIBENZA® PHYTAVERSE® G10 phytase enzyme in feeds (regressions)

11 Discussion

Dry matter was quite similar among samples (87.3%±0.7) and the correction for constant DM (88%) did not greatly change the results; DM did not vary over storage time.

All samples were analyzed in duplicate, and when phytase analysis results presented unexpected values, the back-up samples were also analyzed in duplicate. The back-up samples analyzed were: A250 pellet & B250 mash both at 0, 2 & 3-months, and B250 pellet at 2 months. Where backup samples were analyzed, the results are the average of the original and backup analyses. Except for B250 mash at 0-month, all back-up samples resulted in higher phytase activity than the original samples.

For Batch A, phytase results for A250 mash, A500 mash and A500 pellet over time were quite constant, with the phytase activity at the end of the 3-months storage period 98%, 105% and 108% respectively that of the initial activity. The slope of regression lines of phytase activity over time of storage for these three treatments were not significantly different from 0 (P=0.887, P=0.288 and P=0.790 respectively). For A250 pellet, though, the results varied over time, decreasing to 91% at 1-month, 75% at 2-months and "recovering" to 84% at 3-months; the slope of the regression line was not significantly different from 0 (P=0.213). As increasing the phytase activity over time is unrealistic, this variation is considered to be due to analytical artifacts more than real loss of activity, especially taking into account that A500 pellet and the A250 mash, which was used to produce the A250 pellets, retained 108% and 98%, respectively, of the initial activity at the end of the storage period.

For batch B, phytase results for B250 pellet, B500 mash and B500 pellet slightly varied over time, especially at the 500 U/kg dose. But the phytase activity at the end of storage period was 114%, 98% and 96% respectively that of the initial activity at 0-month. The slope of regression lines of phytase activity over time of storage were not significantly different from 0 (P=0.476, P=0.887 and P=0.883 respectively).

For B250 mash the variation over time was higher, decreasing to 86% at 1-month, 81% at 2-months and 77% at 3-months; the slope of the regression line was not significantly different from 0 (P=0.058). Moreover, taking into account that treatments B500 mash and B250 pellets produced from the B250 mash retained 98% and 114%, respectively, of the initial activity at the end of the storage period, the variations in B250 mash are considered to be the results of analytical variation more than real loss of phytase activity.

For batch C, C250 mash phytase activity was quite constant over time, being 110% at the end of the storage period, and the slope of the regression line over time was not significantly different from 0 (P=0.341). Results varied more for C250 pellet and C500 pellet. For C250 pellet, the 1- and 3-month time points were 127% and 121%, respectively, of the initial activity, while the 2-month time point was 83% of the initial activity. The C500 pellet varied from 102% at 1 month, to 83% at 2 months, to 108% at 3 months. Finally, C500 mash changed from 103% to 99% to 82% of the initial activity over the three months of the study. The slopes of the regression lines for these three treatments were not significantly different from 0 in all cases (P=0.200, P=0.967 and P=0.887). The variability among these three treatments are considered analytical artifacts rather than actual losses of activity. For the C500 mash treatment where 82% of the initial activity remained after 3 months storage, the results are considered to be due more to analytical variation than real loss of activity because the C250 mash (110% retained at 3 months) and C500 pellets (108% retained at 3 months), which was produced from the C500 mash, were both within 10% of their initial phytase activity.

12 Conclusions

According to the results of the present stability study in feeds, CIBENZA® PHYTAVERSE® G10 phytase enzyme:

- Was stable over time (1, 2 and 3-months storage at ambient conditions) for all three batches (A & B & C), at both feed forms (mash & pellet) and at both concentrations tested (250 & 500 U/kg) as demonstrated by slope of linear regressions of phytase activity over time not being significantly different from 0 (flat line, no significant loss of activity).
- Presented good stability (in general ±10% of 0-month value) up to 3-months in pelleted feeds for all three batches (A & B & C), for both feed forms (mash & pellet) and at both concentrations tested (250 & 500 U/kg). Exceptions at three months were: A250 pellet (84%), B250 mash (78%) and C500 mash (82%) on the lower side, and B250 pellet (114%) and C250 pellet (120%) on the upper side. The variation in activity at 3-months was considered to be within the range of expected values, especially considering the other dose/form for the same batches of enzyme did not differ from their respective T=0 activity by more than 14% (A500 pellet [108%] and A250 mash [98%] as references for A250 pellet, B500 mash [98%] and B250 pellet [114%] as references for B250 mash, and C250 mash [110%] and C500 pellet [108%] as references for C500 mash).

13 References

ISO 30024:2009. Animal feeding stuffs – Determination of phytase activity

SAS Institute Inc. 2012. Base SAS® 9.4 Guide to Information Maps. Cary, NC: SAS Institute Inc.

Statutory Instrument 1999 No. 1663. The Feeding Stuffs (Sampling and Analysis) Regulation 1999.

14 List of Appendices

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Appendix 1- *Curricula vitae* of Study Director & Study Monitor

Study Director:
Name: (b) (6)

Study Monitor:
Name: Drew Lichtenstein
Qualifications: B.S. Biochemistry (Michigan State University 1982), PhD Biochemistry (University of Wisconsin-Madison 1990)
Present Position: Research Manager, Specialty Products, Novus International
Experience: Over 35 years research experience in biochemistry and cell biology; more than 8 years of experience in animal feed enzymes.

Appendix 2 - Certificate of analysis of CIBENZA® PHYTAVERSE® G10 phytase enzyme used (3 batches)

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Certificate of Analysis

CIBENZA® PHYTAVERSE™ G10 Phytase Enzyme (Test Article VR005)

Lot number: P23941
Date of Manufacture: October 8, 2014

Specification	Specification Limit	Test Result
Appearance	White to Beige granules	Pass
Bulk Density-untapped (g/cm^3)	≥ 0.50	(b) (4)
Particle size (mesh)	<2% on 20 mesh <10% thru 140 mesh	
Activity (U/g)	NLT 10,000	
Loss on Drying (%)	**≤ 12**	
Lead (mg/kg)	≤ 5	
Arsenic (mg/kg)	< 2	
Cadmium (mg/kg)	< 0.5	
Mercury (mg/kg)	< 0.5	
Total Plate Count (cfu/g)	≤ 50,000	
Total Coliform (MPN/g)	≤ 30	
E. coli (/25g)	Absent	
Salmonella (/25g)	Absent	
Yeast and Mold (CFU/g)	Run and Record	
Staphylococcus aureus (/g)	Absent	
Production Organism (CFU/g)	Absent	
Antibiotic Activity (Zone of Inhibition)	Absent	
Mycotoxin		
Aflatoxin B1	NMT 1.0 ppb	
Aflatoxin B2	NMT 1.0 ppb	
Aflatoxin G1	NMT 1.0 ppb	
Aflatoxin G2	NMT 1.0 ppb	
Fumonisin B1	NMT 0.1 ppm	
Fumonisin B2	NMT 0.1 ppm	
Fumonisin B3	NMT 0.1 ppm	
Ochratoxin A	NMT 2.0 ppb	
Deoxynivalenol	NMT 3.0 ppm	
Acetyldeoxynivalenol	NMT 0.8 ppm	
Fusarenon X	NMT 0.4 ppm	
Nivalenol	NMT 0.6 ppm	
T-2 Toxin	NMT 0.2 ppm	
HT-2 Toxin	NMT 0.2 ppm	
Neosolaniol	NMT 0.4 ppm	
Diacetoxyscirpenol	NMT 0.4 ppm	
Zearalenone	NMT 43.1 ppb	
Sterigmatocystin	NMT 200 ppb	

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PCBs	10,000 pg/g	(b) (4)
Dioxins	1 pg/g	

* Production organism testing was performed on the enzyme concentrate used to produce this dry product.
** Results of retesting performed in March 2017.

Approved by: _____ Date: March 29, 2017

Mark Burcin
Sr. Manager, QA/QC

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CIBENZA® PHYTAVERSE™ G10 Phytase Enzyme (Test Article VR005)

Lot number: P26641
Date of Manufacture: October 8, 2014

Specification	Specification Limit	Test Result
Appearance	White to Beige granules	(b) (4)
Bulk Density-untapped (g/cm^3)	≥ 0.50	
Particle size (mesh)	<2% on 20 mesh <10% thru 140 mesh	
Activity (U/g)	NLT 10,000	
Loss on Drying (%)	**≤ 12**	
Lead (mg/kg)	≤ 5	
Arsenic (mg/kg)	< 2	
Cadmium (mg/kg)	< 0.5	
Mercury (mg/kg)	< 0.5	
Total Plate Count (cfu/g)	≤ 50,000	
Total Coliform (cfu/g)	≤ 30	
E. coli (/25g)	Absent	
Salmonella (/25g)	Absent	
Yeast and Mold (CFU/g)	Run and Record	
Staphylococcus aureus (/g)	Absent	
Production Organism (CFU/g)	Absent	
Antibiotic Activity (Zone of Inhibition)	Absent	
Mycotoxin		
Aflatoxin B1	NMT 1.0 ppb	
Aflatoxin B2	NMT 1.0 ppb	
Aflatoxin G1	NMT 1.0 ppb	
Aflatoxin G2	NMT 1.0 ppb	
Fumonisin B1	NMT 0.1 ppm	
Fumonisin B2	NMT 0.1 ppm	
Fumonisin B3	NMT 0.1 ppm	
Ochratoxin A	NMT 2.0 ppb	
Deoxynivalenol	NMT 3.0 ppm	
Acetyldeoxynivalenol	NMT 0.8 ppm	
Fusarenon X	NMT 0.4 ppm	
Nivalenol	NMT 0.6 ppm	
T-2 Toxin	NMT 0.2 ppm	
HT-2 Toxin	NMT 0.2 ppm	
Neosolaniol	NMT 0.4 ppm	
Diacetoxyscirpenol	NMT 0.4 ppm	
Zearalenone	NMT 43.1 ppb	

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Sterigmatocystin	NMT 200 ppb	(b) (4)
PCBs	10,000 pg/g	
Dioxins	1 pg/g	

* Production organism testing was performed on the enzyme concentrate used to produce this dry product.
** Results of retesting performed in March 2017.

Approved by: _____ Date: March 29, 2017

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Certificate of Analysis

CIBENZA® PHYTAVERSE™ G10 Phytase Enzyme (Test Article VR005)

Lot number: RO15271001
Date of Manufacture: September 28, 2015

Specification	Specification Limit	Test Result
Appearance	White to Beige granules	(b) (4)
Bulk Density-untapped (g/cm^3)	≥ 0.50	
Particle size (mesh)	<2% on 20 mesh <10% thru 140 mesh	
Activity (U/g)	NLT 10,000	
Loss on Drying (%)	**≤ 12**	
Lead (mg/kg)	≤ 5	
Arsenic (mg/kg)	< 2	
Cadmium (mg/kg)	< 0.5	
Mercury (mg/kg)	< 0.5	
Total Plate Count (cfu/g)	≤ 50,000	
Total Coliform (cfu/g)	≤ 30	
E. coli (/25g)	Absent	
Salmonella (/25g)	Absent	
Yeast and Mold (CFU/g)	Run and Record	
Staphylococcus aureus (/g)	Absent	
Production Organism (CFU/g)	Absent	
Antibiotic Activity (Zone of Inhibition)	Absent	
Mycotoxin		
Aflatoxin B1	NMT 1.0 ppb	
Aflatoxin B2	NMT 1.0 ppb	
Aflatoxin G1	NMT 1.0 ppb	
Aflatoxin G2	NMT 1.0 ppb	
Fumonisin B1	NMT 0.1 ppm	
Fumonisin B2	NMT 0.1 ppm	
Fumonisin B3	NMT 0.1 ppm	
Ochratoxin A	NMT 2.0 ppb	
Deoxynivalenol	NMT 3.0 ppm	
Acetyldeoxynivalenol	NMT 0.8 ppm	
Fusarenon X	NMT 0.4 ppm	
Nivalenol	NMT 0.6 ppm	
T-2 Toxin	NMT 0.2 ppm	
HT-2 Toxin	NMT 0.2 ppm	
Neosolaniol	NMT 0.4 ppm	
Diacetoxyscirpenol	NMT 0.4 ppm	
Zearalenone	NMT 43.1 ppb	

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Certificate of Analysis

Sterigmatocystin	NMT 200 ppb	(b) (4)
PCBs	10,000 pg/g	
Dioxins	1 pg/g	

* Production organism testing was performed on the enzyme concentrate used to produce this dry product.
** Results of retesting performed in March 2017.

Approved by: _____ Date: March 29, 2017

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Appendix 3 - Relevant laboratory reports

CERTIFICATE OF ANALYSIS

Company:	Novus International Inc and BASF Enzymes LLC
Type of sample:	F597 feeds
Laboratory ref. :	172006 to 172011 172032 to 172037 180004 to 180015 180069 to 180080 181548 to 181559 181804 to 181010
Reception date:	28th November 2017
Analysis starting date:	1st December 2017
Analysis finishing date:	22nd March 2018

Sample description: See Results section

Analysis performed:

- Moisture -dry matter- by oven drying –method 2 (SOP 0602-L-10001) (AOAC, 2000)
- Nitrogen -crude protein- by combustion -Dumas method (SOP 0602-L-10118) (AOAC, 2000)
- Ether extract on a Soxtec system -method 3B (SOP 0602-L-10003) (AOAC, 2000)
- Ash after muffle furnace incineration -method 12 (SOP 0602-L-10002) (AOAC, 2000)
- Phytase (SOP 0602-L-10143; ISO 30024:2009. Animal feeding stuffs – Determination of phytase activity.)

Results:

LAB. REF.	SAMPLE DESCRIPTION	CRUDE PROTEIN (%)	ETHER EXTRACT (%)	ASH (%)
172032	A250 pellet stab 0 mes	(b) (4)		
172033	A500 pellet stab 0 mes			
172034	B250 pellet stab 0 mes			
172035	B500 pellet stab 0 mes			
172036	C250 pellet stab 0 mes			
172037	C500 pellet stab 0 mes			

LAB. REF.	SAMPLE DESCRIPTION	PHYTASE (U/kg)	DM (%)
172006	A250 mash stab 0 mes	(b) (4)	
172007	A500 mash stab 0 mes		
172008	B250 mash stab 0 mes		
172009	B500 mash stab 0 mes		
172010	C250 mash stab 0 mes		
172011	C500 mash stab 0 mes		
172032	A250 pellet stab 0 mes		
172033	A500 pellet stab 0 mes		
172034	B250 pellet stab 0 mes		
172035	B500 pellet stab 0 mes		
172036	C250 pellet stab 0 mes		
172037	C500 pellet stab 0 mes		

LAB. REF.	SAMPLE DESCRIPTION	PHYTASE (U/kg)	DM (%)
180004	A250 mash stab 1 mes	(b) (4)	
180005	A500 mash stab 1 mes		
180006	B250 mash stab 1 mes		
180007	B500 mash stab 1 mes		
180008	C250 mash stab 1 mes		
180009	C500 mash stab 1 mes		
180010	A250 pellet stab 1 mes		
180011	A500 pellet stab 1 mes		
180012	B250 pellet stab 1 mes		
180013	B500 pellet stab 1 mes		
180014	C250 pellet stab 1 mes		
180015	C500 pellet stab 1 mes		

LAB. REF.	SAMPLE DESCRIPTION	PHYTASE (U/kg)	DM (%)
180069	A250 mash stab 2 mes	(b) (4)	
180072	A500 mash stab 2 mes		
180070	B250 mash stab 2 mes		
180073	B500 mash stab 2 mes		
180071	C250 mash stab 2 mes		
180074	C500 mash stab 2 mes		
180075	A250 pellet stab 2 mes		
180078	A500 pellet stab 2 mes		
180076	B250 pellet stab 2 mes		
180079	B500 pellet stab 2 mes		
180077	C250 pellet stab 2 mes		
180080	C500 pellet stab 2 mes		

LAB. REF.	SAMPLE DESCRIPTION	PHYTASE (U/kg)	DM (%)
181548	A250 mash stab 3 mes	(b) (4)	
181549	A500 mash stab 3 mes		
181550	B250 mash stab 3 mes		
181551	B500 mash stab 3 mes		
181552	C250 mash stab 3 mes		
181553	C500 mash stab 3 mes		

LAB. REF.	SAMPLE DESCRIPTION	PHYTASE (U/kg)	DM (%)
181554	A250 pellet stab 3 mes	(b) (4)	
181555	A500 pellet stab 3 mes		
181556	B250 pellet stab 3 mes		
181557	B500 pellet stab 3 mes		
181558	C250 pellet stab 3 mes		
181559	C500 pellet stab 3 mes		

LAB. REF.	SAMPLE DESCRIPTION	PHYTASE (U/kg)	DM (%)
181804	A250 pellet stab 0 mes	(b) (4)	
181805	A250 pellet stab 2 mes		
181806	A250 pellet stab 3 mes		
181807	B250 mash stab 0 mes		
181808	B250 mash stab 2 mes		
181809	B250 mash stab 3 mes		
181810	B250 pellet stab 2 mes		

(b) (4), (b) (6)

Signature: (b) (4)

Date: 26TH MARCH 2018

Appendix 4 - Raw data

Obs	enzyme	form	Tr	Tr_form	lab_ref	dose	U_kg_as_is	DM_p	month	U_kg_88_pc_DM
1	A	mash	A250	A250mash	172006	250		(b) (4)	0	(b) (4)
2	A	mash	A500	A500mash	172007	500			0	
3	B	mash	B250	B250mash	172008	250			0	
4	B	mash	B500	B500mash	172009	500			0	
5	C	mash	C250	C250mash	172010	250			0	
6	C	mash	C500	C500mash	172011	500			0	
7	A	pellet	A250	A250pellet	172032	250			0	
8	A	pellet	A500	A500pellet	172033	500			0	
9	B	pellet	B250	B250pellet	172034	250			0	
10	B	pellet	B500	B500pellet	172035	500			0	
11	C	pellet	C250	C250pellet	172036	250			0	
12	C	pellet	C500	C500pellet	172037	500			0	
13	A	mash	A250	A250mash	180004	250			1	
14	A	mash	A500	A500mash	180005	500			1	
15	B	mash	B250	B250mash	180006	250			1	
16	B	mash	B500	B500mash	180007	500			1	
17	C	mash	C250	C250mash	180008	250			1	
18	C	mash	C500	C500mash	180009	500			1	
19	A	pellet	A250	A250pellet	180010	250			1	
20	A	pellet	A500	A500pellet	180011	500			1	
21	B	pellet	B250	B250pellet	180012	250			1	
22	B	pellet	B500	B500pellet	180013	500			1	
23	C	pellet	C250	C250pellet	180014	250			1	
24	C	pellet	C500	C500pellet	180015	500			1	
25	A	mash	A250	A250mash	180069	250			2	
26	A	mash	A500	A500mash	180072	500			2	
27	B	mash	B250	B250mash	180070	250			2	
28	B	mash	B500	B500mash	180073	500			2	
29	C	mash	C250	C250mash	180071	250			2	
30	C	mash	C500	C500mash	180074	500			2	
31	A	pellet	A250	A250pellet	180075	250			2	
32	A	pellet	A500	A500pellet	180078	500			2	
33	B	pellet	B250	B250pellet	180076	250			2	
34	B	pellet	B500	B500pellet	180079	500			2	
35	C	pellet	C250	C250pellet	180077	250			2	
36	C	pellet	C500	C500pellet	180080	500			2	
37	A	mash	A250	A250mash	181548	250			3	
38	A	mash	A500	A500mash	181549	500			3	
39	B	mash	B250	B250mash	181550	250			3	
40	B	mash	B500	B500mash	181551	500			3	
41	C	mash	C250	C250mash	181552	250			3	
42	C	mash	C500	C500mash	181553	500			3	
43	A	pellet	A250	A250pellet	181554	250			3	
44	A	pellet	A500	A500pellet	181555	500			3	
45	B	pellet	B250	B250pellet	181556	250			3	
46	B	pellet	B500	B500pellet	181557	500			3	
47	C	pellet	C250	C250pellet	181558	250			3	
48	C	pellet	C500	C500pellet	181559	500			3	
49	A	pellet	A250	A250pellet	181804	250			0	
50	A	pellet	A250	A250pellet	181805	250			2	
51	A	pellet	A250	A250pellet	181806	250			3	
52	B	mash	B250	B250mash	181807	250			0	
53	B	mash	B250	B250mash	181808	250			2	
54	B	mash	B250	B250mash	181809	250			3	
55	B	pellet	B250	B250pellet	181810	250			2	

Appendix 5 - Statistical printouts

		N	U_kg_-as_is	DM_p	U_kg_-88_pc-_DM	pc_Om-_as_is	pc_Om-_88_p-c_DM	pc_Om-_DM
			Mean	Mean	Mean	Mean	Mean	Mean
Tr_form	month							
A250mash	0	1	319	87.3	322	100.0	100.0	100.0
	1	1	(b) (4)					
	2	1						
	3	1						
A250pellet	0	2	299	87.2	302	100.0	100.0	100.0
	1	1	(b) (4)					
	2	2						
	3	2						
A500mash	0	1	624	87.5	628	100.0	100.0	100.0
	1	1	(b) (4)					
	2	1						
	3	1						
A500pellet	0	1	491	87.2	496	100.0	100.0	100.0
	1	1	(b) (4)					
	2	1						
	3	1						
B250mash	0	2	329	87.3	331	100.0	100.0	100.0
	1	1	(b) (4)					
	2	2						
	3	2						
B250pellet	0	1	236	87.2	238	100.0	100.0	100.0
	1	1	(b) (4)					
	2	2						
	3	1						
B500mash	0	1	638	87.3	643	100.0	100.0	100.0
	1	1	(b) (4)					
	2	1						
	3	1						

(Continued)

		N	U_kg_-as_is	DM_p	U_kg_-88_pc-_DM	pc_Om-_as_is	pc_Om-_88_p-c_DM	pc_Om-_DM
			Mean	Mean	Mean	Mean	Mean	Mean
Tr_form	month							
B500pellet	0	1	489	87.1	494	100.0	100.0	100.0
	1	1	(b) (4)					
	2	1						
	3	1						
C250mash	0	1	308	87.7	309	100.0	100.0	100.0
	1	1	(b) (4)					
	2	1						
	3	1						
C250pellet	0	1	232	86.7	235	100.0	100.0	100.0
	1	1	(b) (4)					
	2	1						
	3	1						
C500mash	0	1	541	87.5	544	100.0	100.0	100.0
	1	1	(b) (4)					
	2	1						
	3	1						
C500pellet	0	1	455	86.9	461	100.0	100.0	100.0
	1	1	(b) (4)					
	2	1						
	3	1						

Obs	enzyme	dose	form	month	Tr	Tr_form	_FREQ_	U_kg_ as_is	DM_p	U_kg_88_ pc_DM	pc_Om_ as_is	pc_Om_ 88_pc_DM	pc_Om_DM
1	A	250	mash	0	A250	A250mash	1		(b) (4)	(b) (4)	1	(b) (4)	
2	A	250	mash	1	A250	A250mash	1				1		
3	A	250	mash	2	A250	A250mash	1				1		
4	A	250	mash	3	A250	A250mash	1						
5	A	250	pellet	0	A250	A250pellet	2				1		
6	A	250	pellet	1	A250	A250pellet	1						
7	A	250	pellet	2	A250	A250pellet	2						
8	A	250	pellet	3	A250	A250pellet	2						
9	A	500	mash	0	A500	A500mash	1				1		
10	A	500	mash	1	A500	A500mash	1						
11	A	500	mash	2	A500	A500mash	1				1		
12	A	500	mash	3	A500	A500mash	1				1		
13	A	500	pellet	0	A500	A500pellet	1				1		
14	A	500	pellet	1	A500	A500pellet	1				1		
15	A	500	pellet	2	A500	A500pellet	1						
16	A	500	pellet	3	A500	A500pellet	1				1		
17	B	250	mash	0	B250	B250mash	2				1		
18	B	250	mash	1	B250	B250mash	1						
19	B	250	mash	2	B250	B250mash	2						
20	B	250	mash	3	B250	B250mash	2						
21	B	250	pellet	0	B250	B250pellet	1				1		
22	B	250	pellet	1	B250	B250pellet	1						
23	B	250	pellet	2	B250	B250pellet	2						
24	B	250	pellet	3	B250	B250pellet	1				1		
25	B	500	mash	0	B500	B500mash	1				1		
26	B	500	mash	1	B500	B500mash	1						
27	B	500	mash	2	B500	B500mash	1						
28	B	500	mash	3	B500	B500mash	1						
29	B	500	pellet	0	B500	B500pellet	1				1		
30	B	500	pellet	1	B500	B500pellet	1						
31	B	500	pellet	2	B500	B500pellet	1						
32	B	500	pellet	3	B500	B500pellet	1						
33	C	250	mash	0	C250	C250mash	1				1		
34	C	250	mash	1	C250	C250mash	1				1		
35	C	250	mash	2	C250	C250mash	1						
36	C	250	mash	3	C250	C250mash	1				1		
37	C	250	pellet	0	C250	C250pellet	1				1		
38	C	250	pellet	1	C250	C250pellet	1				1		
39	C	250	pellet	2	C250	C250pellet	1						
40	C	250	pellet	3	C250	C250pellet	1				1		
41	C	500	mash	0	C500	C500mash	1				1		
42	C	500	mash	1	C500	C500mash	1				1		
43	C	500	mash	2	C500	C500mash	1						
44	C	500	mash	3	C500	C500mash	1						
45	C	500	pellet	0	C500	C500pellet	1				1		
46	C	500	pellet	1	C500	C500pellet	1				1		
47	C	500	pellet	2	C500	C500pellet	1						
48	C	500	pellet	3	C500	C500pellet	1				1		

Tr_form=A250mash

The GLM Procedure

Number of Observations Read 4
Number of Observations Used 4

Tr_form=A250mash

The GLM Procedure

Dependent Variable: U_kg_as_is

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	16.2000000	16.2000000	0.05	0.8484
Error	2	688.8000000	344.4000000		
Corrected Total	3	705.0000000			

R-Square	Coeff Var	Root MSE	U_kg_as_is Mean
0.022979	5.649320	18.55802	328.5000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	16.20000000	16.20000000	0.05	0.8484

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	16.20000000	16.20000000	0.05	0.8484

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	331.2000000	15.52675111	21.33	0.0022
month	-1.8000000	8.29939757	-0.22	0.8484

Tr_form=A250mash

The GLM Procedure

Dependent Variable: U_kg_88_pc_DM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	7.7282331	7.7282331	0.03	0.8871
Error	2	599.1118656	299.5559328		
Corrected Total	3	606.8400987			

R-Square	Coeff Var	Root MSE	U_kg_88_pc_DM Mean
0.012735	5.221572	17.30768	331.4650

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	7.72823305	7.72823305	0.03	0.8871

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	7.72823305	7.72823305	0.03	0.8871

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	333.3298984	14.48064753	23.02	0.0019
month	-1.2432404	7.74023169	-0.16	0.8871

Tr_form=A250mash

The GLM Procedure

Dependent Variable: pc_Om_as_is

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	1.59196549	1.59196549	0.05	0.8484
Error	2	67.68801407	33.84400704		
Corrected Total	3	69.27997956			

R-Square	Coeff Var	Root MSE	pc_Om_as_is Mean
0.022979	5.649320	5.817560	102.9781

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	1.59196549	1.59196549	0.05	0.8484

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	1.59196549	1.59196549	0.05	0.8484

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	103.8244514	4.86732010	21.33	0.0022
month	-0.5642633	2.60169203	-0.22	0.8484

---------- Tr_form=A250mash ----------

The GLM Procedure

Dependent Variable: pc_Om_88_pc_DM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.74741459	0.74741459	0.03	0.8871
Error	2	57.94143987	28.97071994		
Corrected Total	3	58.68885446			

R-Square	Coeff Var	Root MSE	pc_Om_88_pc_DM Mean
0.012735	5.221572	5.382446	103.0809

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	0.74741459	0.74741459	0.03	0.8871

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	0.74741459	0.74741459	0.03	0.8871

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	103.6608943	4.50327702	23.02	0.0019
month	-0.3866302	2.40710282	-0.16	0.8871

---------- Tr_form=A250pellet ----------

The GLM Procedure

Number of Observations Read	4
Number of Observations Used	4

---------- Tr_form=A250pellet ----------

The GLM Procedure

Dependent Variable: U_kg_as_is

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	1901.250000	1901.250000	3.15	0.2180
Error	2	1207.500000	603.750000		
Corrected Total	3	3108.750000			

R-Square	Coeff Var	Root MSE	U_kg_as_is Mean
0.611580	9.423327	24.57132	260.7500

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	1901.250000	1901.250000	3.15	0.2180

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	1901.250000	1901.250000	3.15	0.2180

Parameter	Estimate	Standard Error	t Value	Pr > |t|

Intercept	290.0000000	20.55784522	14.11	0.0050
month	-19.5000000	10.98863049	-1.77	0.2180

---------- Tr_form=A250pellet ----------

The GLM Procedure

Dependent Variable: U_kg_88_pc_DM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	2047.843575	2047.843575	3.26	0.2127
Error	2	1256.149102	628.074551		
Corrected Total	3	3303.992677			

R-Square	Coeff Var	Root MSE	U_kg_88_pc_DM Mean
0.619809	9.550053	25.06142	262.4218

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	2047.843575	2047.843575	3.26	0.2127

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	2047.843575	2047.843575	3.26	0.2127

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	292.7784569	20.96788462	13.96	0.0051
month	-20.2378041	11.20780577	-1.81	0.2127

---------- Tr_form=A250pellet ----------

The GLM Procedure

Dependent Variable: pc_Om_as_is

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	212.6654064	212.6654064	3.15	0.2180
Error	2	135.0656033	67.5328016		
Corrected Total	3	347.7310097			

R-Square	Coeff Var	Root MSE	pc_Om_as_is Mean
0.611580	9.423327	8.217834	87.20736

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	212.6654064	212.6654064	3.15	0.2180

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	212.6654064	212.6654064	3.15	0.2180

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	96.98996656	6.87553352	14.11	0.0050
month	-6.52173913	3.67512725	-1.77	0.2180

---------- Tr_form=A250pellet ----------

The GLM Procedure

Dependent Variable: pc_Om_88_pc_DM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	224.7829359	224.7829359	3.26	0.2127
Error	2	137.8820562	68.9410281		
Corrected Total	3	362.6649921			

R-Square	Coeff Var	Root MSE	pc_Om_88_pc_DM Mean
0.619809	9.550053	8.303073	86.94270

Source	DF	Type I SS	Mean Square	F Value	Pr > F

month	1	224.7829359	224.7829359	3.26	0.2127

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	224.7829359	224.7829359	3.26	0.2127

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	97.00014806	6.94684962	13.96	0.0051
month	-6.70496735	3.71324732	-1.81	0.2127

Tr_form=A500mash

The GLM Procedure

Number of Observations Read	4
Number of Observations Used	4

Tr_form=A500mash

The GLM Procedure

Dependent Variable: U_kg_as_is

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	966.050000	966.050000	2.08	0.2863
Error	2	930.700000	465.350000		
Corrected Total	3	1896.750000			

R-Square	Coeff Var	Root MSE	U_kg_as_is Mean
0.509319	3.430930	21.57197	628.7500

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	966.0500000	966.0500000	2.08	0.2863

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	966.0500000	966.0500000	2.08	0.2863

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	607.9000000	18.04840713	33.68	0.0009
month	13.9000000	9.64727941	1.44	0.2863

Tr_form=A500mash

The GLM Procedure

Dependent Variable: U_kg_88_pc_DM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	942.170446	942.170446	2.06	0.2876
Error	2	914.412923	457.206462		
Corrected Total	3	1856.583369			

R-Square	Coeff Var	Root MSE	U_kg_88_pc_DM Mean
0.507475	3.382098	21.38239	632.2225

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	942.1704460	942.1704460	2.06	0.2876

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	942.1704460	942.1704460	2.06	0.2876

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	611.6318150	17.88978823	34.19	0.0009
month	13.7271297	9.56249404	1.44	0.2876

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---------- Tr_form=A500mash ----------

The GLM Procedure

Dependent Variable: pc_Om_as_is

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	24.81020916	24.81020916	2.08	0.2863
Error	2	23.90234632	11.95117316		
Corrected Total	3	48.71255547			

R-Square	Coeff Var	Root MSE	pc_Om_as_is Mean
0.509319	3.430930	3.457047	100.7612

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	24.81020916	24.81020916	2.08	0.2863

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	24.81020916	24.81020916	2.08	0.2863

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	97.41987179	2.89237294	33.68	0.0009
month	2.22756410	1.54603837	1.44	0.2863

---------- Tr_form=A500mash ----------

The GLM Procedure

Dependent Variable: pc_Om_88_pc_DM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	23.90086009	23.90086009	2.06	0.2876
Error	2	23.19671078	11.59835539		
Corrected Total	3	47.09757087			

R-Square	Coeff Var	Root MSE	pc_Om_88_pc_DM Mean
0.507475	3.382098	3.405636	100.6959

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	23.90086009	23.90086009	2.06	0.2876

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	23.90086009	23.90086009	2.06	0.2876

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	97.41640400	2.84935936	34.19	0.0009
month	2.18636045	1.52304664	1.44	0.2876

---------- Tr_form=A500pellet ----------

The GLM Procedure

Number of Observations Read 4
Number of Observations Used 4

---------- Tr_form=A500pellet ----------

The GLM Procedure

Dependent Variable: U_kg_as_is

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	245.000000	245.000000	0.12	0.7655
Error	2	4209.000000	2104.500000		
Corrected Total	3	4454.000000			

R-Square	Coeff Var	Root MSE	U_kg_as_is Mean
0.055007	9.400580	45.87483	488.0000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	245.0000000	245.0000000	0.12	0.7655

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	245.0000000	245.0000000	0.12	0.7655

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	477.5000000	38.38163623	12.44	0.0064
month	7.0000000	20.51584753	0.34	0.7655

---------- Tr_form=A500pellet ----------

The GLM Procedure

Dependent Variable: U_kg_88_pc_DM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	198.512645	198.512645	0.09	0.7902
Error	2	4311.158579	2155.579290		
Corrected Total	3	4509.671224			

R-Square	Coeff Var	Root MSE	U_kg_88_pc_DM Mean
0.044019	9.441518	46.42822	491.7452

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	198.5126454	198.5126454	0.09	0.7902

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	198.5126454	198.5126454	0.09	0.7902

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	482.2937489	38.84463287	12.42	0.0064
month	6.3009943	20.76332964	0.30	0.7902

---------- Tr_form=A500pellet ----------

The GLM Procedure

Dependent Variable: pc_Om_as_is

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	10.1625595	10.1625595	0.12	0.7655
Error	2	174.5886237	87.2943119		
Corrected Total	3	184.7511832			

R-Square	Coeff Var	Root MSE	pc_Om_as_is Mean
0.055007	9.400580	9.343143	99.38900

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	10.16255947	10.16255947	0.12	0.7655

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	10.16255947	10.16255947	0.12	0.7655

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	97.25050916	7.81703386	12.44	0.0064
month	1.42566191	4.17838035	0.34	0.7655

---------- Tr_form=A500pellet ----------

The GLM Procedure

Dependent Variable: pc_Om_88_pc_DM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	8.0759645	8.0759645	0.09	0.7902
Error	2	175.3881407	87.6940704		
Corrected Total	3	183.4641052			

R-Square	Coeff Var	Root MSE	pc_Om_88_pc_DM Mean
0.044019	9.441518	9.364511	99.18438

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	8.07596453	8.07596453	0.09	0.7902

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	8.07596453	8.07596453	0.09	0.7902

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	97.27802532	7.83491220	12.42	0.0064
month	1.27090240	4.18793673	0.30	0.7902

Tr_form=B250mash

The GLM Procedure

Number of Observations Read 4
Number of Observations Used 4

Tr_form=B250mash

The GLM Procedure

Dependent Variable: U_kg_as_is

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	2773.012500	2773.012500	15.68	0.0583
Error	2	353.675000	176.837500		
Corrected Total	3	3126.687500			

R-Square	Coeff Var	Root MSE	U_kg_as_is Mean
0.886885	4.701026	13.29803	282.8750

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	2773.012500	2773.012500	15.68	0.0583

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	2773.012500	2773.012500	15.68	0.0583

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	318.2000000	11.12592693	28.60	0.0012
month	-23.5500000	5.94705810	-3.96	0.0583

Tr_form=B250mash

The GLM Procedure

Dependent Variable: U_kg_88_pc_DM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	2859.890299	2859.890299	15.85	0.0577
Error	2	360.832057	180.416028		
Corrected Total	3	3220.722356			

R-Square	Coeff Var	Root MSE	U_kg_88_pc_DM Mean
0.887965	4.717346	13.43190	284.7344

Source	DF	Type I SS	Mean Square	F Value	Pr > F

```
month                          1     2859.890299     2859.890299      15.85    0.0577

Source                        DF     Type III SS     Mean Square    F Value    Pr > F
month                          1     2859.890299     2859.890299      15.85    0.0577

                                                    Standard
          Parameter         Estimate           Error    t Value    Pr > |t|
          Intercept      320.6084510     11.23793664      28.53      0.0012
          month          -23.9160628      6.00692980      -3.98      0.0577
```



```
---------------------------------------- Tr_form=B250mash ----------------------------------------
                                  The GLM Procedure
                           Dependent Variable: pc_0m_as_is
                                              Sum of
Source                        DF         Squares     Mean Square    F Value    Pr > F
Model                          1     256.9692041     256.9692041      15.68    0.0583
Error                          2      32.7743143      16.3871572
Corrected Total                3     289.7435185

              R-Square     Coeff Var      Root MSE    pc_0m_as_is Mean
              0.886885      4.701026      4.048105            86.11111

Source                        DF       Type I SS     Mean Square    F Value    Pr > F
month                          1     256.9692041     256.9692041      15.68    0.0583

Source                        DF     Type III SS     Mean Square    F Value    Pr > F
month                          1     256.9692041     256.9692041      15.68    0.0583

                                                    Standard
          Parameter         Estimate           Error    t Value    Pr > |t|
          Intercept      96.86453577      3.38688795      28.60      0.0012
          month          -7.16894977      1.81036776      -3.96      0.0583
```



```
---------------------------------------- Tr_form=B250mash ----------------------------------------
                                  The GLM Procedure
                         Dependent Variable: pc_0m_88_pc_DM
                                              Sum of
Source                        DF         Squares     Mean Square    F Value    Pr > F
Model                          1     261.0175868     261.0175868      15.85    0.0577
Error                          2      32.9325613      16.4662807
Corrected Total                3     293.9501481

            R-Square     Coeff Var      Root MSE    pc_0m_88_pc_DM Mean
            0.887965      4.717346      4.057867              86.02013

Source                        DF       Type I SS     Mean Square    F Value    Pr > F
month                          1     261.0175868     261.0175868      15.85    0.0577

Source                        DF     Type III SS     Mean Square    F Value    Pr > F
month                          1     261.0175868     261.0175868      15.85    0.0577

                                                    Standard
          Parameter         Estimate           Error    t Value    Pr > |t|
          Intercept      96.85792561      3.39505471      28.53      0.0012
          month          -7.22520016      1.81473307      -3.98      0.0577
```



```
---------------------------------------- Tr_form=B250pellet ----------------------------------------
                                  The GLM Procedure
                          Number of Observations Read           4
                          Number of Observations Used           4
```

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---------- Tr_form=B250pellet ----------

The GLM Procedure

Dependent Variable: U_kg_as_is

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	437.112500	437.112500	0.80	0.4666
Error	2	1099.075000	549.537500		
Corrected Total	3	1536.187500			

R-Square	Coeff Var	Root MSE	U_kg_as_is Mean
0.284544	9.896450	23.44222	236.8750

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	437.1125000	437.1125000	0.80	0.4666

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	437.1125000	437.1125000	0.80	0.4666

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	222.8500000	19.61316522	11.36	0.0077
month	9.3500000	10.48367779	0.89	0.4666

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---------- Tr_form=B250pellet ----------

The GLM Procedure

Dependent Variable: U_kg_88_pc_DM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	428.519926	428.519926	0.76	0.4759
Error	2	1131.619227	565.809613		
Corrected Total	3	1560.139152			

R-Square	Coeff Var	Root MSE	U_kg_88_pc_DM Mean
0.274668	9.960571	23.78675	238.8091

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	428.5199257	428.5199257	0.76	0.4759

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	428.5199257	428.5199257	0.76	0.4759

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	224.9226627	19.90142531	11.30	0.0077
month	9.2576447	10.63775929	0.87	0.4759

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---------- Tr_form=B250pellet ----------

The GLM Procedure

Dependent Variable: pc_Om_as_is

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	78.4818479	78.4818479	0.80	0.4666
Error	2	197.3346380	98.6673190		
Corrected Total	3	275.8164859			

R-Square	Coeff Var	Root MSE	pc_Om_as_is Mean
0.284544	9.896450	9.933142	100.3708

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	78.48184789	78.48184789	0.80	0.4666

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	78.48184789	78.48184789	0.80	0.4666

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	94.42796610	8.31066323	11.36	0.0077
month	3.96186441	4.44223635	0.89	0.4666

---------- Tr_form=B25Opellet ----------

The GLM Procedure

Dependent Variable: pc_Om_88_pc_DM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	75.4946437	75.4946437	0.76	0.4759
Error	2	199.3634021	99.6817011		
Corrected Total	3	274.8580458			

R-Square	Coeff Var	Root MSE	pc_Om_88_pc_DM Mean
0.274668	9.960571	9.984072	100.2359

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	75.49464366	75.49464366	0.76	0.4759

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	75.49464366	75.49464366	0.76	0.4759

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	94.40734309	8.35327425	11.30	0.0077
month	3.88573400	4.46501290	0.87	0.4759

---------- Tr_form=B50Omash ----------

The GLM Procedure

Number of Observations Read 4
Number of Observations Used 4

---------- Tr_form=B50Omash ----------

The GLM Procedure

Dependent Variable: U_kg_as_is

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	45.000000	45.000000	0.02	0.9071
Error	2	5166.000000	2583.000000		
Corrected Total	3	5211.000000			

R-Square	Coeff Var	Root MSE	U_kg_as_is Mean
0.008636	8.534546	50.82322	595.5000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	45.00000000	45.00000000	0.02	0.9071

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	45.00000000	45.00000000	0.02	0.9071

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	600.0000000	42.52175914	14.11	0.0050
month	-3.0000000	22.72883631	-0.13	0.9071

---------- Tr_form=B50Omash ----------

The GLM Procedure

Dependent Variable: U_kg_88_pc_DM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	68.823839	68.823839	0.03	0.8865
Error	2	5276.798875	2638.399437		
Corrected Total	3	5345.622714			

R-Square	Coeff Var	Root MSE	U_kg_88_pc_DM Mean
0.012875	8.569034	51.36535	599.4299

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	68.82383881	68.82383881	0.03	0.8865

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	68.82383881	68.82383881	0.03	0.8865

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	604.9950674	42.97533719	14.08	0.0050
month	-3.7100900	22.97128398	-0.16	0.8865

---------- Tr_form=B500mash ----------

The GLM Procedure

Dependent Variable: pc_Om_as_is

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	1.1055316	1.1055316	0.02	0.9071
Error	2	126.9150264	63.4575132		
Corrected Total	3	128.0205580			

R-Square	Coeff Var	Root MSE	pc_Om_as_is Mean
0.008636	8.534546	7.966022	93.33856

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	1.10553159	1.10553159	0.02	0.9071

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	1.10553159	1.10553159	0.02	0.9071

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	94.04388715	6.66485253	14.11	0.0050
month	-0.47021944	3.56251353	-0.13	0.9071

---------- Tr_form=B500mash ----------

The GLM Procedure

Dependent Variable: pc_Om_88_pc_DM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	1.6632662	1.6632662	0.03	0.8865
Error	2	127.5244320	63.7622160		
Corrected Total	3	129.1876981			

R-Square	Coeff Var	Root MSE	pc_Om_88_pc_DM Mean
0.012875	8.569034	7.985125	93.18582

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	1.66326615	1.66326615	0.03	0.8865

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	1.66326615	1.66326615	0.03	0.8865

Parameter	Estimate	Standard Error	t Value	Pr > |t|

Intercept	94.05096631	6.68083462	14.08	0.0050
month	-0.57676098	3.57105631	-0.16	0.8865

(b) (4) Trial F597, stability feeds 170
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Tr_form=B50Opellet

The GLM Procedure

Number of Observations Read 4
Number of Observations Used 4

(b) (4) Trial F597, stability feeds 171
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Tr_form=B50Opellet

The GLM Procedure

Dependent Variable: U_kg_as_is

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	31.250000	31.250000	0.02	0.9064
Error	2	3537.500000	1768.750000		
Corrected Total	3	3568.750000			

R-Square	Coeff Var	Root MSE	U_kg_as_is Mean
0.008757	9.289124	42.05651	452.7500

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	31.25000000	31.25000000	0.02	0.9064

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	31.25000000	31.25000000	0.02	0.9064

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	456.5000000	35.18700044	12.97	0.0059
month	-2.5000000	18.80824287	-0.13	0.9064

(b) (4) rial F597, stability feeds 172
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Tr_form=B50Opellet

The GLM Procedure

Dependent Variable: U_kg_88_pc_DM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	49.824305	49.824305	0.03	0.8833
Error	2	3609.305522	1804.652761		
Corrected Total	3	3659.129827			

R-Square	Coeff Var	Root MSE	U_kg_88_pc_DM Mean
0.013616	9.303373	42.48120	456.6215

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	49.82430523	49.82430523	0.03	0.8833

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	49.82430523	49.82430523	0.03	0.8833

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	461.3565965	35.54232593	12.98	0.0059
month	-3.1567168	18.99817234	-0.17	0.8833

(b) (4) Trial F597, stability feeds 173
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Tr_form=B50Opellet

The GLM Procedure

Dependent Variable: pc_Om_as_is

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	1.3068697	1.3068697	0.02	0.9064

```
Error                          2     147.9376550      73.9688275
Corrected Total                3     149.2445247

                R-Square     Coeff Var      Root MSE    pc_Om_as_is Mean
                0.008757      9.289124      8.600513           92.58691

Source                         DF       Type I SS     Mean Square    F Value    Pr > F
month                           1      1.30686974      1.30686974       0.02    0.9064

Source                         DF     Type III SS     Mean Square    F Value    Pr > F
month                           1      1.30686974      1.30686974       0.02    0.9064

                                            Standard
          Parameter         Estimate           Error    t Value    Pr > |t|
          Intercept      93.35378323      7.19570561      12.97      0.0059
          month          -0.51124744      3.84626644      -0.13      0.9064
```



```
---------------------------------------- Tr_form=B500pellet ----------------------------------------
                                The GLM Procedure
                          Dependent Variable: pc_Om_88_pc_DM
                                            Sum of
Source                         DF          Squares     Mean Square    F Value    Pr > F
Model                           1       2.0398364       2.0398364       0.03    0.8833
Error                           2     147.7670968      73.8835484
Corrected Total                 3     149.8069332

            R-Square     Coeff Var      Root MSE    pc_Om_88_pc_DM Mean
            0.013616      9.303373      8.595554               92.39180

Source                         DF       Type I SS     Mean Square    F Value    Pr > F
month                           1      2.03983644      2.03983644       0.03    0.8833

Source                         DF     Type III SS     Mean Square    F Value    Pr > F
month                           1      2.03983644      2.03983644       0.03    0.8833

                                            Standard
          Parameter         Estimate           Error    t Value    Pr > |t|
          Intercept      93.34988386      7.19155643      12.98      0.0059
          month          -0.63872317      3.84404861      -0.17      0.8833
```



```
---------------------------------------- Tr_form=C250mash ----------------------------------------
                                The GLM Procedure
                          Number of Observations Read           4
                          Number of Observations Used           4
```



```
---------------------------------------- Tr_form=C250mash ----------------------------------------
                                The GLM Procedure
                          Dependent Variable: U_kg_as_is
                                            Sum of
Source                         DF          Squares     Mean Square    F Value    Pr > F
Model                           1     336.2000000     336.2000000       1.61    0.3323
Error                           2     417.8000000     208.9000000
Corrected Total                 3     754.0000000

                R-Square     Coeff Var      Root MSE    U_kg_as_is Mean
                0.445889      4.545086      14.45337           318.0000

Source                         DF       Type I SS     Mean Square    F Value    Pr > F
month                           1     336.2000000     336.2000000       1.61    0.3323

Source                         DF     Type III SS     Mean Square    F Value    Pr > F
month                           1     336.2000000     336.2000000       1.61    0.3323
```

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	305.7000000	12.09255970	25.28	0.0016
month	8.2000000	6.46374504	1.27	0.3323

Tr_form=C250mash

The GLM Procedure

Dependent Variable: U_kg_88_pc_DM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	332.1094622	332.1094622	1.53	0.3411
Error	2	432.9635766	216.4817883		
Corrected Total	3	765.0730389			

R-Square	Coeff Var	Root MSE	U_kg_88_pc_DM Mean
0.434089	4.603923	14.71332	319.5822

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	332.1094622	332.1094622	1.53	0.3411

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	332.1094622	332.1094622	1.53	0.3411

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	307.3573043	12.31004678	24.97	0.0016
month	8.1499627	6.57999678	1.24	0.3411

Tr_form=C250mash

The GLM Procedure

Dependent Variable: pc_Om_as_is

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	35.44020914	35.44020914	1.61	0.3323
Error	2	44.04199696	22.02099848		
Corrected Total	3	79.48220611			

R-Square	Coeff Var	Root MSE	pc_Om_as_is Mean
0.445889	4.545086	4.692654	103.2468

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	35.44020914	35.44020914	1.61	0.3323

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	35.44020914	35.44020914	1.61	0.3323

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	99.25324675	3.92615575	25.28	0.0016
month	2.66233766	2.09861852	1.27	0.3323

Tr_form=C250mash

The GLM Procedure

Dependent Variable: pc_Om_88_pc_DM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	34.76275202	34.76275202	1.53	0.3411
Error	2	45.31941171	22.65970585		
Corrected Total	3	80.08216373			

R-Square	Coeff Var	Root MSE	pc_Om_88_pc_DM Mean

```
              0.434089      4.603923      4.760221              103.3949

Source                     DF       Type I SS     Mean Square    F Value    Pr > F
month                       1     34.76275202     34.76275202       1.53    0.3411

Source                     DF     Type III SS     Mean Square    F Value    Pr > F
month                       1     34.76275202     34.76275202       1.53    0.3411

                                          Standard
          Parameter         Estimate           Error    t Value    Pr > |t|
          Intercept      99.43974205      3.98268679      24.97      0.0016
          month           2.63676893      2.12883564       1.24      0.3411
```



```
------------------------------------- Tr_form=C250pellet -------------------------------------
                                  The GLM Procedure
                          Number of Observations Read           4
                          Number of Observations Used           4
```



```
------------------------------------- Tr_form=C250pellet -------------------------------------
                                  The GLM Procedure
                            Dependent Variable: U_kg_as_is
                                          Sum of
Source                     DF         Squares     Mean Square    F Value    Pr > F
Model                       1       92.450000       92.450000       0.03    0.8799
Error                       2     6316.300000     3158.150000
Corrected Total             3     6408.750000

               R-Square     Coeff Var      Root MSE    U_kg_as_is Mean
               0.014426      22.50147      56.19742          249.7500

Source                     DF       Type I SS     Mean Square    F Value    Pr > F
month                       1     92.45000000     92.45000000       0.03    0.8799

Source                     DF     Type III SS     Mean Square    F Value    Pr > F
month                       1     92.45000000     92.45000000       0.03    0.8799

                                          Standard
          Parameter         Estimate           Error    t Value    Pr > |t|
          Intercept     243.3000000     47.01813480       5.17      0.0354
          month           4.3000000     25.13225020       0.17      0.8799
```



```
------------------------------------- Tr_form=C250pellet -------------------------------------
                                  The GLM Procedure
                           Dependent Variable: U_kg_88_pc_DM
                                          Sum of
Source                     DF         Squares     Mean Square    F Value    Pr > F
Model                       1       84.147059       84.147059       0.03    0.8870
Error                       2     6509.670383     3254.835192
Corrected Total             3     6593.817442

               R-Square     Coeff Var      Root MSE    U_kg_88_pc_DM Mean
               0.012762      22.55008      57.05116              252.9976

Source                     DF       Type I SS     Mean Square    F Value    Pr > F
month                       1     84.14705937     84.14705937       0.03    0.8870

Source                     DF     Type III SS     Mean Square    F Value    Pr > F
month                       1     84.14705937     84.14705937       0.03    0.8870

                                          Standard
          Parameter         Estimate           Error    t Value    Pr > |t|
          Intercept     246.8440119     47.73242749       5.17      0.0354
```

```
month            4.1023666     25.51405570       0.16      0.8870
```

(b) (4) Trial F597, stability feeds 183
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```
---------------------------------------- Tr_form=C250pellet ----------------------------------------
                                The GLM Procedure
                          Dependent Variable: pc_Om_as_is
                                              Sum of
Source                         DF          Squares      Mean Square    F Value    Pr > F
Model                           1        17.176353        17.176353       0.03    0.8799
Error                           2      1173.509958       586.754979
Corrected Total                 3      1190.686311

                  R-Square     Coeff Var      Root MSE    pc_Om_as_is Mean
                  0.014426      22.50147      24.22303             107.6509

Source                         DF       Type I SS     Mean Square    F Value    Pr > F
month                           1      17.17635256     17.17635256       0.03    0.8799

Source                         DF     Type III SS     Mean Square    F Value    Pr > F
month                           1      17.17635256     17.17635256       0.03    0.8799

                                             Standard
               Parameter         Estimate           Error    t Value    Pr > |t|
               Intercept      104.8706897     20.26643741       5.17      0.0354
               month            1.8534483     10.83286646       0.17      0.8799
```

(b) (4) Trial F597, stability feeds 184
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```
---------------------------------------- Tr_form=C250pellet ----------------------------------------
                                The GLM Procedure
                          Dependent Variable: pc_Om_88_pc_DM
                                              Sum of
Source                         DF          Squares      Mean Square    F Value    Pr > F
Model                           1        15.178686        15.178686       0.03    0.8870
Error                           2      1174.232864       587.116432
Corrected Total                 3      1189.411550

                  R-Square     Coeff Var      Root MSE    pc_Om_88_pc_DM Mean
                  0.012762      22.55008      24.23049               107.4519

Source                         DF       Type I SS     Mean Square    F Value    Pr > F
month                           1      15.17868597     15.17868597       0.03    0.8870

Source                         DF     Type III SS     Mean Square    F Value    Pr > F
month                           1      15.17868597     15.17868597       0.03    0.8870

                                             Standard
               Parameter         Estimate           Error    t Value    Pr > |t|
               Intercept      104.8383585     20.27267872       5.17      0.0354
               month            1.7423367     10.83620258       0.16      0.8870
```

(b) (4) Trial F597, stability feeds 185
13:26 Wednesday, March 14, 2018

```
---------------------------------------- Tr_form=C500mash ----------------------------------------
                                The GLM Procedure
                          Number of Observations Read           4
                          Number of Observations Used           4
```

(b) (4) Trial F597, stability feeds 186
13:26 Wednesday, March 14, 2018

```
---------------------------------------- Tr_form=C500mash ----------------------------------------
                                The GLM Procedure
                            Dependent Variable: U_kg_as_is
                                              Sum of
Source                         DF          Squares      Mean Square    F Value    Pr > F
Model                           1     4836.050000     4836.050000       3.30    0.2109
Error                           2     2930.700000     1465.350000
```

Corrected Total 3 7766.750000

R-Square	Coeff Var	Root MSE	U_kg_as_is Mean
0.622661	7.372150	38.27989	519.2500

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	4836.050000	4836.050000	3.30	0.2109

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	4836.050000	4836.050000	3.30	0.2109

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	565.9000000	32.02725402	17.67	0.0032
month	-31.1000000	17.11928737	-1.82	0.2109

---------- Tr_form=C50Omash ----------

The GLM Procedure
Dependent Variable: U_kg_88_pc_DM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	5040.859999	5040.859999	3.57	0.1996
Error	2	2827.534988	1413.767494		
Corrected Total	3	7868.394987			

R-Square	Coeff Var	Root MSE	U_kg_88_pc_DM Mean
0.640647	7.213917	37.60010	521.2162

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	5040.859999	5040.859999	3.57	0.1996

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	5040.859999	5040.859999	3.57	0.1996

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	568.8437369	31.45850037	18.08	0.0030
month	-31.7517244	16.81527576	-1.89	0.1996

---------- Tr_form=C50Omash ----------

The GLM Procedure
Dependent Variable: pc_Om_as_is

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	165.2327961	165.2327961	3.30	0.2109
Error	2	100.1329092	50.0664546		
Corrected Total	3	265.3657053			

R-Square	Coeff Var	Root MSE	pc_Om_as_is Mean
0.622661	7.372150	7.075765	95.97967

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	165.2327961	165.2327961	3.30	0.2109

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	165.2327961	165.2327961	3.30	0.2109

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	104.6025878	5.92000999	17.67	0.0032
month	-5.7486137	3.16437844	-1.82	0.2109

---------- Tr_form=C50Omash ----------

The GLM Procedure

Dependent Variable: pc_Om_88_pc_DM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	170.1234330	170.1234330	3.57	0.1996
Error	2	95.4261692	47.7130846		
Corrected Total	3	265.5496022			

R-Square	Coeff Var	Root MSE	pc_Om_88_pc_DM Mean
0.640647	7.213917	6.907466	95.75195

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	170.1234330	170.1234330	3.57	0.1996

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	170.1234330	170.1234330	3.57	0.1996

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	104.5015499	5.77920057	18.08	0.0030
month	-5.8330684	3.08911264	-1.89	0.1996

---------- Tr_form=C50Opellet ----------

The GLM Procedure

Number of Observations Read 4
Number of Observations Used 4

---------- Tr_form=C50Opellet ----------

The GLM Procedure

Dependent Variable: U_kg_as_is

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	14.450000	14.450000	0.00	0.9550
Error	2	7122.300000	3561.150000		
Corrected Total	3	7136.750000			

R-Square	Coeff Var	Root MSE	U_kg_as_is Mean
0.002025	13.35767	59.67537	446.7500

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	14.45000000	14.45000000	0.00	0.9550

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	14.45000000	14.45000000	0.00	0.9550

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	444.2000000	49.92799816	8.90	0.0124
month	1.7000000	26.68763759	0.06	0.9550

---------- Tr_form=C50Opellet ----------

The GLM Procedure

Dependent Variable: U_kg_88_pc_DM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	8.243989	8.243989	0.00	0.9665
Error	2	7355.392316	3677.696158		
Corrected Total	3	7363.636305			

R-Square	Coeff Var	Root MSE	U_kg_88_pc_DM Mean
0.001120	13.42607	60.64401	451.6885

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	8.24398897	8.24398897	0.00	0.9665

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	8.24398897	8.24398897	0.00	0.9665

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	449.7624441	50.73842046	8.86	0.0125
month	1.2840552	27.12082653	0.05	0.9665

Tr_form=C50Opellet

The GLM Procedure

Dependent Variable: pc_Om_as_is

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.6979833	0.6979833	0.00	0.9550
Error	2	344.0309141	172.0154571		
Corrected Total	3	344.7288975			

R-Square	Coeff Var	Root MSE	pc_Om_as_is Mean
0.002025	13.35767	13.11547	98.18681

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	0.69798334	0.69798334	0.00	0.9550

Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	0.69798334	0.69798334	0.00	0.9550

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	97.62637363	10.97318641	8.90	0.0124
month	0.37362637	5.86541485	0.06	0.9550

Tr_form=C50Opellet

The GLM Procedure

Dependent Variable: pc_Om_88_pc_DM

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.3880504	0.3880504	0.00	0.9665
Error	2	346.2235617	173.1117809		
Corrected Total	3	346.6116122			

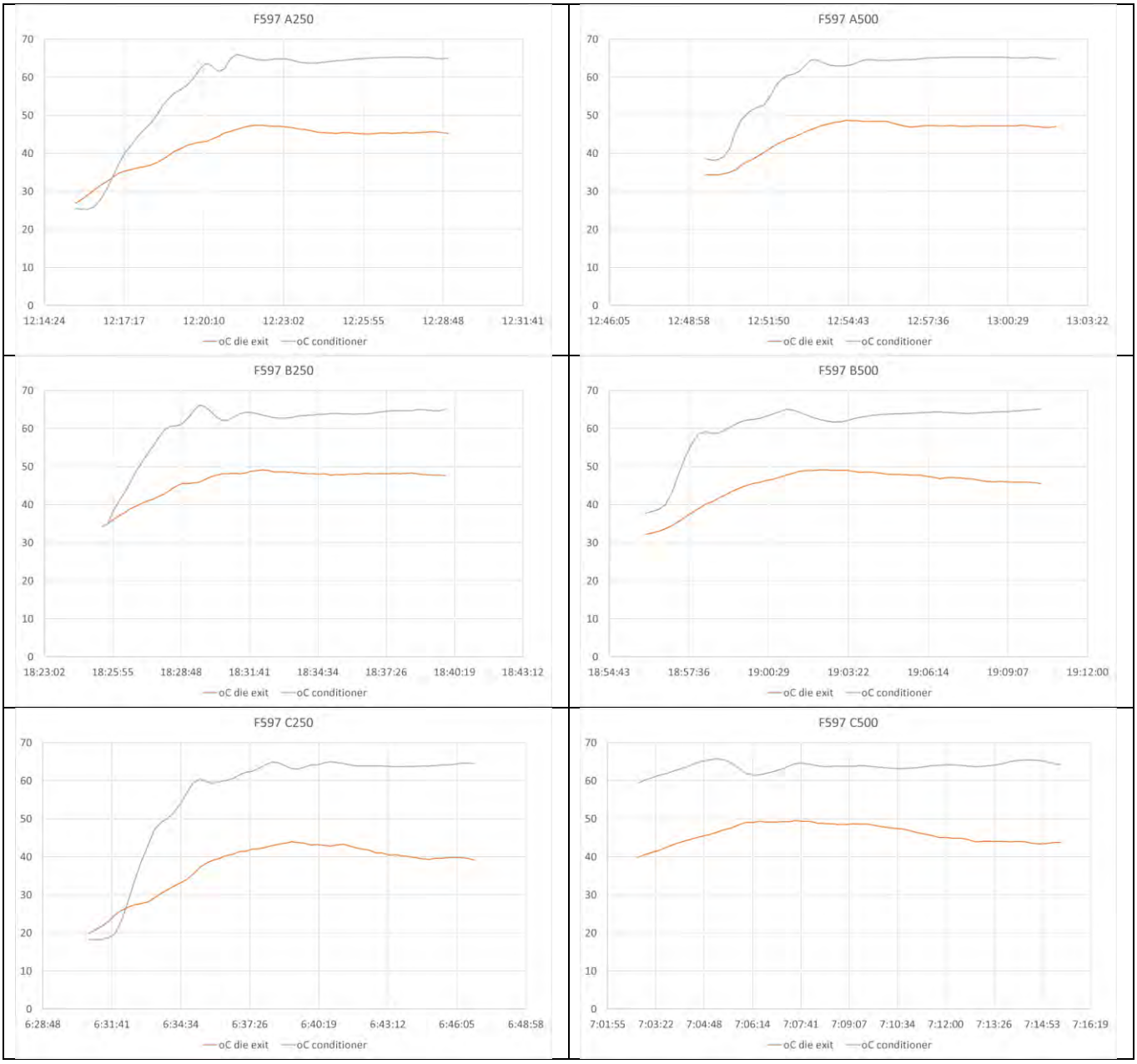
R-Square	Coeff Var	Root MSE	pc_Om_88_pc_DM Mean
0.001120	13.42607	13.15720	97.99738

Source	DF	Type I SS	Mean Square	F Value	Pr > F
month	1	0.38805044	0.38805044	0.00	0.9665

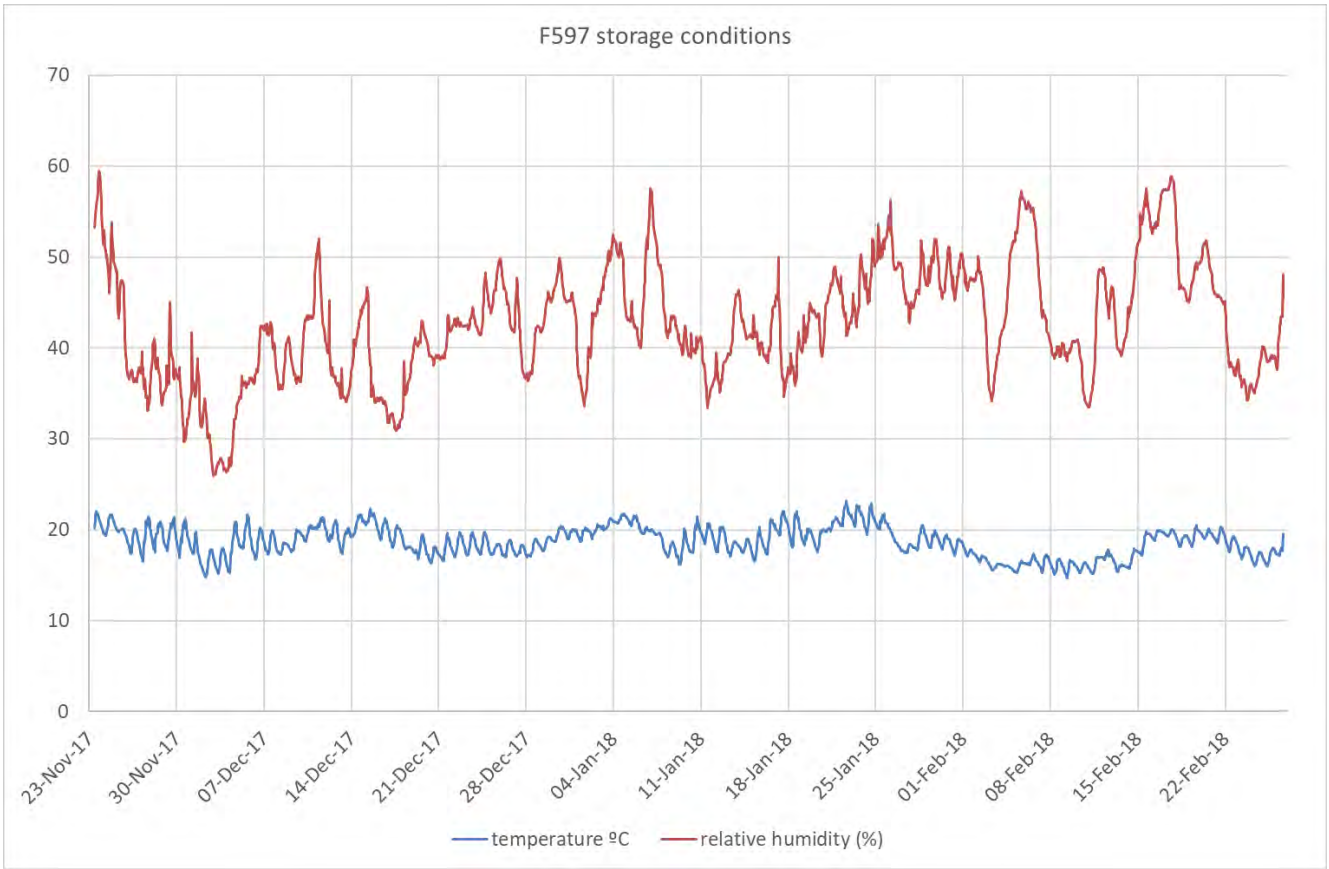
Source	DF	Type III SS	Mean Square	F Value	Pr > F
month	1	0.38805044	0.38805044	0.00	0.9665

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	97.57949751	11.00809914	8.86	0.0125
month	0.27858587	5.88407649	0.05	0.9665

Appendix 6 – Temperature profile in the conditioner during pelleting



Appendix 7 – Temperature and relative humidity during storage of stability samples



Appendix 20: Homogeneity Evaluation of CIBENZA® PHYTAVERSE® G10 Phytase Enzyme in Feed

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

Table of contents

1 Summary

The objective of this study was to evaluate the Homogeneity of CIBENZA® PHYTAVERSE® G10 phytase enzyme in feeds.

For each batch, the homogeneity of the test article was determined by measuring phytase activity in 10 subsamples taken at different location points of the mixer (mash) or at bagging (pelleted).

Results are presented next in Summary Table 1.

Summary Table 1. Homogeneity of CIBENZA® PHYTAVERSE® G10 phytase enzyme in feeds

		U/kg as is						U/kg 88% DM					
		N	Mean	CV	StdDev	Max	Min	N	Mean	CV	StdDev	Max	Min
Tr	form												
A250	mash	10	321				(b) (4)	10	323				(b) (4)
	pellet	10	295					10	298				
B250	mash	10	310					10	311				
	pellet	10	306					10	308				
C250	mash	10	292					10	294				
	pellet	10	269					10	273				

† One phytase unit is the amount of enzyme that releases 1 µmol of inorganic phosphate from phytate per minute under the conditions of the assay (Phytate concentration: 5.0 mM, pH: 5.5, Temperature: 37°C, Incubation time: 30 min) (ISO 30024:2009). Normal analytical variation for the method is 10%.

According the results of the present homogeneity study in feeds, CIBENZA® PHYTAVERSE® G10 phytase enzyme:

- Presented good mixing homogeneity (CV ~7 to 15%), actual CVs below to 2× the CV of the method itself (10%) for all 3 batches tested both in mash and pelleted form

(b) (4)

(b) (4), (b) (6)

3 Study title and unique study code

Homogeneity evaluation of CIBENZA® PHYTAVERSE® G10 phytase enzyme in feed.

Unique study code: F598

4 Study objective

To evaluate the homogeneity of three batches of CIBENZA® PHYTAVERSE® G10 phytase enzyme in mash and pelleted feeds.

5 Study location

(b) (4)

6 Important dates & duration of the study

Date of feeds manufacture: 23rd and 24th November 2017
Duration of study: 2 days at feed mill, 7th December 2017 end of analysis

7 Test products

Table 1. Details of test product

Code	Product	Provider	Lot nº Manufacture Date	Active substance	Activity (U/g)†	
					Guaranteed	Analysed
A	CIBENZA® PHYTAVERSE® G10 Phytase Enzyme	Novus International, Inc.	Lot: P23941 Made: 08 October 2014	6-phytase	10,000	13,951
B	CIBENZA® PHYTAVERSE® G10 Phytase Enzyme	Novus International, Inc.	Lot: P26641 Made: 08 October 2014	6-phytase	10,000	13,742
C	CIBENZA® PHYTAVERSE® G10 Phytase Enzyme	Novus International, Inc.	Lot: RO15271001 Made: 28 September 2015	6-phytase	10,000	13,522

† One phytase unit is the amount of enzyme that releases 1 µmol of inorganic phosphate from phytate per minute under the conditions of the assay (Phytate concentration: 5.0 mM, pH: 5.5, Temperature: 37°C, Incubation time: 30 min) (ISO 30024:2009). Normal analytical variation for the method is 10%.

8 Key study personnel

Study Director: (b) (6), (b) (4)

External Study Monitor: Drew Lichtenstein, Ph.D. Research Manager, Specialty Products, Novus International, Inc., 20 Research Park Dr., St. Charles, MO 63304, United States of America, Tel: +1 314 453-7793, E-mail: drew.lichtenstein@novusint.com

Study Sponsors: 1) Gavin Bowman, Director, Global Regulatory Affairs, Novus International, 20 Research Park Dr., St. Charles, MO 63304, United States of America Tel: +1 636 926 7402, E-mail: gavin.bowman@novusint.com

2) Roxanna Van Dorn, Sr. Regulatory Affairs Specialist, Phone: +1 858 431-8590, Mobile: +1-858-349-7339, Fax: +1-973-307-2549, E-mail: roxanna.vandorn@basf.com. Postal Address: BASF Enzymes LLC, 3550 John Hopkins Court, San Diego, CA 92121 United States

Feed mill & supervision of diet manufacture: (b) (6), (b) (4)

Feed analysis (DM and CIBENZA® PHYTAVERSE® G10 phytase enzyme): (b) (6), (b) (4)

Optional/back-up facility for feed analysis (DM and CIBENZA® PHYTAVERSE® G10 phytase enzyme): Drew Lichtenstein, Novus International, Inc., 20 Research Park Drive, Saint Charles, MO, 63304; United States of America.

9 Material and methods

9.1 Experimental treatments

Number of treated and control groups: Corn/soya based diet was used for homogeneity purposes.

CIBENZA® PHYTAVERSE® G10 phytase enzyme from each batch was added in serial mixing steps to the mash feed to provide 250 and 500 U/kg feed as detailed in Table 2, that was later pelleted.

Table 2. Experimental Treatments

Treatment	Product	CIBENZA® PHYTAVERSE® G10 phytase enzyme		
		U/kg feed	mg/kg feed†	g to add to 200 kg feed†
A250	CIBENZA® PHYTAVERSE® G10 phytase enzyme batch P23941	250	(b) (4)	
A500		500		
B250	CIBENZA® PHYTAVERSE® G10 phytase enzyme batch P26641	250		
B500		500		
C250	CIBENZA® PHYTAVERSE® G10 phytase enzyme batch RO15271001	250		
C500		500		

† inclusion based on actual activity of each batch

9.2 Treatment application

CIBENZA® PHYTAVERSE® G10 phytase enzyme was mixed with a fraction of 10 kg soya in serial mixing steps, mash feed was then produced and later pelleted.

9.3 Detailed study design

Figure 1. Basic study design

For each batch and dose of enzyme:

The homogeneity of the test article in the mash and pelleted feeds was determined by measuring phytase activity in:

- 10 subsamples taken at different places of the mixer for mash feed
- 10 subsamples taken at different times at bagging for pelleted feed

The amount of endogenous phytase in blank feed has been determined in other studies being values below the level of quantitation.

Feeds were produced as follows:

- Firstly, a fraction of 10 kg soya from the feed was mixed in serial mixing steps with the corresponding amount of CIBENZA® PHYTAVERSE® G10 phytase enzyme depending on actual activity of each batch as detailed in Table 2.
- Secondly, a 200 kg batch of mash feed was produced by including the 10 kg soya containing CIBENZA® PHYTAVERSE® G10 phytase enzyme prepared as described above.
- Mash feed was then pelleted and bagged.

9.4 Feed composition

Feeds did not contain any enzymes, antibiotics or any other growth promoters. The ingredients, premix and the calculated and actual analyses of the diets are presented in Table 3 to Table 5.

Table 3. Composition (g/kg) of the basal diet

Corn	577
Soybean meal 48%	373
Fat blend	13.69
Dicalcium phosphate	6.81
Calcium carbonate	12.12
Methionine Hydroxy Analogue	1.75
Premix Min-Vit	10.00
Sodium chloride	1.94
L-lysine HCL	2.91
L-threonine	0.65

Table 4. Composition of vitamin-mineral premix			
	Units	per kg of vitamin-mineral premix	when premix added at 10 kg/ton feed, results in the following values per kg of feed
Vitamins, provitamins and similar			
(b) (4)	IU	1 000 000	(b) (4)
	IU	350 000	
	mg	3 000	
	mg	210	
	mg	855	
	mg	470	
	mg	5	
	mg	300	
	mg	2 000	
	mg	1 520	
	mg	6 710	
	mg	150	
	mg	25	
	mg	70 000	
	mg	6 500	
	mg	150	
	mg	1 500	
	mg	8 000	
	mg	8 500	
	mg	20	
	g	50	
	g	150	
	mg	5 000	
	ate	up to 1 kg	

Table 5. Calculated analyses of the basal diet (g/kg)	
Metabolizable Energy kcal/kg	2864
Dry Matter	868
Ash	58
Crude Fiber	27
Ether Extract	41
Crude Protein	227
Ca	9.6
P	5.0
Dig lysine	14.1
Dig SAA	9.4
Dig threonine	8.4

9.5 Feeds manufacture

All the process is automated and controlled by a computer provided with software from (b) (4) so that the incorporation of ingredients and the functioning of the equipment is regulated and recorded by the software. The addition of manual ingredients (vitamins, amino acids and oligo minerals, as well as test products) is made by means of a bar code system.

Feed ingredients were ground through a 40HP hammer mill (Rosal VRE-40) with a horizontal axis and a 3 mm sieve, provided with an automatic feeder.

The feed mixer was a 500 L Rosal mixer with a double horizontal ribbon, which is sufficient for 200 to 250 kg of feed. The amount of feed prepared was 200 kg per treatment. Fat was added by means of a dosing device provided by three nozzles (b) (4) The mixing time was 6 min. The calculated amount of product for each CIBENZA® PHYTAVERSE® G10 phytase enzyme batch and dose (Table 2) was manually premixed with the corresponding vitamin-mineral premix and amino acids and then added to 10 kg of soya in a commercial mixer and mixed for 6 minutes. Then this premix was incorporated into the final mixture and mixed for 6 minutes.

Mash feed was then pelleted in a pelleting press (MABRIK PVR-40) provided with a die of 280 mm of internal diameter with holes of 3×36 mm. The compression group consists of 2 rollers. The feeder is of stainless steel of progressive opening and is moved by a reducing engine. The conditioner is of stainless steel with adjustable blades, prepared for the reception of water and steam. The steam generator has a manometer to reduce the pressure to 2.5-3 kg/cm^2 and a flux regulator valve. Pelleting is automatically regulated by the software of the system which adjusts the temperature of the mash feed at the end of the conditioner (approximately 30 to 40 seconds of conditioning time). The pelletization temperature was adjusted to a mean temperature of 65ºC and the actual maximum temperature was 66.2ºC. Temperatures were recorded at fixed intervals (i.e. 10 seconds) in the outlet of the conditioner and outlet of die. The vertical cooler (MABRIK, S.A) works by air aspiration provided by a 7.5 HP turbine.

9.5.1 Short description of the process

Under (b) (4)

9.6 Feeds samples at manufacture

For each CIBENZA® PHYTAVERSE® G10 phytase enzyme batch:

- 10 grab samples of mash feed (~1.1 kg each) were taken from several points of the mixer. From these 10 grab mash feed samples:
  - Triplicate (NOVUS, (b) (4) backup) (b) (4)
- 10 grab samples of pelleted feed (~1.1 kg each) were taken at bagging. From these 10 grab pelleted feed samples:
  - Triplicate (NOVUS, (b) (4) backup) (b) (4))

Homogeneity samples were placed in zip-lock plastic bags labelled with the unique study code (F598), treatment code (A250 / A500 / B250 / B500 / C250 / C500), feed form (mash / pellet), date of manufacture and the analysis required (DM, phytase activity).

9.7 Feed sampling plan

Table 6. Sampling plan				
Treatment	Feed form	n at sampling	Final Samples	
			NOVUS	(b) (4)
A250	mash	10 × ~1.1 kg	10 × 250g	(b) (4)
	pellet	10 × ~1.1 kg	10 × 250g	
A500	mash	10 × ~1.1 kg	10 × 250g	
	pellet	10 × ~1.1 kg	10 × 250g	
B250	mash	10 × ~1.1 kg	10 × 250g	
	pellet	10 × ~1.1 kg	10 × 250g	
B500	mash	10 × ~1.1 kg	10 × 250g	
	pellet	10 × ~1.1 kg	10 × 250g	
C250	mash	10 × ~1.1 kg	10 × 250g	
	pellet	10 × ~1.1 kg	10 × 250g	
C500	mash	10 × ~1.1 kg	10 × 250g	
	pellet	10 × ~1.1 kg	10 × 250g	

For homogeneity analysis, A250, B250 and C250 samples were analyzed in IRTA's lab within 10 working days after production of the feeds containing CIBENZA® PHYTAVERSE® G10 phytase enzyme; the A500, B500 and C500 homogeneity samples were kept frozen serving as back up samples. The 250 U/kg samples were refrigerated (4°C) until tested to make sure they reflected accurate activity values at the time the feed was manufactured. One set of samples was dispatched to NOVUS (Reus, Spain) as backup samples. A second set of samples was sent to (b) (4) lab for analysis. A third set of samples was sent (b) (4) lab for storage as backup samples.

9.8 Statistics

For each CIBENZA® PHYTAVERSE® G10 phytase enzyme batch:

- Homogeneity: Mean CIBENZA® PHYTAVERSE® G10 phytase enzyme activity (arithmetic mean) and variation (standard deviation) was used to express the result as a unique value described as the coefficient of variation.

Calculations:

$$\%CV = \frac{s}{\bar{y}} \times 100$$

$$\bar{y} = \frac{\Sigma y_i}{n}$$

$$s = \sqrt{s^2}$$

$$s^2 = \frac{\Sigma (y_i - \bar{y})^2}{n-1}$$

where:

%CV= coefficient of variation

Σ= summation

s= standard deviation

y_i= individual result from each sample

s^2= variance

n= total number of samples

$\bar{y}$ = mean

10 Results

The results are summarized in Table 7 and Table 8. Values from proximate analysis were within expected ranges.

Table 7. Analyzed values of experimental diets

Sample	Dry matter (%)	Crude protein (%)	Ether extract (%)	Ash (%)
A250 pellet	87.2	22.8	4.1	5.5
A500 pellet	87.2	22.9	4.0	5.5
B250 pellet	87.2	23.0	4.0	5.5
B500 pellet	87.1	23.0	3.9	5.4
C250 pellet	86.7	23.2	3.8	5.4
C500 pellet	86.9	23.0	3.6	5.5

Table 8. Homogeneity of CIBENZA® PHYTAVERSE® G10 phytase enzyme in feeds

		U/kg as is						U/kg 88% DM					
		N	Mean	CV	StdDev	Max	Min	N	Mean	CV	StdDev	Max	Min
Tr	form												
A250	mash	10	321	[redacted]			(b) (4)	10	323	[redacted]			(b) (4)
	pellet	10	295					10	298				
B250	mash	10	310					10	311				
	pellet	10	306					10	308				
C250	mash	10	292					10	294				
	pellet	10	269					10	273				

† One phytase unit is the amount of enzyme that releases 1 µmol of inorganic phosphate from phytate per minute under the conditions of the assay (Phytate concentration: 5.0 mM, pH: 5.5, Temperature: 37°C, Incubation time: 30 min) (ISO 30024:2009). Normal analytical variation for the method is 10%.

11 Discussion

Dry matter was quite similar among samples (87.3%±0.7) and the correction for constant DM (88%) did not change the results of the coefficients of variation for homogeneity. Mean phytase activity ranged from 292 to 321 U/kg (as-is) for mash feeds and from 269 to 306 U/kg (as-is) for pelleted feeds. Considering mash and pellets for each enzyme batch, the average activities were: 308 U/kg (as-is) for both A250 and B250, and 281 U/kg (as-is) for C250.

The overall, homogeneity of mixing for the three CIBENZA® PHYTAVERSE® G10 phytase enzyme batches tested expressed as Coefficients of Variation were on average 9%, 12% and 10% when standardized at 88% DM content. The average CV values for mash feeds were 12% and that for pelleted feeds was 9%. Individually per enzyme batch and feed form, the CV values were 8%, 15% and 12% for mash A250, B250 and C250 respectively, and 11%, 8% and 8% for pelleted A250, B250 and C250 respectively. CV of homogeneity slightly increased by pelleting for A250, while it decreased for B250 and C250. All these small variations are considered within the expected fluctuations due to the method variability itself.

All these CVs of the homogeneity were close to 1× and always <1.5× the CV of the normal analytical variation of the method itself (normal analytical CV is 10%), and therefore the CVs of the homogeneity are considered good ($CV<2\times_{analytical}CV$).

Per the protocol, back up samples of A500, B500, and C500 were not tested, because the lowest inclusion rate of 250 U/kg demonstrated good homogeneity.

12 Conclusions

According the results of the present homogeneity study in feeds, CIBENZA® PHYTAVERSE® G10 phytase enzyme:

- Presented good mixing homogeneity (CV ~7 to 15%), actual CVs below to 2× the CV of the method itself (10%) for all 3 batches tested, and both in mash and pelleted form.

13 References

ISO 30024:2009. Animal feeding stuffs – Determination of phytase activity

SAS Institute Inc. 2012. Base SAS® 9.4 Guide to Information Maps. Cary, NC: SAS Institute Inc.

Statutory Instrument 1999 No. 1663. The Feeding Stuffs (Sampling and Analysis) Regulation 1999.

14 List of Appendices

Appendix 1 - Curricula vitae of Study Director & Study Monitor
Appendix 2 - Certificate of analysis of CIBENZA® PHYTAVERSE® G10 phytase enzyme used (3 batches)
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Appendix 6 – Temperature profile in the conditioner during pelleting

Appendix 1- *Curricula vitae* of Study Director & Study Monitor

Study Director:

(b) (6)

Study Monitor:
Name: Drew Lichtenstein
Qualifications: B.S. Biochemistry (Michigan State University 1982), PhD Biochemistry (University of Wisconsin-Madison 1990)
Present Position: Research Manager, Specialty Products, Novus International
Experience: Over 35 years research experience in biochemistry and cell biology; more than 8 years of experience in animal feed enzymes.

Appendix 2 - Certificate of analysis of CIBENZA® PHYTAVERSE® G10 phytase enzyme used (3 batches)

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Certificate of Analysis

CIBENZA® PHYTAVERSE™ G10 Phytase Enzyme (Test Article VR005)

Lot number: P23941
Date of Manufacture: October 8, 2014

Specification	Specification Limit	Test Result
Appearance	White to Beige granules	(b) (4)
Bulk Density-untapped (g/cm^3)	≥ 0.50	
Particle size (mesh)	<2% on 20 mesh <10% thru 140 mesh	
Activity (U/g)	NLT 10,000	
Loss on Drying (%)	≤ 12	
Lead (mg/kg)	≤ 5	
Arsenic (mg/kg)	< 2	
Cadmium (mg/kg)	< 0.5	
Mercury (mg/kg)	< 0.5	
Total Plate Count (cfu/g)	≤ 50,000	
Total Coliform (MPN/g)	≤ 30	
E. coli (/25g)	Absent	
Salmonella (/25g)	Absent	
Yeast and Mold (CFU/g)	Run and Record	
Staphylococcus aureus (/g)	Absent	
Production Organism (CFU/g)	Absent	
Antibiotic Activity (Zone of Inhibition)	Absent	
Mycotoxin		
Aflatoxin B1	NMT 1.0 ppb	
Aflatoxin B2	NMT 1.0 ppb	
Aflatoxin G1	NMT 1.0 ppb	
Aflatoxin G2	NMT 1.0 ppb	
Fumonisin B1	NMT 0.1 ppm	
Fumonisin B2	NMT 0.1 ppm	
Fumonisin B3	NMT 0.1 ppm	
Ochratoxin A	NMT 2.0 ppb	
Deoxynivalenol	NMT 3.0 ppm	
Acetyldeoxynivalenol	NMT 0.8 ppm	
Fusarenon X	NMT 0.4 ppm	
Nivalenol	NMT 0.6 ppm	
T-2 Toxin	NMT 0.2 ppm	
HT-2 Toxin	NMT 0.2 ppm	
Neosolaniol	NMT 0.4 ppm	
Diacetoxyscirpenol	NMT 0.4 ppm	
Zearalenone	NMT 43.1 ppb	
Sterigmatocystin	NMT 200 ppb	

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PCBs	10,000 pg/g	(b) (4)
Dioxins	1 pg/g	

* Production organism testing was performed on the enzyme concentrate used to produce this dry product.
** Results of retesting performed in March 2017.

Approved by: _____ Date: March 29, 2017

Mark Burcin
Sr. Manager, QA/QC

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Certificate of Analysis

CIBENZA® PHYTAVERSE™ G10 Phytase Enzyme (Test Article VR005)

Lot number: P26641
Date of Manufacture: October 8, 2014

Specification	Specification Limit	Test Result
Appearance	White to Beige granules	(b) (4)
Bulk Density-untapped (g/cm^3)	≥ 0.50	
Particle size (mesh)	<2% on 20 mesh <10% thru 140 mesh	
Activity (U/g)	NLT 10,000	
Loss on Drying (%)	**≤ 12**	
Lead (mg/kg)	≤ 5	
Arsenic (mg/kg)	< 2	
Cadmium (mg/kg)	< 0.5	
Mercury (mg/kg)	< 0.5	
Total Plate Count (cfu/g)	≤ 50,000	
Total Coliform (cfu/g)	≤ 30	
E. coli (/25g)	Absent	
Salmonella (/25g)	Absent	
Yeast and Mold (CFU/g)	Run and Record	
Staphylococcus aureus (/g)	Absent	
Production Organism (CFU/g)	Absent	
Antibiotic Activity (Zone of Inhibition)	Absent	
Mycotoxin		
Aflatoxin B1	NMT 1.0 ppb	
Aflatoxin B2	NMT 1.0 ppb	
Aflatoxin G1	NMT 1.0 ppb	
Aflatoxin G2	NMT 1.0 ppb	
Fumonisin B1	NMT 0.1 ppm	
Fumonisin B2	NMT 0.1 ppm	
Fumonisin B3	NMT 0.1 ppm	
Ochratoxin A	NMT 2.0 ppb	
Deoxynivalenol	NMT 3.0 ppm	
Acetyldeoxynivalenol	NMT 0.8 ppm	
Fusarenon X	NMT 0.4 ppm	
Nivalenol	NMT 0.6 ppm	
T-2 Toxin	NMT 0.2 ppm	
HT-2 Toxin	NMT 0.2 ppm	
Neosolaniol	NMT 0.4 ppm	
Diacetoxyscirpenol	NMT 0.4 ppm	
Zearalenone	NMT 43.1 ppb	

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Certificate of Analysis

Sterigmatocystin	NMT 200 ppb	(b) (4)
PCBs	10,000 pg/g	
Dioxins	1 pg/g	

* Production organism testing was performed on the enzyme concentrate used to produce this dry product.
** Results of retesting performed in March 2017.

Approved by: _____ Date: March 29, 2017

Mark Burcin
Sr. Manager, QA/QC

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Certificate of Analysis

CIBENZA® PHYTAVERSE™ G10 Phytase Enzyme (Test Article VR005)

Lot number: RO15271001
Date of Manufacture: September 28, 2015

Specification	Specification Limit	Test Result
Appearance	White to Beige granules	(b) (4)
Bulk Density-untapped (g/cm^3)	≥ 0.50	
Particle size (mesh)	<2% on 20 mesh <10% thru 140 mesh	
Activity (U/g)	NLT 10,000	
Loss on Drying (%)	≤ 12	
Lead (mg/kg)	≤ 5	
Arsenic (mg/kg)	< 2	
Cadmium (mg/kg)	< 0.5	
Mercury (mg/kg)	< 0.5	
Total Plate Count (cfu/g)	≤ 50,000	
Total Coliform (cfu/g)	≤ 30	
E. coli (/25g)	Absent	
Salmonella (/25g)	Absent	
Yeast and Mold (CFU/g)	Run and Record	
Staphylococcus aureus (/g)	Absent	
Production Organism (CFU/g)	Absent	
Antibiotic Activity (Zone of Inhibition)	Absent	
Mycotoxin		
Aflatoxin B1	NMT 1.0 ppb	
Aflatoxin B2	NMT 1.0 ppb	
Aflatoxin G1	NMT 1.0 ppb	
Aflatoxin G2	NMT 1.0 ppb	
Fumonisin B1	NMT 0.1 ppm	
Fumonisin B2	NMT 0.1 ppm	
Fumonisin B3	NMT 0.1 ppm	
Ochratoxin A	NMT 2.0 ppb	
Deoxynivalenol	NMT 3.0 ppm	
Acetyldeoxynivalenol	NMT 0.8 ppm	
Fusarenon X	NMT 0.4 ppm	
Nivalenol	NMT 0.6 ppm	
T-2 Toxin	NMT 0.2 ppm	
HT-2 Toxin	NMT 0.2 ppm	
Neosolaniol	NMT 0.4 ppm	
Diacetoxyscirpenol	NMT 0.4 ppm	
Zearalenone	NMT 43.1 ppb	

BASF Enzymes LLC
3550 John Hopkins Court
San Diego, CA 92121
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www.basf.us

Certificate of Analysis

Sterigmatocystin	NMT 200 ppb	(b) (4)
PCBs	10,000 pg/g	
Dioxins	1 pg/g	

* Production organism testing was performed on the enzyme concentrate used to produce this dry product.
** Results of retesting performed in March 2017.

Approved by: _____ Date: March 29, 2017

Mark Burcin
Sr. Manager, QA/QC

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San Diego, CA 92121
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Appendix 3 - Relevant laboratory reports

CERTIFICATE OF ANALYSIS

Company:	Novus International Inc and BASF Enzymes LLC
Type of sample:	F598 feeds
Laboratory ref. :	172032 to 172037 172012 to 172021 172022 to 172031 172059 to 172068 172069 to 172078 172087 to 172096 172097 to 172106
Reception date:	28th November 2017
Analysis starting date:	1st December 2017
Analysis finishing date:	22th March 2018

Sample description: See Results section

Analysis performed:

- Moisture -dry matter- by oven drying –method 2 (SOP 0602-L-10001) (AOAC, 2000)
- Nitrogen -crude protein- by combustion -Dumas method (SOP 0602-L-10118) (AOAC, 2000)
- Ether extract on a Soxtec system -method 3B (SOP 0602-L-10003) (AOAC, 2000)
- Ash after muffle furnace incineration -method 12 (SOP 0602-L-10002) (AOAC, 2000)
- Phytase (SOP 0602-L-10143; ISO 30024:2009. Animal feeding stuffs – Determination of phytase activity.)

Results:

LAB. REF.	SAMPLE DESCRIPTION	CRUDE PROTEIN (%)	ETHER EXTRACT (%)	ASH (%)
172032	A250 pellet stab 0 mes	(b) (4)		
172033	A500 pellet stab 0 mes			
172034	B250 pellet stab 0 mes			
172035	B500 pellet stab 0 mes			
172036	C250 pellet stab 0 mes			
172037	C500 pellet stab 0 mes			

LAB. REF.	DESCRIPTION	PHYTASE U/kg	DM %
172012	A250 MASH 1	349	(b) (4)
172013	A250 MASH 2	270	
172014	A250 MASH 3	305	
172015	A250 MASH 4	328	
172016	A250 MASH 5	315	
172017	A250 MASH 6	354	
172018	A250 MASH 7	303	
172019	A250 MASH 8	333	
172020	A250 MASH 9	319	
172021	A250 MASH 10	335	
172022	A250 PELLET 1	303	
172023	A250 PELLET 2	310	
172024	A250 PELLET 3	268	
172025	A250 PELLET 4	295	
172026	A250 PELLET 5	310	
172027	A250 PELLET 6	342	
172028	A250 PELLET 7	321	
172029	A250 PELLET 8	235	
172030	A250 PELLET 9	268	
172031	A250 PELLET 10	295	

LAB. REF.	DESCRIPTION	PHYTASE U/kg	DM %
172059	B250 MASH 1	242	(b) (4)
172060	B250 MASH 2	292	
172061	B250 MASH 3	287	
172062	B250 MASH 4	286	
172063	B250 MASH 5	341	
172064	B250 MASH 6	284	
172065	B250 MASH 7	332	
172066	B250 MASH 8	402	
172067	B250 MASH 9	279	
172068	B250 MASH 10	350	
172069	B250 PELLET 1	259	
172070	B250 PELLET 2	323	
172071	B250 PELLET 3	314	
172072	B250 PELLET 4	313	
172073	B250 PELLET 5	306	
172074	B250 PELLET 6	320	
172075	B250 PELLET 7	350	
172076	B250 PELLET 8	303	
172077	B250 PELLET 9	283	
172078	B250 PELLET 10	285	

LAB. REF.	DESCRIPTION	PHYTASE U/kg	DM %
172087	C250 MASH 1	318	(b) (4)
172088	C250 MASH 2	270	
172089	C250 MASH 3	323	
172090	C250 MASH 4	251	
172091	C250 MASH 5	291	
172092	C250 MASH 6	320	
172093	C250 MASH 7	289	
172094	C250 MASH 8	224	
172095	C250 MASH 9	312	
172096	C250 MASH 10	326	
172097	C250 PELLET 1	269	
172098	C250 PELLET 2	269	
172099	C250 PELLET 3	281	
172100	C250 PELLET 4	298	
172101	C250 PELLET 5	246	
172102	C250 PELLET 6	301	
172103	C250 PELLET 7	241	
172104	C250 PELLET 8	244	
172105	C250 PELLET 9	269	
172106	C250 PELLET 10	276	

(b) (4), (b) (6)

Signature: (b) (6)

Date: 26TH MARCH 2018

Appendix 4 - Raw data

Obs	enzyme	form	homogeneity	Trt	lab_ref	dose	Tr	location	U_kg_as_is	DM_p	U_kg_88_p_DM
1	A	mash	yes	A250mash	172012	250	A250	1	349		(b) (4)
2	A	mash	yes	A250mash	172013	250	A250	2	270		
3	A	mash	yes	A250mash	172014	250	A250	3	305		
4	A	mash	yes	A250mash	172015	250	A250	4	328		
5	A	mash	yes	A250mash	172016	250	A250	5	315		
6	A	mash	yes	A250mash	172017	250	A250	6	354		
7	A	mash	yes	A250mash	172018	250	A250	7	303		
8	A	mash	yes	A250mash	172019	250	A250	8	333		
9	A	mash	yes	A250mash	172020	250	A250	9	319		
10	A	mash	yes	A250mash	172021	250	A250	10	335		
11	A	pellet	yes	A250pellet	172022	250	A250	1	303		
12	A	pellet	yes	A250pellet	172023	250	A250	2	310		
13	A	pellet	yes	A250pellet	172024	250	A250	3	268		
14	A	pellet	yes	A250pellet	172025	250	A250	4	295		
15	A	pellet	yes	A250pellet	172026	250	A250	5	310		
16	A	pellet	yes	A250pellet	172027	250	A250	6	342		
17	A	pellet	yes	A250pellet	172028	250	A250	7	321		
18	A	pellet	yes	A250pellet	172029	250	A250	8	235		
19	A	pellet	yes	A250pellet	172030	250	A250	9	268		
20	A	pellet	yes	A250pellet	172031	250	A250	10	295		
21	B	mash	yes	B250mash	172059	250	B250	1	242		
22	B	mash	yes	B250mash	172060	250	B250	2	292		
23	B	mash	yes	B250mash	172061	250	B250	3	287		
24	B	mash	yes	B250mash	172062	250	B250	4	286		
25	B	mash	yes	B250mash	172063	250	B250	5	341		
26	B	mash	yes	B250mash	172064	250	B250	6	284		
27	B	mash	yes	B250mash	172065	250	B250	7	332		
28	B	mash	yes	B250mash	172066	250	B250	8	402		
29	B	mash	yes	B250mash	172067	250	B250	9	279		
30	B	mash	yes	B250mash	172068	250	B250	10	350		
31	B	pellet	yes	B250pellet	172069	250	B250	1	259		
32	B	pellet	yes	B250pellet	172070	250	B250	2	323		
33	B	pellet	yes	B250pellet	172071	250	B250	3	314		
34	B	pellet	yes	B250pellet	172072	250	B250	4	313		
35	B	pellet	yes	B250pellet	172073	250	B250	5	306		
36	B	pellet	yes	B250pellet	172074	250	B250	6	320		
37	B	pellet	yes	B250pellet	172075	250	B250	7	350		
38	B	pellet	yes	B250pellet	172076	250	B250	8	303		
39	B	pellet	yes	B250pellet	172077	250	B250	9	283		
40	B	pellet	yes	B250pellet	172078	250	B250	10	285		
41	C	mash	yes	C250mash	172087	250	C250	1	318		
42	C	mash	yes	C250mash	172088	250	C250	2	270		
43	C	mash	yes	C250mash	172089	250	C250	3	323		
44	C	mash	yes	C250mash	172090	250	C250	4	251		
45	C	mash	yes	C250mash	172091	250	C250	5	291		
46	C	mash	yes	C250mash	172092	250	C250	6	320		
47	C	mash	yes	C250mash	172093	250	C250	7	289		
48	C	mash	yes	C250mash	172094	250	C250	8	224		
49	C	mash	yes	C250mash	172095	250	C250	9	312		
50	C	mash	yes	C250mash	172096	250	C250	10	326		
51	C	pellet	yes	C250pellet	172097	250	C250	1	269		
52	C	pellet	yes	C250pellet	172098	250	C250	2	269		
53	C	pellet	yes	C250pellet	172099	250	C250	3	281		
54	C	pellet	yes	C250pellet	172100	250	C250	4	298		
55	C	pellet	yes	C250pellet	172101	250	C250	5	246		
56	C	pellet	yes	C250pellet	172102	250	C250	6	301		
57	C	pellet	yes	C250pellet	172103	250	C250	7	241		
58	C	pellet	yes	C250pellet	172104	250	C250	8	244		
59	C	pellet	yes	C250pellet	172105	250	C250	9	269		
60	C	pellet	yes	C250pellet	172106	250	C250	10	276		

Appendix 5 - Statistical printouts

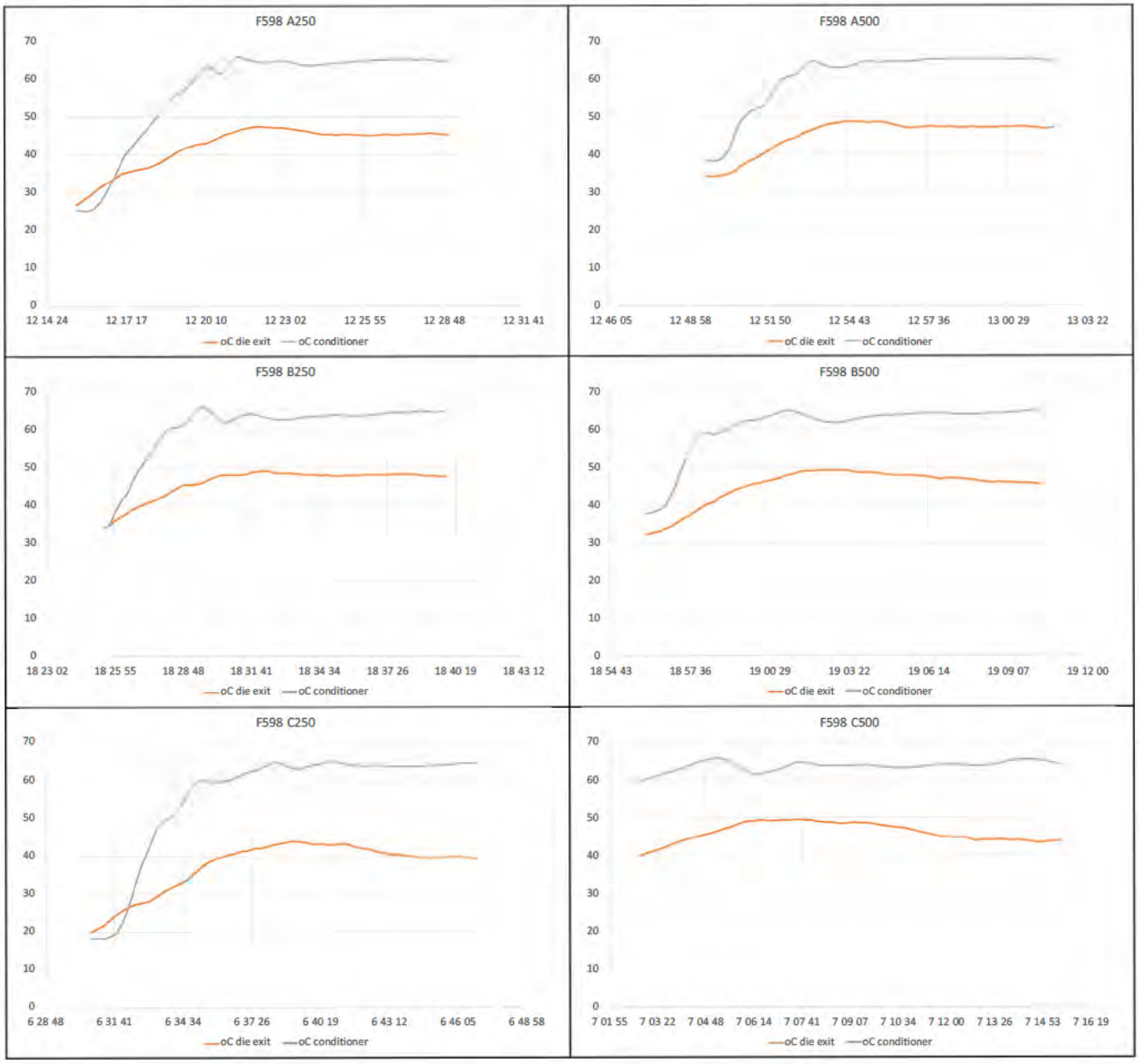
Obs	enzyme	form	homogeneity	Trt	lab_ref	dose	Tr	location	U_kg_as_is	U_kg_DM_p	U_kg_88_p_DM
1	A	mash	yes	A250mash	172012	250	A250	1	349		
2	A	mash	yes	A250mash	172013	250	A250	2	270		
3	A	mash	yes	A250mash	172014	250	A250	3	305		
4	A	mash	yes	A250mash	172015	250	A250	4	328		
5	A	mash	yes	A250mash	172016	250	A250	5	315		
6	A	mash	yes	A250mash	172017	250	A250	6	354		
7	A	mash	yes	A250mash	172018	250	A250	7	303		
8	A	mash	yes	A250mash	172019	250	A250	8	333		
9	A	mash	yes	A250mash	172020	250	A250	9	319		
10	A	mash	yes	A250mash	172021	250	A250	10	335		
11	A	pellet	yes	A250pellet	172022	250	A250	1	303		
12	A	pellet	yes	A250pellet	172023	250	A250	2	310		
13	A	pellet	yes	A250pellet	172024	250	A250	3	268		
14	A	pellet	yes	A250pellet	172025	250	A250	4	295		
15	A	pellet	yes	A250pellet	172026	250	A250	5	310		
16	A	pellet	yes	A250pellet	172027	250	A250	6	342		
17	A	pellet	yes	A250pellet	172028	250	A250	7	321		
18	A	pellet	yes	A250pellet	172029	250	A250	8	235		
19	A	pellet	yes	A250pellet	172030	250	A250	9	268		
20	A	pellet	yes	A250pellet	172031	250	A250	10	295		
21	B	mash	yes	B250mash	172059	250	B250	1	242		
22	B	mash	yes	B250mash	172060	250	B250	2	292		
23	B	mash	yes	B250mash	172061	250	B250	3	287		
24	B	mash	yes	B250mash	172062	250	B250	4	286		
25	B	mash	yes	B250mash	172063	250	B250	5	341		
26	B	mash	yes	B250mash	172064	250	B250	6	284		
27	B	mash	yes	B250mash	172065	250	B250	7	332		
28	B	mash	yes	B250mash	172066	250	B250	8	402		
29	B	mash	yes	B250mash	172067	250	B250	9	279		
30	B	mash	yes	B250mash	172068	250	B250	10	350		
31	B	pellet	yes	B250pellet	172069	250	B250	1	259		
32	B	pellet	yes	B250pellet	172070	250	B250	2	323		
33	B	pellet	yes	B250pellet	172071	250	B250	3	314		
34	B	pellet	yes	B250pellet	172072	250	B250	4	313		
35	B	pellet	yes	B250pellet	172073	250	B250	5	306		
36	B	pellet	yes	B250pellet	172074	250	B250	6	320		
37	B	pellet	yes	B250pellet	172075	250	B250	7	350		
38	B	pellet	yes	B250pellet	172076	250	B250	8	303		
39	B	pellet	yes	B250pellet	172077	250	B250	9	283		
40	B	pellet	yes	B250pellet	172078	250	B250	10	285		
41	C	mash	yes	C250mash	172087	250	C250	1	318		
42	C	mash	yes	C250mash	172088	250	C250	2	270		
43	C	mash	yes	C250mash	172089	250	C250	3	323		
44	C	mash	yes	C250mash	172090	250	C250	4	251		
45	C	mash	yes	C250mash	172091	250	C250	5	291		
46	C	mash	yes	C250mash	172092	250	C250	6	320		
47	C	mash	yes	C250mash	172093	250	C250	7	289		
48	C	mash	yes	C250mash	172094	250	C250	8	224		
49	C	mash	yes	C250mash	172095	250	C250	9	312		
50	C	mash	yes	C250mash	172096	250	C250	10	326		
51	C	pellet	yes	C250pellet	172097	250	C250	1	269		
52	C	pellet	yes	C250pellet	172098	250	C250	2	269		
53	C	pellet	yes	C250pellet	172099	250	C250	3	281		
54	C	pellet	yes	C250pellet	172100	250	C250	4	298		
55	C	pellet	yes	C250pellet	172101	250	C250	5	246		
56	C	pellet	yes	C250pellet	172102	250	C250	6	301		
57	C	pellet	yes	C250pellet	172103	250	C250	7	241		
58	C	pellet	yes	C250pellet	172104	250	C250	8	244		
59	C	pellet	yes	C250pellet	172105	250	C250	9	269		
60	C	pellet	yes	C250pellet	172106	250	C250	10	276		

(b) (4)

		U_kg_as_is						U_kg_88_p_DM					
		N	Mean	CV	StdDev	Max	Min	N	Mean	CV	StdDev	Max	Min
Tr	form												
A250	mash	10	321	7.7	24.7	354	270	10	323	7.7	24.8	356	272
	pellet	10	295	10.4	30.7	342	235	10	298	10.5	31.4	346	237
B250	mash	10	310	14.9	46.2	402	242	10	311	14.9	46.4	404	243
	pellet	10	306	8.2	25.2	350	259	10	308	8.3	25.5	352	260
C250	mash	10	292	11.8	34.6	326	224	10	294	11.9	35.0	327	225
	pellet	10	269	7.8	21.1	301	241	10	273	7.8	21.3	306	244

		U_kg_as_is		U_kg_as_i-s_CV		U_kg_88_p-_DM		U_kg_88_p-_DM_CV		DM_p		DM_p_CV	
		N	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean
Tr													
A250		2	308	2	9	2	310	2	9	2	87	2	0
B250		2	308	2	12	2	310	2	12	2	87	2	0
C250		2	281	2	10	2	283	2	10	2	87	2	0
form													
mash		3	308	3	11	3	309	3	12	3	88	3	0
pellet		3	290	3	9	3	293	3	9	3	87	3	0
Tr	form		(b) (4)		(b) (4)		(b) (4)		(b) (4)		(b) (4)		
A250	mash	1		1		1		1		1		1	0
	pellet	1		1		1		1		1		1	0
B250	mash	1		1		1		1		1		1	0
	pellet	1		1		1		1		1		1	0
C250	mash	1		1		1		1		1		1	0
	pellet	1		1		1		1		1		1	0
All		6		6		6		6		6		6	0

Appendix 6 – Temperature profile in the conditioner during pelleting



Appendix 21: Evaluation of the Thermostability of CIBENZA® PHYTAVERSE® G10 Phytase Enzyme in Pelleted Poultry Feed

(b) (4)

(b) (4)

Evaluation of the thermostability of CIBENZA® PHYTAVERSE® G10 Phytase Enzyme in pelleted poultry feed. Study 01-17.

(b) (4)

Title:
Evaluation of the thermostability of CIBENZA® PHYTAVERSE® G10 Phytase Enzyme in pelleted poultry feed. Study 01-17.

Prepared for: Novus International, Inc.

Prepared by:
(b) (4)

March 2018
Author: (b) (4), (b) (6)

(b) (4), (b) (6)

Study Sponsors		Study Monitor
Gavin Bowman, Director, Global Regulatory Affairs, Novus International 20 Research Park Dr., St. Charles, MO 63304, United States of America	Roxanna Van Dom Senior Regulatory Affairs Specialist BASF Enzymes LLC 3550 John Hopkins Court, San Diego, CA 92121, United States of America	Drew Lichtenstein Research Manager, Specialty Products Novus International 20 Research Park Dr., St. Charles, MO 63304, United States of America

Table of Contents

1. Summary

The objective of this study was to evaluate the thermostability of 6-phytase enzyme activity in feeds supplemented with CIBENZA® PHYTAVERSE® G10 phytase enzyme pelleted at varying temperatures during feed production.

Results (Summary Table 1) from this thermostability (pelleting) trial with CIBENZA® PHYTAVERSE® G10 phytase enzyme showed that:

- Both the 250 U/kg and 500 U/kg doses retained over 80% of the initial (mash) phytase activity at pelleting temperatures up to 80°C with a conditioning time of approximately 60 seconds.
- Overall average phytase activity at the 85°C pelleting temperature with a conditioning time (also known as retention time) of approximately 60 seconds was greater than 85% of the initial phytase activity.
- The 250 U/kg dose retained more than 80% of the initial phytase activity at pelleting temperatures up to 88°C with a conditioning time of approximately 60 seconds.
- Overall average of the phytase activity was reduced to approximately 60% when the pelleting temperature was 90°C with a conditioning time of approximately 60 seconds.

Summary Table 1 Phytase Activity in Pelleted Feed

Condition	Dose Averages		Overall Average
	250 U/kg	500 U/kg	
Mash	(b) (4)		100%
Pellet 65°C			99%
Pellet 75°C			93%
Pellet 80°C			91%
Pellet 85°C			87%
Pellet 88°C			76%
Pellet 90°C			61%

2. Study Locations

2.1. Feed Production

(b) (4)

2.2. Enzyme Testing Laboratory

Samples for testing were sent to (b) (4)

(b) (4)

3. Identification of Test Article

3.1. Classification

Feed enzyme preparation used in poultry and swine feed.

3.2. Source Organism

Pseudomonas fluorescens BD50104.

3.3. Trade Name

CIBENZA® PHYTAVERSE® G10 phytase enzyme.

3.4. Active Ingredient

6-phytase (E.C. 3.1.3.26)
Guaranteed Activity: 10,000 U/g

3.5. Safety/Hazard Warning

See SDS.

3.6. Lot Number and Manufacturing Date

Batch/Lot no.: P26641
Manufacturing date: October 8, 2014

Batch/Lot no.: P23941
Manufacturing date: October 8, 2014

Batch/Lot no.: RO15271001
Manufacturing date: September 28, 2015

3.7. Manufacturing Date of Feeds

Feed was manufactured on October 8, 2017 at the [redacted](b)(4). Phytase analyses were performed at [redacted](b)(4) in [redacted](b)(4) beginning on October 30, 2017.

4. Start Date

October 8, 2017

5. End Date

October 9, 2017

6. Purpose

The purpose of this study is to evaluate the thermostability of 6-phytase enzyme activity in feeds supplemented with CIBENZA® PHYTAVERSE® G10 phytase enzyme at pelleted at varying temperatures during feed production. The resulting data will be used to establish recommended

temperature conditions when pelleting feed containing CIBENZA® PHYTAVERSE® G10 phytase enzyme.

7. Scope

The phytase activity data was collected and evaluated according to established international standards.

8. Experimental Design

The CIBENZA® PHYTAVERSE® G10 phytase enzyme Test Article lots chosen for the thermostability study (i.e. Lot n°: P26641, P23941, and RO15271001) comply with all applicable specifications and Standard Operating Procedures (SOPs).

The vitamin and mineral premix was tested for phytase activity prior to being used in manufacturing the feed to ensure that it was negative for phytase activity.

8.1. Composition of the Mash Feed

The composition of the feed is found in Table 1. The composition of the vitamin-mineral premix is found in Table 2. The calculated content of the vitamin-mineral premix is found in Table 3. The calculated nutritional content of each diet is listed in Table 4.

Table 1. Composition of Poultry Feed

Ingredients	Inclusion, %
Corn	66.50
Soybean meal 48	26.20
Soy oil	4.40
Salt	0.40
Limestone	1.00
Mono calcium Phosphate	0.50
Vitamin premix	1.00
CIBENZA® PHYTAVERSE® G10 Phytase Enzyme	-
Total	100.0

Table 2. Composition of vitamin-mineral premix

Component	Units	per kg of vitamin-mineral premix	per kg Feed at 10 kg/MT
Vitamins, provitamins and similar			
(b) (4)	IU	1000000	(b) (4)
	IU	350000	
	mg	3000	
	mg	210	
	mg	855	
	mg	470	
	mg	5	
	mg	300	
	mg	2000	
	mg	1520	
	mg	6710	
	mg	150	
	mg	25	
	mg	70000	
	mg	6500	
	mg	150	

Table 2. Composition of vitamin-mineral premix			
Component	Units	per kg of vitamin-mineral premix	per kg Feed at 10 kg/MT
(b) (4)	mg	1500	(b) (4)
	mg	8000	
	mg	8500	
	mg	20	
	g	50	
	g	150	
	mg	5000	
		up to 1 kg	

Table 3. Calculated Analyses of Vitamin/Mineral Premix		
Calculated Analyses	Units	Results
Crude protein	%	2.024
Ash	%	81.660
Dry matter	%	83.680
Calcium	%	20.000
Phosphorous	%	0.053
Sodium	%	6.776
Chloride	%	6.078
Potassium	%	0.011
Sulphur	%	0.599

Table 4. Calculated Analyses of Experimental Rations		
Calculated Analyses	Units	Broiler diet
Crude protein	%	17.78
Crude fat	%	7.45
Crude fiber	%	2.11
Calcium	%	0.75
Phosphorus-Total	%	0.46
Phosphorus available	%	0.22
Sodium	%	0.23
Chloride	%	0.23
Potassium	%	0.79
Met	%	0.28
Cys	%	0.30
Me+Cys	%	0.58
Lys	%	0.91
His	%	0.48
Tryp	%	0.20
Thr	%	0.67
Arg	%	0.29
Iso	%	0.73
Leu	%	1.61
Phe	%	0.87
Tyr	%	0.66
Val	%	0.84
Phe+Tyr	%	1.95
Linoleic acid	%	2.57
Sulphur	%	0.16
Magnesium	%	0.18
Betaine	%	0.15

8.2. Preparation of the Mash Feed

The feed mash was prepared in two batches of 1200 kg. The raw materials were weighted and milled on a Champion hammer mill mounted with a 3.5 mm screen. Afterwards, the feed mash, soy oil, and vitamin premix were mixed in a 2500-liter horizontal mixer for 10 minutes. Feed with no added CIBENZA® PHYTAVERSE® G10 phytase enzyme is referred to as Batch D (control). Feed that was supplemented with CIBENZA® PHYTAVERSE® G10 phytase enzyme is referred as Batch A, B, or C depending on the batch of CIBENZA® PHYTAVERSE® G10 phytase enzyme added to the feed (Table 6). CIBENZA® PHYTAVERSE® G10 phytase enzyme was supplemented into feed at a dose of 250 U/kg (Batches A2, B2, and C2) or 500 U/kg (Batches A5, B5, and C5) (Table 6).

8.3. Mixing of enzyme

A premix of 10 kg mash feed and enzyme (250 or 500 U/kg) (see Table 6) was mixed in a 70-liter ribbon mixer for 10 minutes. The premix and 310 kg mash feed (320 kg and no premix for control sample) was transferred to a 600-liter horizontal mixer (Figure 1) and mixed for 10 minutes. Upon completion of the mixing cycle, 10 samples of 200 g each were taken from the mash feed of each batch from different locations within the mixer. The 10 samples were mixed together thoroughly to create a single composite sample, which was then split with a sample riffler until two 1 kg portions were obtained. The portions were labeled with the form ("M" for mash), Batch number (D, A2, A5, etc.) and portion designation ("A" or "B) (see Table 7 for a detailed breakout of samples collected and their labeling). One portion was subjected to testing (see Section 13), while the other portion was held in reserve under refrigerated conditions at (b) (4) as a backup. Remaining feed was pelleted as described below.

8.4. Pelletizing

9. Test Product

Table 5 Details of test product

Code	Product	Provider	Lot nº Manufacture Date	Active substance	Activity (U/g)†	
					Guaranteed	Analyzed
A	CIBENZA® PHYTAVERSE® G10 Phytase Enzyme	Novus International, Inc.	Lot: P23941 Made: 08 October 2014	6-phytase	10,000	13,951
B	CIBENZA® PHYTAVERSE® G10 Phytase Enzyme	Novus International, Inc.	Lot: P26641 Made: 08 October 2014	6-phytase	10,000	13,742
C	CIBENZA® PHYTAVERSE® G10 Phytase Enzyme	Novus International, Inc.	Lot: RO15271001 Made: 28 September 2015	6-phytase	10,000	13,522

10. Calculation Section

The minimum phytase activity in CIBENZA® PHYTAVERSE® G10 phytase enzyme is 10,000 U/g.

In this study, a sufficient quantity from each of the three lots of CIBENZA® PHYTAVERSE® G10

phytase enzyme was incorporated into two batches of feed so that the low and high target concentration of 6-phytase in the final feed was present at the intended rate of 250 U/kg or at 500 U/kg, respectively.

Calculations for Batch A2 with low inclusion level in feed:

Calculations for Batch A5 with high inclusion level in feed:

Calculations for Batch B2 with low inclusion level in feed:

Calculations for Batch B5 with high inclusion level in feed:

Calculations for Batch C2 with low inclusion level in feed:

Calculations for Batch C5 with high inclusion level in feed:

The amount of CIBENZA® PHYTAVERSE® G10 Phytase Enzyme added to each 320 kg batch is summarized in Table 6.

Table 6. Amount of CIBENZA® PHYTAVERSE® G10 Phytase Enzyme added to each diet			
Feed Batch nº	**CIBENZA® PHYTAVERSE® G10 Phytase Enzyme Lot nº**	**Inclusion level**	**CIBENZA® PHYTAVERSE® G10 phytase enzyme added to 320 kg**
Batch D – control	not applicable	0 U/kg	0.00 g
Batch A2 – low inclusion level	P23941	250 U/kg	5.73 g
Batch A5 – high inclusion level	P23941	500 U/kg	11.47 g
Batch B2 – low inclusion level	P26641	250 U/kg	5.82 g
Batch B5 – high inclusion level	P26641	500 U/kg	11.64 g
Batch C2 – low inclusion level	RO15271001	250 U/kg	5.92 g
Batch C5 – high inclusion level	RO15271001	500 U/kg	11.83 g

11. Sample Packaging/Justification for Simulator Bags

11.1. Justification for Simulator Bags

Feed is typically delivered in bulk to storage bins prior to being consumed by the intended animal species. If not delivered in bulk, the feed would be typically packaged in paper bags or a suitable bag that would physically contain the feed but would not offer much protection against moisture or vapor transmission. Approximately 1 kg of feed samples containing CIBENZA® PHYTAVERSE® G10 Phytase Enzyme from each of the three lots were packaged in sample bags which are representative of the ones used for commercial purposes.

11.2. Sample Packaging

In this thermostability study, (b) (4) packaged the feed samples containing each of the three lots of CIBENZA® PHYTAVERSE® G10 phytase enzyme and the control feed into sample bags and closed them. Two portions of mash for each batch of feed (total of 14 portions) and 12 pellet portions for each batch of feed (total 84 portions) were packaged into sample bags. One portion of mash and one portion of pellets at each pelleting condition were shipped to the enzyme testing facility for analysis. Table 7 outlines the details of samples that were collected.

12. Sample Labeling

Bags for the enzyme activity analysis were labeled appropriately for evaluating phytase activity at different pelleting temperatures. The samples were labeled with a unique label containing information relevant to the study and the sample. The following information was placed on each label:

- Study Number
- Compound name and concentration
- CIBENZA® PHYTAVERSE® lot no.
- Feed Form
- Batch Number
- Portion Designation
- Conditioning (Pelleting) Temperature
- Feed Mfg. Date

13. Sampling

13.1. Control Feed without CIBENZA® PHYTAVERSE® G10 phytase enzyme

For the control batch of feed, portion A (1 kg) of the mash and for each pelleting condition was shipped to the testing site for analysis and portion B (1 kg) was stored refrigerated at (b) (4) as backup. Portion A was evaluated for phytase activity, loss on drying, and physical appearance. Four independent 50 g sub-portions were analyzed for phytase activity. The average of all four analyses were used to state the phytase activity in each feed portion. A single sub-portion was analyzed for moisture content. No abnormalities in physical appearance were noted.

The purpose of assaying the feed without added enzyme was to determine whether there is any feed matrix interference in the assay procedure. The phytase activity of all control mash and pellet samples was below the limit of quantification (LOQ) of the method (60 U/kg) for all samples (Table 8).

Proximate analysis was performed on the mash feed including fat, crude fiber, crude protein, phosphorus and calcium. A sample was also analysed for moisture content by loss on drying.

13.2. Samples Containing CIBENZA® PHYTAVERSE® G10 phytase enzyme

For every batch of feed containing CIBENZA® PHYTAVERSE® phytase enzyme, portion A (1 kg) of the mash and each pelleting condition was shipped to the testing site for analysis and portion B (1 kg) was stored refrigerated at (b) (4) as backup. Portion A was evaluated for phytase activity, loss on drying, and physical appearance. Four independent 50 g sub-portions were analyzed for phytase activity. The average of all four analyses was used to state the phytase activity in each feed portion. No abnormalities in physical appearance were noted.

13.3. Overview of Sampling

Table 7 Sampling and Labelling of Feed Portions

CIBENZA® PHYTAVERSE® G10 Lot no.	Batch Number	PHYTAVERSE® G10 Target Dose	Feed Form	Pelleting Temperature	Labeling Nomenclature
-	D	None, Control	Mash	N/A	MDA & MDB
-	D	None, Control	Pellet	65°C	P65DA & P65DB
-	D	None, Control	Pellet	75°C	P75DA & P5D7B
-	D	None, Control	Pellet	80°C	P80DA & P80DB
-	D	None, Control	Pellet	85°C	P85DA & P85DB
-	D	None, Control	Pellet	88°C	P88DA & P88DB
-	D	None, Control	Pellet	90°C	P90DA & P90DB
P23941	A250	250 FTU/ kg feed	Mash	N/A	MA2A & MA2B
P23941	A250	250 FTU/ kg feed	Pellet	65°C	P65A2A & P65A2B
P23941	A250	250 FTU/ kg feed	Pellet	75°C	P75A2A & P75A2B
P23941	A250	250 FTU/ kg feed	Pellet	80°C	P80A2A & P80A2B
P23941	A250	250 FTU/ kg feed	Pellet	85°C	P85A2A & P85A2B
P23941	A250	250 FTU/ kg feed	Pellet	88°C	P88A2A & P88A2B
P23941	A250	250 FTU/ kg feed	Pellet	90°C	P90A2A & P90A2B
P23941	A500	500 FTU/ kg feed	Mash	N/A	MA5A & MA5B
P23941	A500	500 FTU/ kg feed	Pellet	65°C	P65A5A & P65A5B
P23941	A500	500 FTU/ kg feed	Pellet	75°C	P75A5A & P75A5B
P23941	A500	500 FTU/ kg feed	Pellet	80°C	P80A5A & P80A5B
P23941	A500	500 FTU/ kg feed	Pellet	85°C	P85A5A & P85A5B
P23941	A500	500 FTU/ kg feed	Pellet	88°C	P88A5A & P88A5B

Table 7 Sampling and Labelling of Feed Portions					
CIBENZA® PHYTAVERSE® G10 Lot no.	**Batch Number**	**PHYTAVERSE® G10 Target Dose**	**Feed Form**	**Pelleting Temperature**	**Labeling Nomenclature**
P23941	A500	500 FTU/ kg feed	Pellet	90°C	P90A5A & P90A5B
P26641	B250	250 FTU/ kg feed	Mash	N/A	MB2A & MB2B
P26641	B250	250 FTU/ kg feed	Pellet	65°C	P65B2A & P65B2B
P26641	B250	250 FTU/ kg feed	Pellet	75°C	P75B2A & P75B2B
P26641	B250	250 FTU/ kg feed	Pellet	80°C	P80B2A & P80B2B
P26641	B250	250 FTU/ kg feed	Pellet	85°C	P85B2A & P85B2B
P26641	B250	250 FTU/ kg feed	Pellet	88°C	P88B2A & P88B2B
P26641	B250	250 FTU/ kg feed	Pellet	90°C	P90B2A & P90B2B
P26641	B500	500 FTU/ kg feed	Mash	N/A	MB5A & MB5B
P26641	B500	500 FTU/ kg feed	Pellet	65°C	P65B5A & P65B5B
P26641	B500	500 FTU/ kg feed	Pellet	75°C	P75B5A & P75B5B
P26641	B500	500 FTU/ kg feed	Pellet	80°C	P80B5A & P80B5B
P26641	B500	500 FTU/ kg feed	Pellet	85°C	P85B5A & P85B5B
P26641	B500	500 FTU/ kg feed	Pellet	88°C	P88B5A & P88B5B
P26641	B500	500 FTU/ kg feed	Pellet	90°C	P90B5A & P90B5B
RO15271001	C250	250 FTU/ kg feed	Mash	N/A	MC2A & MC2B
RO15271001	C250	250 FTU/ kg feed	Pellet	65°C	P65C2A & P65C2B
RO15271001	C250	250 FTU/ kg feed	Pellet	75°C	P75C2A & P75C2B
RO15271001	C250	250 FTU/ kg feed	Pellet	80°C	P80C2A & P80C2B
RO15271001	C250	250 FTU/ kg feed	Pellet	85°C	P85C2A & P85C2B
RO15271001	C250	250 FTU/ kg feed	Pellet	88°C	P88C2A & P88C2B
RO15271001	C250	250 FTU/ kg feed	Pellet	90°C	P90C2A & P90C2B
RO15271001	C500	500 FTU/ kg feed	Mash	N/A	MC5A & MC5B
RO15271001	C500	500 FTU/ kg feed	Pellet	65°C	P65C5A & P65C5B
RO15271001	C500	500 FTU/ kg feed	Pellet	75°C	P75C5A & P75C5B
RO15271001	C500	500 FTU/ kg feed	Pellet	80°C	P80C5A & P80C5B
RO15271001	C500	500 FTU/ kg feed	Pellet	85°C	P85C5A & P85C5B
RO15271001	C500	500 FTU/ kg feed	Pellet	88°C	P88C5A & P88C5B
RO15271001	C500	500 FTU/ kg feed	Pellet	90°C	P90C5A & P90C5B

14. Analytical Methods

14.1. Phytase enzyme assay method

Mash and pelleted feed were assayed for 6-phytase activity using the validated and verified method ISO 30024 (“Animal feeding stuffs - Determination of phytase activity”).

14.2. Physical appearance

Samples were checked for mold, insect infestation, and other changes by visual inspection and the observations were recorded.

14.3. Loss on Drying

Mash and pelleted feed were analyzed for loss on drying using method AOAC 934.01 (“Loss on Drying [Moisture] at 95-100°C for Feeds”).

15. Phytase Activity Doses

Feed containing three doses were prepared.

- Control diet, (0 units of Phytase activity per kg feed)
- Low inclusion level in feed (250 units of Phytase activity per kg feed).

- High inclusion level in feed (500 units of Phytase activity per kg feed)

16. Additional Test and Acceptance Limits

16.1. Physical Appearance

Material shows no visible presence of mold growth or other situation that renders the sample unacceptable for enzyme thermostability evaluation.

17. Sample Disposal

Backup samples remaining at (b) (4) may be disposed of in an appropriate manner after testing is completed or terminated, and with authorization by the Sponsors or his/her representatives. If authorization for disposal is not received by the storage laboratory within 2 months after the testing is completed, the storage laboratory is to contact the Study Sponsors.

18. Changes to the Protocol

There protocol was amended to change the Novus Sponsor to Gavin Bowman, Director, Global Regulatory Affairs. An amendment to the protocol was issued (Appendix 3).

19. Results

Certificates of Analysis for proximate composition and enzyme activity are found in Appendix 2 .

19.1. Phytase Results

Phytase activity in mash and pelleted samples shown in Table 8 is the average of quadruplicate analyses except where noted in the text (see Section 20). The conditioning time (retention time) for all pelleted feeds was approximately 60 seconds.

Table 8 Phytase Activity in Mash and Pelleted Samples

Lab Reference	Sample Description	Average Phytase Activity (U/kg)	RSD	Percent of Corresponding Mash Activity
171843	D NONE CONTROL MASH MDA	<LOQ	10%	N/A
171844	D NONE CONTROL PELLET 65ºC P65DA	<LOQ	13%	N/A
171845	D NONE CONTROL PELLET 75ºC P75DA	<LOQ	10%	N/A
171851	D NONE CONTROL PELLET 80ºC P80DA	<LOQ	40%	N/A
171852	D NONE CONTROL PELLET 85ºC P85DA	<LOQ	14%	N/A
171853	D NONE CONTROL PELLET 88ºC P88DA	<LOQ	24%	N/A
171854	D NONE CONTROL PELLET 90ºC P90DA	<LOQ	5%	N/A
171831	P23941 A250 MASH MA2A	**320**	**14%**	N/A
171831	P23941 A250 MASH MA2A **(repeat)**	**265**	**9%**	N/A
171832	P23941 A250 PELLET 65ºC P65A2A	237	4%	89%
171833	P23941 A250 PELLET 75ºC P75A2A	275	6%	104%
171834	P23941 A250 PELLET 80ºC P80A2A	284	7%	107%
171835	P23941 A250 PELLET 85ºC P85A2A	211	5%	80%
171836	P23941 A250 PELLET 88ºC P88A2A	211	8%	80%
171842	P23941 A250 PELLET 90ºC P90A2A	114	4%	43%
171859	P23941 A500 MASH MA5A	519	4%	N/A
171860	P23941 A500 PELLET 65ºC P65A5A	476	5%	92%
171861	P23941 A500 PELLET 75ºC P75A5A	448	6%	86%
171862	P23941 A500 PELLET 80ºC P80A5A	424	8%	82%
171869	P23941 A500 PELLET 85ºC P85A5A	444	5%	86%
171864	P23941 A500 PELLET 88ºC P88A5A	369	5%	71%

Table 8 Phytase Activity in Mash and Pelleted Samples				
Lab Reference	**Sample Description**	**Average Phytase Activity (U/kg)**	**RSD**	**Percent of Corresponding Mash Activity**
171865	P23941 A500 PELLET 90ºC P90A5A	287	6%	55%
171866	P26641 B250 MASH MB2A	233	6%	N/A
171867	P26641 B250 PELLET 65ºC P65B2A	281	10%	121%
171868	P26641 B250 PELLET 75ºC P75B2A	233	3%	100%
171886	P26641 B250 PELLET 80ºC P80B2A	218	3%	93%
171887	P26641 B250 PELLET 85ºC P85B2A	244	9%	105%
171888	P26641 B250 PELLET 88ºC P88B2A	199	12%	85%
171889	P26641 B250 PELLET 90ºC P90B2A	174	6%	75%
171909	P26641 B500 MASH MB5A	**435**	5%	N/A
171909	P26641 B500 MASH MB5A **(Repeat)**	**578**	2%	82%
171910	P26641 B500 PELLET 65ºC P65B5A	474	4%	78%
171911	P26641 B500 PELLET 75ºC P75B5A	449	10%	73%
171912	P26641 B500 PELLET 80ºC P80B5A	419	5%	72%
171913	P26641 B500 PELLET 85ºC P85B5A	416	3%	59%
171933	P26641 B500 PELLET 88ºC P88B5A	343	3%	59%
171934	P26641 B500 PELLET 90ºC P90B5A	342	4%	82%
172168	RO15271001 C250 MASH MC2A	251	12%	N/A
172169	RO15271001 C250 PELLET 65ºC P65C2A	259	12%	103%
172170	RO15271001 C250 PELLET 75ºC P75C2A	235	8%	94%
172171	RO15271001 C250 PELLET 80ºC P80C2A	231	6%	92%
172172	RO15271001 C250 PELLET 85ºC P85C2A	243	5%	97%
172191	RO15271001 C250 PELLET 88ºC P88C2A	204	9%	81%
172192	RO15271001 C250 PELLET 90ºC P90C2A	181	5%	72%
172189	RO15271001 C500 MASH MC5A	530	6%	N/A
172190	RO15271001 C500 PELLET 65ºC P65C5A	496	3%	93%
172200	RO15271001 C500 PELLET 75ºC P75C5A	450	2%	85%
172201	RO15271001 C500 PELLET 80ºC P80C5A	470	7%	89%
172202	RO15271001 C500 PELLET 85ºC P85C5A	392	7%	74%
172203	RO15271001 C500 PELLET 88ºC P88C5A	368	4%	69%
172204	RO15271001 C500 PELLET 90ºC P90C5A	291	6%	55%

19.2. Proximate Analysis Results

Values from the proximate analysis were within the expected ranges (Table 9).

Table 9 Proximate Analysis Results	
Analyte	**Value (%)**
Crude protein	17.02
Ether Extract	8.01
Ash	4.47
Crude fiber	1.60
Phosphorus	0.40
Calcium	0.70

19.3. Loss on drying

Loss on drying was measured at 11.9 % resulting in a dry matter content of 88.1%.

20. Discussion

Phytase activity in mash was generally within 7% of the targeted dose (Table 10). The exceptions were feed batches A250 (enzyme batch P23941) and B500 (enzyme batch P26641) where phytase activity was 128% and 87%, respectively, of the dose (Table 8). Therefore, these two samples were retested in quadruplicate. Because the mean phytase activity for

batch A250 was nearly 30% higher than the expected dose, the original quadruplicate analysis was excluded from the results. Phytase activity for batch A250 was 106% (265 U/kg) of the expected dose after being reanalyzed (Table 8). Both sets of analyses (original and repeat) were included for batch B500 because the original analysis was only 13% lower than the expected level. Average activity for B500 mash was 506 U/kg including all eight replicates.

Table 10 Phytase Activity in Mash

Parameter	P23941		P26641		RO15271001	
	A250	A500	B250	B500	C250	C500
Expected (U/kg)	(b) (4)					
Actual (U/kg)						
% of Expected	106%	104%	93%	101%	100%	106%

At pelleting temperatures from 65°C to 80°C, average phytase activity in pellets, as a percentage of the activity measured in the corresponding mash feed, was approximately 100% at the 250 U/kg dose and varied from 93% to 84% for the 500 U/kg dose (Table 11**Error! Reference source not found.**). Average phytase activity in pelleted feed was greater than 80% at a pelleting temperature of 85°C for both doses (Table 11**Error! Reference source not found.**). Recovery of phytase activity for individual enzyme batches ranged from 80% to 105% at 85°C for the 250 dose, while that for the 500 U/kg dose was 74% to 86% (Table 11**Error! Reference source not found.**). The average phytase activity for the 250 U/kg dose was 82% of the corresponding mash sample at 88°C, while that for the high dose was 69% at the same pelleting temperature. The range for the 250 U/kg dose at 88°C was 80% to 85%, while that for the 500 U/kg dose was 68% to 71% (Table 11**Error! Reference source not found.**). Percent activity retained at the 90°C pelleting temperature was relatively constant for the 500 U/kg dose (55%, 55%, and 68%), but varied for the 250 U/kg dose (43%, 72% and 75%) (Table 11). The conditioning time (retention time) for all pelleted feed was approximately 60 seconds

Table 11 Phytase Activity (% from analyzed value in mash)

Condition	P23941		P26641		RO15271001		Dose Averages		Overall Average
	A250	A500	B250	B500	C250	C500	250 U/kg	500 U/Kg	
Mash	100%	100%	100%	100%	100%	100%	100%	100%	100%
Pellet 65°C	(b) (4)								99%
Pellet 75°C									93%
Pellet 80°C									91%
Pellet 85°C									87%
Pellet 88°C									76%
Pellet 90°C									61%

A trend for lower percent activity retained was noticed when comparing the 500 U/kg dose to the 250 U/kg dose (Table 11). An investigation conducted to determine root cause for this observation was inconclusive. Analysis of the raw data showed there were no clerical or calculation errors. Similarly, a technical review of the raw absorbance data and standard curve data (slopes and intercepts) did not reveal a source for the observed phytase activity results. Another possible explanation for this observation is that the percent recovery values were biased low by higher-than-expected phytase activity in the corresponding mash samples. However, this explanation was ruled out as well because there no substantial difference between the 250 U/kg and 500 U/kg doses with respect to the percent of expected phytase activity measured in the mash (Table 10).

21. Conclusions

Results from this thermostability (pelleting) trial with CIBENZA® PHYTAVERSE® G10 phytase enzyme showed that:

- Both the 250 U/kg and 500 U/kg doses retained over 80% of the initial (mash) phytase activity at pelleting temperatures up to 80°C with a conditioning time (also known as retention time) of approximately 60 seconds.
- Overall average phytase activity at the 85°C pelleting temperature with a conditioning time of approximately 60 seconds, 85% of the initial phytase activity was retained.
- The 250 U/kg dose retained more than 80% of the initial phytase activity at pelleting temperatures up to 88°C.
- Overall average of the phytase activity was reduced to approximately 60% when the pelleting temperature was 90°C with a conditioning time of approximately 60 seconds.

22. Key Study Personnel

Study Director: (b) (4), (b) (6)

External Study Monitor: Drew Lichtenstein, Ph.D. Research Manager, Specialty Products, Novus International, Inc., 20 Research Park Dr., St. Charles, MO 63304, United States of America, Tel: +1 314 453-7793, E-mail: drew.lichtenstein@novusint.com

Study Sponsors: 1) Gavin Bowman, Director, Global Regulatory Affairs, Phone: +1 636 926 7402, E-mail: gavin.bowman@novusint.com. Postal Address: Novus International, 20 Research Park Drive, St. Charles, MO 63304 United States.

2) Roxanna Van Dorn, Sr. Regulatory Affairs Specialist, Phone: +1 858 431-8590, Mobile: +1-858-349-7339, Fax: +1-973-307-2549, E-mail: roxanna.vandorn@basf.com. Postal Address: BASF Enzymes LLC, 3550 John Hopkins Court, San Diego, CA 92121 United States

Feed mill supervisor: (b) (4), (b) (6)

Enzyme analysis laboratory manager: (b) (4), (b) (6)

Appendix 1 Trial Documents

FIRM: Novus International Inc.

Trial No. 1, Control **Study no. 01-17** **Mixture: Corn based diet**

Test product: Cibenza Phytaverse G10 **Inclusion rate: 0 g/ 320 kg diet**

Pellet Press: Simon Heesen Die: Ø 3x 35 mm

Test at: [redacted] (b) (4) - Pilot Plant [redacted] (b) (6), (b) (4) Date: October 9, 2017

Composition of mixture:	**Moisture in mash: 11.9 %**
66.5 % Corn	1.0 % Vitamins/minerals, T&V
26.2 % Hipro Soya 48	0.5 % Monocalcium Phosfate
4.4 % Soya Oil	0.4 % Salt
1.0 % Limestone	

Hammer milling, mm	3,5
Fat addition	Horizontal mixer
Fat temperature, °C	20
Steam addition	Cascade Mixer (155 rpm)
Steam pressure, bars	2

Test Temperature.		65	75	80	85	88	90
Meal temperature, °C		64.8-65.3	74.8-74.9	79.9-80.0	84.9-85.1	87.9-88.1	90.0-90.2
Capacity, kg/h		300	300	300	300	300	300
Pellet press, amp.		9	9	9	9	9	9
Meal,kg. + enzyme, g	0						
Meal, kg	320						
Mixing time,							
Meal. + enzyme, min.	0						
Feed mixture, min.	10						
Cooling time, min.	15-17	15-17	15-17	15-17	15-17	15-17	15-17
Pelleting time, min		0-10.0	10.0-21	21-31.5	31.5-42	42-52.5	52.5-63
Sample collect, min		8.5-10	19.5-21	30-31.5	40.5-42	51-52.5	61.5-63

Cold meal 21 c. samples: Blank, no enzyme, Meal from Horizontal mixer + Pellets of each temp. (65-75-80-88-90 C), 1,0 kg of each
Cascade mixer: Filling 5500- 5570 g feed Retention time 64-65 sec
Open steam valve: no. 1
Pallet adjustment no: 2

FIRM: Novus International Inc.

Trial No. 2, Batch A250 **Study no. 01-17** **Mixture: Corn based diet**

Test product: Cibenza Phytaverse G10 Lot P23941 Inclusion rate: 5.73 g/ 320 kg diet

Pellet Press: Simon Heesen Die: Ø 3x 35 mm

Test at: [redacted] (b) (4) - Pilot Plant [redacted] (b) (4), (b) (6) Date: October 9, 2017

Composition of mixture:	**Moisture in mash: 11.9 %**
66.5 % Corn	1.0 % Vitamins/minerals, T&V
26.2 % Hipro Soya 48	0.5 % Monocalcium Phosfate
4.4 % Soya Oil	0.4 % Salt
1.0 % Limestone	

Hammer milling, mm	3,5
Fat addition	Horizontal mixer
Fat temperature, °C	20
Steam addition	Cascade Mixer (155 rpm)
Steam pressure, bars	2

Test Temperature.		65	75	80	85	88	90
Meal temperature, °C		65.0-66.0	74.9-75.1	79.9-80.1	85.0-85.2	87.9-88.0	90.1-90.2
Capacity, kg/h		300	300	300	300	300	300
Pellet press, amp.		9	9	9	9	9	9
Meal,kg. + enzyme, g	10+5,74						
Meal, kg	310						
Mixing time,							
Meal. + enzyme, min.	10						
Feed mixture, min.	10						
Cooling time, min.	15-17	15-17	15-17	15-17	15-17	15-17	15-17
Pelleting time, min		0-10.5	10.5-21	21-31.5	31.5-42	42-52.5	52.5-63
Sample collect, min		9-10.5	19.5-21	30-31.5	40.5-42	51-52.5	61.5-63

Cold meal 21 c. samples: Blank, no enzyme, Meal from Horizontal mixer + Pellets of each temp. (65-75-80-88-90 C), 1,0 kg of each
Cascade mixer: Filling 5500- 5570 g feed Retention time 64-65 sec
Open steam valve: no. 1
Pallet adjustment no: 2

FIRM: Novus International Inc.

Trial No. 3, Batch A500 **Study no. 01-17** **Mixture: Corn based diet**

Test product: Cibenza Phytaverse G10 Lot P23941 Inclusion rate: 11.48 g/ 320 kg diet

Pellet Press: Simon Heesen Die: Ø 3x 35 mm

Test at: [redacted] (b) (4) - Pilot Plant [redacted] (b) (4), (b) (6) Date: October 9, 2017

Composition of mixture:	**Moisture in mash: 11.9 %**
66.5 % Corn	1.0 % Vitamins/minerals, T&V
26.2 % Hipro Soya 48	0.5 % Monocalcium Phosfate
4.4 % Soya Oil	0.4 % Salt
1.0 % Limestone	

Hammer milling, mm	3,5
Fat addition	Horizontal mixer
Fat temperature, °C	20
Steam addition	Cascade Mixer (155 rpm)
Steam pressure, bars	2

Test Temperature.		65	75	80	85	88	90
Meal temperature, °C		65.4-65.7	74.9-75.2	79.7-80.0	84.9-85.0	88.0-88.2	90.0-90.1
Capacity, kg/h		300	300	300	300	300	300
Pellet press, amp.		9	9	9	9	9	9
Meal,kg. + enzyme, g	10+11.48						
Meal, kg	310						
Mixing time,							
Meal. + enzyme, min.	10						
Feed mixture, min.	10						
Cooling time, min.	15-17	15-17	15-17	15-17	15-17	15-17	15-17
Pelleting time, min		0-10.5	10.5-21	21-31.5	31.5-42	42-52.5	52.5-63
Sample collect, min		9-10.5	19.5-21	30-31.5	40.5-42	51-52.5	61.5-63

Cold meal 21 c. samples: Blank, no enzyme, Meal from Horizontal mixer + Pellets of each temp. (65-75-80-88-90 C), 1,0 kg of each
Cascade mixer: Filling 5500- 5570 g feed Retention time 64-65 sec
Open steam valve: no. 1
Pallet adjustment no: 2

FIRM: Novus International Inc.

Trial No. 4, Batch B250 **Study no. 01-17** **Mixture: Corn based diet**

Test product: Cibenza Phytaverse G10 Lot P26641 **Inclusion rate: 5.83 g/ 320 kg diet**

Pellet Press: Simon Heesen Die: Ø 3x 35 mm

Test at: [redacted] (b) (4) - Pilot Plant [redacted] (b) (4), (b) (6) Date: October 9, 2017

Composition of mixture:	**Moisture in mash: 11.9 %**
66.5 % Corn	1.0 % Vitamins/minerals, T&V
26.2 % Hipro Soya 48	0.5 % Monocalcium Phosfate
4.4 % Soya Oil	0.4 % Salt
1.0 % Limestone	

Hammer milling, mm	3,5
Fat addition	Horizontal mixer
Fat temperature, °C	20
Steam addition	Cascade Mixer (155 rpm)
Steam pressure, bars	2

Test Temperaturs.		65	75	80	85	88	90
Meal temperature, °C		65.0-65.3	74.9-75.2	80.0-80.2	84.9-85.2	87.5-87.8	90.2
Capacity, kg/h		300	300	300	300	300	300
Pellet press, amp.		9	9	9	9	9	9
Meal, kg + enzyme, g	10+5.83						
Meal, kg	310						
Mixing time,							
Meal. + enzyme, min.	10						
Feed mixture, min.	10						
Cooling time, min.	15-17	15-17	15-17	15-17	15-17	15-17	15-17
Pelleting time, min		0-10.5	10.5-21	21-31.5	31.5-42	42-52.5	52.5-63
Sample collect, min		9-10.5	19.5-21	30-31.5	40.5-42	51-52.5	61.5-63

Cold meal 21 c. samples: Blank, no enzyme, Meal from Horizontal mixer + Pellets of each temp. (65-75-80-88-90 C), 1,0 kg of each
Cascade mixer: Filling 5500- 5570 g feed Retention time 64-65 sec
Open steam valve: no. 1
Pallet adjustment no: 2

FIRM: Novus International Inc.

Trial No. 5, Batch B500 **Study no. 01-17** **Mixture: Corn based diet**

Test product: Cibenza Phytaverse G10 Lot P26641 Inclusion rate: 11.64 g/ 320 kg diet

Pellet Press: Simon Heesen Die: Ø 3x 35 mm

Test at: [redacted] (b) (4) - Pilot Plant [redacted] (b) (4), (b) (6) Date: October 9, 2017

Composition of mixture:	**Moisture in mash: 11.9 %**
66.5 % Corn	1.0 % Vitamins/minerals, T&V
26.2 % Hipro Soya 48	0.5 % Monocalcium Phosfate
4.4 % Soya Oil	0.4 % Salt
1.0 % Limestone	

Hammer milling, mm	3,5
Fat addition	Horizontal mixer
Fat temperature, °C	20
Steam addition	Cascade Mixer (155 rpm)
Steam pressure, bars	2

Test Temperaturs.		65	75	80	85	88	90
Meal temperature, °C		64.5-65.0	75.0-75.1	79.9-80.0	85.0-85.3	87.9-88.0	90.1-90.2
Capacity, kg/h		300	300	300	300	300	300
Pellet press, amp.		9	9	9	9	9	9
Meal, kg + enzyme, g	10+11.64						
Meal, kg	310						
Mixing time,							
Meal. + enzyme, min.	10						
Feed mixture, min.	10						
Cooling time, min.	15-17	15-17	15-17	15-17	15-17	15-17	15-17
Pelleting time, min		0-10.5	10.5-21	21-31.5	31.5-42	42-52.5	52.5-63
Sample collect, min		9-10.5	19.5-21	30-31.5	40.5-42	51-52.5	61.5-63

Cold meal 21 c. samples: Blank, no enzyme, Meal from Horizontal mixer + Pellets of each temp. (65-75-80-88-90 C), 1,0 kg of each
Cascade mixer: Filling 5500- 5570 g feed Retention time 64-65 sec
Open steam valve: no. 1
Pallet adjustment no: 2

FIRM: Novus International Inc.

Trial No. 6, Batch C250 **Study no. 01-17** **Mixture: Corn based diet**

Test product: Cibenza Phytaverse G10 Lot R015271001 Inclusion rate: 5.94 g/ 320 kg diet

Pellet Press: Simon Heesen Die: Ø 3x 35 mm

Test at: [redacted] (b) (4) - Pilot Plant [redacted] (b) (4), (b) (6) Date: October 9, 2017

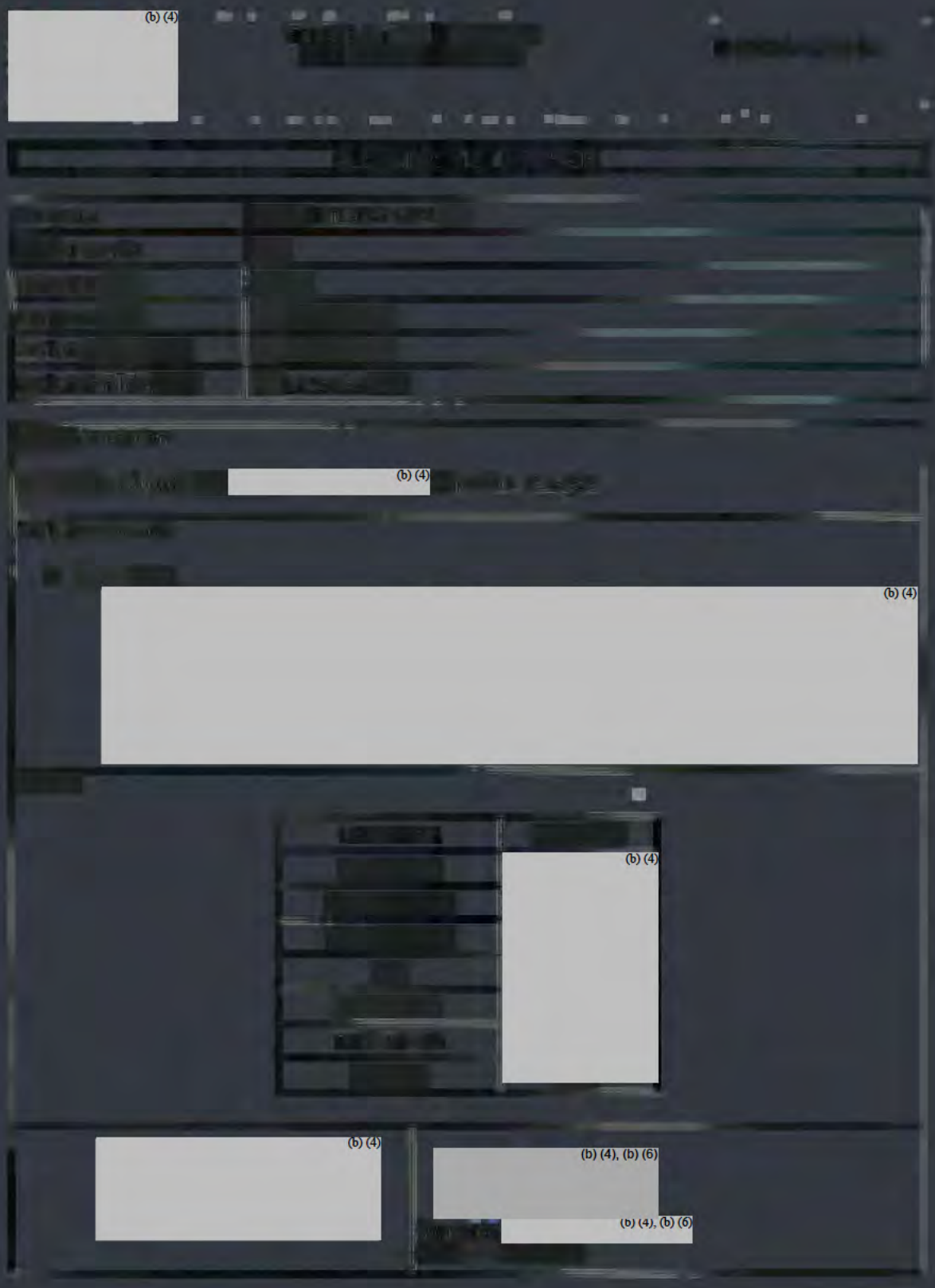
Composition of mixture:	**Moisture in mash: 11.9 %**
66.5 % Corn	1.0 % Vitamins/minerals, T&V
26.2 % Hipro Soya 48	0.5 % Monocalcium Phosfate
4.4 % Soya Oil	0.4 % Salt
1.0 % Limestone	

Hammer milling, mm	3,5
Fat addition	Horizontal mixer
Fat temperature, °C	20
Steam addition	Cascade Mixer (155 rpm)
Steam pressure, bars	2

Test Temperaturs.		65	75	80	85	88	90
Meal temperature, °C		64.9-65.2	74.7-75.4	79.7-79.9	85.1-85.3	88.1	90.0-90.2
Capacity, kg/h		300	300	300	300	300	300
Pellet press, amp.		9	9	9	9	9	9
Meal, kg + enzyme, g	10+5.94						
Meal, kg	310						
Mixing time,							
Meal. + enzyme, min.	10						
Feed mixture, min.	10						
Cooling time, min.	15-17	15-17	15-17	15-17	15-17	15-17	15-17
Pelleting time, min		0-10.5	10.5-21	21-31.5	31.5-42	42-52.5	52.5-63
Sample collect, min		9-10.5	19.5-21	30-31.5	40.5-42	51-52.5	61.5-63

Cold meal 21 c. samples: Blank, no enzyme, Meal from Horizontal mixer + Pellets of each temp. (65-75-80-88-90 C), 1,0 kg of each
Cascade mixer: Filling 5500- 5570 g feed Retention time 64-65 sec
Open steam valve: no. 1
Pallet adjustment no: 2

Appendix 2 Certificates of Analysis



CERTIFICATE OF ANALYSIS

Company:	NOVUS INTERNATIONAL, Inc.
Type of sample:	Feeds
Laboratory ref. :	171831 to 171836, 171842 to 171845, 171851 to 171854, 171859 to 171862, 171864 to 171869, 171886 to 171889, 171909 to 171913, 171933 to 171934, 172168 to 172172, 172189 to 172192, 172200 to 172204
Reception date:	16st October 2017
Analysis starting date:	30th October 2017
Analysis finishing date:	16th January 2018

Sample description:
Forty nine feeds produced at the (b) (4) for phytase, dry matter and physical appearance evaluation, internally identified as (b) (4) rial L-220.

Analysis performed:
Determination of phytase activity, according to ISO 30024:2009 spectrophotometric method (b) (4) method 0602-L-10143).
Determination of dry matter (DM) content according to AOAC (2000) gravimetric method nº 925.09.

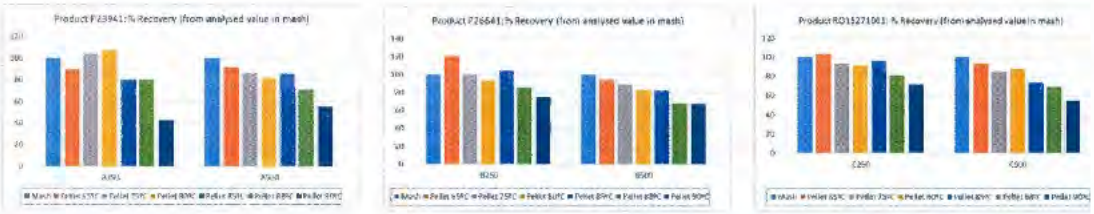
Results:

LAB. REF.	SAMPLE DESCRIPTION	DM (%)	PHYSICAL ASPECT	PHYTASE (FTU/kg)	PHYTASE (FTU/kg)	PHYTASE (FTU/kg)	PHYTASE (FTU/kg)	PHYTASE (FTU/kg) (average)	st.dv.	CV (%)
171843	D NONE CONTROL MASH MDA	(b) (4)	OK				(b) (4)	29	2.9	(b) (4)
171844	D NONE CONTROL PELLET 65ºC P65DA		OK					33	4.2	
171845	D NONE CONTROL PELLET 75ºC P75DA		OK					37	3.6	
171851	D NONE CONTROL PELLET 80ºC P80DA		OK					22	8.5	
171852	D NONE CONTROL PELLET 85ºC P85DA		OK					25	3.5	
171853	D NONE CONTROL PELLET 88ºC P88DA		OK					25	5.8	
171854	D NONE CONTROL PELLET 90ºC P90DA		OK					24	1.3	
171831	P23941 A250 MASH MA2A		OK					320	46.2	
171831	P23941 A250 MASH MA2A (repetition)		OK					265	22.8	
171832	P23941 A250 PELLET 65ºC P65A2A		OK					237	9.6	
171833	P23941 A250 PELLET 75ºC P75A2A		OK					275	16.5	
171834	P23941 A250 PELLET 80ºC P80A2A		OK					284	19.2	
171835	P23941 A250 PELLET 85ºC P85A2A		OK					211	10.8	
171836	P23941 A250 PELLET 88ºC P88A2A		OK					211	17.1	
171842	P23941 A250 PELLET 90ºC P90A2A		OK					114	4.6	
171859	P23941 A500 MASH MA5A		OK					519	19.5	
171860	P23941 A500 PELLET 65ºC P65A5A		OK					476	26.0	
171861	P23941 A500 PELLET 75ºC P75A5A		OK					448	27.5	
171862	P23941 A500 PELLET 80ºC P80A5A		OK					424	34.2	
171869	P23941 A500 PELLET 85ºC P85A5A		OK					444	20.1	
171864	P23941 A500 PELLET 88ºC P88A5A		OK					369	18.8	
171865	P23941 A500 PELLET 90ºC P90A5A		OK					287	17.1	
171866	P26641 B250 MASH MB2A		OK					233	14.8	
171867	P26641 B250 PELLET 65ºC P65B2A		OK					281	27.8	
171868	P26641 B250 PELLET 75ºC P75B2A		OK					233	6.4	
171886	P26641 B250 PELLET 80ºC P80B2A		OK					218	6.6	
171887	P26641 B250 PELLET 85ºC P85B2A		OK					244	21.2	
171888	P26641 B250 PELLET 88ºC P88B2A		OK					199	24.1	
171889	P26641 B250 PELLET 90ºC P90B2A		OK					174	11.2	
171909	P26641 B500 MASH MB5A		OK					435	23.1	
171909	P26641 B500 MASH MB5A (repetition)		OK					578	14.4	
171910	P26641 B500 PELLET 65ºC P65B5A		OK					474	17.9	
171911	P26641 B500 PELLET 75ºC P75B5A		OK					449	44.2	
171912	P26641 B500 PELLET 80ºC P80B5A		OK					419	19.1	
171913	P26641 B500 PELLET 85ºC P85B5A		OK					416	11.0	
171933	P26641 B500 PELLET 88ºC P88B5A		OK					343	11.3	
171934	P26641 B500 PELLET 90ºC P90B5A		OK					342	14.2	
172168	RO15271001 C250 MASH MC2A		OK					251	29.0	
172169	RO15271001 C250 PELLET 65ºC P65C2A		OK					259	30.1	
172170	RO15271001 C250 PELLET 75ºC P75C2A		OK					235	18.3	

172171	RO15271001 C250 PELLET 80°C P80C2A	(b) (4)	OK	(b) (4)	231	12.8	(b) (4)
172172	RO15271001 C250 PELLET 85°C P85C2A		OK		243	12.6	
172191	RO15271001 C250 PELLET 88°C P88C2A		OK		204	18.2	
172192	RO15271001 C250 PELLET 90°C P90C2A		OK		181	9.5	
172189	RO15271001 C500 MASH MC5A		OK		530	31.3	
172190	RO15271001 C500 PELLET 65°C P65C5A		OK		496	13.4	
172200	RO15271001 C500 PELLET 75°C P75C5A		OK		450	7.9	
172201	RO15271001 C500 PELLET 80°C P80C5A		OK		470	33.6	
172202	RO15271001 C500 PELLET 85°C P85C5A		OK		392	25.7	
172203	RO15271001 C500 PELLET 88°C P88C5A		OK		368	14.2	
172204	RO15271001 C500 PELLET 90°C P90C5A		OK		291	17.7	

Phytase Recovery (% from analysed value in mash)

	P23941		P26641		RO15271001	
	A250	A500	B250	B500	C250	C500
Mash	100	100	100	100	100	100
Pellet 65°C	(b) (4)					
Pellet 75°C						
Pellet 80°C						
Pellet 85°C						
Pellet 88°C						
Pellet 90°C						



(b) (4)

(b) (4), (b) (6)

(b) (4), (b) (6)

Signature:

Date: 26th April 2018

Appendix 3 Protocol Amendment

The Novus sponsor of the Study was changed. The protocol was amended accordingly as shown below.

Evaluation of the thermostability of CIBENZA® PHYTAVERSE® G10 Phytase Enzyme in pelleted poultry feed

Study no. 01-17

Amendment to Protocol

It is hereby stated that Mr. Gavin Bowman replaces Study Sponsor from Novus International, Mr. Sanjay Nimkar.

Signed by Study Director, Study Sponsors and Study Monitor:

(b) (4)			
March 9, 2018	FEB 28, 2018	28 Feb 2018	March 6, 2018
Study Director	Study Sponsors		Study Monitor
(b) (4)	Gavin Bowman Director, Global Regulatory Affairs Novus International 20 Research Park Dr. St. Charles, MO 63304, United States of America	Roxanna Van Dorn Senior Regulatory Affairs Specialist BASF Enzymes LLC 3550 John Hopkins Court, San Diego, CA 92121, United States of America	Drew Lichtenstein Research Manager, Specialty Products Novus International 20 Research Park Dr., St. Charles, MO 63304, United States of America

Appendix 22: Sources of Vitamins and Minerals used in the Thermostability Study

(b) (4)

Date **27th March 2018**
Product: **CIBENZA® PHYTAVERSE® G10 Phytase Enzyme**

TO WHOM IT MAY CONCERN:

The table below provides source and regulatory status for the ingredients in the vitamin-mineral premix used in "Evaluation of the thermostability of CIBENZA PHYTAVERSE G10 Phytase Enzyme in pelleted poultry feed" (Study no. 01-17; (b) (4) code L220) conducted at the (b) (4) (b) (4). (b) (4) provided the vitamin-mineral premix used in this study.

#	Vitamin/Mineral	Source	Regulatory status to support ingredient use in US
1	(b) (4)		
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			

C.I.F. Q5855049B

(b) (4)

#	Vitamin/Mineral	Source	Regulatory status to support ingredient use in US
18	(b) (4)		
19			
20			
21			
22			
23			
24			
25			
26			
27			

Sincerely,

(b) (4), (b) (6)

(b) (4), (b) (6)

(b) (4)

Appendix 23: Evaluation of Increasing Levels of a Microbial Phytase in Phosphorus Deficient Broiler Diets Via Live Broiler Performance, Tibia Bone Ash, Apparent Metabolizable Energy, and Amino Acid Digestibility

Evaluation of increasing levels of a microbial phytase in phosphorus deficient broiler diets via live broiler performance, tibia bone ash, apparent metabolizable energy, and amino acid digestibility

J. Pieniazek,[*] K. A. Smith,[*] M. P. Williams,[*] M. K. Manangi,[†] M. Vazquez-Anon,[†] A. Solbak,[‡] M. Miller,[‡] and J. T. Lee[*,1]

[*]*Poultry Science Department, Texas A&M AgriLife Research and Extension, Texas A&M System, College Station, TX, USA;* [†]*Novus International, St. Louis, MO; and* [‡]*Verenium Corporation, San Diego, CA*

ABSTRACT The objective was to investigate increasing concentrations of an evolved microbial phytase on male broiler performance, tibia bone ash, AME, and amino acid digestibility when fed diets deficient in available phosphorus (aP). Experiment 1 evaluated the effects of phytase during a 21 d battery cage study and Experiment 2 was a 42 d grow-out. Experiment 1 included six treatments; negative control (NC) with an aP level of 0.23% (starter) and 0.19% (grower), two positive controls (PC) consisting of an additional 0.12% and 0.22% aP (PC 1 and PC 2), and the NC supplemented with three levels of phytase (250, 500, and 2,000 U/kg). The NC diet reduced ($P < 0.05$) FC, BW, and bone ash. Phytase increased ($P < 0.05$) BW with 2,000 U/kg phytase yielding similar results to the PC2, and improved FCR and increased bone ash was observed at all phytase levels. Amino acid digestibility coefficients were increased ($P < 0.05$) with phytase at 250 U/kg. Phytase at all rates increased ($P < 0.05$) AME to levels similar level as PC diets. Linear regression analysis indicated average P equivalency values for BW and bone ash of 0.137, 0.147, and 0.226 for phytase inclusion of 250, 500, and 2000 U/kg, respectively. Experiment 2 included a PC consisting of 0.45%, 0.41%, and 0.38% aP for the starter, grower, and finisher, respectively; NC with reduced aP of 0.17%; and phytase at 500 and 2,000 U/kg. Phytase increased BW ($P < 0.05$) compared to the NC as 2,000 U/kg phytase resulted in further BW increases compared to the PC (starter and grower). Phytase improved FCR to levels comparable to the PC, with supplementation at 2,000 U/kg resulting in improvements beyond the PC in the starter phase. Amino acid digestibility coefficients were increased with phytase at 2,000 U/kg to levels comparable to that of the PC. These data confirm that the inclusion of phytase improves broiler performance and bone mineralization in aP reduced diets and levels beyond the traditional 500 U/kg can result in further improvements.

Key words: phytase, broiler, digestibility, bone ash, performance

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http://dx.doi.org/10.3382/ps/pew225

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Appendix 24: The Effects of CIBENZA® PHYTAVERSE® G10 Phytase Enzyme on Bone Ash of Broilers Fed Reduced Phosphorus Diets

Final Report Amendment

Study Number	NV-13-2
Effective Date	Date of Study Investigator's signature
Amendment Number	1
Author	(b) (4), (b) (6)
Final Report Section Affected	14. Results and Evaluation

Changes are shown here with strikethroughs, and additions are highlighted.

14. Results and Evaluation

The main effect of treatment on percent tibia ash was statistically significant (Table 1). The percent tibia ash in the PC group was significantly higher than that observed NC and 250 U groups (53.50% vs. 44.75% and 51.24% respectively), but not significantly different from the 500 U group (52.86%). Both the 250 and 500 U groups had significantly higher ash values than the negative control group (51.24% and 52.86% vs. 44.75% respectively). Additionally, ash values in the 500 U group were significantly higher than values in the 250 U group (52.86% vs. 51.24% respectively).

The main effect of treatment on magnesium and phosphorus % values was statistically significant (Table 1). For phosphorus and magnesium values, values in the PC group were significantly higher than the NC and 250 U group (17.92%, 0.79% vs. 16.98%, 0.64% and 17.31%, 0.71% respectively). Phosphorus and magnesium values for the 250 and 500 U groups were significantly higher than the NC (17.31%, 0.71% and 17.76%, 0.75% vs. 16.98%, 0.64% respectively). Calcium values were not affected by treatment (Table 1). The additional necropsy and bone assessment in the NC birds at study end resulted in an average hip pop-out score of 1.10 out of 2.00 and an average of 0.82 out of 2.00 for bone softening on gross evaluations. No joint abnormalities were noted on examination of this group.

The main effect of treatment on average body weight gain was statistically significant for each time period (Table 1). During Days 0 – 14, gain in the PC group was not significantly different from the gain seen in the NC group. Gain in both the 250 and 500 U groups was significantly higher than both the PC and NC groups (0.304 kg, 0.310 kg and 0.292 kg, 0.282 kg respectively). During Days 14 – 28 and overall (Day 0 – 28), gain in the PC group was significantly higher than the gain seen in the NC group (0.928 kg vs. 0.751 kg and 1.221 kg vs. 1.033 kg respectively). Gain in the 250 and 500 U groups was significantly higher than the gain in the NC group (0.940 kg and 0.973 kg, vs. 0.751 kg respectively for days 14-28 and 1.244 kg, 1.283 kg vs. 1.033 kg respectively for 0-28 days). Gain in the 500 U dose group was also significantly higher than the gain in PC group (~~2.04~~ 0.973 kg vs ~~1.96~~ 0.928 kg for days 14-28 and 1.283 kg vs. 1.221 kg for days 0-28).

Reason for Amendment:

This amendment is necessary to amend inadvertent typographical errors in the original final report.

Anticipated Impact on the Study:

None

Study Investigator: (b) (4), (b) (6) Date 03MAR20

Study Representative: [signature] Date 3/3/2020

[redacted] (b) (4)

INVESTIGATOR FINAL REPORT

[redacted]

The effects of CIBENZA® PHYTAVERSE™ G10 Phytase Enzyme on bone ash of broilers fed reduced phosphorus diets.

[redacted]

Project No.:

NV-13-2

[redacted]

STUDY SPONSOR

Novus International, Inc.
20 Research park Drive
St. Charles, MO 63304

TEST FACILITY

[redacted] (b) (4)

November 2016

[redacted]

Table of Contents

1. **Title**

The effects of CIBENZA® PHYTAVERSE™ G10 Phytase Enzyme on bone ash of broilers fed reduced phosphorus diets.

1.1. Study Number

NV-13-2

2. **Study Objective**

The study was used to demonstrate utility of the additive and thus to build the regulatory dossier of CIBENZA® PHYTAVERSE™ G10 for its use as a feed ingredient. The study evaluated the utility of adding CIBENZA® PHYTAVERSE™ G10 at two potential minimum doses (250 and 500 U/kg diet) in broilers fed diets containing sub-optimum levels of non-phytate phosphorus by assessing tibia ash levels, as an indicator of phosphorous availability.

2.1. Study Pivotal vs. non-Pivotal

The study was pivotal. The study was used to demonstrate the utility of the additive and thus to build the regulatory dossier of CIBENZA® PHYTAVERSE™ G10 for its use as a feed ingredient.

2.2. Standards Applied to Study Conduct

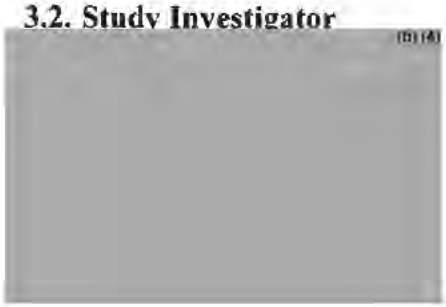
The study was conducted consistent with good clinical practice guidance as provided by the FDA's Guidance for Industry – Good Clinical Practice (VICH GL 9) GFI No. 85.

3. **Key Study Personnel**

3.1. Sponsor

Megharaja Manangi
Novus International, Inc.
20 Research park Drive
St. Charles, MO 63304
Phone: 314-576-8886
E-mail: Megharaja.Manangi@novusint.com

3.2. Study Investigator



3.3. General Study Personnel

Megharaja Manangi, PhD Novus International, Inc.	Sponsor Representative
(b) (4)	Study Monitor
(b) (4)	(b) (4)
(b) (4) **PhD**	Study Statistician

3.4. Test Facility Personnel

(b) (4)	Study investigator, bird evaluation, bird randomization, tibia collection
	Test facility management
(b) (4)	Feed manufacture, weigh birds, verify data
	Feed manufacture
	Bird placement, bird identification, verify data
(b) (4)	Data recording; Data management
	Pen observations, bird identification
	Feed manufacture
	Pen observations, data recording
(b) (4)	Bird placement, weigh birds, weigh feed
	Bird placement, weigh birds, weigh feed

(b) (4)

Bird placement, weigh birds, weigh feed

Assist with randomization

4. Study Locations

Test Facility/Live Phase: (b) (4)

Test Article and Feed Mill/Feed Storage Facility: (b) (4)

Study Monitor: (b) (4), (b) (6)

Statistical Analysis/Statistician: (b) (4)

Analytical Laboratory – Feed Analysis: (b) (4)

Analytical Laboratory – Feed Analysis:
BASF Enzymes, LLC.
3550 John Hopkins Court
San Diego, CA 92121

Analytical Laboratory – Tibia Analysis: (b) (4)

5. **Key Study Dates**

Study Days	Calendar Date(s)	Activities
NA	17FEB15	Study initiation (Protocol Signature).
NA	18FEB15 to 20MAR15	Feed manufacture.
Day 0	20MAR15	Start of live phase. Assessed chick health. Determined average chick weight and assured compliance. Neck tagged birds. Random assignment of chicks to pens. Weighed birds by pen. Administration of starter treatment diets.
Day 14	03APR15	Collected and weighed uneaten starter feed per pen. Weighed birds by pen. Fed grower treatment diets.
Day 28	17APR15	Collected and weighed uneaten grower feed per pen. Weighed birds by pen. Fed finisher treatment diets. Collected right and left legs from selected birds. Mortality evaluation on select birds. Euthanized and dispose of remaining study birds. End of study.

6. **Experimental Materials**

6.1.1. Test Article

CIBENZA® PHYTAVERSE™ G10

Generic Name:	Phytase
Active/Inactive Ingredient:	*Pseudomonas fluorescens* fermentation extract, with a wheat flour carrier. The phytase liquid concentrate contains sucrose, sodium citrate, sodium chloride, sodium propionate, potassium sorbate, and sodium benzoate. The liquid concentrate was dried onto food grade wheat flour for a dry preparation.
Trade Name:	CIBENZA® PHYTAVERSE™ G10
Chemical Name:	6-phytase
Lot Number:	P26641
Formulation:	Dry Granule
Concentration:	13813 U/g
Expiry Date:	TBD

The test article was supplied by the Sponsor packaged in plastic nalgene bottles. The test article was stored in a secured, temperature –controlled, dry area out of direct sunlight. CIBENZA® PHYTAVERSE™ G10 is stable at 25° C for a minimum of 6 months. The sponsor provided the testing facility with a Material Safety Data Sheet (MSDS) for CIBENZA® PHYTAVERSE™ G10. The MSDS is included in the study records. All test article use was recorded and included in the study records.

7. Materials and Methods

7.1. Study Design

On study day 0 birds were randomly assigned to one of four (4) treatment groups (Trt A, Trt, B, Trt C, and Trt D).

The treatment groups consisted of the following:

- Positive control – The diet met or exceeded the NRC 1994 and industry standards.
- Negative control – The diet met or exceeded the NRC 1994 standards with the exception of non-phytate phosphorus formulated to 0.3% non-phytate P (NPP) for starter (days 0-14), and 0.26% NPP for grower (days 14-28).
- Negative control diet with 250 U CIBENZA® PHYTAVERSE™ G10 per kg feed
- Negative control diet with 500 U CIBENZA® PHYTAVERSE™ G10 per kg feed

U was defined as the amount of enzyme that catalyzes the release of one micromole phosphate from the phytate per minute at 37°C at pH 5.5 in accordance to the assay.

7.2. Blinding of Study

Pens within each block were randomly assigned to one (1) of four (4) letter blinding codes (A, B, C, & D). The sponsor held the treatment code that related the treatment number to the blinded treatment letter code. All investigators and lab personnel at the testing facility were blinded to treatment levels and did not have access to the treatment codes. The feed mill manger, feed mill technician, and the data manager were not blinded.

Test articles were provided by the sponsor in pre-measured bottles labeled with the treatment letter code and were added to the mixed treatment diets according to the treatment code. Wheat flour was used as a placebo in order to protect blinding.

7.3. Randomization and Blocking

The experimental design was a randomized complete block design. The blocking factor was the pen location within the house. The test facility was divided into twelve blocks containing four pens each. The random assignment of blinding code/diets to pens was conducted using a computer random number generator (Microsoft Excel) as depicted in the blocking table (Appendix 1 – Blocking Table). Blinding codes/diets were randomly assigned to pens within the block such that one pen was fed each diet/treatment.

Birds were allocated to individual pens randomly according to [redacted].

7.4. Animal Selection and Identification

960 Male Cobb 500 birds (20 birds per pen, 48 pens) were purchased as day-of-hatch chicks from [redacted]. Chicks were a commercial strain. Chicks hatched from eggs laid by young breeders were avoided. All birds were visually evaluated upon arrival at the test facility. Only birds that appeared healthy and alert were assigned to the study.

Birds were identified with a unique tag number attached to the neck. Any tags lost during the study were immediately replaced with a tag with the same number.

Birds were weighed by pen on study day 0 prior to placement on experimental diets and the chick average weight per pen was between 40 grams and 44 grams. Birds were placed on study at approximately one day of age. No acclimation period was utilized.

7.5. Housing and Management

Housing

Birds were housed in an environmentally controlled facility that was adjusted as necessary to maintain bird comfort. Environmental conditions of space, temperature, lighting, bird density, feeder space, and waterer space were similar for all treatment groups. Containment was in accordance with **The Guide for the Care and Use of Agricultural Animals in Research and Teaching** (Ag Guide), Federation of Animal Science Societies, third edition, January 2010.

Birds were placed in floor pens with concrete floors containing an appropriate depth of clean wood shavings to provide a comfortable environment for the chicks. Additional shavings were added to pens if they became to damp for comfortable conditions for the birds during the study. Each pen was approximately 3'X 5' providing approximately 0.75ft^2 per bird (excluding feeder and water space).

Heat was provided to the facility via 4 house has heaters located on the south side of the building. Cooling was provided to the facility by evaporative cooling cell pads with negative pressure ventilation. Negative pressure ventilation was provided by exhaust fans, air circulating tubes and a plenum.

Lighting was provided via incandescent lights and a commercial lighting program was used.

Feed and Watering Method

Feed was provided by a feeder tray for each pen for the first 4 days of the study. Feed was provided *ad libitum* throughout the study via one hanging tube feeder per pen. Water was provided *ad libitum* by one (1) automatic nipple drinker (4 nipples each drinker) per pen. Drinkers were checked twice daily and cleaned as needed to ensure a clean and constant water supply to the birds.

Feed Manufacture

Feed manufacture was according to [redacted]. All experimental diets were manufactured at the [redacted]. A 500 pound capacity vertical mixer (Seedburo Equipment Company) and a 4,000 pound vertical mixer (Prater Industries) were used to prepare the starter, grower and finisher diets. Mixing time ranged from 8-12 minutes depending upon batch size [redacted]

Basal diets were stored in bulk storage bins labeled with study number and diet type. Test articles in pre-measured bottles labeled with the blinded treatment letter code were added to the appropriate diet according to [redacted] Treatment diets were further identified with the appropriate blinded treatment letter code and diets phase type and were stored in separate bulk storage boxes and/or bags. All treatment diets were stored in the feed mill storage facility at ambient conditions after manufacture.

Animal Observations

The test facility, pens and birds were observed at least twice daily for general flock conditions, lighting, water, feed, ventilation and unanticipated events. All Animals were observed regularly by qualified personnel and any adverse effects recorded.

Environmental/Weather Recording Devices

A digital thermometer/hygrometer was located at approximately the center of the testing facility near animal height. High/low reading of temperature and humidity were recorded once daily. Details of the recording device used and location were included in the study records.

7.6. Animal Disposal

Birds in poor condition, unlikely to survive, in pain, distress or requiring therapy, were removed from the study and necropsied by the investigator or technicians blinded to treatment identification. When sex-slips were noted they were removed, euthanized, weighed and recorded on the pen mortality records. Removed birds and mortalities were necropsied to the extent necessary to determine the probable cause of death. The date and of results of the necropsy were recorded on the pen mortality record. Any excessive, unexplained mortality was reported immediately to the sponsor.

Birds did not enter the food and feed chain. Birds were euthanized by carbon dioxide inhalation. Carcasses were disposed of by landfill via commercial dumpster. Reconciliation of test animals is documented in the study records.

Medications and Vaccinations

Birds were vaccinated for Mareks at the hatchery. Upon receipt (Day 0), birds were also vaccinated for Newcastle and Infectious Bronchitis via a spray cabinet. The vaccine was obtained from [redacted] identified as Newcastle-Bronchitis Vaccine, B1 type, B1 strain, Massachusetts type, Live virus (lot number 1401371, expiration date 30JUN15). [redacted] (lot number ESB334, expiration date OCT 2015). No additional vaccinations or medications were used.

7.7. Treatments

7.7.1. Treatment Descriptions

Treatment	Blinding Code	Diet	CIBENZA® PHYTAVERSE™ G10 (U/kg diet)
1	D	Positive Control	0
2	A	Negative Control	0
3	B	Negative Control	250
4	C	Negative Control	500

7.7.2. Control Groups

Two control treatment groups were used. One a positive control treatment group was fed diets containing or exceeding NRC recommended levels for all nutrients, and a negative control treatment group that was fed diets containing or exceeding NRC recommended levels of all nutrients with the exception of non-phytate phosphorus.

7.7.3. Test Article Administration

The experimental test article was homogenously mixed into the daily feed rations as outlined in the treatment description. The test article was administered by oral consumption of feed. There was no withdrawal period and the birds and excess feed did not enter the food or feed chain.

7.8. Diets

Starter and grower diets were fed in mash form. The starter diet was feed from study day 0 to study day 14. The grower diet was feed from study days 14 to study day 28. Diet changes were conducted at the same time for all treatment groups and pens.

Positive Control Diet

The positive control diets comprised primarily of corn and soybean meal with macro- and micro- mineral and vitamin supplementation to meet or exceed the NRC (1994) and industry broiler nutrient requirements.

Negative Control Diet

The negative control diets consisted of the same characteristics as the positive control with available or non-phytate phosphorus formulated 0.15% less than the positive control diet in the corresponding phase. The negative control diets met all other NRC (1994) and industry broiler nutrient requirements.

7.8.1. Feed Sampling

For each phase and treatment a composite sample (approximately 2,500 grams) was collected according to (b) (4). Each composite sample was split into 3 sub-samples: two ~1000 grams each and one, ~500 grams. Each sub-sample was labeled with the study number, blinding code, diet phase, and mixing date.

7.8.2. Feed Analysis

Feed was analyzed prior to commencement of the study. Acceptable feed was within 15% of the intended level for assessment of crude protein, methionine, calcium, total phosphorus, and non-phytate phosphorus. The results of feed analysis were approved by the sponsor prior to the initiation of the study.

- One ~500 gram sub-sample was sent to [redacted] for analysis. Results are included in (Appendix 5- Analysis of Feed (Eurofins)) and the final study records.
- One ~1,000 gram sub-sample was stored at [redacted] at -20° C until the official FDA review is complete.

7.8.2.1. Nutrient Analysis

- For each phase and treatment one ~500 gram sub-sample was sent to [redacted] for analysis. Results are included in (Appendix 4 – Analysis of Ingredients (Eurofins)) and the final study records.

Assays Performed and Method
Moisture (AOAC 930.15)
Crude Protein (AOAC 990.03; 992.15 Mod)
Methionine (AOAC 994.12)
Calories by bomb calorimeter (Parr instrument method)
Ash (AOAC 942.05)
Calcium and Total Phosphorus (AOAC 965.17/985.01 Mod)

7.8.2.2. Enzyme Analysis

For each phase and treatment one ~1,000 gram sub-sample was sent to BASF Enzymes LLC for analysis. Results are included in (Appendix 6— Dose Confirmation Analysis Report (BASF)) and the final study records.

Assays Performed
Phytase for evaluation of CIBENZA® PHYTAVERSE™ G10 activity.

Phytic Acid
Phytic acid in feed was determined by mathematical calculation of phytate bound phosphorus levels in feed.

Phytic acid levels were used to calculate the non-phytate bound phosphorus (NPP)

Non-Phytate Phosphorus (NPP) = Total P – phytate P

7.8.3. Feeding Program

Feed added and removed was weighed and recorded for each pen. Diet changes were conducted at the same time for all pens. The feeding period for the starter diet was from study days 0 – 14, and the grower diet from study das 14 – 28.

7.9. Bone Ash (Tibia) Sample Analysis

Percent tibia bone ash is a direct indicator of poultry phosphorus status and the efficacy of CIBENZA® PHYTAVERSE™ G10 in animals fed reduced non-phytate phosphorus. Results are included in (Appendix 8 – Tibia Analysis Data Summary) and the final study records. AOAC 968.08 Section Db and AOAC 985.01 procedures for analyzing ash minerals using an ICP instrument

7.9.1. Tibia Sample Collection

On study day 28 at the end of the study, the five (5) surviving birds within each pen with the lowest neck tag numbers were selected for bone-ash measurements. Selected birds were euthanized by carbon dioxide. Both the right and left legs (tibias) were harvested from each selected bird.

Each tibia was labeled with the bird number, study number, collection date, and sample description (right or left tibia). Both tibias were frozen at approximately -20° C. Once frozen, the right legs (tibias) were shipped to the [redacted] for bone ash analysis. The left legs (tibias) remained frozen as a backup at [redacted]. until the results were received from the right leg (tibia) until bone ash analysis.

In addition, all remaining birds in the NC group (Treatment A) were euthanized at study day 28 and each bird evaluated for femoral bone pliability, hip pop-out, and femorotibial joint gross examination. For hip pop-out they were given a score of 0 if both hip joints were normal, a score of 1 if one hip was affected by femoral head epiphyseal slipping, or a score of 2 if both hip joints were affected. For joint score they were given a 0 if both femorotibial joints were normal on gross examination, a score of 1 if one joint was affected, and a score of 2 if both joints had evidence of joint pathology. For femoral bone pliability a score of 0 was given for normal bone pliability, a score of 1 if one femur had reduced breaking/bending strength by subjective evaluation, and a score of 2 if both femurs displayed evidence of reduced bone strength.

7.9.2. Percent Bone Ash

The [redacted] conducted analysis by thawing the right leg (tibia) samples and manually removing adhering tissue from the tibia after boiling. The individual bone samples were fat-extracted by use of a mixture of ether and methanol (90% and 10%, respectively). The individual bone samples were labeled and dried at 100° C overnight to determine drone bone weight (AOAC, 1990) then ashed in a muffle furnace at 600° C for 16 hours to determine fat-free dried bone ash. The percentage bone ash was determined by the ratio of remaining ash weight to fat-free dry bone weight multiplied by 100. For each pen

the results for all 5 right tibia samples were averaged so the pen served as the experimental unit.

7.9.3. Tibia Ash Calculations

Percent tibia ash was determined by the ratio of remaining ash weight to fat free dry bone weight multiplied by 100.

Fat-free dry bone ash % = [Fat-free dried bone ash (FFBA)/Fat-free dried bone weight (FFBW)]*100

7.9.4. Tibia Mineral Analysis

The tibia ash was further analyzed for calcium, phosphorus and magnesium by [redacted] utilizing AOAC 968.08 Section Db and AOAC 985.01 procedures for analyzing ash minerals using an ICP instrument.

8. Animal Variables

8.1. Scales

Scales used in weighting feed, feed additives, and birds were licensed by the [redacted] At each use, the scales were checked using standard weights according to [redacted].

8.2. Units of Measure

Weights were recorded in kilogram (kg) or gram (g) and were recorded on the data collection form.

8.3. Bird Weights

Birds were weighed by pen at placement (study day 0), study days 14, and 28.

8.3.1. Average Weight Gain

Average bird weigh gain by pen was calculated for study days 0-14, 0-28, and 14-28.

8.4. Feed Consumption

Feed offered was weighed in by pen. Feed removed was weighed by pen on study day 14, and study day 28.

8.4.1. Average Feed Intake

Average feed intake was calculated as the difference between feed offered and feed per pen calculated for study days 0-14, 0-28, and 14-28.

8.5. Mortality and Removal Weights

Weights of birds that died or were removed was recorded on the pen mortality record. Mortality and removal weights were used to calculate the adjusted Feed Conversion Ratio (FCR).

8.6. Performance Data

Results are included in (Appendix 7 – Performance Data) and the final study records.

8.6.1. Average Feed Conversion Ratio

Average Feed Conversion Ratio was calculated by dividing the total feed consumption in a pen divided by the total weight of surviving birds from that pen.

8.6.2. Adjusted Feed Conversion Ratio

Adjusted Feed Conversion Ratio was calculated by dividing the total feed consumption in a pen divided by the total weight of surviving birds and the weight of removed or mortality birds from that pen.

9. Accountability and Disposition of Test Article, Feed and Animals

All unused test article, unused feed, and animals were documented and those documents were included in the final study records.

10. Statistical Methods

Pen was considered the experimental unit for all outcomes.

The data were analyzed using the following model:

$$Yijk = \mu + Bi + Tj + Eijk$$

Where: μ = the overall mean,
Bi = the effect of the ith block,
Tj = the effect of the jth dietary treatment, and
Eijk = residual error.

Data were analyzed using ANOVA (the GLM procedure in SAS, SAS Institute, Cary NC; version 9.4) and means were separated by LSDs, with the threshold for statistical significance set at the customary 5% level.

The Statistical Analysis Report is included in (Appendix 3 – Statistical Analysis Report) and the final study records.

11. Protocol Amendments and Deviations

All planned changes to the final approved protocol were documented as amendments. All unplanned changes to the approved protocol were documented as deviations. The amendment/deviation contained, but was not limited to: the study number, amendment/deviation number, name of Study Investigator, identification of the protocol section and page number affected, reason(s) for the protocol amendment/deviation, how the change affected the study, and effective date. Protocol changes were discussed and agreed upon by the Study Monitor. Protocol amendments were signed and dated by the Study Investigator and Sponsor Representative. Copies of amendments/deviations were provided to the Study Monitor. Amendments/deviations were appended to the protocol and were addressed as follows:

Amendments

There were four amendments during this study. The amendments are summarized below and are included in the study records.

Amendment Number	Purpose & Sections Effected	Impact on Study
1	The birds arrived the evening prior to placement and were placed study day 0 at approximately one day of age. (Protocol section 8.4.1)	None
2	The study was ended early on day 28 for humane reasons. (Protocol sections 6.2, 7.2, 8.8.4, 9.1.1, 9.1.2, 9.2.1, and 9.2.3)	Little to no impact
3	Additional assessment for femoral pliability, hip pop-out and femoro-tibial joint gross exam on treatment A tibia collection birds and birds that displayed lameness during the study. (Protocol sections 9.2 and 9.2.5)	Little to no impact
4	Addition of the analysis of calcium, phosphorus and magnesium content of the tibias. (Protocol sections 8.6.1.2, 9.1 and 9.1.2)	Improves study
5	Release of feed Treatment 3. Novus released the feed for the study even though Treatment 3 feed did not meet the intended level of 250 units +/- 15% (Protocol sections 8.8.2.2.1)	Little to no impact

Deviations

There were no deviations during the study.

12. Archives

Data and records generated by outside laboratories and consultants were archived at the respective facility. All original data and records generated by outside consultants and laboratories were retained at the facilities for a minimum of three years. Full data sets (copies of all raw data) were submitted to (b) (4) and were utilized and included in investigator final report.

Upon study completion, the study Investigator's final study report, original data and study records, statistician's report, sponsors' data and reported will be stored by the sponsor. An exact copy of the final report and all study records will be kept for five years in the (b) (4) archive following submission to CVM. The (b) (4).

13. Institutional Animal Care and Use Committee Information

Studies with livestock species including studies with poultry, the nature described herein, are not regulated under the Animal Welfare Act (United States Code, Title 7, Sections 2131-2156), and therefore do not require oversight by an Institutional Animal Care and Use Committee.

14. Results and Evaluation

The main effect of treatment on percent tibia ash was statistically significant (Table 1). The percent tibia ash in the PC group was significantly higher than that observed NC and 250 U groups (53.50% vs. 44.75% and 51.24% respectively), but not significantly different from the 500 U group (52.86%). Both the 250 and 500 U groups had significantly higher ash values than the negative control group (51.24% and 52.86% vs. 44.75% respectively). Additionally, ash values in the 500 U group were significantly higher than values in the 250 U group (52.86% vs. 51.24% respectively).

The main effect of treatment on magnesium and phosphorus % values was statistically significant (Table 1). For phosphorus and magnesium values, values in the PC group were significantly higher than the NC and 250 U group (17.92%, 0.79% vs. 16.98%, 0.64% and 17.31%, 0.71% respectively). Phosphorus and magnesium values for the 250 and 500 U groups were significantly higher than the NC (17.31%, 0.71% and 17.76%, 0.75% vs. 16.98%, 0.64% respectively). Calcium values were not affected by treatment (Table 1). The additional necropsy and bone assessment in the NC birds at study end resulted in an average hip pop-out score of 1.10 out of 2.00 and an average of 0.82 out of 2.00 for bone softening on gross evaluations. No joint abnormalities were noted on examination of this group.

The main effect of treatment on average body weight gain was statistically significant for each time period (Table 1). During Days 0 – 14, gain in the PC group was not significantly different from the gain seen in the NC group. Gain in both the 250 and 500 U groups was significantly higher than both the PC and NC groups (0.304 kg, 0.310 kg and 0.292 kg, 0.282 kg respectively). During Days 14 – 28 and overall (Day 0 – 28), gain in the PC group was significantly higher than the gain seen in the NC group (0.928 kg vs. 0.751 kg and 1.221 kg vs. 1.033 kg respectively). Gain in the 250 and 500 U groups was significantly higher than the gain in the NC group (0.940 kg and 0.973 kg, vs. 0.751 kg respectively for days 14-28 and 1.244 kg, 1.283 kg vs. 1.033 kg respectively for 0-28 days). Gain in the 500 U dose group was also significantly higher than the gain in PC group (2.04 kg vs 1.96 kg for days 14-28 and 1.283 kg vs. 1.221 kg for days 0-28).

The main effect of treatment and average daily feed intake was statistically significant for Days 14 – 28 and 0 - 28 (Table 1). No differences between groups were detected during the first 2 weeks of the treatment period. During Days 14 – 28, feed intake in the PC group was significantly higher than the intake seen in the NC group (1.96 kg vs. 1.54 kg respectively). Intake in the 250 and 500 U groups was significantly higher than the intake in the NC group (1.99 kg and 2.04 kg vs. 1.54 kg respectively). Intake in the 500 U dose group was also significantly higher than the intake in PC group and the 250 U group (2.04 kg vs. 1.96 kg and 1.99 kg respectively). Overall (Day 0 – 28), intake in the PC group was significantly higher than the intake seen in the NC group (1.27 kg vs 1.06 kg respectively). Intake in the 250 and

500 U groups was significantly higher than the intake in the NC group (1.30 kg and 1.32 kg vs. 1.06 kg respectively). Intake in the 500 U dose group was also significantly higher than the intake in PC group (1.32 kg vs. 1.27 kg respectively).

The main effect of treatment on average feed intake per bird was statistically significant for Days 14 – 28 and 0 - 28 (Table 1). No differences between groups were detected during the first 2 weeks of the treatment period. During Days 14 – 28, feed intake in the PC group was significantly higher than the intake seen in the NC group (1.387 kg vs. 1.213 kg respectively). Intake in the 250 and 500 U groups was significantly higher than the intake in the NC group (1.401 kg and 1.449 kg vs. 1.213 kg respectively). Intake in the 500 U dose group was also significantly higher than the gain in PC group and the 250 U group (1.449 kg vs. 1.387 kg and 1.401 kg respectively). Overall (Day 0 – 28), intake in the PC group was significantly higher than the intake seen in the NC group (1.798 kg vs. 1.671 kg respectively). Intake in the 250 and 500 U groups was significantly higher than the intake in the NC group (1.822 kg and 1.873 kg vs. 1.671 kg respectively). Intake in the 500 U dose group was also significantly higher than the intake in PC group (1.873 kg vs. 1.798 kg respectively).

The main effect of treatment on feed to gain ration (FCR, adjusted for mortality and culls) was statistically significant for Days 0 – 14, Days 14 – 28 and 0 - 28 (Table 1). During Days 0 – 14 and overall (Day 0 – 28), FCR in the PC group was significantly improved as compared to the FCR seen in the NC group (1.4939 vs. 1.5744 for days 0-14 and 1.4721 vs. 1.5403 for 0-28 days respectively). FCR in the 250 and 500 U groups was significantly improved versus the FCR in the NC group (1.3849 and 1.3573 vs. 1.4572 respectively for days 0-14, 1.4902 and 1.4806 vs. 1.5744 for days 14-28, and 1.4643 and 1.4504 vs. 1.5403 for days 0-28 respectively). FCR in the 500 U dose group was also significantly improved as compared to the FCR in PC group (1.3573 vs. 1.4038 for days 0-14, 1.4806 vs. 1.4939 for days 14-28, and 1.4504 vs. 1.4721 for days 0-28 respectively). During Days 14 – 28, FCR in the PC group was significantly improved as compared to the FCR seen in the NC group (1.4939 vs. 1.5744 respectively). FCR in the 250 and 500 U groups was significantly improved versus the FCR in the NC group (1.4902 and 1.4806 vs. 1.5744 respectively). FCR in the 250 U and 500 U dose groups was not significantly different from the FCR in PC group.

No statistically significant treatment differences were seen for mortality during the starter phase (Table 1). During the grower phase, and subsequently overall, mortality rates were significantly higher in the negative control group as compared to the other 3 groups (Table 1).

Table 1. Least squares means and square errors

Variable	Positive Control	Negative Control	NC + 250U	NC + 500U	SEM	Overall P-value for treatment
Tibia Ash, %	53.50a	44.75c	51.24b	52.86a	0.5315	<0.0001
Tibia Ash Calcium, %	37.80	37.93	37.55	38.24	0.3448	0.4197
Tibia Ash Magnesium, %	0.79a	0.64d	0.71c	0.75b	0.0084	<0.0001
Tibia Ash Phosphorus, %	17.92a	16.98c	17.31b	17.76a	0.1514	<0.0001
Average Pen Weight Gain, kg (bird basis)						
0-14d	0.292b	0.282b	0.304a	0.310a	0.0038	<0.0001
14-28d	0.928b	0.751c	0.940b	0.973a	0.0075	<0.0001
0-28d	1.221b	1.033c	1.244b	1.283a	0.0101	<0.0001
Pen Daily Feed Intake, kg						
0-14d	0.58	0.58	0.60	0.60	0.0079	0.2576
14-28d	1.96b	1.54c	1.99b	2.04a	0.0165	<0.0001
0-28d	1.27b	1.06c	1.30ab	1.32a	0.0112	<0.0001
Average Feed Intake, bird basis, kg						
0-14d	0.411	0.413	0.421	0.421	0.0055	0.4747
14-28d	1.387b	1.213c	1.401b	1.449a	0.0137	<.0001
0-28d	1.798b	1.671c	1.822ab	1.873a	0.0195	<.0001
Feed Conversion Ratio†						
0-14d	1.4038b	1.4572a	1.3849bc	1.3573c	0.0137	0.0001
14-28d	1.4939b	1.5744a	1.4902b	1.4806b	0.0052	<0.0001
0-28d	1.4721b	1.5403a	1.4643bc	1.4504c	0.0050	<0.0001
Mortality						
0-14d	0.83%	1.25%	0.41%	0.41%	0.47	0.5495
14-28d	0.00%b	9.70%a	0.00%b	0.83%b	0.80	<0.0001
0-28d	0.83%b	10.83%a	0.42%b	1.25%b	0.95	<0.0001

abcd: within a row, values with no letters in common are significantly different at $P < 0.05$
†Adjusted for mortality and culls

15. Conclusions:

The results of this study indicate and support the addition of CIBENZA® PHYTAVERSE™ G10 at either 250 or 500 U/kg of feed to diets containing sub-optimal levels of non-phytate phosphorous.

This trial was terminated prematurely on study 28 due to progressive lameness and the inability to obtain feed and water in the phosphorous deficient diet fed birds. In order to maintain humane and ethical treatment of the study birds, the trial was terminated following data collection on study day 28. The significant increase in mortality identified in the deficient phosphorous diet fed group from days 14 to 28 is likely the result of the inability to maintain homeostasis secondary to a non-ambulatory state resulting in a reduction in feed and water intake.

The bone assessment evaluations in the NC group at study end are indicative of loss of bone strength and integrity due to the decrease levels of available phosphorous in the tibia ash results in this treatment group. The incidence of hip pop-out and soft bone identified in this group is due to the reduced phosphorous availability in the diet resulting in deficient levels of phosphorous and magnesium deposition in the bone and subsequent lameness. The most severely affected pens tended to have the lowest phosphorous and magnesium levels on bone ash analysis of the remaining birds. The most severely affected pens in the NC group had lower numbers of birds remaining at study day 28 for evaluation due to the more severely affected birds having already been euthanized or died therefore pen to pen comparisons of gross bone and joint pathology in the NC group is subjective. Since no other treatment groups had bone assessments performed at study end no comparisons across treatments were made.

In this study, the addition of either 250 or 500 U of CIBENZA® PHYTAVERSE™ G10 per kg of phosphorous deficient feed resulted in improved growth performance evidenced by increases in average feed intake, average body weight gain, and a lower average feed conversion ratio in a dose-dependent manner, with the higher dose resulting in better performance compared to birds fed a phosphorous deficient diet alone from 0 to 28 days of age. Bone parameters for birds were also improved at both inclusion levels compared to the birds fed the phosphorous deficient diet alone. In addition, the inclusion of CIBENZA® PHYTAVERSE™ G10 at the higher level of 500 U/kg of phosphorous deficient feed also significantly improved performance parameters compared to a diet supplying a standard level of phosphorous from 0 to 28 days of age.

These findings support the addition of CIBENZA® PHYTAVERSE™ G10 at either 250 or 500 U/kg of feed to ameliorate negative performance effects secondary to a diet that contains sub-optimal levels of non-phytate phosphorous.

16. Accuracy of Report Statement

This report is a complete and accurate representation of all study observations as provided by the Study Investigator.

(b) (4), (b) (6)

[redacted]

08 Nov 16

Date

17. References

The use of percent bone ash as an indicator of phytase efficacy is supported by the following peer-reviewed literature.

Li Y. C., D. R. Ledoux, T. L. Veum, V. Raboy, and D. S. Ertl. 2000. Effects of low phytic acid corn on phosphorus utilization, performance, and bone mineralization in broiler chicks. Poultry Science 79:1444-1450.

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Rousseau, X., M. P. Letourneau-Montminy, N. Meme, M. Magnin, Y. Nys, and A. Narcy. 2012. Phosphorus utilization in finishing broiler chickens: Effect of dietary calcium and microbial phytase. Poult. Sci. 11:2829-2837.

Quantitative Determination of Phytate in the Presence of High Inorganic Phosphate Analytical Biochemistry Vol. 77:536-539 (1977).

Walk, C. L., C. L. Wyatt, R. Upton, and A. P. McElroy. 2011. Effect of diet and phytase on the performance and tibia ash of broilers exposed to live coccidia oocyst vaccine. J. Appl. Poult. Res. 20 (2):153-161.

18. List of Appendices

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19. List of Records

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Sample Collection Form & Mortality Evaluations
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Resumes & CV's
Relevant SOP's
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Appendix 1 – Blocking Table

Trt	Blocks & Pens											
	1	2	3	4	5	6	7	8	9	10	11	12
A	85	87	92	96	103	108	111	115	123	129	133	135
B	84	88	91	97	105	109	113	118	124	128	134	138
C	86	90	94	98	104	107	112	117	125	130	132	136
D	83	89	93	95	106	110	114	116	126	127	131	137

Appendix 2 – Diet Formulations

	(+) control Starter	(-) control Starter	(+) control Grower	(-) control Grower	(+) control Finisher	(-) control Finisher
Corn	56.795%	56.795%	61.811%	61.811%	66.764%	66.764%
Soybean Meal	35.810%	35.810%	31.602%	31.602%	26.190%	26.190%
Soy Oil	1.947%	1.947%	2.114%	2.114%	2.401%	2.401%
Dicalcium Phosphate	1.821%	1.006%	1.632%	0.817%	1.512%	0.697%
Sand	1.401%	1.674%	0.742%	1.013%	1.270%	1.540%
CQR Limestone	0.994%	1.534%	0.905%	1.448%	0.849%	1.392%
CQR Salt, Plain	0.440%	0.442%	0.443%	0.444%	0.446%	0.448%
DL-Methionine	0.299%	0.299%	0.263%	0.263%	0.214%	0.214%
Choline Chloride 60%	0.196%	0.196%	0.207%	0.207%	0.114%	0.114%
CQR Poultry NRC Mineral Premix	0.140%	0.140%	0.140%	0.140%	0.140%	0.140%
CQR Poultry NRC Vitamin Premix	0.100%	0.100%	0.100%	0.100%	0.100%	0.100%
Salinomycin	0.041%	0.041%	0.041%	0.041%	0.000%	0.000%
Threonine	0.008%	0.008%	0.000%	0.000%	0.000%	0.000%
L-Lysine	0.008%	0.008%	0.000%	0.000%	0.000%	0.000%

Statistical Analysis Report

The effects of CIBENZA® PHYTAVERSE™ G10 Phytase Enzyme on bone ash of broilers fed reduced phosphorus diets

Study No. NV-13-2

Sponsor:

Novus International, Inc.
20 Research park Drive
St. Charles, MO 63304

Study location:

(b) (4)

Prepared By:

(b) (4)

(b) (4), (b) (6)

12 Apr 2016
Date

Study Design:

Nine hundred and sixty (960) chicks were randomized to 48 pens of 20 birds each. The experimental design was a randomized complete block design. The blocking factor was pen location within the house. The test facility was divided into twelve blocks of four pens in each block.

Treatments were as follows:

1. Positive control – This diet was designed to meet or exceed NRC 1994 and industry standards.
2. Negative control (NC) – This diet was designed to meet or exceed NRC 1994 standards with the exception of non-phytate phosphorus which was formulated to 0.3%non-phytate P (NPP) for starter (0-14d) and 0.26%NPP for grower (14-28d).
3. NC with 250 U CIBENZA® PHYTAVERSE™ G10 per kg diet
4. NC with 500 U CIBENZA® PHYTAVERSE™ G10 per kg diet

Pen weight weights were obtained on Day 0, 14 and 28. Feed weighbacks were collected on Days 14 and 28. Feed issue was as needed.

At the end of the study, the 5 surviving birds within each pen with the lowest neck numbers were used for bone-ash measurement.

Statistical Methods:

Pen was considered the experimental unit for all outcomes.

The data were analyzed using the following model:

$$Y_{ijk} = \mu + B_i + T_j + E_{ijk}$$

Where; μ = the overall mean,
B_i = the effect of the i^{th} block,
T_j = the effect of the j^{th} dietary treatment, and
E_{ijk} = residual error.

Data were analyzed using ANOVA (the GLM procedure in SAS, SAS Institute, Cary NC; version 9.4) and means were separated by LSDs, with the threshold for statistical significance set at the customary 5% level.

Results:

Tibia percent ash: The main effect of treatment was statistically significant (Table 1). The percent tibia ash in the PC group was significantly higher than that observed NC and 250 U groups, but not significantly different from the 500 U group. Both the 250 and 500 U groups had significantly higher ash values than the negative control group. Additionally, ash values in the 500 U group were significantly higher than values in the 250 U group.

Bone minerals: The main effect of treatment on magnesium and phosphorus % values was statistically significant (Table 1). For phosphorus and magnesium values, values in the PC group were significantly

higher than the NC and 250 U group. Phosphorus and magnesium values for the 250 and 500 U groups were significantly higher than the NC. Calcium values were not affected by treatment (Table 1).

Gain: The main effect of treatment was statistically significant for each time period (Table 1).

During Days 0 – 14, gain in the PC group was not significantly different from the gain seen in the NC group. Gain in both the 250 and 500 U groups was significantly higher than both the PC and NC groups.

During Days 14 – 28 and overall (Day 0 – 28), gain in the PC group was significantly higher than the gain seen in the NC group. Gain in the 250 and 500 U groups was significantly higher than the gain in the NC group. Gain in the 500 U dose group was also significantly higher than the gain in PC group.

Average Daily Feed Intake: The main effect of treatment was statistically significant for Days 14 – 28 and 0 - 28 (Table 1). No differences between groups were detected during the first 2 weeks of the treatment period.

During Days 14 – 28, feed intake in the PC group was significantly higher than the intake seen in the NC group. Intake in the 250 and 500 U groups was significantly higher than the intake in the NC group. Intake in the 500 U dose group was also significantly higher than the gain in PC group and the 250 U group.

Overall (Day 0 – 28), intake in the PC group was significantly higher than the intake seen in the NC group. Intake in the 250 and 500 U groups was significantly higher than the intake in the NC group. Intake in the 500 U dose group was also significantly higher than the intake in PC group.

Average Feed Intake per bird: The main effect of treatment was statistically significant for Days 14 – 28 and 0 - 28 (Table 1). No differences between groups were detected during the first 2 weeks of the treatment period.

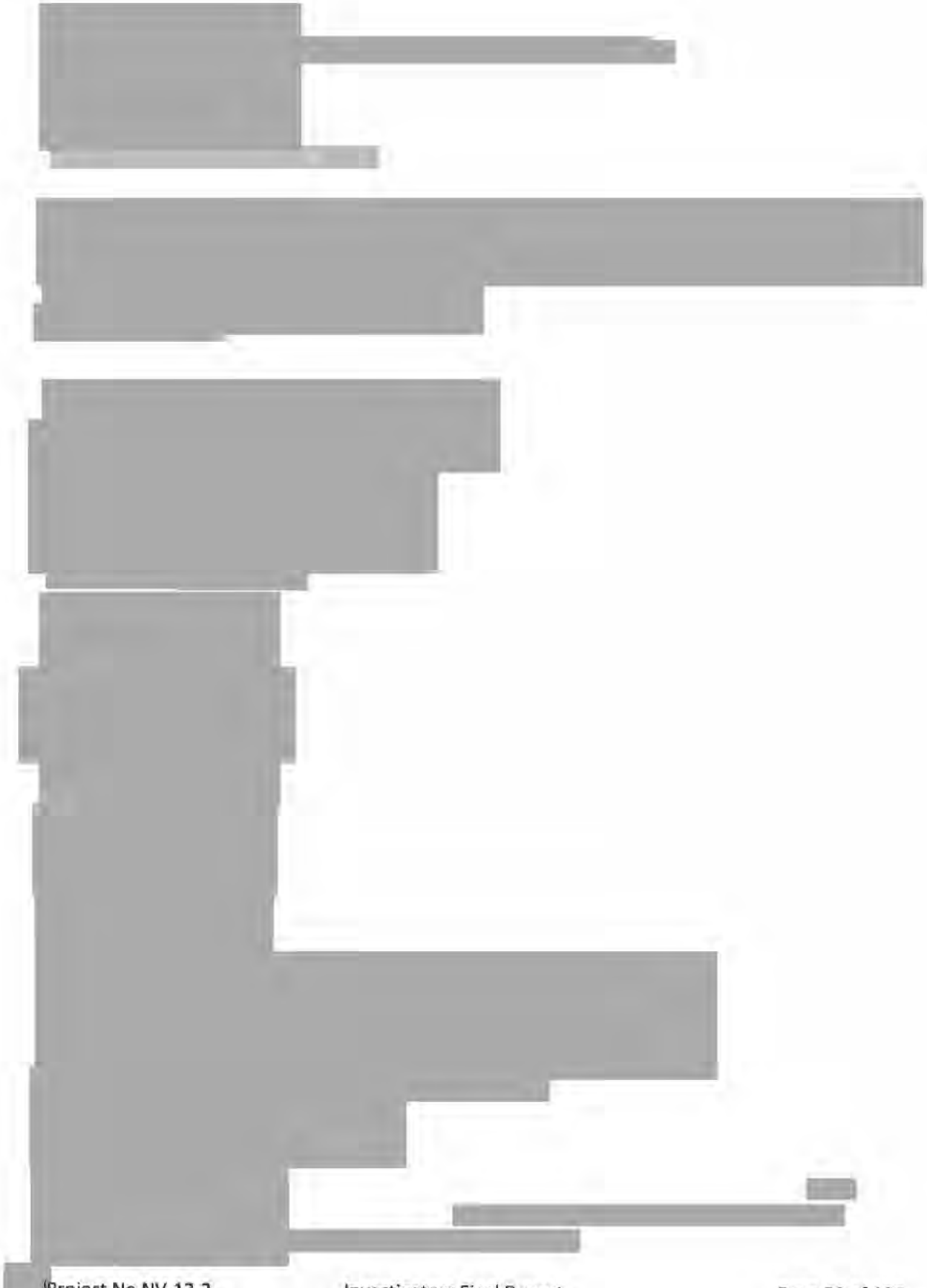
During Days 14 – 28, feed intake in the PC group was significantly higher than the intake seen in the NC group. Intake in the 250 and 500 U groups was significantly higher than the intake in the NC group. Intake in the 500 U dose group was also significantly higher than the intake in PC group and the 250 U group.

Overall (Day 0 – 28), intake in the PC group was significantly higher than the intake seen in the NC group. Intake in the 250 and 500 U groups was significantly higher than the intake in the NC group. Intake in the 500 U dose group was also significantly higher than the intake in PC group.

Feed to Gain ratio (FCR, adjusted for mortality and culls): The main effect of treatment was statistically significant for Days 0 – 14, Days 14 – 28 and 0 - 28 (Table 1).

During Days 0 – 14 and overall (Day 0 – 28), FCR in the PC group was significantly improved as compared to the FCR seen in the NC group. FCR in the 250 and 500 U groups was significantly improved versus the FCR in the NC group. FCR in the 500 U dose group was also significantly improved as compared to the FCR in PC group.

During Days 14 – 28, FCR in the PC group was significantly improved as compared to the FCR seen in the NC group. FCR in the 250 and 500 U groups was significantly improved versus the FCR in the NC group. FCR in the 250 U and 500 U dose groups was not significantly different from the FCR in PC group.



Mortality: No statistically significant treatment differences were seen for mortality during the starter phase (Table 1). During the grower phase, and subsequently overall, mortality rates were significantly higher in the negative control group as compared to the other 3 groups (Table 1).

Table 1: Least squares means and square errors

Variable	Positive Control	Negative Control	NC + 250U	NC + 500U	SEM	Overall P-value for treatment
Tibia Ash, %	53.50a	44.75c	51.24b	52.86a	0.5315	<0.0001
Tibia Ash Calcium, %	37.80	37.93	37.55	38.24	0.3448	0.4197
Tibia Ash Magnesium, %	0.79a	0.64d	0.71c	0.75b	0.0084	<0.0001
Tibia Ash Phosphorus, %	17.92a	16.98c	17.31b	17.76a	0.1514	<0.0001
Average Pen Weight Gain, kg (bird basis)						
0-14d	0.292b	0.282b	0.304a	0.310a	0.0038	<0.0001
14-28d	0.928b	0.751c	0.940b	0.973a	0.0075	<0.0001
0-28d	1.221b	1.033c	1.244b	1.283a	0.0101	<0.0001
Pen Daily Feed Intake, kg						
0-14d	0.58	0.58	0.60	0.60	0.0079	0.2576
14-28d	1.96b	1.54c	1.99b	2.04a	0.0165	<0.0001
0-28d	1.27b	1.06c	1.30ab	1.32a	0.0112	<0.0001
Average Feed Intake, bird basis, kg						
0-14d	0.411	0.413	0.421	0.421	0.0055	0.4747
14-28d	1.387b	1.213c	1.401b	1.449a	0.0137	<.0001
0-28d	1.798b	1.671c	1.822ab	1.873a	0.0195	<.0001
Feed Conversion Ratio†						
0-14d	1.4038b	1.4572a	1.3849bc	1.3573c	0.0137	0.0001
14-28d	1.4939b	1.5744a	1.4902b	1.4806b	0.0052	<0.0001
0-28d	1.4721b	1.5403a	1.4643bc	1.4504c	0.0050	<0.0001
Mortality						
0-14d	0.83%	1.25%	0.41%	0.41%	0.47	0.5495
14-28d	0.00%b	9.70%a	0.00%b	0.83%b	0.80	<0.0001
0-28d	0.83%b	10.83%a	0.42%b	1.25%b	0.95	<0.0001

abcd: within a row, values with no letters in common are significantly different at $P < 0.05$
†Adjusted for mortality and culls

Appendix 4 – Analysis of Ingredients (Eurofins)

(b) (4)

(b) (4)

CERTIFICATE OF ANALYSIS

Test	Result
Moisture by Forced Draft Oven	(b) (4)
Protein, Combustion	
Crude Fat	
Ash	
Calcium	
Phosphorus	
Phytic Acid	

Method Reference
Ash - AOAC 942.05
Calcium by ICP in Feed Samples - AOAC 985.17 / 985.01 mod
Crude Fat By Ethyl Ether Extraction - AOAC 920.39
Moisture - Forced Draft Oven - AOAC 930.15
Phosphorus by ICP - AOAC 985.17 / 985.01 mod.
Phytic Acid - Analytical Biochemistry Vol. 77 536-539 (1977)
Protein - Combustion - AOAC 992.15; AOAC 990.03, AOCS Ba 4e-93

Respectfully Submitted,

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CERTIFICATE OF ANALYSIS

Test	Result
Moisture - Forced Draft Oven	(b) (4)
Protein, Combustion	
Crude Fat	
Ash	
Calcium	
Phosphorus	
Phytic Acid	

Method Reference

Ash - AOAC 942.05
Calcium by ICP in Feed Samples - AOAC 965.17 / 985.01 mod
Crude Fat by Petroleum Ether Extraction - AOCS Ba 3-38 Mod
Moisture - Forced Draft Oven - AOCS Ba 2a-38
Phosphorus by ICP - AOAC 965.17 / 985.01 mod.
Phytic Acid - Analytical Biochemistry Vol. 77:536-539 (1977)
Protein - Combustion - AOAC 992.15; AOAC 990.03; AOCS Ba 4e-93

(b) (4)

Appendix 5 – Analysis of Feed (Eurofins)

(b) (4)

[redacted]

Sample Reference: Project No NV-13-2

CERTIFICATE OF ANALYSIS

(b) (4)

Test	Result	
QD146 - Moisture : Forced Draft Oven	(b) (4)	Completed: 02/26/2015
AOAC 930.15 * Moisture by Forced Draft Oven		
QD052 - Protein - Combustion		Completed: 02/26/2015
AOAC 992.15; AOAC 990.03; AOCS Ba 4e-93 * Protein, Combustion		
QD025 - Ash		Completed: 02/26/2015
AOAC 942.05 * Ash		
QD034 - Calories by Bomb Calorimeter		Completed: 03/04/2015
Parr Instruments Calories By Bomb Calorimeter		
QD033 - Calcium by ICP in Feed Samples		Completed: 02/27/2015
AOAC 965.17 / 985.01 mod. * Calcium		
QD175 - Phosphorus by ICP		Completed: 02/27/2015
AOAC 965.17 / 985.01 mod * Phosphorus		
QD495 - Phytic Acid		Completed: 03/07/2015
Analytical Biochemistry Vol. 77 536-539 (1977) * Phytic Acid		
QQ177 - Cystine & Methionine (AOAC, Meat Matrices)		Completed: 03/04/2015
AOAC 994.12 mod. * Cystine * Methionine		

*The test result is covered by our current A2LA accreditation

(b) (4)

[redacted]


(b) (4)


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Sample Reference: Project No. NV-13-2

CERTIFICATE OF ANALYSIS

(b) (4)

Test	Result	
QD146 - Moisture - Forced Draft Oven		**Completed: 02/26/2015**
AOAC 930.15 * Moisture by Forced Draft Oven	(b) (4)	
QD052 - Protein - Combustion		**Completed: 02/26/2015**
AOAC 992.15, AOAC 990.03, AOCS Ba 4e-93 * Protein, Combustion		
QD025 - Ash		**Completed: 02/26/2015**
AOAC 942.05 * Ash		
QD034 - Calories by Bomb Calorimeter		**Completed: 03/04/2015**
Parr Instruments Calories By Bomb Calorimeter		
QD033 - Calcium by ICP in Feed Samples		**Completed: 02/27/2015**
AOAC 965.17 / 985.01 mod * Calcium		
QD175 - Phosphorus by ICP		**Completed: 02/27/2015**
AOAC 965.17 / 985.01 mod * Phosphorus		
QD495 - Phytic Acid		**Completed: 03/07/2015**
Analytical Biochemistry Vol. 77:536-539 (1977) * Phytic Acid		
QQ177 - Cystine & Methionine (AOAC, Most Matrices)		**Completed: 03/04/2015**
AOAC 994.12 mod * Cystine * Methionine		

*The test result is covered by our current A2LA accreditation.

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Sample Reference: Project No. NV-13-2

CERTIFICATE OF ANALYSIS

(b) (4)

Test	Result	
QD146 - Moisture - Forced Draft Oven		**Completed: 02/26/2015**
AOAC 930.15	(b) (4)	
* Moisture by Forced Draft Oven		
QD052 - Protein - Combustion		**Completed: 02/27/2015**
AOAC 992.15, AOAC 990.03, AOCS Ba 4e-93		
* Protein, Combustion		
QD025 - Ash		**Completed: 02/26/2015**
AOAC 942.05		
* Ash		
QD034 - Calories by Bomb Calorimeter		**Completed: 03/04/2015**
Parr Instruments		
Calories By Bomb Calorimeter		
QD033 - Calcium by ICP in Feed Samples		**Completed: 02/27/2015**
AOAC 985.17 / 985.01 mod.		
* Calcium		
QD175 - Phosphorus by ICP		**Completed: 02/27/2015**
AOAC 985.17 / 985.01 mod		
* Phosphorus		
QD495 - Phytic Acid		**Completed: 03/07/2015**
Analytical Biochemistry Vol. 77:536-539 (1977)		
* Phytic Acid		
QD177 - Cystine & Methionine (AOAC, Most Matrices)		**Completed: 03/04/2015**
AOAC 994.12 mod.		
* Cystine		
* Methionine		

*The test result is covered by our current A2LA accreditation.

(b) (4)


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Sample Reference: Project No. NV-13-2

CERTIFICATE OF ANALYSIS

(b) (4)

Test	Result	
QD146 - Moisture - Forced Draft Oven		**Completed: 02/26/2015**
AOAC 930.15 * Moisture by Forced Draft Oven	(b) (4)	
QD052 - Protein - Combustion		**Completed: 02/27/2015**
AOAC 992.15; AOAC 990.03; AOCS Ba 4e-93 * Protein, Combustion		
QD025 - Ash		**Completed: 02/26/2015**
AOAC 942.05 * Ash		
QD034 - Calories by Bomb Calorimeter		**Completed: 03/04/2015**
Parr Instruments Calories By Bomb Calorimeter		
QD033 - Calcium by ICP in Feed Samples		**Completed: 02/27/2015**
AOAC 965.17 / 985.01 mod. * Calcium		
QD175 - Phosphorus by ICP		**Completed: 02/27/2015**
AOAC 965.17 / 985.01 mod. * Phosphorus		
QD495 - Phytic Acid		**Completed: 03/07/2015**
Analytical Biochemistry Vol. 77:536-539 (1977) * Phyto Acid		
QD177 - Cystine & Methionine (AOAC, Most Matrices)		**Completed: 03/04/2015**
AOAC 994.12 mod. * Cystine * Methionine		

*The test result is covered by our current A2LA accreditation.

(b) (4)

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Sample Reference: Project No. NV-13-2

CERTIFICATE OF ANALYSIS

(b) (4)

Test	Result	
QD146 - Moisture - Forced Draft Oven		Completed: 02/26/2015
AOAC 930.15		
* Moisture by Forced Draft Oven	(b) (4)	
QD052 - Protein - Combustion		Completed: 02/27/2015
AOAC 992.15; AOAC 990.03; AOCS Ba 4e-93		
* Protein, Combustion		
QD005 - Ash		Completed: 02/26/2015
AOAC 942.05		
* Ash		
QD034 - Calories by Bomb Calorimeter		Completed: 03/04/2015
Parr Instruments		
Calories By Bomb Calorimeter		
QD033 - Calcium by ICP in Feed Samples		Completed: 02/27/2015
AOAC 985.17 / 985.01 mod		
* Calcium		
QD175 - Phosphorus by ICP		Completed: 02/27/2015
AOAC 985.17 / 985.01 mod		
* Phosphorus		
QD495 - Phytic Acid		Completed: 03/07/2015
Analytical Biochemistry Vol. 77.536-539 (1977)		
* Phytic Acid		
QQ177 - Cystine & Methionine (AOAC, Most Matrices)		Completed: 03/04/2015
AOAC 994.12 mod.		
* Cystine		
* Methionine		

*The test result is covered by our current A2LA accreditation.

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Sample Reference: Project No. NV-13-2

CERTIFICATE OF ANALYSIS

(b) (4)

Test	Result	
QD148 - Moisture - Forced Draft Oven	(b) (4)	Completed: 02/26/2015
AOAC 930.15 * Moisture by Forced Draft Oven		
QD052 - Protein - Combustion		Completed: 02/27/2015
AOAC 992.15; AOAC 990.03; AOCS Ba 4e-93 * Protein, Combustion		
QD025 - Ash		Completed: 02/26/2015
AOAC 942.05 * Ash		
QD034 - Calories by Bomb Calorimeter		Completed: 03/04/2015
Parr Instruments Calories By Bomb Calorimeter		
QD033 - Calcium by ICP in Feed Samples		Completed: 02/27/2015
AOAC 985.17 / 985.01 mod * Calcium		
QD175 - Phosphorus by ICP		Completed: 02/27/2015
AOAC 985.17 / 985.01 mod * Phosphorus		
QD493 - Phytic Acid		Completed: 03/07/2015
Analytical Biochemistry Vol. 77:536-539 (1977) * Phytic Acid		
QD177 - Cystine & Methionine (AOAC, Most Matrices)		Completed: 03/04/2015
AOAC 994.12 mod * Cystine * Methionine		

*The test result is covered by our current A2LA accreditation.

(b) (4)

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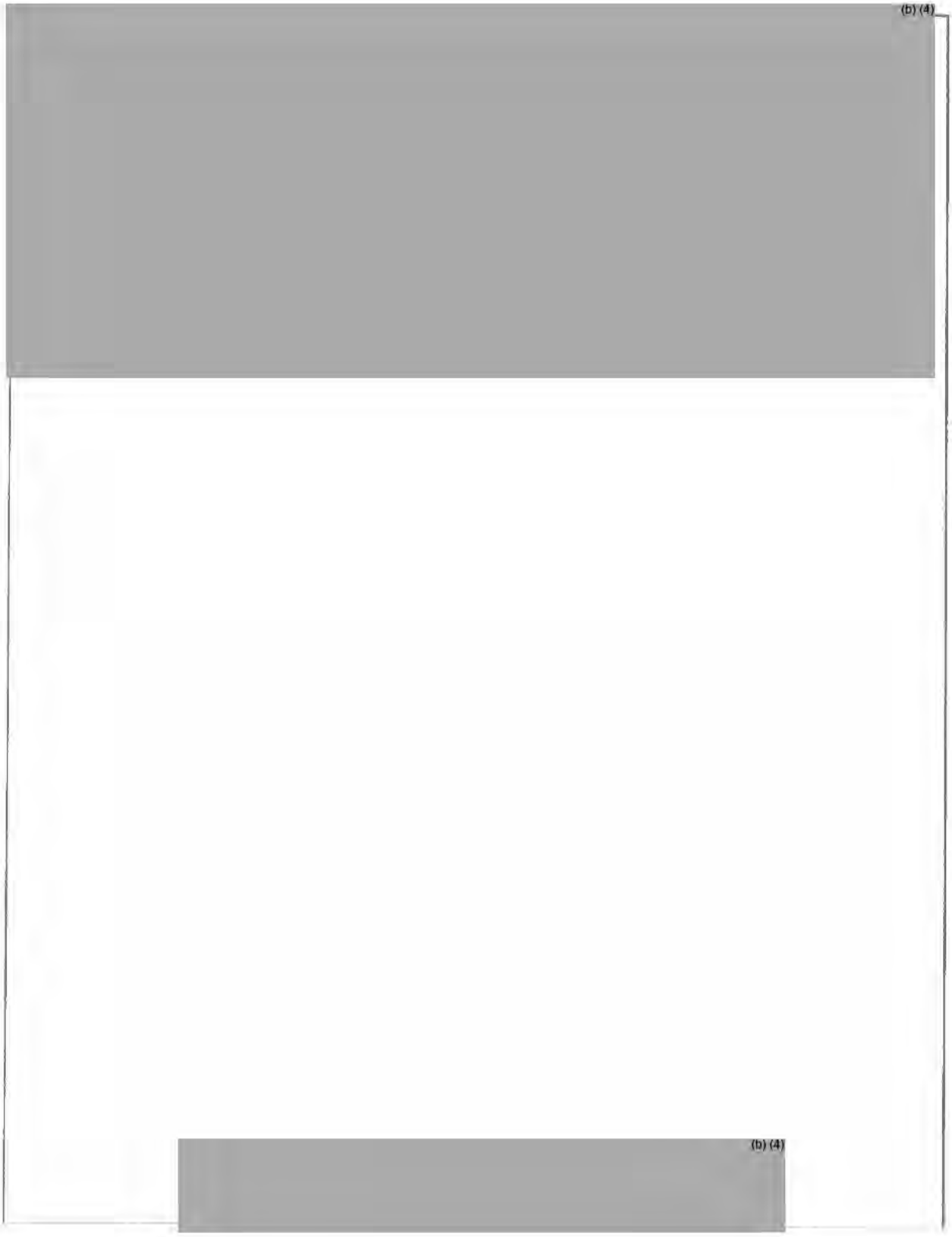
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Sample Reference: Project No NV-13-2

CERTIFICATE OF ANALYSIS

(b) (4)

Test	Result	
QD146 - Moisture - Forced Draft Oven		**Completed: 02/26/2015**
AOAC 930.15 * Moisture by Forced Draft Oven	(b) (4)	
QD052 - Protein - Combustion		**Completed: 02/26/2015**
AOAC 992.15; AOAC 990.03; AOCS Ba 4e-93 * Protein, Combustion		
QD025 - Ash		**Completed: 02/26/2015**
AOAC 942.05 * Ash		
QD034 - Calories by Bomb Calorimeter		**Completed: 03/04/2015**
Parr Instruments Calories By Bomb Calorimeter		
QD033 - Calcium by ICP in Feed Samples		**Completed: 02/27/2015**
AOAC 965.17 / 985.01 mod * Calcium		
QD175 - Phosphorus by ICP		**Completed: 02/27/2015**
AOAC 965.17 / 985.01 mod * Phosphorus		
QD495 - Phytic Acid		**Completed: 03/07/2015**
Analytical Biochemistry Vol. 77:536-539 (1977) * Phytic Acid		
QQ177 - Cystine & Methionine (AOAC, Most Matrices)		**Completed: 03/04/2015**
AOAC 994.12 mod * Cystine * Methionine		

*The test result is covered by our current A2LA accreditation.

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CERTIFICATE OF ANALYSIS

(b) (4)

Test	Result	
QD146 - Moisture - Forced Draft Oven	(b) (4)	Completed: 02/26/2015
AOAC 930.15 * Moisture by Forced Draft Oven		
QD052 - Protein - Combustion		Completed: 02/26/2015
AOAC 992.15; AOAC 990.03; AOCS Ba 4e-93 * Protein, Combustion		
QD025 - Ash		Completed: 02/26/2015
AOAC 942.05 * Ash		
QD034 - Calories by Bomb Calorimeter		Completed: 03/04/2015
Parr Instruments Calories By Bomb Calorimeter		
QD033 - Calcium by ICP in Feed Samples		Completed: 02/27/2015
AOAC 965.17 / 985.01 mod. * Calcium		
QD175 - Phosphorus by ICP		Completed: 02/27/2015
AOAC 965.17 / 985.01 mod. * Phosphorus		
QD495 - Phytic Acid		Completed: 03/07/2015
Analytical Biochemistry Vol. 77:536-539 (1977) * Phytic Acid		
QD177 - Cystine & Methionine (AOAC, Most Matrices)		Completed: 03/04/2015
AOAC 994.12 mod. * Cystine * Methionine		

*The test result is covered by our current A2LA accreditation.

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Sample Reference: Project No. NV-13-2

CERTIFICATE OF ANALYSIS

(b) (4)

Test	Result	
QD148 - Moisture - Forced Draft Oven		**Completed: 02/26/2015**
AOAC 930.15 * Moisture by Forced Draft Oven	(b) (4)	
QD052 - Protein - Combustion		**Completed: 02/26/2015**
AOAC 992.15; AOAC 990.03; AOCS Ba 4e-93 * Protein, Combustion		
QD025 - Ash		**Completed: 02/26/2015**
AOAC 942.05 * Ash		
QD014 - Calories by Bomb Calorimeter		**Completed: 03/04/2015**
Parr Instruments Calories By Bomb Calorimeter		
QD033 - Calcium by ICP in Feed Samples		**Completed: 02/27/2015**
AOAC 985.17 / 985.01 mod * Calcium		
QD175 - Phosphorus by ICP		**Completed: 02/27/2015**
AOAC 985.17 / 985.01 mod. * Phosphorus		
QD495 - Phytic Acid		**Completed: 03/07/2015**
Analytical Biochemistry Vol. 77 536-539 (1977) * Phytic Acid		
QQ177 - Cystine & Methionine (AOAC, Most Matrices)		**Completed: 03/04/2015**
AOAC 994.12 mod * Cystine * Methionine		

*The test result is covered by our current A2LA accreditation.

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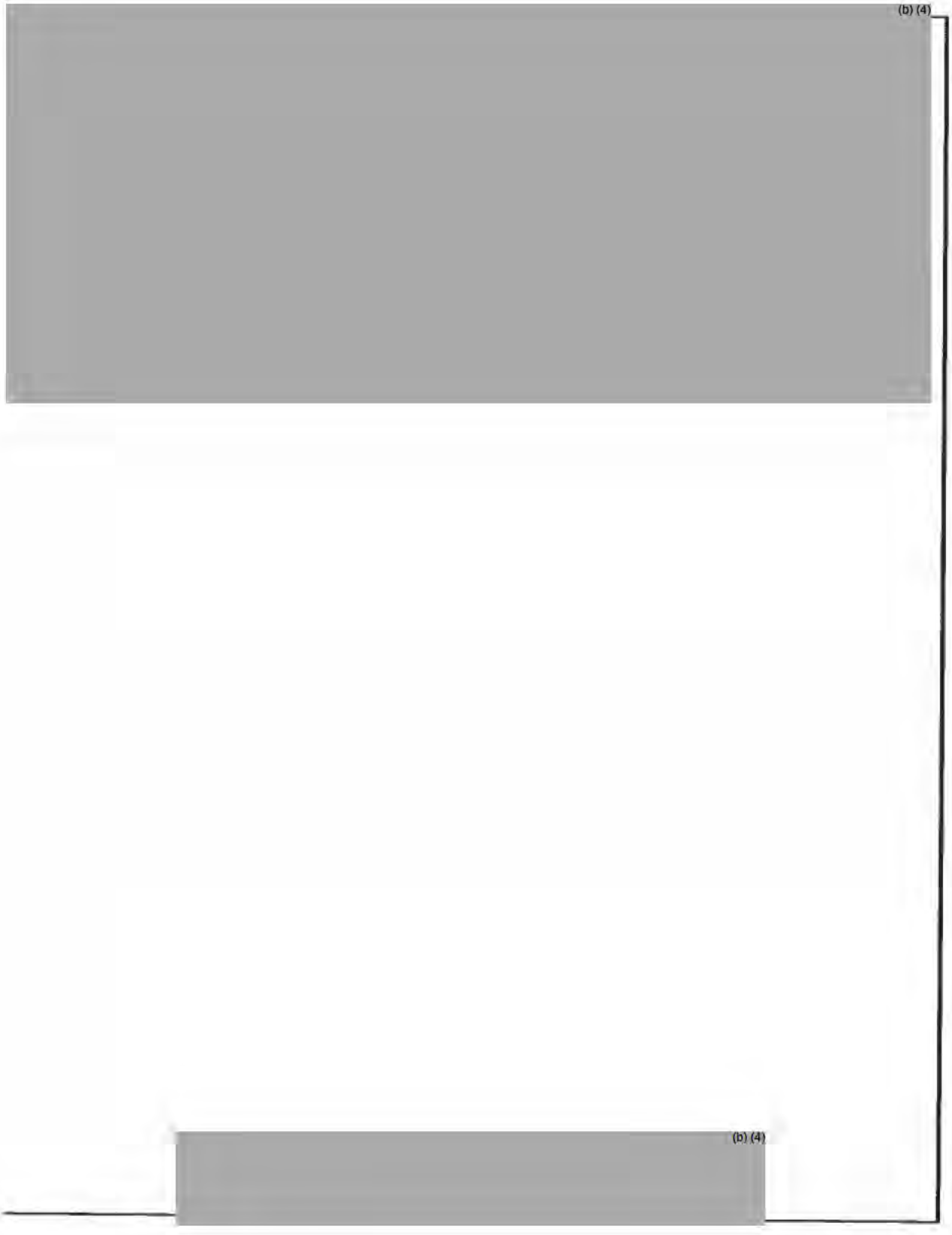
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CERTIFICATE OF ANALYSIS

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Test	Result	
QD146 - Moisture - Forced Draft Oven	(b) (4)	**Completed: 02/26/2015**
AOAC 930.15		
* Moisture by Forced Draft Oven		
QD052 - Protein - Combustion		**Completed: 02/26/2015**
AOAC 992.15, AOAC 990.03, AOCS Ba 4e-93		
* Protein, Combustion		
QD025 - Ash		**Completed: 02/26/2015**
AOAC 942.05		
* Ash		
QD034 - Calories by Bomb Calorimeter		**Completed: 03/04/2015**
Parr Instruments		
Calories By Bomb Calorimeter		
QD033 - Calcium by ICP in Feed Samples		**Completed: 02/27/2015**
AOAC 965.17 / 985.01 mod.		
* Calcium		
QD175 - Phosphorus by ICP		**Completed: 02/27/2015**
AOAC 965.17 / 985.01 mod.		
* Phosphorus		
QD495 - Phytic Acid		**Completed: 03/07/2015**
Analytical Biochemistry Vol. 77 536-539 (1977)		
* Phytic Acid		
QD177 - Cystine & Methionine (AOAC, Most Matrices)		**Completed: 03/04/2015**
AOAC 994.12 mod.		
* Cystine		
* Methionine		

*The test result is covered by our current A2LA accreditation


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Sample Reference: Project No. NV-13-2

CERTIFICATE OF ANALYSIS

(b) (4)

Test	Result	
QD146 - Moisture - Forced Draft Oven		Completed: 02/26/2015
AOAC 930.15 ' Moisture by Forced Draft Oven	(b) (4)	
QD052 - Protein - Combustion		Completed: 02/26/2015
AOAC 992.15, AOAC 990.03; AOCS Ba 4e-93 ' Protein, Combustion		
QD025 - Ash		Completed: 02/26/2015
AOAC 942.05 ' Ash		
QD034 - Calories by Bomb Calorimeter		Completed: 03/04/2015
Parr Instruments Calories By Bomb Calorimeter		
QD038 - Calcium by ICP in Feed Samples		Completed: 02/27/2015
AOAC 985.17 / 985.01 mod ' Calcium		
QD175 - Phosphorus by ICP		Completed: 02/27/2015
AOAC 985.17 / 985.01 mod ' Phosphorus		
QD495 - Phytic Acid		Completed: 03/07/2015
Analytical Biochemistry Vol 77 536-539 (1977) ' Phytic Acid		
QQ177 - Cystine & Methionine (AOAC, Most Matrices)		Completed: 03/04/2015
AOAC 994.12 mod. ' Cystine ' Methionine		

*'The test result is covered by our current A2LA accreditation

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Sample Reference: Project No. NV-13-2

CERTIFICATE OF ANALYSIS

(b) (4)

Test	Result	
QD146 - Moisture - Forced Draft Oven	(b) (4)	Completed: 02/26/2015
AOAC 968.15		
* Moisture by Forced Draft Oven		
QD052 - Protein - Combustion		Completed: 02/26/2015
AOAC 992.15; AOAC 990.03; AOCS Ba 4e-93		
* Protein, Combustion		
QD025 - Ash		Completed: 02/26/2015
AOAC 942.05		
* Ash		
QD034 - Calories by Bomb Calorimeter		Completed: 03/04/2015
Parr Instruments		
Calories By Bomb Calorimeter		
QD033 - Calcium by ICP in Feed Samples		Completed: 02/27/2015
AOAC 985.17 / 985.01 mod.		
* Calcium		
QD175 - Phosphorus by ICP		Completed: 02/27/2015
AOAC 985.17 / 985.01 mod.		
* Phosphorus		
QD455 - Phytic Acid		Completed: 03/07/2015
Analytical Biochemistry Vol. 77:536-539 (1977)		
* Phytic Acid		
QD177 - Cystine & Methionine (AOAC; Most Matrices)		Completed: 03/04/2015
AOAC 994.12 mod		
* Cystine		
* Methionine		

*The test result is covered by our current A2LA accreditation.

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Dose Confirmation Analysis Report

Trial Title: The effects of CIBENZA® PHYTAVERSE™ G10 Phytase Enzyme on bone ash of broilers fed reduced phosphorus diets

Protocol Number: NV-13-2

Sample Analysis Date: March 2, 2015

Sample	Description	Dose (U/kg)	Result (U/kg)*
Starter A	Negative Control	0	(b) (4)
Starter B	CIBENZA® PHYTAVERSE™ G10 Phytase Enzyme	250	
Starter C	CIBENZA® PHYTAVERSE™ G10 Phytase Enzyme	500	
Starter D	Positive Control	0	
Grower A	Negative Control	0	
Grower B	CIBENZA® PHYTAVERSE™ G10 Phytase Enzyme	250	
Grower C	CIBENZA® PHYTAVERSE™ G10 Phytase Enzyme	500	
Grower D	Positive Control	0	
Finisher A	Negative Control	0	
Finisher B	CIBENZA® PHYTAVERSE™ G10 Phytase Enzyme	250	
Finisher C	CIBENZA® PHYTAVERSE™ G10 Phytase Enzyme	500	
Finisher D	Positive Control	0	

*Results reported as <60 U/kg are above the limit of detection (LOD=20 U/kg) and below the limit of quantitation (LOQ = 60 U/kg) of the ISO 30024 phytase analytical method used to determine phytase activity.

Approved by: (b) (4), (b) (6) Date: September 28, 2015

Manager, QC

BASF Enzymes LLC
3550 John Hopkins Court
San Diego, CA 92121
www.basf.us

Table 1. Mortality and Removal Weights of Cobb 500 Broilers Days 0 - 20
(b) (4) Project No. NV-13-2
BLDG 7

			Study Days 0 - 14 (28MAR13 - 01APR13)							
Treatment	Sex	Pen No	No. Birds started	Removals	Mortality	Cause of Death	R	M	No. Birds day 14	Wt. Birds M/R Days 0-14
1	M	83	20	0	0		0.000	0.000	20	0.000
3	M	84	20	0	0		0.000	0.000	20	0.000
2	M	85	20	0	0		0.000	0.000	20	0.000
4	M	86	20	0	0		0.000	0.000	20	0.000
2	M	87	20	0	0		0.000	0.000	20	0.000
3	M	88	20	0	0		0.000	0.000	20	0.000
1	M	89	20	0	0		0.000	0.000	20	0.000
4	M	90	20	0	0		0.000	0.000	20	0.000
3	M	91	20	0	0		0.000	0.000	20	0.000
2	M	92	20	0	0		0.000	0.000	20	0.000
1	M	93	20	0	0		0.000	0.000	20	0.000
4	M	94	20	0	0		0.000	0.000	20	0.000
1	M	96	20	0	0		0.000	0.000	20	0.000
2	M	96	20	0	0		0.000	0.000	20	0.000
3	M	97	20	0	0		0.000	0.000	20	0.000
4	M	98	20	0	0		0.000	0.000	20	0.000
2	M	103	20	0	0		0.000	0.000	20	0.000
4	M	104	20	0	0		0.000	0.000	20	0.000
3	M	105	20	0	1	SDS #2997	0.000	0.145	19	0.145
1	M	106	20	0	1	SDS #6974	0.000	0.153	19	0.153
4	M	107	20	0	0		0.000	0.000	20	0.000
2	M	108	20	0	0		0.000	0.000	20	0.000
3	M	109	20	0	0		0.000	0.000	20	0.000
1	M	110	20	0	0		0.000	0.000	20	0.000
2	M	111	20	0	1	SDS #7345	0.000	0.082	19	0.082
4	M	112	20	0	0		0.000	0.000	20	0.000
3	M	113	20	0	0		0.000	0.000	20	0.000
1	M	114	20	0	0		0.000	0.000	20	0.000
2	M	115	20	0	0		0.000	0.000	20	0.000
1	M	116	20	0	0		0.000	0.000	20	0.000
4	M	117	20	0	0		0.000	0.000	20	0.000
3	M	118	20	0	0		0.000	0.000	20	0.000
2	M	123	20	0	0		0.000	0.000	20	0.000
3	M	124	20	0	0		0.000	0.000	20	0.000
4	M	125	20	0	0		0.000	0.000	20	0.000
1	M	126	20	0	0		0.000	0.000	20	0.000
1	M	127	20	0	0		0.000	0.000	20	0.000
3	M	128	20	0	0		0.000	0.000	20	0.000
2	M	129	20	1	0	C - BL #7504	0.113	0.000	19	0.113
4	M	130	20	0	1	BAC #1926	0.000	0.043	19	0.043
1	M	131	20	0	0		0.000	0.000	20	0.000
4	M	132	20	0	0		0.000	0.000	20	0.000
2	M	133	20	0	0		0.000	0.000	20	0.000
3	M	134	20	0	0		0.000	0.000	20	0.000
2	M	135	20	1	0	C - BL #7550	0.200	0.000	19	0.200
4	M	136	20	0	0		0.000	0.000	20	0.000
1	M	137	20	0	1	SDS #7573	0.000	0.097	19	0.097
3	M	138	20	0	0		0.000	0.000	20	0.000

Table 1 Mortality and Removal Weights of Cobb 500 Broilers Days 0 - 28
Project No. NV-13-2
BLDG 7

Treatment	Sex	Pen No.	Removed	Mortality	Cause of Death	R	M	No. Birds day 28	Wt. Birds M/F Days 14-28	Total Wt. (kg) Days 0-28
1	M	83	0	0		0.000	0.000	20	0.000	0.000
3	M	84	0	0		0.000	0.000	20	0.000	0.000
2	M	85	1	0	C-BL #2824	0.355	0.000	19	0.355	0.355
4	M	86	0	0		0.000	0.000	20	0.000	0.000
2	M	87	2	0	C-BL #2949, C-BL #2950	0.868	0.000	18	0.868	0.868
3	M	88	0	0		0.000	0.000	20	0.000	0.000
1	M	89	0	0		0.000	0.000	20	0.000	0.000
4	M	90	0	0		0.000	0.000	20	0.000	0.000
3	M	91	0	0		0.000	0.000	20	0.000	0.000
2	M	92	0	1	SDS #6866	0.000	0.478	19	0.478	0.478
1	M	93	0	0		0.000	0.000	20	0.000	0.000
4	M	94	0	0		0.000	0.000	20	0.000	0.000
1	M	95	0	0		0.000	0.000	20	0.000	0.000
2	M	96	1	1	SDS #6926, C-BL #6925	0.510	0.553	18	1.063	1.063
3	M	97	0	0		0.000	0.000	20	0.000	0.000
4	M	98	0	0		0.000	0.000	20	0.000	0.000
2	M	103	0	1	SDS #2978	0.000	0.545	19	0.545	0.545
4	M	104	0	0		0.000	0.000	20	0.000	0.000
3	M	105	0	0		0.000	0.000	19	0.000	0.143
1	M	106	0	0		0.000	0.000	19	0.000	0.153
4	M	107	0	0		0.000	0.000	20	0.000	0.000
2	M	108	2	1	C-BL #7005, C-BL#6994, ACT-BL #6996	1.247	0.937	17	2.184	2.184
3	M	109	0	0		0.000	0.000	20	0.000	0.000
1	M	110	0	0		0.000	0.000	20	0.000	0.000
2	M	111	1	0	C-BL #7353	0.579	0.000	18	0.579	0.661
4	M	112	0	0		0.000	0.000	20	0.000	0.000
3	M	113	0	0		0.000	0.000	20	0.000	0.000
1	M	114	0	0		0.000	0.000	20	0.000	0.000
2	M	115	3	1	C-BL #7398, SDS #7402, C-BL #7393, C-BL #7352	1.258	0.493	16	1.751	1.751
1	M	116	0	0		0.000	0.000	20	0.000	0.000
4	M	117	0	0		0.000	0.000	20	0.000	0.000
3	M	118	0	0		0.000	0.000	20	0.000	0.000
2	M	123	2	0	C-BL #7440, C-BL #7433	1.096	0.000	18	1.096	1.096
3	M	124	0	0		0.000	0.000	20	0.000	0.000
4	M	125	0	1	SDS #7459	0.000	1.227	19	1.227	1.227
1	M	126	0	0		0.000	0.000	20	0.000	0.000
1	M	127	0	0		0.000	0.000	20	0.000	0.000
3	M	128	0	0		0.000	0.000	20	0.000	0.000
2	M	129	3	0	C-BL #7480, C-BL #7502, C-BL #7500	1.425	0.000	16	1.425	1.538
4	M	130	0	0		0.000	0.000	19	0.000	0.040
1	M	131	0	0		0.000	0.000	20	0.000	0.000
4	M	132	0	1	SDS #1952	0.000	0.676	19	0.676	0.676
2	M	133	1	0	C-BL #7541	0.348	0.000	19	0.348	0.348
3	M	134	0	0		0.000	0.000	20	0.000	0.000
2	M	135	1	1	C-BL #7553, SDS #7549	0.325	0.474	17	0.799	0.899
4	M	136	0	0		0.000	0.000	20	0.000	0.000
1	M	137	0	0		0.000	0.000	19	0.000	0.057
3	M	138	0	0		0.000	0.000	20	0.000	0.000

Table 2. Summary of Mortalities & Removals of Cobb 500 Broilers Days 0 - 28

(b)Project No. NV-13-2

BLDG 7

Treatment	Sex	Pen No.	Study Days 0 - 14 (28MAR13 - 03APR13): No. Birds Started	Removed	Mortality	Cause of Death	% Removed	% Mortality	No. Birds day 14	Total % M & R 0-14
1	M	83	20	0	0		0.0%	0.0%	20	0.0%
1	M	89	20	0	0		0.0%	0.0%	20	0.0%
1	M	93	20	0	0		0.0%	0.0%	20	0.0%
1	M	96	20	0	0		0.0%	0.0%	20	0.0%
1	M	106	20	0	1	SDS #6974	0.0%	5.0%	19	5.0%
1	M	110	20	0	0		0.0%	0.0%	20	0.0%
1	M	114	20	0	0		0.0%	0.0%	20	0.0%
1	M	116	20	0	0		0.0%	0.0%	20	0.0%
1	M	126	20	0	0		0.0%	0.0%	20	0.0%
1	M	127	20	0	0		0.0%	0.0%	20	0.0%
1	M	131	20	0	0		0.0%	0.0%	20	0.0%
1	M	137	20	0	1	SDS #7573	0.0%	5.0%	19	5.0%
Totals & Averages			240	0	2		0.0%	0.8%	232	0.8%
2	M	85	20	0	0		0.0%	0.0%	20	0.0%
2	M	87	20	0	0		0.0%	0.0%	20	0.0%
2	M	92	20	0	0		0.0%	0.0%	20	0.0%
2	M	95	20	0	0		0.0%	0.0%	20	0.0%
2	M	103	20	0	0		0.0%	0.0%	20	0.0%
2	M	108	20	0	0		0.0%	0.0%	20	0.0%
2	M	111	20	0	1	SDS #7345	0.0%	5.0%	19	5.0%
2	M	118	20	0	0		0.0%	0.0%	20	0.0%
2	M	123	20	0	0		0.0%	0.0%	20	0.0%
2	M	129	20	1	0	C - BL #7504	5.0%	0.0%	19	5.0%
2	M	133	20	0	0		0.0%	0.0%	20	0.0%
2	M	135	20	1	0	C - BL #7550	5.0%	0.0%	19	5.0%
Totals & Averages			240	2	1		0.8%	0.4%	237	1.3%
3	M	84	20	0	0		0.0%	0.0%	20	0.0%
3	M	88	20	0	0		0.0%	0.0%	20	0.0%
3	M	91	20	0	0		0.0%	0.0%	20	0.0%
3	M	97	20	0	0		0.0%	0.0%	20	0.0%
3	M	105	20	0	1	SDS #2997	0.0%	5.0%	19	5.0%
3	M	109	20	0	0		0.0%	0.0%	20	0.0%
3	M	113	20	0	0		0.0%	0.0%	20	0.0%
3	M	116	20	0	0		0.0%	0.0%	20	0.0%
3	M	124	20	0	0		0.0%	0.0%	20	0.0%
3	M	128	20	0	0		0.0%	0.0%	20	0.0%
3	M	134	20	0	0		0.0%	0.0%	20	0.0%
3	M	136	20	0	0		0.0%	0.0%	20	0.0%
Totals & Averages			240	0	1		0.0%	0.4%	239	0.4%
4	M	86	20	0	0		0.0%	0.0%	20	0.0%
4	M	90	20	0	0		0.0%	0.0%	20	0.0%
4	M	94	20	0	0		0.0%	0.0%	20	0.0%
4	M	98	20	0	0		0.0%	0.0%	20	0.0%
4	M	104	20	0	0		0.0%	0.0%	20	0.0%
4	M	107	20	0	0		0.0%	0.0%	20	0.0%
4	M	112	20	0	0		0.0%	0.0%	20	0.0%
4	M	117	20	0	0		0.0%	0.0%	20	0.0%
4	M	125	20	0	0		0.0%	0.0%	20	0.0%
4	M	130	20	0	1	BAC #1935	0.0%	5.0%	19	5.0%
4	M	132	20	0	0		0.0%	0.0%	20	0.0%
4	M	136	20	0	0		0.0%	0.0%	20	0.0%
Totals & Averages			240	0	1		0.0%	0.4%	239	0.4%

Table 2. Summary of Mortalities & Removals of Cobb 500 Broilers Days 0 - 28
(b) Project No. NV-13-2
BLDG 7

Treatment	Sex	Ped. No.	Removed	Mortality	Cause of Death	% Removed	% Mortality	No. Birds day 28	Total % M & R 14-28	Study Total % M & R 0-28
1	M	83	0	0		0.0%	0.0%	20	0.0%	0.0%
1	M	89	0	0		0.0%	0.0%	20	0.0%	0.0%
1	M	93	0	0		0.0%	0.0%	20	0.0%	0.0%
1	M	98	0	0		0.0%	0.0%	20	0.0%	0.0%
1	M	106	0	0		0.0%	0.0%	19	0.0%	5.0%
1	M	110	0	0		0.0%	0.0%	20	0.0%	0.0%
1	M	114	0	0		0.0%	0.0%	20	0.0%	0.0%
1	M	116	0	0		0.0%	0.0%	20	0.0%	0.0%
1	M	126	0	0		0.0%	0.0%	20	0.0%	0.0%
1	M	127	0	0		0.0%	0.0%	20	0.0%	0.0%
1	M	131	0	0		0.0%	0.0%	20	0.0%	0.0%
1	M	137	0	0		0.0%	0.0%	19	0.0%	5.0%
Totals & Averages			0	0		0.0%	0.0%	238	0.0%	0.8%
2	M	85	1	0	C-BL #6821	5.0%	0.0%	19	5.0%	5.0%
2	M	87	2	0	C-BL #2849, C-BL #3860	10.0%	0.0%	18	10.0%	10.0%
2	M	92	0	1	SDS #6886	0.0%	5.0%	19	5.0%	5.0%
2	M	96	1	1	SDS #6926, C-BL #6926	5.0%	5.0%	18	10.0%	10.0%
2	M	103	0	1	SDS #6978	0.0%	5.0%	19	5.0%	5.0%
2	M	108	2	1	C-BL #7005; C-BL#6994; ACT-BL #6996	10.0%	5.0%	17	15.0%	15.0%
2	M	111	1	0	C-BL #7383	5.3%	0.0%	18	5.3%	10.0%
2	M	115	3	1	C-BL #7398, SDS #7402, C-BL #7382, C-BL #7382	15.0%	5.0%	16	20.0%	20.0%
2	M	123	2	0	C-BL #7440, C-BL #7433	10.0%	0.0%	18	10.0%	10.0%
2	M	129	3	0	C-BL #7460, C-BL #7502, C-BL #7500	15.8%	0.0%	16	15.8%	20.0%
2	M	133	1	0	C-BL #7541	5.0%	0.0%	19	5.0%	5.0%
2	M	135	1	1	C-BL #7553, SDS #7549	5.3%	5.3%	17	10.5%	15.0%
Totals & Averages			17	6		7.2%	2.5%	214	9.7%	10.8%
3	M	84	0	0		0.0%	0.0%	20	0.0%	0.0%
3	M	88	0	0		0.0%	0.0%	20	0.0%	0.0%
3	M	91	0	0		0.0%	0.0%	20	0.0%	0.0%
3	M	97	0	0		0.0%	0.0%	20	0.0%	0.0%
3	M	105	0	0		0.0%	0.0%	19	0.0%	5.0%
3	M	109	0	0		0.0%	0.0%	20	0.0%	0.0%
3	M	113	0	0		0.0%	0.0%	20	0.0%	0.0%
3	M	118	0	0		0.0%	0.0%	20	0.0%	0.0%
3	M	124	0	0		0.0%	0.0%	20	0.0%	0.0%
3	M	128	0	0		0.0%	0.0%	20	0.0%	0.0%
3	M	134	0	0		0.0%	0.0%	20	0.0%	0.0%
3	M	138	0	0		0.0%	0.0%	20	0.0%	0.0%
Totals & Averages			0	0		0.0%	0.0%	239	0.0%	0.4%
4	M	86	0	0		0.0%	0.0%	20	0.0%	0.0%
4	M	90	0	0		0.0%	0.0%	20	0.0%	0.0%
4	M	94	0	0		0.0%	0.0%	20	0.0%	0.0%
4	M	98	0	0		0.0%	0.0%	20	0.0%	0.0%
4	M	104	0	0		0.0%	0.0%	20	0.0%	0.0%
4	M	107	0	0		0.0%	0.0%	20	0.0%	0.0%
4	M	112	0	0		0.0%	0.0%	20	0.0%	0.0%
4	M	117	0	0		0.0%	0.0%	20	0.0%	0.0%
4	M	125	0	1	SDS #7458	0.0%	5.0%	19	5.0%	5.0%
4	M	130	0	0		0.0%	0.0%	19	0.0%	0.0%
4	M	132	0	1	SDS #1952	0.0%	5.0%	19	5.0%	5.0%
4	M	136	0	0		0.0%	0.0%	20	0.0%	0.0%
Totals & Averages			0	2		0.0%	0.8%	237	0.8%	1.3%

Table 3 Feed Added and Weighed Back by Pen Study Days 0 - 28
(b) Project No. NV-13-2
BLDG 7

			STARTER			GROWER				
Treatment	Sex	Pen	3/17 Feed 1	4/3 WB	0-14 Consumption	4/3 Feed 2	4/15 Feed 3	4/17 WB	14-28 Consumption	0-28 Consumption
1	M	83	14.00	5.96	8.02	20.00	15.00	6.88	28.12	36.14
3	M	84	14.00	6.36	7.64	20.00	15.00	6.12	25.88	34.52
2	M	85	14.00	5.54	8.46	20.00	15.00	11.85	23.12	31.58
1	M	86	14.00	5.80	8.20	20.00	15.00	5.90	29.10	37.30
2	M	87	14.00	6.06	7.94	20.00	15.00	13.90	21.10	29.04
3	M	88	14.00	5.96	8.04	20.00	15.00	6.70	28.30	36.34
1	M	89	14.00	6.42	7.58	20.00	15.00	6.12	25.88	34.46
2	M	90	14.00	5.72	8.28	20.00	15.00	6.20	25.80	37.08
3	M	91	14.00	5.52	8.48	20.00	15.00	6.70	28.30	36.78
2	M	92	14.00	5.46	8.54	20.00	15.00	12.56	22.44	30.98
1	M	93	14.00	6.26	7.74	20.00	15.00	8.42	26.58	34.32
4	M	94	14.00	5.58	8.42	20.00	15.00	6.08	28.92	37.34
1	M	95	14.00	5.36	8.64	20.00	15.00	7.12	27.88	36.52
2	M	96	14.00	6.32	7.68	20.00	15.00	14.22	20.78	28.46
3	M	97	14.00	5.44	8.56	20.00	15.00	7.30	27.70	36.26
4	M	98	14.00	5.60	8.40	20.00	15.00	5.92	29.08	37.48
2	M	103	14.00	5.66	8.34	20.00	15.00	13.14	21.86	30.20
4	M	104	14.00	5.74	8.26	20.00	15.00	6.52	28.48	36.74
3	M	105	14.00	5.88	8.12	20.00	15.00	7.84	27.16	35.28
1	M	106	14.00	5.54	8.46	20.00	15.00	8.24	26.76	35.22
4	M	107	14.00	6.00	8.00	20.00	15.00	7.32	27.68	35.68
2	M	108	14.00	5.84	8.16	20.00	15.00	12.66	22.34	30.50
3	M	109	14.00	5.22	8.78	20.00	15.00	7.10	27.90	36.68
1	M	110	14.00	5.42	8.58	20.00	15.00	7.60	27.40	35.98
2	M	111	14.00	5.86	8.14	20.00	15.00	13.60	21.40	29.54
4	M	112	14.00	5.32	8.68	20.00	15.00	6.66	28.34	37.02
3	M	113	14.00	5.84	8.16	20.00	15.00	6.78	28.22	36.38
1	M	114	14.00	5.92	8.08	20.00	15.00	7.80	27.20	35.28
2	M	115	14.00	5.56	8.44	20.00	15.00	14.26	20.74	29.18
1	M	116	14.00	6.34	7.66	20.00	15.00	7.18	27.82	35.48
4	M	117	14.00	5.98	8.02	20.00	15.00	7.42	27.58	35.60
3	M	118	14.00	5.32	8.68	20.00	15.00	7.50	27.50	36.18
2	M	123	14.00	5.76	8.24	20.00	15.00	12.42	22.58	30.82
3	M	124	14.00	5.20	8.80	20.00	15.00	6.74	28.26	37.06
4	M	128	14.00	4.98	9.02	20.00	15.00	5.76	29.24	38.26
1	M	126	14.00	5.56	8.42	20.00	15.00	6.34	28.66	37.08
1	M	127	14.00	5.82	8.18	20.00	15.00	7.00	28.00	36.18
3	M	128	14.00	5.22	8.78	20.00	15.00	6.06	28.94	37.72
2	M	129	14.00	6.66	7.34	20.00	15.00	15.56	19.44	26.78
4	M	130	14.00	6.12	7.88	20.00	15.00	7.78	27.22	35.10
1	M	131	14.00	5.18	8.82	20.00	15.00	6.78	28.22	37.04
4	M	132	14.00	5.38	8.62	20.00	15.00	5.64	29.46	38.08
2	M	133	14.00	5.92	8.08	20.00	15.00	13.86	21.14	29.22
3	M	134	14.00	5.38	8.62	20.00	15.00	6.28	28.72	37.34
2	M	135	14.00	5.56	8.44	20.00	15.00	12.94	22.06	30.50
4	M	136	14.00	5.22	8.78	20.00	15.00	6.78	28.22	37.00
1	M	137	14.00	6.32	7.68	20.00	15.00	6.50	28.50	36.18
3	M	138	14.00	6.12	7.88	20.00	15.00	6.10	28.90	36.78

Table 4. Summary by Treatment of Feed Added and Weighed Back by Pen Study Days 0 - 28
(b)Project No. NV-13-2
BLOC 7

			STARTER			GROWER				
			Study Days 0 - 14 [illegible] 15 - 03APR13)			[illegible]				
			3/17	4/3	0-14	4/3	4/13	4/17	14-28	0-28
Treatment	Sex	Pen	Feed 1	WB	Consumption	Feed 2	Feed 3	WB	Consumption	Consumption
1	M	83	14.00	5.98	8.02	20.00	15.00	6.88	28.12	36.14
1	M	89	14.00	6.42	7.58	20.00	15.00	5.12	26.88	34.46
1	M	93	14.00	6.26	7.74	20.00	15.00	8.42	26.58	34.32
1	M	95	14.00	5.36	8.64	20.00	15.00	7.12	27.88	36.52
1	M	105	14.00	5.54	8.46	20.00	15.00	8.24	26.76	35.22
1	M	110	14.00	5.42	8.58	20.00	15.00	7.60	27.40	35.98
1	M	114	14.00	5.92	8.08	20.00	15.00	7.80	27.20	35.28
1	M	116	14.00	6.34	7.66	20.00	15.00	7.18	27.82	35.48
1	M	120	14.00	5.58	8.42	20.00	15.00	6.34	28.66	37.08
1	M	127	14.00	5.82	8.18	20.00	15.00	7.00	28.00	36.18
1	M	131	14.00	5.18	8.82	20.00	15.00	6.78	28.22	37.04
1	M	137	14.00	6.32	7.68	20.00	15.00	8.50	26.50	34.18
Total			168.00	70.14	97.86	240.00	180.00	89.98	330.02	427.88
2	M	85	14.00	5.54	8.46	20.00	15.00	11.88	23.12	31.58
2	M	87	14.00	6.06	7.94	20.00	15.00	13.90	21.10	29.04
2	M	92	14.00	5.46	8.54	20.00	15.00	12.56	22.44	30.98
2	M	96	14.00	6.32	7.68	20.00	15.00	14.22	20.78	28.46
2	M	103	14.00	5.66	8.34	20.00	15.00	13.14	21.86	30.20
2	M	108	14.00	5.84	8.16	20.00	15.00	12.66	22.34	30.50
2	M	111	14.00	5.86	8.14	20.00	15.00	13.60	21.40	29.54
2	M	115	14.00	5.56	8.44	20.00	15.00	14.26	20.74	29.18
2	M	123	14.00	5.76	8.24	20.00	15.00	12.42	22.58	30.82
2	M	129	14.00	6.66	7.34	20.00	15.00	15.56	19.44	26.78
2	M	133	14.00	5.92	8.08	20.00	15.00	13.86	21.14	29.22
2	M	135	14.00	5.56	8.44	20.00	15.00	12.94	22.06	30.50
Total			168.00	70.20	97.80	240.00	180.00	161.00	259.00	356.80
3	M	84	14.00	6.36	7.64	20.00	15.00	6.12	28.88	36.52
3	M	88	14.00	5.96	8.04	20.00	15.00	6.70	28.30	36.34
3	M	91	14.00	5.52	8.48	20.00	15.00	6.70	28.30	36.78
3	M	97	14.00	5.44	8.56	20.00	15.00	7.30	27.70	36.26
3	M	105	14.00	5.88	8.12	20.00	15.00	7.84	27.16	35.28
3	M	109	14.00	5.22	8.78	20.00	15.00	7.10	27.90	36.68
3	M	113	14.00	5.84	8.16	20.00	15.00	6.78	28.22	36.38
3	M	118	14.00	5.32	8.68	20.00	15.00	7.50	27.50	36.18
3	M	124	14.00	5.20	8.80	20.00	15.00	6.74	28.26	37.06
3	M	128	14.00	5.22	8.78	20.00	15.00	6.06	28.94	37.72
3	M	134	14.00	5.38	8.62	20.00	15.00	6.28	28.72	37.34
3	M	138	14.00	6.12	7.88	20.00	15.00	8.10	26.90	34.78
Total			168.00	67.46	100.54	240.00	180.00	85.22	334.78	435.32
4	M	86	14.00	5.80	8.20	20.00	15.00	5.90	29.10	37.30
4	M	90	14.00	5.72	8.28	20.00	15.00	6.20	28.80	37.08
4	M	94	14.00	5.58	8.42	20.00	15.00	5.08	29.92	37.34
4	M	98	14.00	5.60	8.40	20.00	15.00	5.92	29.08	37.48
4	M	104	14.00	5.74	8.26	20.00	15.00	6.52	28.48	36.74
4	M	107	14.00	6.00	8.00	20.00	15.00	7.32	27.68	35.68
4	M	112	14.00	5.32	8.68	20.00	15.00	6.66	28.34	37.02
4	M	117	14.00	5.98	8.02	20.00	15.00	7.42	27.58	35.60
4	M	125	14.00	4.98	9.02	20.00	15.00	5.76	29.24	38.26
4	M	130	14.00	6.12	7.88	20.00	15.00	7.78	27.22	35.10
4	M	132	14.00	5.38	8.62	20.00	15.00	5.54	29.46	38.08
4	M	136	14.00	5.22	8.78	20.00	15.00	5.78	29.22	38.00
Total			168.00	67.44	100.56	240.00	180.00	76.88	343.12	443.68

Table 5. Day 0 Pen Weights of Cobb 500 Broilers (20MAR15)
(b) (4)Project No. NV-13-2
BLDG 7

Treatment	Sex	Pen No.	No. Birds Weighed	Day 0 Pen Wt.	Bird Average Wt.
1	M	83	20	0.857	0.043
3	M	84	20	0.848	0.042
2	M	85	20	0.878	0.044
4	M	86	20	0.854	0.043
2	M	87	20	0.861	0.043
3	M	88	20	0.862	0.043
1	M	89	20	0.844	0.042
4	M	90	20	0.870	0.044
3	M	91	20	0.847	0.042
2	M	92	20	0.862	0.043
1	M	93	20	0.886	0.044
4	M	94	20	0.834	0.042
1	M	95	20	0.841	0.042
2	M	96	20	0.820	0.041
3	M	97	20	0.830	0.042
4	M	98	20	0.831	0.042
2	M	103	20	0.864	0.043
4	M	104	20	0.840	0.042
3	M	105	20	0.839	0.042
1	M	106	20	0.835	0.042
4	M	107	20	0.851	0.043
2	M	108	20	0.826	0.041
3	M	109	20	0.836	0.042
1	M	110	20	0.857	0.043
2	M	111	20	0.826	0.041
4	M	112	20	0.838	0.042
3	M	113	20	0.809	0.040
1	M	114	20	0.813	0.041
2	M	115	20	0.836	0.042
1	M	116	20	0.840	0.042
4	M	117	20	0.834	0.042
3	M	118	20	0.839	0.042
2	M	123	20	0.865	0.043
3	M	124	20	0.865	0.043
4	M	125	20	0.870	0.044
1	M	126	20	0.840	0.042
1	M	127	20	0.844	0.042
3	M	128	20	0.857	0.043
2	M	129	20	0.860	0.043
4	M	130	20	0.868	0.043
1	M	131	20	0.846	0.042
4	M	132	20	0.855	0.043
2	M	133	20	0.865	0.043
3	M	134	20	0.855	0.043
2	M	135	20	0.848	0.042
4	M	136	20	0.861	0.043
1	M	137	20	0.843	0.042
3	M	138	20	0.857	0.043

Table 6 Day 0 Pen Weights of Cobb 500 Broilers Summarized by Treatment (20MAR15)
(b) (4) Project No. NV-13-2
BLDG 7

Treatment	Sex	Pen No.	No. Birds Weighed	Day 0 Pen Wt.	Bird Average Wt.
1	M	83	20	0.857	0.043
1	M	89	20	0.844	0.042
1	M	93	20	0.866	0.044
1	M	95	20	0.841	0.042
1	M	106	20	0.835	0.042
1	M	110	20	0.857	0.043
1	M	114	20	0.813	0.041
1	M	116	20	0.840	0.042
1	M	126	20	0.840	0.042
1	M	127	20	0.844	0.042
1	M	131	20	0.846	0.042
1	M	137	20	0.843	0.042
Total/Averages			240	0.846	0.042
Standard Deviations				0.017	0.001
CVs				2.01%	2.01%

Treatment	Sex	Pen No.	No. Birds Weighed	Day 0 Pen Wt.	Bird Average Wt.
2	M	85	20	0.878	0.044
2	M	87	20	0.861	0.043
2	M	92	20	0.862	0.043
2	M	96	20	0.820	0.041
2	M	103	20	0.864	0.043
2	M	108	20	0.828	0.041
2	M	111	20	0.826	0.041
2	M	115	20	0.836	0.042
2	M	123	20	0.865	0.043
2	M	129	20	0.860	0.043
2	M	133	20	0.865	0.043
2	M	135	20	0.848	0.042
Total/Averages			240	0.851	0.043
Standard Deviations				0.019	0.001
CVs				2.22%	2.22%

Treatment	Sex	Pen No.	No. Birds Weighed	Day 0 Pen Wt.	Bird Average Wt.
3	M	84	20	0.846	0.042
3	M	88	20	0.852	0.043
3	M	91	20	0.847	0.042
3	M	97	20	0.830	0.042
3	M	105	20	0.839	0.042
3	M	109	20	0.836	0.042
3	M	113	20	0.809	0.040
3	M	118	20	0.839	0.042
3	M	124	20	0.865	0.043
3	M	128	20	0.857	0.043
3	M	134	20	0.856	0.043
3	M	136	20	0.857	0.043
Total/Averages			240	0.845	0.042
Standard Deviations				0.015	0.001
CVs				1.81%	1.81%

Treatment	Sex	Pen No.	No. Birds Weighed	Day 0 Pen Wt.	Bird Average Wt.
4	M	86	20	0.854	0.043
4	M	90	20	0.870	0.044
4	M	94	20	0.834	0.042
4	M	98	20	0.831	0.042
4	M	104	20	0.840	0.042
4	M	107	20	0.851	0.043
4	M	112	20	0.830	0.042
4	M	117	20	0.834	0.042
4	M	125	20	0.870	0.044
4	M	130	20	0.866	0.043
4	M	132	20	0.855	0.043
4	M	136	20	0.861	0.043
Total/Averages			240	0.850	0.042
Standard Deviations				0.015	0.001
CVs				1.80%	1.80%

Table 7 Day 14 Pen Weights of Cobb 500 Broilers (03APR15)
(b) (4) Project No. NV-13-2
BLDG 7

Treatment	Sex	Pen No.	No. Birds Weighed	Day 14 Pen Wt.	Bird Average Wt.
1	M	83	20	6.68	0.334
3	M	84	20	6.40	0.320
2	M	85	20	6.72	0.336
4	M	86	20	7.04	0.352
2	M	87	20	6.18	0.309
3	M	88	20	6.88	0.344
1	M	89	20	6.28	0.314
4	M	90	20	7.04	0.352
3	M	91	20	6.96	0.348
2	M	92	20	6.68	0.334
1	M	93	20	6.52	0.326
4	M	94	20	6.76	0.338
1	M	95	20	6.80	0.340
2	M	96	20	6.30	0.315
3	M	97	20	6.82	0.341
4	M	98	20	7.12	0.356
2	M	103	20	6.62	0.331
4	M	104	20	7.04	0.352
3	M	105	19	6.76	0.356
1	M	106	19	6.56	0.345
4	M	107	20	6.52	0.326
2	M	108	20	6.48	0.324
3	M	109	20	7.36	0.368
1	M	110	20	6.44	0.322
2	M	111	19	6.04	0.318
4	M	112	20	6.96	0.348
3	M	113	20	7.04	0.352
1	M	114	20	6.78	0.339
2	M	115	20	6.48	0.324
1	M	116	20	6.50	0.325
4	M	117	20	6.76	0.338
3	M	118	20	6.76	0.338
2	M	123	20	6.80	0.340
3	M	124	20	7.02	0.351
4	M	125	20	7.38	0.369
1	M	126	20	7.06	0.353
1	M	127	20	6.78	0.339
3	M	128	20	7.28	0.364
2	M	129	19	5.94	0.313
4	M	130	19	6.66	0.351
1	M	131	20	7.12	0.356
4	M	132	20	7.48	0.374
2	M	133	20	6.26	0.313
3	M	134	20	6.82	0.341
2	M	135	19	6.46	0.340
4	M	136	20	7.32	0.366
1	M	137	19	6.12	0.322
3	M	138	20	6.52	0.326

Table 8. Weights and Performance of Cobb 500 Broilers Study Days 0-14 (02APR15)
(b) (4) Project No. NV-13-2
BLDG 7

Treatment	Sex	Pen No.	Number of Birds				Day 14 Pen Weight	Day 0 Pen Weight	Pen Gain	Day 14 Bird Average	Day 0 Bird Average	Day 0 - 13 Bird Average	Ret. a. Weight	Total Pen Gain	Day 0 - 14 Feed Consump	Day 0 - 14 Av. Feed Intake per Bird	Feed Gain	Adjusted Feed Gain
			Started	Removed	Mortality	Weighed	(kg)	(kg)	(kg)	Wt. (kg)	Wt. (kg)	Gain (kg)	(kg)	= Ret	(kg)	(kg)	(kg/bird)	(kg/bird)
1	M	83	20	0	0	20	6.68	0.857	5.823	0.334	0.043	0.291	0.000	5.823	8.02	0.401	1.377	1.377
3	M	84	20	0	0	20	6.40	0.848	5.552	0.320	0.042	0.278	0.000	5.552	7.64	0.382	1.376	1.376
2	M	85	20	0	0	20	6.72	0.678	5.842	0.336	0.034	0.292	0.000	5.842	8.46	0.423	1.448	1.448
4	M	86	20	0	0	20	7.04	0.854	6.186	0.352	0.043	0.309	0.000	6.186	8.20	0.410	1.326	1.326
2	M	87	20	0	0	20	6.16	0.841	5.319	0.308	0.042	0.266	0.000	5.319	7.54	0.377	1.493	1.493
3	M	88	20	0	0	20	6.88	0.852	6.028	0.344	0.043	0.301	0.000	6.028	8.04	0.402	1.334	1.334
1	M	89	20	0	0	20	6.29	0.853	5.436	0.314	0.042	0.272	0.000	5.436	7.58	0.379	1.394	1.394
4	M	90	20	0	0	20	7.04	0.870	6.170	0.352	0.044	0.309	0.000	6.170	8.28	0.414	1.342	1.342
3	M	91	20	0	0	20	6.96	0.847	6.113	0.348	0.042	0.306	0.000	6.113	8.48	0.424	1.387	1.387
2	M	92	20	0	0	20	6.68	0.862	5.818	0.334	0.043	0.291	0.000	5.818	8.54	0.427	1.468	1.468
1	M	93	20	0	0	20	6.52	0.888	5.634	0.326	0.044	0.282	0.000	5.634	7.74	0.387	1.374	1.374
4	M	94	20	0	0	20	6.76	0.834	5.926	0.338	0.042	0.296	0.000	5.926	8.42	0.421	1.421	1.421
1	M	95	20	0	0	20	6.80	0.841	5.959	0.340	0.042	0.298	0.000	5.959	8.64	0.432	1.450	1.450
2	M	96	20	0	0	20	6.30	0.820	5.480	0.315	0.041	0.274	0.000	5.480	7.88	0.394	1.403	1.403
3	M	97	20	0	0	20	6.87	0.880	5.990	0.344	0.044	0.300	0.000	5.990	8.56	0.428	1.429	1.429
4	M	98	20	0	0	20	7.12	0.831	6.289	0.356	0.042	0.314	0.000	6.289	8.40	0.420	1.336	1.336
2	M	103	20	0	0	20	6.62	0.864	5.756	0.331	0.043	0.288	0.000	5.756	8.34	0.417	1.449	1.449
4	M	104	20	0	0	20	7.04	0.840	6.200	0.352	0.042	0.310	0.000	6.200	8.26	0.413	1.332	1.332
3	M	105	20	0	1	19	6.76	0.836	5.921	0.356	0.044	0.314	0.145	6.066	8.12	0.427	1.371	1.339
1	M	106	20	0	1	19	6.56	0.836	5.725	0.345	0.044	0.304	0.153	5.878	8.46	0.445	1.478	1.439
3	M	107	20	0	0	20	6.52	0.851	5.669	0.326	0.043	0.283	0.000	5.669	8.00	0.400	1.411	1.411
2	M	108	20	0	0	20	6.48	0.828	5.652	0.324	0.041	0.283	0.000	5.652	8.16	0.408	1.444	1.444
3	M	109	20	0	0	20	7.36	0.835	6.525	0.368	0.042	0.326	0.000	6.525	8.78	0.439	1.346	1.346
1	M	110	20	0	0	20	6.44	0.857	5.583	0.322	0.043	0.279	0.000	5.583	8.58	0.429	1.537	1.537
2	M	111	20	0	1	19	6.04	0.826	5.214	0.318	0.043	0.277	0.062	5.296	8.14	0.428	1.561	1.537
4	M	112	20	0	0	20	6.96	0.830	6.130	0.348	0.042	0.307	0.000	6.130	8.68	0.434	1.416	1.416
3	M	113	20	0	0	20	7.04	0.809	6.231	0.352	0.040	0.312	0.000	6.231	8.16	0.408	1.310	1.310
3	M	114	20	0	0	20	6.76	0.813	5.967	0.338	0.041	0.296	0.000	5.967	8.08	0.404	1.354	1.354
2	M	115	20	0	0	20	6.48	0.836	5.644	0.324	0.042	0.282	0.000	5.644	8.44	0.422	1.495	1.495
1	M	116	20	0	0	20	6.50	0.840	5.660	0.325	0.042	0.283	0.000	5.660	7.66	0.383	1.353	1.353
4	M	117	20	0	0	20	6.76	0.834	5.926	0.338	0.042	0.296	0.000	5.926	8.02	0.401	1.353	1.353
3	M	118	20	0	0	20	6.76	0.839	5.921	0.338	0.042	0.296	0.000	5.921	8.88	0.434	1.466	1.466
2	M	123	20	0	0	20	6.80	0.865	5.935	0.340	0.043	0.297	0.000	5.935	8.24	0.412	1.388	1.388
3	M	124	20	0	0	20	7.02	0.865	6.155	0.351	0.043	0.308	0.000	6.155	8.80	0.440	1.430	1.430
4	M	125	20	0	0	20	7.38	0.870	6.510	0.369	0.044	0.326	0.000	6.510	9.02	0.451	1.386	1.386
1	M	126	20	0	0	20	7.06	0.840	6.220	0.353	0.042	0.311	0.000	6.220	8.42	0.421	1.354	1.354
1	M	127	20	0	0	20	6.76	0.844	5.916	0.338	0.042	0.297	0.000	5.858	8.18	0.409	1.379	1.378
3	M	128	20	0	0	20	7.26	0.857	6.423	0.364	0.043	0.321	0.000	6.423	8.78	0.439	1.367	1.367
2	M	129	20	1	0	19	5.94	0.860	5.080	0.313	0.043	0.270	0.113	5.193	7.34	0.386	1.445	1.413
4	M	130	20	0	1	19	6.86	0.865	5.995	0.361	0.043	0.318	0.043	6.038	7.84	0.415	1.314	1.306
1	M	131	20	0	0	20	7.12	0.846	6.274	0.356	0.042	0.314	0.000	6.274	8.82	0.441	1.406	1.406
4	M	132	20	0	0	20	7.48	0.855	6.625	0.374	0.043	0.331	0.000	6.625	8.62	0.431	1.301	1.301
2	M	133	20	0	0	20	6.26	0.865	5.395	0.313	0.043	0.270	0.000	5.395	8.08	0.404	1.496	1.496
3	M	134	20	0	0	20	6.82	0.858	5.964	0.341	0.043	0.298	0.000	5.964	8.62	0.431	1.445	1.445
1	M	135	20	1	0	19	6.46	0.848	5.612	0.340	0.042	0.298	0.200	5.812	8.44	0.444	1.504	1.462

Table 8. Weights and Performance of Cobb 500 Broilers Study Days 0-14 (03APR16)
(b) (4)Project No. NV-13-2
Study 7

Treatment	Sex	Pen No	Number of Birds				Day 14 Pen Weight (kg)	Day 0 Pen Weight (kg)	Pen Gain (kg)	Day 14 Bird Average Wt. (kg)	Day 0 Bird Average Wt. (kg)	Day 0 - 14 Bird Average Gain (kg)	R.M.-A. Weight (kg)	Total Pen Gain - R.M.	Day 0 - 14 Feed Consump (kg)	Day 0 - 14 Av. Feed intake per Bird (kg)	Feed Gain (kg/bird)	Adjusted Feed Gain (kg/bird)
			Started	Removed	Mortality	Weighed												
4	M	136	20	0	0	20	7.32	0.861	6.459	0.366	0.043	0.323	0.000	6.459	8.78	0.439	1.359	1.359
1	M	137	20	0	1	19	6.12	0.843	5.277	0.322	0.042	0.280	0.097	5.374	7.68	0.404	1.456	1.429
3	M	138	20	0	0	20	6.52	0.857	5.663	0.326	0.043	0.283	0.000	5.663	7.88	0.394	1.391	1.391

Table 9. Weights and Performance of Cobb 500 Broilers by Treatment Study Days 0-14 (03APR15)
(b) (4) Project No. NV-13-2
BLDG 7

Treatment	Sex	Pen No	Number of Birds				Day 14 Pen Weight	Pen Gain	Day 14 Bird Average	Day 0 - 14 Bird Average	Day 0 - 14 Feed Consump	Day 0 - 14 Av. Feed Intake per Bird	Feed Gain	Adjusted Feed Gain
			Started	Removed	Mortality	Weighed	(kg)	(kg)	Wt. (kg)	Gain (kg)	(kg)	(kg)	(kg/bird)	(kg/bird)
1	M	83	20	0	0	20	6.68	5.823	0.334	0.291	8.02	0.401	1.377	1.377
1	M	89	20	0	0	20	6.26	5.436	0.314	0.272	7.58	0.379	1.394	1.394
1	M	93	20	0	0	20	6.52	5.634	0.326	0.282	7.74	0.387	1.374	1.374
1	M	95	20	0	0	20	6.80	5.959	0.340	0.298	8.64	0.432	1.450	1.450
1	M	105	20	0	1	19	6.56	5.725	0.345	0.304	8.48	0.445	1.478	1.439
1	M	110	20	0	0	20	6.44	5.583	0.322	0.279	8.58	0.429	1.537	1.537
1	M	114	20	0	0	20	6.78	5.967	0.339	0.298	8.08	0.404	1.354	1.354
1	M	116	20	0	0	20	6.50	5.660	0.325	0.283	7.66	0.383	1.353	1.353
1	M	126	20	0	0	20	7.06	6.220	0.353	0.311	8.42	0.421	1.354	1.354
1	M	127	20	0	0	20	6.78	5.936	0.339	0.297	8.18	0.409	1.378	1.378
1	M	131	20	0	0	20	7.12	6.274	0.356	0.314	8.82	0.441	1.406	1.406
1	M	137	20	0	1	19	6.12	5.277	0.322	0.280	7.68	0.404	1.455	1.429
Total/Averages			240	0	2	238	6.637	5.791	0.336	0.292	8.155	0.411	1.469	1.404
Standard Deviations							0.294	0.298	0.013	0.013	0.429	0.022	0.058	0.054
CVs							4.44%	5.15%	3.91%	4.58%	5.25%	5.45%	4.14%	3.82%
2	M	85	20	0	0	20	6.72	5.842	0.336	0.292	8.46	0.423	1.448	1.448
2	M	87	20	0	0	20	6.18	5.319	0.309	0.266	7.94	0.397	1.493	1.493
2	M	92	20	0	0	20	6.68	5.818	0.334	0.291	8.54	0.427	1.468	1.468
2	M	96	20	0	0	20	6.30	5.460	0.315	0.274	7.68	0.384	1.401	1.401
2	M	103	20	0	0	20	6.62	5.756	0.331	0.288	8.34	0.417	1.449	1.449
2	M	108	20	0	0	20	6.48	5.652	0.324	0.283	8.16	0.408	1.444	1.444
2	M	111	20	0	1	19	6.04	5.214	0.318	0.277	8.14	0.428	1.561	1.537
2	M	115	20	0	0	20	6.48	5.644	0.324	0.282	8.44	0.422	1.495	1.495
2	M	123	20	0	0	20	6.80	5.935	0.340	0.297	8.24	0.412	1.388	1.388
2	M	129	20	1	0	19	5.94	5.080	0.313	0.270	7.34	0.386	1.445	1.413
2	M	133	20	0	0	20	6.26	5.395	0.313	0.270	8.08	0.404	1.498	1.498
2	M	135	20	1	0	19	6.48	5.612	0.340	0.298	8.44	0.444	1.504	1.452
Total/Averages			240	2	1	237	6.413	5.562	0.325	0.282	8.150	0.413	1.466	1.457
Standard Deviations							0.273	0.267	0.011	0.011	0.355	0.018	0.048	0.044
CVs							4.26%	4.80%	3.46%	3.90%	4.36%	4.33%	3.24%	3.00%
3	M	84	20	0	0	20	6.40	5.552	0.320	0.278	7.64	0.382	1.376	1.376
3	M	88	20	0	0	20	6.88	6.028	0.344	0.301	8.04	0.402	1.334	1.334
3	M	91	20	0	0	20	6.96	6.113	0.348	0.306	8.48	0.424	1.387	1.387
3	M	97	20	0	0	20	6.82	5.990	0.341	0.300	8.56	0.428	1.429	1.429
3	M	105	20	0	1	19	6.76	5.921	0.356	0.314	8.12	0.427	1.371	1.339
3	M	109	20	0	0	20	7.36	6.525	0.368	0.326	8.78	0.439	1.346	1.346
3	M	113	20	0	0	20	7.04	6.231	0.352	0.312	8.16	0.408	1.310	1.310
3	M	118	20	0	0	20	6.76	5.921	0.338	0.296	8.68	0.434	1.466	1.466
3	M	124	20	0	0	20	7.02	6.155	0.351	0.308	8.80	0.440	1.430	1.430
3	M	128	20	0	0	20	7.28	6.423	0.364	0.321	8.78	0.439	1.367	1.367
3	M	134	20	0	0	20	6.82	5.964	0.341	0.298	8.62	0.431	1.445	1.445
3	M	138	20	0	0	20	6.52	5.663	0.326	0.283	7.88	0.394	1.391	1.391
Total/Averages			240	0	1	239	6.885	6.041	0.346	0.304	8.376	0.421	1.388	1.385
Standard Deviations							0.276	0.279	0.014	0.014	0.395	0.019	0.047	0.049
CVs							4.01%	4.61%	4.05%	4.67%	4.71%	4.62%	3.40%	3.55%
4	M	86	20	0	0	20	7.04	6.186	0.352	0.309	8.20	0.410	1.326	1.326
4	M	90	20	0	0	20	7.04	6.170	0.352	0.309	8.28	0.414	1.342	1.342
4	M	94	20	0	0	20	6.76	5.926	0.338	0.296	8.42	0.421	1.421	1.421
4	M	98	20	0	0	20	7.12	6.289	0.356	0.314	8.40	0.420	1.336	1.336
4	M	104	20	0	0	20	7.04	6.200	0.352	0.310	8.26	0.413	1.332	1.332
4	M	107	20	0	0	20	6.52	5.669	0.326	0.283	8.00	0.400	1.411	1.411
4	M	112	20	0	0	20	6.96	6.130	0.348	0.307	8.68	0.434	1.416	1.416
4	M	117	20	0	0	20	6.76	5.926	0.338	0.296	8.02	0.401	1.353	1.353
4	M	125	20	0	0	20	7.38	6.510	0.369	0.326	9.02	0.451	1.386	1.386
4	M	130	20	0	1	19	6.86	5.995	0.361	0.318	7.88	0.415	1.314	1.305
4	M	132	20	0	0	20	7.48	6.625	0.374	0.331	8.62	0.431	1.301	1.301
4	M	136	20	0	0	20	7.32	6.459	0.366	0.323	8.78	0.439	1.359	1.359
Total/Averages			240	0	1	239	7.023	6.174	0.353	0.310	8.380	0.421	1.358	1.357
Standard Deviations							0.279	0.274	0.014	0.014	0.344	0.015	0.041	0.042
CVs							3.97%	4.43%	3.96%	4.38%	4.10%	3.66%	3.02%	3.10%

Graph 1. Body Weights and Performance Study of Cobb 500 Broilers Study Days 0 - 14 (03APR13)
(b) (4) Project No. NV-13-2
BLDG 7

Treatment	Average Bird Wt Gain (kg)	Adjusted Feed Gain	Treatment Description
1	0.292	1.404	Positive Control (PC)
2	0.282	1.457	Negative Control (NC)
3	0.304	1.385	NC with 250 U CIBENZA® PHYTAVERSE™ G10 per kg diet
4	0.310	1.317	NC with 500 U CIBENZA® PHYTAVERSE™ G10 per kg diet

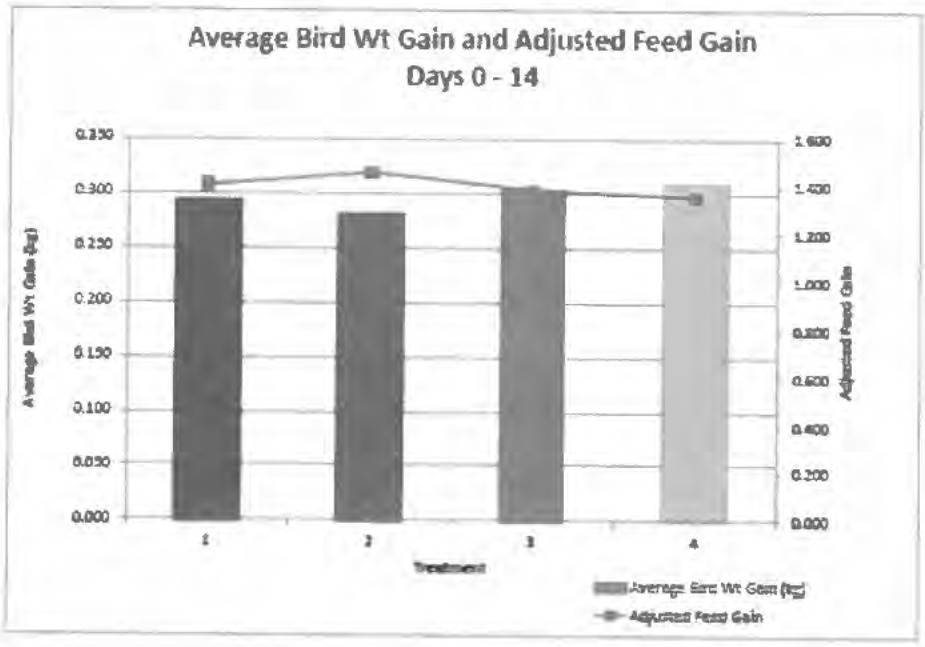


Table 10. Day 28 Pen Weights of Cobb 500 Broilers (17APR15)
(b) (4) Project No. NV-13-2
BLDG 7

Treatment	Sex	Pen No.	No. Birds Weighed	Day 28 Pen Wt.	Bird Average Wt.
1	M	83	20	25.62	1.281
3	M	84	20	24.48	1.224
2	M	85	19	21.16	1.114
4	M	86	20	26.58	1.329
2	M	87	18	18.42	1.023
3	M	88	20	26.06	1.303
1	M	89	20	24.10	1.205
4	M	90	20	26.26	1.313
3	M	91	20	25.80	1.290
2	M	92	19	20.34	1.071
1	M	93	20	24.22	1.211
4	M	94	20	26.34	1.317
1	M	95	20	25.16	1.258
2	M	96	18	18.36	1.020
3	M	97	20	25.32	1.266
4	M	98	20	26.60	1.330
2	M	103	19	19.86	1.045
4	M	104	20	26.66	1.334
3	M	105	19	25.02	1.317
1	M	106	19	24.56	1.293
4	M	107	20	25.80	1.290
2	M	108	17	18.86	1.109
3	M	109	20	25.98	1.299
1	M	110	20	25.08	1.254
2	M	111	18	19.12	1.062
4	M	112	20	26.16	1.308
3	M	113	20	25.18	1.309
1	M	114	20	25.08	1.254
2	M	115	16	17.88	1.118
1	M	116	20	25.20	1.260
4	M	117	20	25.72	1.286
3	M	118	20	25.18	1.259
2	M	123	18	20.26	1.126
3	M	124	20	25.72	1.286
4	M	125	19	25.68	1.352
1	M	126	20	26.18	1.309
1	M	127	20	25.46	1.273
3	M	128	20	26.48	1.324
2	M	129	16	16.80	1.050
4	M	130	19	25.22	1.327
1	M	131	20	25.82	1.291
4	M	132	19	26.40	1.389
2	M	133	19	19.34	1.018
3	M	134	20	26.26	1.313
2	M	135	17	19.62	1.154
4	M	136	20	26.68	1.334
1	M	137	19	24.08	1.267
3	M	138	20	24.80	1.240

Table 11. Weights and Performance of Cobb 500 Broilers Study Days 0 - 28 (17APR15)
(b) Project No. NV-13-2
BLDG 7

Treatment	Sex	Pen No	Number of Birds				Day 28 Pen Weight (kg)	Day 0 Pen Weight (kg)	Pen Gain (kg)	Day 28 Bird Average Wt. (kg)	Day 0 Bird Average Wt. (kg)	Day 0-28 Bird Average Gain (kg)	R.M.A. Weight (kg)	Total Pen Gain = R.M.A.	Day 0-28 Feed Consuming (kg)	Day 0-28 Av. Feed Intake per Bird (kg)	Feed Gain (kg/bird)	Adjusted Feed Gain (kg/bird)
			Started	Removed	Mortality	Weighed												
1	M	83	20	0	0	20	25.62	0.857	24.763	1.281	0.043	1.238	0.000	24.763	36.14	1.807	1.459	1.459
3	M	84	20	6	0	20	24.48	0.848	23.632	1.224	0.042	1.182	0.000	23.632	34.52	1.726	1.461	1.461
2	M	85	20	1	0	19	21.18	0.878	20.283	1.114	0.044	1.070	0.355	20.637	31.58	1.662	1.557	1.530
4	M	86	20	0	0	20	26.58	0.854	25.726	1.329	0.043	1.286	0.000	25.726	37.30	1.865	1.450	1.450
2	M	87	20	2	0	18	18.42	0.861	17.559	1.023	0.048	0.960	0.898	18.447	29.04	1.613	1.654	1.574
1	M	88	20	0	0	20	26.06	0.852	25.208	1.303	0.043	1.260	0.000	25.208	36.34	1.817	1.442	1.442
4	M	90	20	0	0	20	24.10	0.844	23.256	1.205	0.042	1.163	0.000	23.256	34.46	1.723	1.482	1.482
3	M	91	20	0	0	20	26.26	0.870	25.390	1.313	0.044	1.270	0.000	25.390	37.08	1.854	1.460	1.460
2	M	92	20	0	0	20	25.60	0.847	24.753	1.280	0.042	1.238	0.000	24.753	36.78	1.839	1.474	1.474
1	M	93	20	0	1	19	20.34	0.862	19.478	1.071	0.043	1.027	0.478	19.956	30.98	1.631	1.591	1.552
4	M	94	20	0	0	20	24.22	0.886	23.334	1.211	0.044	1.167	0.000	23.334	34.32	1.716	1.471	1.471
1	M	95	20	0	0	20	26.34	0.834	25.506	1.317	0.042	1.275	0.000	25.506	37.34	1.867	1.464	1.464
2	M	96	20	0	0	20	25.16	0.841	24.319	1.258	0.042	1.215	0.000	24.319	36.52	1.826	1.502	1.502
3	M	97	20	1	1	18	18.36	0.820	17.540	1.020	0.041	0.979	1.083	18.603	29.46	1.581	1.623	1.530
4	M	98	20	0	0	20	26.60	0.831	25.769	1.330	0.042	1.288	0.000	25.769	37.48	1.874	1.454	1.454
2	M	103	20	0	1	19	19.86	0.864	18.996	1.045	0.043	1.003	0.545	19.541	30.20	1.589	1.590	1.545
4	M	104	20	0	0	20	26.66	0.840	25.840	1.334	0.042	1.292	0.000	25.840	36.74	1.837	1.422	1.422
3	M	105	20	0	1	19	25.02	0.839	24.181	1.317	0.042	1.275	0.145	24.326	35.28	1.857	1.459	1.450
1	M	106	20	0	1	19	24.56	0.835	23.725	1.293	0.042	1.251	0.153	23.878	35.22	1.854	1.485	1.475
4	M	107	20	0	0	20	25.80	0.851	24.949	1.290	0.043	1.247	0.000	24.949	35.66	1.784	1.430	1.430
2	M	108	20	2	1	17	18.85	0.828	18.032	1.109	0.041	1.068	2.184	20.216	30.50	1.794	1.691	1.509
3	M	109	20	0	0	20	25.98	0.835	25.145	1.299	0.042	1.257	0.000	25.145	36.68	1.834	1.459	1.459
1	M	110	20	0	0	20	25.08	0.857	24.223	1.254	0.043	1.211	0.000	24.223	35.98	1.799	1.485	1.485
2	M	111	20	1	1	18	19.12	0.826	18.294	1.062	0.041	1.021	0.661	18.955	29.54	1.641	1.616	1.558
4	M	112	20	0	0	20	26.15	0.830	25.330	1.308	0.042	1.267	0.000	25.330	37.02	1.851	1.462	1.462
3	M	113	20	0	0	20	26.18	0.809	25.371	1.309	0.040	1.269	0.000	25.371	36.38	1.819	1.434	1.434
1	M	114	20	0	0	20	25.08	0.813	24.267	1.254	0.041	1.213	0.000	24.267	35.28	1.764	1.454	1.454
2	M	115	20	3	1	16	17.88	0.836	17.044	1.118	0.042	1.076	1.751	18.795	29.18	1.824	1.712	1.550
1	M	116	20	0	0	20	25.20	0.840	24.360	1.260	0.042	1.218	0.000	24.360	35.48	1.774	1.456	1.456
4	M	117	20	0	0	20	25.72	0.834	24.886	1.286	0.042	1.244	0.000	24.886	35.60	1.780	1.431	1.431
2	M	118	20	0	0	20	25.18	0.839	24.341	1.259	0.042	1.217	0.000	24.341	36.18	1.809	1.486	1.486
2	M	123	20	2	0	18	20.26	0.865	19.395	1.126	0.043	1.082	1.096	20.491	30.82	1.712	1.589	1.504
3	M	124	20	0	0	20	25.72	0.865	24.856	1.286	0.043	1.243	0.000	24.856	37.06	1.853	1.491	1.491
4	M	125	20	0	1	19	25.68	0.870	24.810	1.352	0.044	1.308	1.227	26.037	38.26	2.014	1.542	1.469
1	M	126	20	0	0	20	26.18	0.840	25.340	1.309	0.042	1.267	0.000	25.340	37.08	1.854	1.463	1.463
1	M	127	20	0	0	20	25.48	0.844	24.636	1.273	0.042	1.231	0.000	24.636	36.18	1.809	1.470	1.470
3	M	128	20	0	0	20	26.48	0.857	25.623	1.324	0.043	1.281	0.000	25.623	37.72	1.886	1.472	1.472
2	M	129	20	4	0	16	16.80	0.860	15.940	1.050	0.043	1.007	1.538	17.478	26.76	1.674	1.680	1.532
4	M	130	20	0	1	19	25.22	0.866	24.356	1.327	0.043	1.284	0.043	24.398	35.10	1.847	1.441	1.436
1	M	131	20	0	0	20	25.62	0.646	24.974	1.281	0.042	1.239	0.000	24.974	37.04	1.852	1.483	1.483
4	M	132	20	0	1	19	26.40	0.855	25.545	1.389	0.043	1.347	0.678	26.223	38.08	2.004	1.491	1.452
2	M	133	20	1	0	19	19.34	0.865	18.475	1.018	0.043	0.975	0.346	18.821	29.22	1.538	1.562	1.553
3	M	134	20	0	0	20	26.26	0.856	25.404	1.313	0.043	1.270	0.000	25.404	37.34	1.867	1.470	1.470
2	M	135	20	2	1	17	19.62	0.848	18.772	1.154	0.043	1.112	0.999	19.771	30.50	1.794	1.625	1.543

Table 19. Weights and Performance of Cobb 500 Broilers Study Days 0 - 28 (17APR15)
(b) (4)Project No. NV-13-2
BLDG 7

Treatment	Sex	Pen No.	Number of Birds				Day 28 Pen Weight (kg)	Day 0 Pen Weight (kg)	Pen Gain (kg)	Day 28 Bird Average Wt. (kg)	Day 0 Bird Average Wt. (kg)	Day 0-28 Bird Average Gain (kg)	R.M.A Weight (kg)	Total Pen Gain - R.M.A	Day 0-28 Feed Consump. (kg)	Day 0-28 Av. Feed Intake per Bird (kg)	Feed Gain (kg/bird)	Adjusted Feed Gain (kg/bird)
			Started	Removed	Mortality	Weighed												
4	M	136	20	0	0	20	26.68	0.851	25.819	1.334	0.043	1.291	0.000	25.819	36.00	1.800	1.472	1.472
1	M	137	20	0	1	19	24.08	0.843	23.237	1.267	0.042	1.225	0.097	23.334	34.16	1.799	1.471	1.465
3	M	138	20	0	0	20	24.80	0.857	23.943	1.240	0.043	1.197	0.000	23.943	34.78	1.739	1.453	1.453

Table 12. Weights and Performance of Cobb 500 Broilers by Treatment Study Days 0 - 28 (17APR15)
(b) (4) Project No. NV-13-2
BLDG 7

Treatment	Sex	Pen No	Number of Birds				Day 28 Pen Weight	Pen Gain	Day 28 Bird Average	Day 0-28 Bird Average	Day 0-28 Feed Consump	Day 0-28 Av. Feed Intake per Bird	Feed Gain	Adjusted Feed Gain
			Started	Removed	Mortality	Weighed	(kg)	(kg)	Wt. (kg)	Gain (kg)	(kg)	(kg)	(kg/bird)	(kg/bird)
1	M	83	20	0	0	20	25.62	24.783	1.281	1.239	36.14	1.807	1.459	1.459
1	M	89	20	0	0	20	24.10	23.256	1.205	1.163	34.46	1.723	1.482	1.482
1	M	93	20	0	0	20	24.22	23.334	1.211	1.167	34.32	1.716	1.471	1.471
1	M	95	20	0	0	20	25.16	24.319	1.258	1.216	36.52	1.826	1.502	1.502
1	M	106	20	0	1	19	24.56	23.725	1.293	1.251	35.22	1.854	1.485	1.475
1	M	110	20	0	0	20	25.08	24.223	1.254	1.211	35.98	1.799	1.485	1.485
1	M	114	20	0	0	20	25.08	24.257	1.254	1.213	35.28	1.764	1.454	1.454
1	M	116	20	0	0	20	25.20	24.360	1.260	1.218	35.48	1.774	1.456	1.456
1	M	126	20	0	0	20	26.18	25.340	1.309	1.267	37.08	1.854	1.463	1.463
1	M	127	20	0	0	20	25.46	24.616	1.273	1.231	36.18	1.809	1.470	1.470
1	M	131	20	0	0	20	25.82	24.974	1.291	1.249	37.04	1.852	1.483	1.483
1	M	137	20	0	1	19	24.08	23.237	1.267	1.225	34.18	1.799	1.471	1.465
Total/Averages			240	0	2	238	25.047	24.201	1.263	1.221	35.657	1.798	1.473	1.472
Standard Deviations							0.685	0.689	0.031	0.031	1.003	0.047	0.014	0.014
CVs							2.74%	2.85%	2.44%	2.56%	2.81%	2.61%	0.97%	0.95%
2	M	85	20	1	0	19	21.16	20.282	1.114	1.070	31.58	1.662	1.557	1.530
2	M	87	20	2	0	18	18.42	17.559	1.023	0.980	29.04	1.613	1.654	1.574
2	M	92	20	0	1	19	20.34	19.478	1.071	1.027	30.98	1.631	1.591	1.562
2	M	96	20	1	1	18	18.36	17.540	1.020	0.979	28.46	1.581	1.623	1.530
2	M	103	20	0	1	19	19.86	18.996	1.045	1.002	30.20	1.589	1.590	1.545
2	M	108	20	2	1	17	18.86	18.032	1.109	1.068	30.50	1.794	1.691	1.509
2	M	111	20	1	1	18	19.12	18.294	1.062	1.021	29.54	1.641	1.615	1.556
2	M	115	20	3	1	16	17.88	17.044	1.118	1.076	29.18	1.824	1.712	1.553
2	M	123	20	2	0	18	20.26	19.395	1.126	1.082	30.82	1.712	1.589	1.504
2	M	129	20	4	0	16	16.80	15.940	1.050	1.007	26.78	1.674	1.680	1.532
2	M	133	20	1	0	19	19.34	18.475	1.018	0.975	29.27	1.538	1.582	1.553
2	M	135	20	2	1	17	19.62	18.772	1.154	1.112	30.50	1.794	1.625	1.543
Total/Averages			240	19	7	214	19.168	18.317	1.076	1.033	29.733	1.671	1.626	1.549
Standard Deviations							1.199	1.191	0.047	0.047	1.313	0.092	0.049	0.020
CVs							6.26%	6.50%	4.34%	4.52%	4.42%	5.52%	3.00%	1.32%
3	M	84	20	0	0	20	24.48	23.632	1.224	1.182	34.52	1.726	1.461	1.461
3	M	88	20	0	0	20	26.06	25.208	1.303	1.260	36.34	1.817	1.442	1.442
3	M	91	20	0	0	20	25.80	24.953	1.290	1.248	36.78	1.839	1.474	1.474
3	M	97	20	0	0	20	25.32	24.490	1.266	1.225	36.26	1.813	1.481	1.481
3	M	105	20	0	1	19	25.02	24.181	1.317	1.275	35.28	1.857	1.459	1.450
3	M	109	20	0	0	20	25.98	25.145	1.299	1.257	36.68	1.834	1.459	1.459
3	M	113	20	0	0	20	26.18	25.371	1.309	1.269	36.38	1.819	1.434	1.434
3	M	118	20	0	0	20	25.18	24.341	1.259	1.217	36.18	1.809	1.486	1.486
3	M	124	20	0	0	20	25.72	24.865	1.286	1.243	37.06	1.853	1.491	1.491
3	M	126	20	0	0	20	26.48	25.623	1.324	1.281	37.72	1.886	1.472	1.472
3	M	134	20	0	0	20	26.26	25.404	1.313	1.270	37.34	1.867	1.470	1.470
3	M	138	20	0	0	20	24.80	23.943	1.240	1.197	34.78	1.739	1.453	1.453
Total/Averages			240	0	1	239	25.607	24.767	1.286	1.244	36.277	1.822	1.465	1.464
Standard Deviations							0.635	0.636	0.032	0.032	0.881	0.048	0.017	0.018
CVs							2.48%	2.57%	2.48%	2.57%	2.70%	2.62%	1.18%	1.21%
4	M	86	20	0	0	20	26.58	25.726	1.329	1.286	37.30	1.865	1.450	1.450
4	M	90	20	0	0	20	26.26	25.390	1.313	1.270	37.08	1.854	1.460	1.460
4	M	94	20	0	0	20	26.34	25.506	1.317	1.275	37.34	1.867	1.464	1.464
4	M	98	20	0	0	20	26.60	25.769	1.330	1.288	37.48	1.874	1.454	1.454
4	M	104	20	0	0	20	26.68	25.840	1.334	1.292	36.74	1.837	1.422	1.422
4	M	107	20	0	0	20	25.80	24.949	1.290	1.247	35.68	1.784	1.430	1.430
4	M	112	20	0	0	20	26.16	25.330	1.308	1.267	37.02	1.851	1.462	1.462
4	M	117	20	0	0	20	25.72	24.886	1.286	1.244	35.60	1.780	1.431	1.431
4	M	125	20	0	1	19	25.68	24.810	1.352	1.308	38.26	2.014	1.542	1.469
4	M	130	20	0	1	19	25.22	24.355	1.327	1.284	35.10	1.847	1.441	1.439
4	M	132	20	0	1	19	26.40	25.545	1.389	1.347	38.08	2.004	1.491	1.452
4	M	136	20	0	0	20	26.68	25.819	1.334	1.291	38.00	1.900	1.472	1.472
Total/Averages			240	0	3	237	26.177	25.327	1.326	1.283	36.973	1.873	1.460	1.458
Standard Deviations							0.471	0.476	0.027	0.027	1.026	0.072	0.032	0.017
CVs							1.80%	1.88%	2.07%	2.12%	2.77%	3.85%	2.22%	1.14%

Graph 2. Body Weights and Performance Study of Cobb 500 Broilers Study Days 0-28 (17APR15)
(b) (4) Project No. NV-13-2
BLDG 7

Treatment	Average Bird Wt Gain (kg)	Adjusted Feed Gain	Treatment Description
1	1.221	1.472	Positive Control (PC)
2	1.033	1.540	Negative Control (NC)
3	1.244	1.464	NC with 250 U CIBENZA® PHYTAVERSE™ G10 per kg diet
4	1.288	1.450	NC with 500 U CIBENZA® PHYTAVERSE™ G10 per kg diet

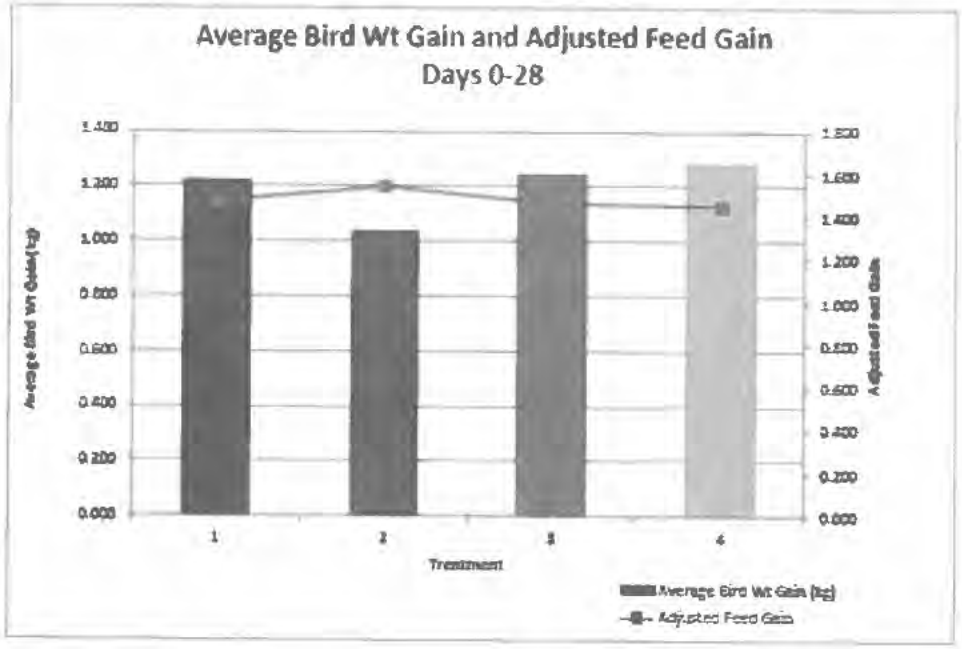


Table 13 Weights and Performance of Cobb 500 Broilers Study Days 14-28 (17APR16)
(b) Project No. NV-13-2
SLDG 7

Treatment	Sex	Pen No	Number of Birds				Day 28 Pen Weight	Day 14 Pen Weight	Pen Gain	Day 28 Bird Average	Day 14 Bird Average	Day 14-28 Bird Average	RSM A Weight	Total Pen Gain	Day 14-28 Feed Consump	Day 14-28 Av. Feed Intake per Bird	Feed Gain	Adjusted Feed Gain
			Started	Removed	Mortality	Weighed	(kg)	(kg)	(kg)	Wt. (kg)	Wt. (kg)	Gain (kg)	(kg)	+ RSM	(kg)	(kg)	(kg/bird)	(kg/bird)
1	M	83	20	0	0	20	25.62	6.98	18.64	1.281	0.334	0.947	0.000	18.940	28.12	1.406	1.485	1.485
3	M	84	20	0	0	20	24.48	6.40	18.08	1.224	0.320	0.904	0.000	18.080	26.88	1.344	1.487	1.487
2	M	85	20	1	0	19	21.16	6.72	14.44	1.114	0.336	0.778	0.356	14.796	23.12	1.217	1.601	1.563
4	M	86	20	0	0	20	26.58	7.04	19.54	1.329	0.352	0.977	0.000	19.540	29.10	1.455	1.489	1.489
2	M	87	20	2	0	18	18.42	6.18	12.24	1.023	0.309	0.714	0.888	13.128	21.10	1.172	1.724	1.607
3	M	88	20	0	0	20	26.06	6.88	19.18	1.303	0.344	0.959	0.000	19.180	28.30	1.415	1.475	1.475
1	M	89	20	0	0	20	24.10	6.28	17.82	1.205	0.314	0.891	0.000	17.820	26.88	1.344	1.508	1.508
3	M	90	20	0	0	20	26.26	7.04	19.22	1.313	0.352	0.961	0.000	19.220	28.80	1.440	1.498	1.498
3	M	91	20	0	0	20	25.80	6.96	18.84	1.290	0.348	0.942	0.000	18.840	28.30	1.415	1.502	1.502
2	M	92	20	0	1	19	20.34	6.68	13.66	1.071	0.334	0.737	0.478	14.138	22.44	1.181	1.643	1.587
1	M	93	20	0	0	20	24.22	6.52	17.70	1.211	0.326	0.885	0.000	17.700	26.58	1.329	1.502	1.502
4	M	94	20	0	0	20	26.34	6.78	19.56	1.317	0.339	0.979	0.000	19.580	28.92	1.446	1.477	1.477
1	M	95	20	0	0	20	25.16	6.80	18.36	1.258	0.340	0.918	0.000	18.360	27.88	1.394	1.519	1.519
2	M	96	20	1	1	18	18.36	6.30	12.06	1.020	0.315	0.705	1.003	13.123	20.78	1.154	1.723	1.583
3	M	97	20	0	0	20	25.32	6.82	18.50	1.266	0.341	0.925	0.000	18.500	27.70	1.385	1.497	1.497
4	M	98	20	0	0	20	26.60	7.12	19.48	1.330	0.356	0.974	0.000	19.480	29.06	1.453	1.493	1.493
2	M	103	20	0	1	19	19.85	6.62	13.24	1.045	0.331	0.714	0.545	13.785	21.86	1.151	1.651	1.580
4	M	104	20	0	0	20	26.68	7.04	19.64	1.334	0.352	0.982	0.000	19.640	28.48	1.424	1.450	1.450
3	M	105	19	0	0	19	25.02	6.78	18.26	1.317	0.358	0.961	0.000	18.260	27.16	1.429	1.487	1.487
1	M	106	19	0	0	19	24.56	6.56	18.00	1.293	0.345	0.947	0.000	18.000	26.76	1.408	1.487	1.487
4	M	107	20	0	0	20	25.80	6.52	19.28	1.290	0.326	0.964	0.000	19.280	27.68	1.384	1.436	1.436
2	M	108	20	2	1	17	18.86	6.48	12.38	1.109	0.324	0.785	2.184	14.564	22.34	1.314	1.806	1.534
3	M	109	20	0	0	20	25.98	7.36	18.62	1.299	0.368	0.931	0.000	18.620	27.90	1.395	1.498	1.498
1	M	110	20	0	0	20	25.08	6.44	18.64	1.254	0.322	0.932	0.000	18.640	27.40	1.370	1.470	1.470
2	M	111	19	1	0	18	19.12	6.04	13.08	1.062	0.318	0.744	0.575	13.655	21.40	1.189	1.636	1.567
4	M	112	20	0	0	20	26.16	6.96	19.20	1.308	0.348	0.960	0.000	19.200	28.34	1.417	1.476	1.476
3	M	113	20	0	0	20	25.18	7.04	18.14	1.259	0.352	0.957	0.000	18.140	28.22	1.411	1.474	1.474
4	M	114	20	0	0	20	25.08	6.78	18.30	1.254	0.339	0.915	0.000	18.300	27.20	1.360	1.486	1.486
2	M	115	20	3	1	16	17.89	6.48	11.40	1.118	0.324	0.754	1.751	13.151	20.74	1.296	1.819	1.577
1	M	116	20	0	0	20	25.20	6.50	18.70	1.260	0.325	0.935	0.000	18.700	27.82	1.391	1.488	1.488
4	M	117	20	0	0	20	25.72	6.76	18.96	1.286	0.338	0.948	0.000	18.960	27.58	1.379	1.455	1.455
3	M	118	20	0	0	20	25.18	6.76	18.42	1.259	0.338	0.921	0.000	18.420	27.50	1.375	1.493	1.493
2	M	123	20	2	0	18	20.26	6.80	13.46	1.126	0.340	0.748	1.086	14.556	22.58	1.254	1.678	1.551
3	M	124	20	0	0	20	25.72	7.02	18.70	1.286	0.351	0.935	0.000	18.700	28.26	1.413	1.511	1.511
4	M	125	20	0	1	19	25.68	7.38	18.30	1.352	0.369	0.963	1.227	19.527	29.34	1.539	1.598	1.497
1	M	126	20	0	0	20	26.12	7.06	19.12	1.306	0.353	0.956	0.000	19.120	28.66	1.433	1.499	1.499
1	M	127	20	0	0	20	25.46	6.78	18.68	1.273	0.339	0.934	0.000	18.680	28.00	1.400	1.499	1.499
3	M	128	20	0	0	20	26.46	7.28	19.20	1.324	0.364	0.960	0.000	19.200	28.94	1.447	1.507	1.507
2	M	129	19	3	0	16	15.80	5.94	10.86	1.050	0.313	0.737	1.405	12.265	19.44	1.215	1.790	1.587
4	M	130	19	0	0	19	25.22	6.85	18.36	1.327	0.361	0.966	0.000	18.360	27.22	1.433	1.483	1.483
1	M	131	20	0	0	20	25.82	7.12	18.70	1.291	0.356	0.935	0.000	18.700	28.22	1.411	1.509	1.509
4	M	132	20	0	1	19	26.40	7.48	18.92	1.389	0.374	1.015	0.676	19.596	29.46	1.551	1.567	1.503
2	M	133	20	1	0	19	19.34	6.26	13.08	1.018	0.313	0.705	0.346	13.426	21.14	1.113	1.616	1.575
3	M	134	20	0	0	20	26.26	6.82	19.44	1.313	0.341	0.972	0.000	19.440	28.72	1.436	1.477	1.477

Table 13. Weights and Performance of Cobb 500 Broilers Study Days 14-28 (17APR15)
(b) (4) Project No. NV-13-2
BLDG 7

Treatment	Sex	Pen No	Number of Birds				Day 28 Pen Weight (kg)	Day 14 Pen Weight (kg)	Pen Gain (kg)	Day 28 Bird Average Wt. (kg)	Day 14 Bird Average Wt. (kg)	Day 14-28 Bird Average Gain (kg)	RMA Weight (kg)	Total Pen Gain (kg)	Day 14-28 Feed Consump (kg)	Day 14-28 Av. Feed Intake per Bird (kg)	Feed Gain (kg/bird)	Adjusted Feed Gain (kg/bird)
			Started	Removed	Mortality	Weighed												
2	M	135	19		1	17	19.62	6.46	13.16	1.154	0.340	0.814	0.798	13.956	22.06	1.258	1.676	1.580
4	M	136	20		0	20	26.68	7.32	19.36	1.334	0.366	0.968	0.000	19.360	29.22	1.461	1.509	1.509
1	M	137	19	0	0	19	24.08	6.12	17.96	1.267	0.322	0.945	0.000	17.960	26.50	1.395	1.476	1.476
3	M	138	20	0	0	20	24.80	6.52	18.28	1.240	0.326	0.914	0.000	18.280	26.90	1.345	1.472	1.472

Table 14. Weights and Performance of Cobb 500 Broilers by Treatment Study Days 14-28 (17APR15)
BLDG 7

Treatment	Sex	Pen No	Number of Birds				Day 28 Pen Weight (kg)	Pen Gain (kg)	Day 28 Bird Average Wt. (kg)	Day 14-28 Bird Average Gain (kg)	Day 14-28 Feed Consump (kg)	Day 14-28 Av. Feed Intake per Bird (kg)	Feed Gain (kg/bird)	Adjusted Feed Gain (kg/bird)
			Started	Removed	Mortality	Weighed								
1	M	83	20	0	0	20	25.82	18.94	1.291	0.947	28.12	1.406	1.485	1.485
1	M	89	20	0	0	20	24.10	17.82	1.205	0.891	26.88	1.344	1.508	1.508
1	M	93	20	0	0	20	24.22	17.70	1.211	0.885	26.58	1.329	1.502	1.502
1	M	95	20	0	0	20	25.16	18.36	1.258	0.918	27.88	1.394	1.519	1.519
1	M	106	19	0	0	19	24.56	18.00	1.293	0.947	26.76	1.408	1.487	1.487
1	M	110	20	0	0	20	25.08	18.64	1.254	0.932	27.40	1.370	1.470	1.470
1	M	114	20	0	0	20	25.08	18.30	1.254	0.915	27.20	1.360	1.486	1.486
1	M	116	20	0	0	20	25.20	18.70	1.260	0.935	27.82	1.391	1.488	1.488
1	M	126	20	0	0	20	26.18	19.12	1.309	0.956	28.66	1.433	1.499	1.499
1	M	127	20	0	0	20	25.46	18.68	1.273	0.934	28.00	1.400	1.499	1.499
1	M	131	20	0	0	20	25.82	18.70	1.291	0.935	28.22	1.411	1.509	1.509
1	M	137	19	0	0	19	24.08	17.96	1.267	0.945	26.50	1.395	1.476	1.476
Total/Averages			238	0	0	238	25.047	18.410	1.253	0.928	27.502	1.387	1.494	1.494
Standard Deviations							0.686	0.459	0.031	0.022	0.715	0.030	0.014	0.014
CVs							2.74%	2.49%	2.44%	2.40%	2.60%	2.18%	0.97%	0.97%
2	M	85	20	1	0	19	21.16	14.44	1.114	0.778	23.12	1.217	1.601	1.563
2	M	87	20	2	0	18	18.42	12.24	1.023	0.714	21.10	1.172	1.724	1.607
2	M	92	20	0	1	19	20.34	13.66	1.071	0.737	22.44	1.181	1.643	1.587
2	M	96	20	1	1	18	18.36	12.06	1.020	0.705	20.78	1.154	1.723	1.583
2	M	103	20	0	1	19	19.86	13.24	1.045	0.714	21.86	1.151	1.651	1.586
2	M	108	20	2	1	17	18.86	12.38	1.109	0.785	22.34	1.314	1.805	1.534
2	M	111	19	1	0	18	19.12	13.08	1.062	0.744	21.40	1.189	1.636	1.567
2	M	115	20	3	1	16	17.88	11.40	1.118	0.794	20.74	1.296	1.819	1.577
2	M	123	20	2	0	18	20.28	13.46	1.126	0.786	22.58	1.254	1.678	1.551
2	M	129	19	3	0	16	16.80	10.86	1.050	0.737	19.44	1.215	1.790	1.582
2	M	133	20	1	0	19	19.34	13.08	1.018	0.705	21.14	1.113	1.616	1.575
2	M	135	19	1	1	17	19.62	13.16	1.154	0.814	22.06	1.298	1.676	1.580
Total/Averages			237	17	6	214	19.168	12.755	1.076	0.751	21.583	1.213	1.697	1.574
Standard Deviations							1.199	1.004	0.047	0.038	1.017	0.065	0.075	0.018
CVs							6.26%	7.87%	4.34%	5.12%	4.71%	5.37%	4.42%	1.20%
3	M	84	20	0	0	20	24.48	18.08	1.224	0.904	26.88	1.344	1.487	1.487
3	M	88	20	0	0	20	26.06	19.18	1.303	0.959	28.30	1.415	1.475	1.475
3	M	91	20	0	0	20	25.80	18.84	1.290	0.942	28.30	1.415	1.502	1.502
3	M	97	20	0	0	20	25.32	18.50	1.266	0.925	27.70	1.385	1.497	1.497
3	M	105	19	0	0	19	25.02	18.26	1.317	0.961	27.16	1.429	1.487	1.487
3	M	109	20	0	0	20	25.98	18.62	1.299	0.931	27.90	1.395	1.498	1.498
3	M	113	20	0	0	20	26.18	19.14	1.309	0.957	28.22	1.411	1.474	1.474
3	M	118	20	0	0	20	25.18	18.42	1.259	0.921	27.50	1.375	1.493	1.493
3	M	124	20	0	0	20	25.72	18.70	1.286	0.935	28.26	1.413	1.511	1.511
3	M	128	20	0	0	20	26.48	19.20	1.324	0.960	28.94	1.447	1.507	1.507
3	M	134	20	0	0	20	26.26	19.44	1.313	0.972	28.72	1.436	1.477	1.477
3	M	138	20	0	0	20	24.80	18.28	1.240	0.914	26.90	1.345	1.472	1.472
Total/Averages			239	0	0	239	25.607	18.722	1.286	0.940	27.898	1.401	1.490	1.490
Standard Deviations							0.635	0.438	0.032	0.022	0.681	0.033	0.013	0.013
CVs							2.48%	2.34%	2.48%	2.31%	2.44%	2.37%	0.90%	0.90%
4	M	86	20	0	0	20	26.58	19.54	1.329	0.977	29.10	1.455	1.489	1.489
4	M	90	20	0	0	20	26.26	19.22	1.313	0.961	28.80	1.440	1.498	1.498
4	M	94	20	0	0	20	26.34	19.58	1.317	0.979	28.92	1.446	1.477	1.477
4	M	98	20	0	0	20	26.60	19.48	1.330	0.974	29.08	1.454	1.493	1.493
4	M	104	20	0	0	20	26.68	19.64	1.334	0.982	28.48	1.424	1.450	1.450
4	M	107	20	0	0	20	25.80	19.28	1.290	0.964	27.68	1.384	1.436	1.436
4	M	112	20	0	0	20	26.16	19.20	1.308	0.960	28.34	1.417	1.476	1.476
4	M	117	20	0	0	20	25.72	18.96	1.286	0.948	27.58	1.379	1.455	1.455
4	M	125	20	0	1	19	25.68	18.30	1.352	0.983	29.24	1.539	1.598	1.497
4	M	130	19	0	0	19	25.22	18.36	1.327	0.966	27.22	1.433	1.483	1.483
4	M	132	20	0	1	19	26.40	18.92	1.389	1.015	29.46	1.551	1.557	1.503
4	M	136	20	0	0	20	26.68	19.36	1.334	0.968	29.22	1.461	1.509	1.509
Total/Averages			239	0	2	237	26.177	19.153	1.326	0.973	28.593	1.449	1.493	1.481
Standard Deviations							0.471	0.446	0.027	0.017	0.740	0.052	0.045	0.023
CVs							1.80%	2.33%	2.07%	1.73%	2.59%	3.59%	3.04%	1.55%

Graph 3. Body Weights and Performance Study of Cobb 500 Broilers Study Days 14-28 (17APR15)
(b) (4) Project No. NV-13-2
BLDG 7

Treatment	Average Bird Wt Gain (kg)	Adjusted Feed Gain	Treatment Description
1	0.928	1.494	Positive Control (PC)
2	0.751	1.574	Negative Control (NC)
3	0.940	1.490	NC with 250 U QUBENZA® PHYTAVERSE™ G10 per kg diet
4	0.973	1.481	NC with 500 U QUBENZA® PHYTAVERSE™ G10 per kg diet

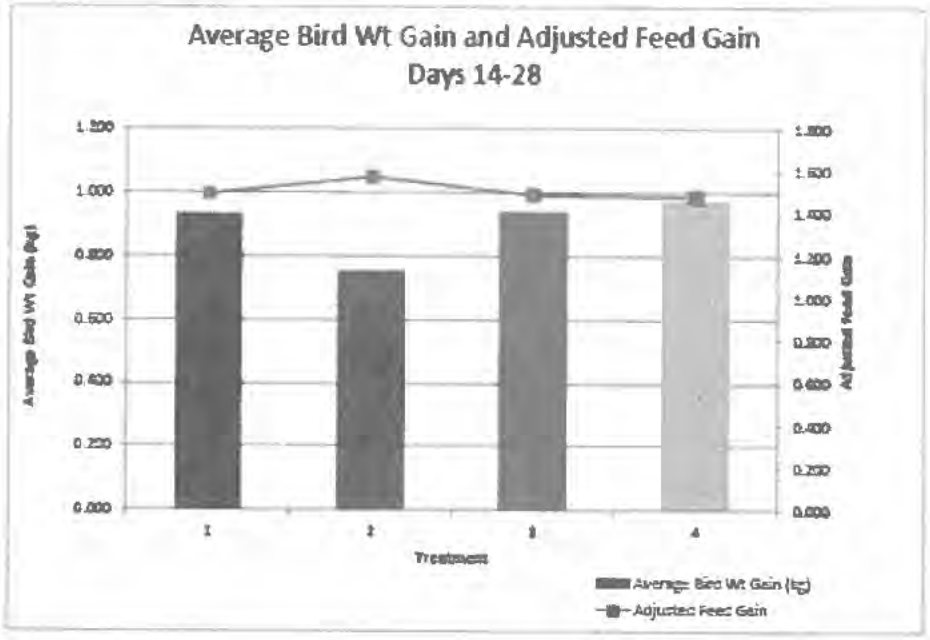


Table 15 Tibia Ash Weights of Cobb 500 Broilers
(b) (4) Project No. NV-13-2
BLDG 7

Treatment	Sex	Pen No.	Bone No.	% Ash (600°C)	Dry bone Wt (105°C) (grams)	Ash Wt (600°C) (grams)
1	M	83	2800	55.31	2.7031	1.4952
1	M	83	2801	53.85	2.7256	1.4678
1	M	83	2802	54.97	2.8442	1.5634
1	M	83	2803	53.05	2.1170	1.1231
1	M	83	2804	53.14	2.8888	1.5349
3	M	84	6791	48.05	2.5369	1.2189
3	M	84	6792	49.86	2.4174	1.2053
3	M	84	6793	48.67	2.3648	1.1509
3	M	84	6794	54.26	2.0342	1.1037
3	M	84	6795	49.72	2.4974	1.2416
2	M	85	2820	50.76	2.7269	1.3843
2	M	85	2821	47.68	2.1667	1.0330
2	M	85	2822	41.27	2.0233	0.8350
2	M	85	2823	47.07	2.0609	0.9700
2	M	85	2825	44.53	2.2301	0.9952
4	M	86	6811	53.46	2.2779	1.2176
4	M	86	6812	56.09	2.5335	1.3958
4	M	86	6813	52.74	3.0109	1.5879
4	M	86	6814	52.96	2.7655	1.4730
4	M	86	6815	54.07	3.0836	1.6673
2	M	87	2840	45.47	1.9931	0.9063
2	M	87	2841	46.77	1.6986	0.7944
2	M	87	2842	52.05	2.0839	1.0846
2	M	87	2843	44.66	1.9225	0.8590
2	M	87	2844	45.21	2.0050	0.9066
3	M	88	6831	38.72	3.9010	1.5106
3	M	88	6832	49.52	2.5556	1.2656
3	M	88	6833	49.66	2.7142	1.3478
3	M	88	6834	50.60	2.8026	1.4182
3	M	88	6835	52.90	2.0756	1.0979
1	M	89	2860	56.22	1.9841	1.0957
1	M	89	2861	50.92	2.4671	1.2563
1	M	89	2862	54.32	2.1947	1.1922
1	M	89	2863	52.22	2.7223	1.4215
1	M	89	2864	52.71	2.5585	1.3470
4	M	90	6851	54.39	2.7901	1.5176
4	M	90	6852	51.76	2.6543	1.3736
4	M	90	6853	56.36	2.7086	1.4956
4	M	90	6854	52.56	2.4406	1.2826
4	M	90	6855	53.08	2.6300	1.3959
3	M	91	2850	52.77	2.3927	1.2626
3	M	91	2851	51.86	2.7104	1.4051
3	M	91	2852	51.83	2.3555	1.2206
3	M	91	2853	52.50	2.2598	1.1863
3	M	91	2854	54.59	2.5544	1.3945
2	M	92	6871	51.73	1.9374	1.0022
2	M	92	6872	43.32	1.9062	0.8257

Table 15. Tibia Ash Weights of Cobb 500 Broilers
(b) (4) Project No. NV-13-2
BLDG 7

Treatment	Sex	Pen No.	Bone No.	% Ash (600°C)	Dry bone Wt (105°C) (grams)	Ash Wt (600°C) (grams)
2	M	92	6873	40.23	2.3142	0.9310
2	M	92	6874	45.22	1.8082	0.8176
2	M	92	6875	47.56	2.1572	1.0307
1	M	93	2900	54.72	3.0426	1.6650
1	M	93	2901	53.61	2.0720	1.1109
1	M	93	2902	52.62	2.5027	1.3169
1	M	93	2903	52.98	2.3984	1.2706
1	M	93	2904	54.52	2.3139	1.2615
4	M	94	6891	52.74	2.6373	1.3909
4	M	94	6892	51.91	2.5421	1.3197
4	M	94	6893	52.47	2.1056	1.1046
4	M	94	6894	50.97	2.8143	1.4345
4	M	94	6895	52.70	2.3393	1.2329
1	M	95	2920	53.13	2.8997	1.5406
1	M	95	2921	54.04	2.6526	1.4335
1	M	95	2922	56.52	2.4489	1.3842
1	M	95	2923	52.26	2.6701	1.3955
1	M	95	2924	50.18	2.2150	1.1114
2	M	96	6911	45.17	2.0661	0.9332
2	M	96	6912	47.23	1.8976	0.8962
2	M	96	6913	40.10	1.6675	0.6686
2	M	96	6914	48.40	1.3734	0.6647
2	M	96	6915	38.20	1.9247	0.7352
3	M	97	2940	51.40	2.6523	1.3633
3	M	97	2941	51.37	2.3575	1.2111
3	M	97	2942	53.63	2.9441	1.5789
3	M	97	2943	53.86	2.6184	1.4103
3	M	97	2944	46.39	1.9567	0.9077
4	M	98	6931	51.81	2.6858	1.3916
4	M	98	6932	52.22	2.3328	1.2182
4	M	98	6933	54.01	2.9548	1.6012
4	M	98	6934	51.79	3.2365	1.6762
4	M	98	6935	53.70	2.6372	1.4162
2	M	103	2960	38.83	2.4272	0.9424
2	M	103	2961	45.20	1.8557	0.8388
2	M	103	2962	37.23	2.7694	1.0311
2	M	103	2963	40.45	1.7301	0.6998
2	M	103	2964	39.77	1.7453	0.6941
4	M	104	6951	52.56	2.8390	1.4922
4	M	104	6952	52.41	2.6857	1.4076
4	M	104	6953	51.53	2.8827	1.4855
4	M	104	6954	51.25	2.0469	1.0491
4	M	104	6955	52.92	2.3989	1.2694
3	M	105	2980	53.06	2.5069	1.3301
3	M	105	2981	52.85	2.6124	1.4864
3	M	105	2982	52.62	2.2427	1.1802
3	M	105	2983	51.99	2.7458	1.4275

Table 15. Tibia Ash Weights of Cobb 500 Broilers
(b) (4) Project No. NV-13-2
BLDG 7

Treatment	Sex	Pen No.	Bone No.	% Ash (600°C)	Dry bone Wt (105°C) (grams)	Ash Wt (600°C) (grams)
3	M	105	2984	54.07	2.5123	1.3583
1	M	106	6971	53.25	2.4263	1.2920
1	M	106	6972	53.83	2.8475	1.5329
1	M	106	6973	53.97	2.6380	1.4237
1	M	106	6975	52.53	2.5365	1.3567
1	M	106	6976	55.46	2.2796	1.2643
4	M	107	3000	53.21	2.3682	1.2601
4	M	107	3001	53.52	2.7344	1.4634
4	M	107	3002	54.81	2.7585	1.5120
4	M	107	3003	51.01	2.8361	1.4467
4	M	107	3004	53.86	2.5535	1.3752
2	M	108	6991	45.06	2.1518	0.9695
2	M	108	6992	51.97	2.4363	1.2661
2	M	108	6993	41.36	2.3412	0.9684
2	M	108	6995	47.94	2.1261	1.0193
2	M	108	6997	43.19	1.9543	0.8440
3	M	109	7325	53.16	2.3036	1.2246
3	M	109	7326	52.90	2.6656	1.4100
3	M	109	7327	50.41	2.5636	1.2923
3	M	109	7328	52.00	2.7532	1.4369
3	M	109	7329	48.23	2.5129	1.2119
1	M	110	7011	55.14	2.4732	1.3636
1	M	110	7012	51.33	2.3824	1.2230
1	M	110	7013	53.25	2.5512	1.3586
1	M	110	7014	54.39	2.7910	1.5180
1	M	110	7015	53.80	2.6621	1.4321
2	M	111	7346	44.04	1.8710	0.8240
2	M	111	7347	44.31	1.9219	0.8515
2	M	111	7348	43.32	2.4051	1.0420
2	M	111	7349	47.75	2.2203	1.0603
2	M	111	7350	40.41	1.9283	0.7792
4	M	112	7031	52.53	2.5180	1.3227
4	M	112	7032	53.58	2.6913	1.4419
4	M	112	7033	50.96	2.1788	1.1103
4	M	112	7034	53.57	2.6806	1.4361
4	M	112	7035	53.23	3.0142	1.6045
3	M	113	7365	53.29	2.3355	1.2447
3	M	113	7366	50.93	2.3880	1.2162
3	M	113	7367	54.56	2.3034	1.2568
3	M	113	7368	52.95	2.4369	1.2904
3	M	113	7369	51.36	2.4756	1.2720
1	M	114	1798	53.16	2.3633	1.2564
1	M	114	1799	54.49	2.6053	1.4196
1	M	114	1800	56.48	2.4767	1.4000
1	M	114	1801	54.26	2.2127	1.2006
1	M	114	1802	53.77	2.5361	1.3636
2	M	115	7355	50.82	2.3195	1.1787

Table 15. Tibia Ash Weights of Cobb 500 Broilers
(b) (4) Project No. NV-13-2
BLDG 7

Treatment	Sex	Pen No.	Bone No.	% Ash (600°C)	Dry bone wt (105°C) (grams)	Ash wt (600°C) (grams)
2	M	115	7366	45.66	2.0190	0.9439
2	M	115	7367	43.71	1.6281	0.7391
2	M	115	7368	47.01	2.1811	1.0253
2	M	115	7369	43.71	2.0795	0.9259
1	M	116	1818	55.15	2.6299	1.4503
1	M	116	1819	54.16	2.6190	1.4193
1	M	116	1820	53.74	2.3580	1.2568
1	M	116	1821	53.83	2.5886	1.3934
1	M	116	1822	52.17	2.5331	1.3214
4	M	117	7405	52.83	2.2453	1.1861
4	M	117	7406	55.45	2.5279	1.4016
4	M	117	7407	51.16	2.4302	1.2432
4	M	117	7408	54.52	2.3662	1.2925
4	M	117	7409	50.57	2.7499	1.3906
3	M	118	1838	47.55	2.2941	1.0909
3	M	118	1839	52.76	2.1341	1.1260
3	M	118	1840	49.35	2.8582	1.4091
3	M	118	1841	52.92	2.7271	1.4431
3	M	118	1842	51.16	2.3260	1.1905
2	M	123	7425	41.33	1.8743	0.7747
2	M	123	7426	46.54	2.3251	1.0820
2	M	123	7427	49.35	2.8382	1.4254
2	M	123	7428	53.96	2.6073	1.4066
2	M	123	7429	39.55	1.6735	0.6618
3	M	124	1858	52.44	2.8520	1.4956
3	M	124	1859	49.57	2.3485	1.1642
3	M	124	1860	49.84	3.1403	1.5652
3	M	124	1861	50.51	2.7085	1.3708
3	M	124	1862	54.00	2.4691	1.3334
4	M	125	7445	54.11	2.4646	1.3336
4	M	125	7446	53.00	2.8532	1.5123
4	M	125	7447	52.74	2.6856	1.5219
4	M	125	7448	53.15	2.4783	1.3171
4	M	125	7449	50.78	3.0749	1.5615
1	M	126	1878	52.23	2.3777	1.2418
1	M	126	1879	50.89	2.6017	1.3241
1	M	126	1880	56.05	3.1047	1.7092
1	M	126	1881	53.71	2.3856	1.2813
1	M	126	1882	55.64	2.7734	1.5430
1	M	127	7465	53.18	2.4496	1.3027
1	M	127	7466	54.37	2.2373	1.2164
1	M	127	7467	54.12	2.7291	1.4771
1	M	127	7468	54.20	2.1346	1.1569
1	M	127	7469	53.44	3.0581	1.6342
3	M	128	1898	51.08	2.4974	1.2756
3	M	128	1899	50.66	2.4723	1.2525
3	M	128	1900	52.49	2.3981	1.2587

Table 15. Tibia Ash Weights of Cobb 500 Broilers

(b) (4) Project No. NV-13-2

BLDG 7

Treatment	Sex	Pen No.	Boss No.	% Ash (600°C)	Dry bone Wt (105°C) (grams)	Ash Wt (600°C) (grams)
3	M	128	1901	53.51	2.8552	1.5278
3	M	128	1902	53.84	2.6962	1.4516
2	M	129	7485	38.25	2.1580	0.8254
2	M	129	7486	43.55	2.1510	0.9390
2	M	129	7487	45.89	2.1378	0.9810
2	M	129	7488	44.86	2.0546	0.9221
2	M	129	7489	45.30	1.8861	0.8854
4	M	130	1918	52.86	2.6367	1.3948
4	M	130	1919	52.91	2.4180	1.2793
4	M	130	1920	53.61	3.1046	1.6643
4	M	130	1921	50.57	2.9318	1.4826
4	M	130	1922	50.39	2.4207	1.2198
1	M	131	7505	49.06	2.4196	1.1870
1	M	131	7506	53.02	2.7621	1.4645
1	M	131	7507	53.26	2.5526	1.3589
1	M	131	7508	52.58	2.5094	1.3212
1	M	131	7509	52.58	2.5879	1.3607
4	M	132	1938	52.22	2.6916	1.4056
4	M	132	1939	52.58	2.5754	1.3567
4	M	132	1940	53.66	3.0904	1.6582
4	M	132	1941	53.04	3.2828	1.7304
4	M	132	1942	53.25	2.5786	1.3730
2	M	133	7525	41.72	2.3217	0.9685
2	M	133	7526	36.91	1.5878	0.5860
2	M	133	7527	50.72	2.3540	1.1940
2	M	133	7528	41.77	1.7222	0.7193
2	M	133	7529	41.38	1.9816	0.8200
3	M	134	1952	49.02	2.4651	1.2083
3	M	134	1958	58.89	2.6261	1.5464
3	M	134	1959	54.17	3.0068	1.6287
3	M	134	1960	48.96	2.4059	1.1298
3	M	134	1961	52.81	2.3210	1.2257
2	M	135	7545	51.82	2.4479	1.2684
2	M	135	7546	47.17	2.4330	1.1429
2	M	135	7547	43.08	1.8997	0.8181
2	M	135	7548	42.61	1.7654	0.7522
2	M	135	7551	43.64	2.4130	1.0530
4	M	136	1978	52.93	2.3127	1.2241
4	M	136	1979	54.89	2.4615	1.3511
4	M	136	1980	53.09	2.7504	1.4603
4	M	136	1981	54.83	2.5796	1.4593
4	M	136	1982	51.89	2.4617	1.2773
1	M	137	7562	51.35	2.7649	1.4196
1	M	137	7565	51.23	2.2010	1.1276
1	M	137	7566	54.00	2.4420	1.3187
1	M	137	7568	53.74	2.5153	1.3518
1	M	137	7569	53.99	2.2324	1.2053

Table 15. Tibia Ash Weights of Cobb 500 Broilers
(b) (4) Project No. NV-13-2
BLDG 7

Treatment	Sex	Pen No.	Bone No.	% Ash (600°C)	Dry bone Wt (105°C) (grams)	Ash Wt (600°C) (grams)
3	M	138	1998	50.89	2.7911	1.4203
3	M	138	1999	47.12	2.2756	1.0722
3	M	138	2000	52.06	2.5665	1.3377
3	M	138	2001	52.78	2.2805	1.2036
3	M	138	2002	41.52	1.8987	0.7883

Table 16. Tibia Ash Weights of Cobb 500 Broilers Summarized by Pen
(b) (4) Project No. NV-13-2
BLDG 7

Treatment	Sex	Pen No.	Bone No.	% Ash (600°C)	Dry bone Wt (105°C) (grams)	Ash Wt (600°C) (grams)
1	M	83	2800	55.31	2.7031	1.4952
1	M	83	2801	53.85	2.7256	1.4678
1	M	83	2802	54.97	2.8442	1.5634
1	M	83	2803	53.05	2.1170	1.1231
1	M	83	2804	53.14	2.8586	1.5349
Total/Averages				54.06	2.6697	1.4969
Standard Deviations				1.04	0.31	0.13
CVs				1.93%	11.71%	12.47%
3	M	84	6791	48.05	2.5369	1.2189
3	M	84	6792	49.86	2.4174	1.2053
3	M	84	6793	48.67	2.3648	1.1509
3	M	84	6794	54.26	2.0342	1.1037
3	M	84	6795	49.72	2.4974	1.2418
Total/Averages				50.11	2.3701	1.1841
Standard Deviations				2.44	0.20	0.06
CVs				4.89%	8.42%	4.73%
2	M	85	2820	50.76	2.7269	1.3843
2	M	85	2821	47.68	2.1667	1.0330
2	M	85	2822	41.27	2.0239	0.8350
2	M	85	2823	47.07	2.0609	0.9700
2	M	85	2825	44.63	2.2301	0.9952
Total/Averages				46.28	2.2418	1.0435
Standard Deviations				3.65	0.28	0.20
CVs				7.89%	12.50%	18.81%
4	M	86	6811	53.46	2.2779	1.2176
4	M	86	6812	55.09	2.5335	1.3958
4	M	86	6813	52.74	3.0109	1.5879
4	M	86	6814	52.88	2.7855	1.4730
4	M	86	6815	54.07	3.0836	1.6673
Total/Averages				53.85	2.7823	1.4884
Standard Deviations				0.99	0.34	0.17
CVs				1.82%	12.24%	11.59%
2	M	87	2840	45.47	1.9931	0.9063
2	M	87	2841	46.77	1.6986	0.7944
2	M	87	2842	52.05	2.0836	1.0846
2	M	87	2843	44.58	1.9225	0.8590
2	M	87	2844	45.21	2.0052	0.9066
Total/Averages				46.84	1.9406	0.9102
Standard Deviations				2.91	0.15	0.11
CVs				6.42%	7.57%	11.84%

Table 16. Tibia Ash Weights of Cobb 500 Broilers Summarized by Pen

(b) (4) Project No. NV-13-2

BLDG 7

Treatment	Sex	Pen No.	Bone No.	% Ash (600°C)	Dry Bone Wt (105°C) (grams)	Ash Wt (600°C) (grams)
3	M	88	6831	36.72	3.9010	1.5105
3	M	88	6832	49.52	2.5555	1.2656
3	M	88	6833	49.66	2.7142	1.3478
3	M	88	6834	50.50	2.8026	1.4182
3	M	88	6835	52.90	2.6756	1.0979
Total/Averages				43.26	2.8298	1.2280
Standard Deviations				6.61	0.87	0.16
CVs				11.42%	23.90%	11.69%
1	M	89	2860	55.22	1.9841	1.0957
1	M	89	2861	50.92	2.4671	1.2563
1	M	89	2862	54.32	2.1947	1.1922
1	M	89	2863	52.22	2.7223	1.4215
1	M	89	2864	52.71	2.5555	1.3470
Total/Averages				53.08	2.5047	1.2625
Standard Deviations				1.71	0.29	0.15
CVs				3.23%	12.54%	10.11%
4	M	90	6851	54.39	2.7901	1.5176
4	M	90	6852	51.76	2.6543	1.3736
4	M	90	6853	55.36	2.7086	1.4996
4	M	90	6854	52.55	2.4406	1.2825
4	M	90	6856	53.08	2.6300	1.3959
Total/Averages				53.43	2.8447	1.4139
Standard Deviations				1.45	0.13	0.10
CVs				2.70%	4.80%	6.89%
3	M	91	2880	52.77	2.3927	1.2626
3	M	91	2881	51.58	2.7104	1.4061
3	M	91	2882	51.83	2.3556	1.2206
3	M	91	2883	52.50	2.2596	1.1863
3	M	91	2884	54.59	2.5544	1.3945
Total/Averages				52.79	2.4648	1.2941
Standard Deviations				1.12	0.18	0.10
CVs				2.13%	7.29%	7.79%
2	M	92	6871	51.73	1.9374	1.0022
2	M	92	6872	43.32	1.9062	0.8257
2	M	92	6873	40.23	2.3142	0.9310
2	M	92	6874	45.22	1.8082	0.8176
2	M	92	6875	47.56	2.1672	1.0307
Total/Averages				45.81	2.0266	0.8214
Standard Deviations				4.35	0.21	0.10
CVs				9.53%	10.26%	10.65%

Table 16. Tibia Ash Weights of Cobb 500 Broilers Summarized by Pen
(b) (4) Project No. NV-13-2
BLDG 7

Treatment	Sex	Pen No.	Bone No.	% Ash (600°C)	Dry Bone Wt (105°C) (grams)	Ash Wt (600°C) (grams)
1	M	93	2900	54.72	3.0426	1.6650
1	M	93	2901	53.61	2.0720	1.1109
1	M	93	2902	52.62	2.5027	1.3169
1	M	93	2903	52.98	2.3964	1.2706
1	M	93	2904	54.52	2.3139	1.2615
Total/Averages				53.68	2.4855	1.3250
Standard Deviations				0.92	0.26	0.21
CVs				1.72%	14.58%	16.49%
4	M	94	6891	52.74	2.6373	1.3909
4	M	94	6892	51.91	2.5421	1.3197
4	M	94	6893	52.47	2.1056	1.1048
4	M	94	6894	50.97	2.8143	1.4345
4	M	94	6895	52.70	2.3393	1.2329
Total/Averages				52.16	2.4877	1.2966
Standard Deviations				0.74	0.27	0.13
CVs				1.42%	11.01%	10.15%
1	M	95	2920	53.13	2.8997	1.5405
1	M	95	2921	54.04	2.6526	1.4335
1	M	95	2922	56.52	2.4489	1.3842
1	M	95	2923	52.26	2.6701	1.3955
1	M	95	2924	50.18	2.2150	1.1114
Total/Averages				53.23	2.5773	1.3730
Standard Deviations				2.33	0.26	0.16
CVs				4.38%	10.01%	11.66%
2	M	96	6911	45.17	2.0661	0.9332
2	M	96	6912	47.23	1.8976	0.8962
2	M	96	6913	40.10	1.6675	0.6686
2	M	96	6914	48.40	1.3734	0.6647
2	M	96	6915	38.20	1.9247	0.7352
Total/Averages				43.82	1.7859	0.7796
Standard Deviations				4.47	0.27	0.13
CVs				10.20%	15.19%	16.31%
3	M	97	2940	51.40	2.6523	1.3633
3	M	97	2941	51.37	2.3575	1.2111
3	M	97	2942	53.63	2.9441	1.5789
3	M	97	2943	53.86	2.6164	1.4103
3	M	97	2944	46.39	1.9567	0.9077
Total/Averages				51.33	2.5058	1.2943
Standard Deviations				3.08	0.37	0.26
CVs				5.86%	14.79%	19.63%

Table 16. Tibia Ash Weights of Cobb 500 Broilers Summarized by Pen

(b) (4) Project No. NV-13-2

BLDG 7

Treatment	Sex	Pen No.	Bone No.	% Ash (600°C)	Dry Bone Wt (105°C) (grams)	Ash Wt (600°C) (grams)
4	M	96	6931	51.81	2.6858	1.3916
4	M	96	6932	52.22	2.3329	1.2182
4	M	96	6933	54.01	2.9849	1.6012
4	M	96	6934	51.79	3.2365	1.6762
4	M	96	6935	53.70	2.6372	1.4162
Totals/Averages				52.71	2.7714	1.4607
Standard Deviations				1.07	0.24	0.18
CVs				2.02%	12.28%	12.42%
2	M	103	2960	38.83	2.4272	0.9424
2	M	103	2961	46.20	1.8537	0.8388
2	M	103	2962	37.23	2.7694	1.0311
2	M	103	2963	40.45	1.7301	0.6996
2	M	103	2964	39.77	1.7453	0.6941
Totals/Averages				40.30	2.1052	0.8412
Standard Deviations				3.03	0.47	0.15
CVs				7.44%	22.54%	17.83%
4	M	104	6951	52.56	2.6390	1.4922
4	M	104	6952	52.41	2.6857	1.4076
4	M	104	6953	51.53	2.8227	1.4355
4	M	104	6954	51.25	2.0469	1.0491
4	M	104	6955	52.92	2.3969	1.2594
Totals/Averages				52.13	2.5782	1.3488
Standard Deviations				0.71	0.35	0.19
CVs				1.36%	13.57%	13.89%
3	M	105	2950	53.06	2.5069	1.3301
3	M	105	2951	52.35	2.8124	1.4864
3	M	105	2952	52.62	2.2427	1.1802
3	M	105	2953	51.99	2.7458	1.4275
3	M	105	2954	54.07	2.5123	1.3583
Totals/Averages				52.82	2.5840	1.3565
Standard Deviations				0.78	0.23	0.12
CVs				1.48%	8.81%	8.84%
1	M	106	6971	53.25	2.4253	1.2920
1	M	106	6972	53.83	2.8475	1.5329
1	M	106	6973	53.97	2.6380	1.4237
1	M	106	6975	52.53	2.5855	1.3587
1	M	106	6976	55.46	2.2795	1.2643
Totals/Averages				53.81	2.5552	1.3743
Standard Deviations				1.03	0.22	0.11
CVs				1.92%	8.43%	7.88%

Table 16. Tibia Ash Weights of Cobb 500 Broilers Summarized by Pen

(b) (4) Project No. NV-13-2

BLDG 7

Treatment	Sex	Pen No.	Bone No.	% Ash (600°C)	Dry bone Wt (105°C) (grams)	Ash Wt (600°C) (grams)
4	M	107	3000	53.21	2.3682	1.2601
4	M	107	3001	53.52	2.7344	1.4634
4	M	107	3002	54.81	2.7585	1.5120
4	M	107	3003	51.91	2.8361	1.4457
4	M	107	3004	53.86	2.5535	1.3752
Total/Averages				53.39	2.6501	1.4116
Standard Deviations				1.49	0.19	0.10
CVs				2.84%	7.12%	6.93%
2	M	108	6991	45.06	2.1516	0.9696
2	M	108	6992	51.97	2.4363	1.2661
2	M	108	6993	41.35	2.3412	0.9684
2	M	108	6996	47.94	2.1261	1.0193
2	M	108	6997	43.19	1.9543	0.8440
Total/Averages				45.90	2.2019	1.0135
Standard Deviations				4.17	0.19	0.16
CVs				9.09%	8.61%	15.83%
3	M	109	7325	53.16	2.3036	1.2246
3	M	109	7326	52.90	2.6656	1.4100
3	M	109	7327	50.41	2.5636	1.2923
3	M	109	7328	52.00	2.7632	1.4369
3	M	109	7329	48.23	2.5129	1.2119
Total/Averages				51.34	2.5818	1.3151
Standard Deviations				2.04	0.17	0.10
CVs				3.98%	6.77%	7.80%
1	M	110	7011	56.14	2.4752	1.3838
1	M	110	7012	51.33	2.3824	1.2230
1	M	110	7013	53.25	2.5512	1.3586
1	M	110	7014	54.39	2.7910	1.5180
1	M	110	7015	53.50	2.6621	1.4321
Total/Averages				53.72	2.5724	1.3791
Standard Deviations				1.44	0.16	0.11
CVs				2.69%	6.23%	7.97%
2	M	111	7346	44.04	1.5710	0.6240
2	M	111	7347	44.31	1.9219	0.8515
2	M	111	7348	43.32	2.4051	1.0420
2	M	111	7349	47.75	2.2203	1.0603
2	M	111	7350	40.41	1.9283	0.7792
Total/Averages				43.97	2.0693	0.9714
Standard Deviations				2.62	0.23	0.13
CVs				5.97%	11.24%	14.00%

Table 16. Tibia Ash Weights of Cobb 500 Broilers Summarized by Pen
(b) (4) Project No NV-13-2
BLDG 7

Treatment	Sex	Pen No.	Bone No.	% Ash (600°C)	Dry bone Wt (105°C) (grams)	Ash Wt (600°C) (grams)
4	M	112	7031	52.53	2.5180	1.3227
4	M	112	7032	53.58	2.6913	1.4419
4	M	112	7033	50.96	2.1786	1.1103
4	M	112	7034	53.57	2.6806	1.4361
4	M	112	7035	53.23	3.0142	1.6045
Total/Averages				52.77	2.6169	1.3831
Standard Deviations				1.10	0.30	0.18
CVs				2.09%	11.61%	13.20%
3	M	113	7365	53.29	2.3355	1.2447
3	M	113	7366	50.93	2.3880	1.2162
3	M	113	7367	54.56	2.3034	1.2568
3	M	113	7368	52.95	2.4369	1.2904
3	M	113	7369	51.36	2.4756	1.2720
Total/Averages				52.62	2.3879	1.2560
Standard Deviations				1.43	0.07	0.04
CVs				2.81%	2.96%	2.23%
1	M	114	1798	53.16	2.3633	1.2564
1	M	114	1799	54.49	2.6053	1.4196
1	M	114	1800	56.48	2.4787	1.4000
1	M	114	1801	54.26	2.2127	1.2006
1	M	114	1802	53.77	2.5361	1.3636
Total/Averages				54.43	2.4392	1.3280
Standard Deviations				1.26	0.16	0.10
CVs				2.30%	6.34%	7.18%
2	M	115	7365	50.82	2.3195	1.1787
2	M	115	7366	46.66	2.0190	0.9420
2	M	115	7367	43.71	1.8251	0.7991
2	M	115	7368	47.01	2.1811	1.0253
2	M	115	7369	43.71	2.0795	0.9090
Total/Averages				46.38	2.0854	0.9708
Standard Deviations				2.93	0.18	0.14
CVs				6.32%	8.80%	14.95%
1	M	116	1818	55.15	2.6299	1.4503
1	M	116	1819	54.19	2.6190	1.4193
1	M	116	1820	53.74	2.3550	1.2655
1	M	116	1821	53.83	2.5886	1.3934
1	M	116	1822	52.17	2.5331	1.3214
Total/Averages				53.81	2.5451	1.3700
Standard Deviations				1.08	0.11	0.08
CVs				2.00%	4.43%	5.60%

Table 16. Tibia Ash Weights of Cobb 500 Broilers Summarized by Pen

(b) (4) Project No. NV-13-2

BLDG 7

Treatment	Sex	Pen No.	Bone No.	% Ash (600°C)	Dry bone Wt (105°C) (grams)	Ash Wt (600°C) (grams)
4	M	117	7405	52.83	2.2453	1.1861
4	M	117	7406	55.45	2.5279	1.4016
4	M	117	7407	51.16	2.4302	1.2432
4	M	117	7408	54.52	2.3662	1.2905
4	M	117	7409	50.57	2.7499	1.3906
Total/Averages				52.91	2.4639	1.3024
Standard Deviations				2.12	0.18	0.09
CVs				4.00%	7.71%	7.16%
3	M	118	1838	47.56	2.2941	1.0909
3	M	118	1839	52.76	2.1341	1.1260
3	M	118	1840	49.35	2.8552	1.4091
3	M	118	1841	52.92	2.7271	1.4431
3	M	118	1842	51.18	2.3260	1.1905
Total/Averages				50.76	2.4873	1.2519
Standard Deviations				2.20	0.31	0.16
CVs				4.33%	12.47%	13.05%
2	M	123	7425	41.33	1.8743	0.7747
2	M	123	7426	46.54	2.3251	1.0820
2	M	123	7427	49.35	2.8862	1.4254
2	M	123	7428	53.99	2.6073	1.4068
2	M	123	7429	39.58	1.6736	0.6618
Total/Averages				46.16	2.2733	1.0501
Standard Deviations				5.87	0.49	0.36
CVs				12.73%	22.11%	32.89%
3	M	124	1858	52.44	2.6520	1.4956
3	M	124	1859	49.57	2.3485	1.1642
3	M	124	1860	49.84	3.1403	1.5652
3	M	124	1861	50.51	2.7085	1.3708
3	M	124	1862	54.00	2.4691	1.3334
Total/Averages				51.39	2.7037	1.3858
Standard Deviations				1.89	0.31	0.16
CVs				3.67%	11.51%	11.33%
4	M	125	7445	54.11	2.4646	1.3335
4	M	125	7446	53.00	2.8532	1.5123
4	M	125	7447	52.74	2.6858	1.5219
4	M	125	7448	53.15	2.4783	1.3171
4	M	125	7449	50.76	3.0749	1.5615
Total/Averages				52.75	2.7514	1.4493
Standard Deviations				1.22	0.27	0.11
CVs				2.31%	9.78%	7.82%

Table 16. Tibia Ash Weights of Cobb 500 Broilers Summarized by Pen
(b) (4) Project No. NV-13-2

BLDG 7

Treatment	Sex	Pen No.	Bone No.	% Ash (600°C)	Dry bone Wt (105°C) (grams)	Ash Wt (600°C) (grams)
1	M	126	1878	52.23	2.3777	1.2418
1	M	126	1879	50.89	2.6017	1.3241
1	M	126	1880	55.05	3.1047	1.7092
1	M	126	1881	53.71	2.3856	1.2813
1	M	126	1882	55.64	2.7734	1.5430
Total/Averages				53.50	2.6486	1.4199
Standard Deviations				1.96	0.30	0.20
CVs				3.67%	11.48%	14.04%
1	M	127	7465	53.18	2.4496	1.3027
1	M	127	7466	54.37	2.2373	1.2164
1	M	127	7467	54.12	2.7291	1.4771
1	M	127	7468	54.20	2.1346	1.1569
1	M	127	7469	53.44	3.0581	1.6342
Total/Averages				53.86	2.5217	1.3575
Standard Deviations				0.52	0.38	0.20
CVs				0.96%	14.93%	14.68%
3	M	128	1898	51.08	2.4974	1.2756
3	M	128	1899	50.66	2.4723	1.2525
3	M	128	1900	52.49	2.3981	1.2587
3	M	128	1901	53.51	2.8552	1.5278
3	M	128	1902	53.84	2.6962	1.4516
Total/Averages				52.31	2.5838	1.3532
Standard Deviations				1.42	0.19	0.13
CVs				2.71%	7.28%	9.44%
2	M	129	7485	38.25	2.1580	0.8254
2	M	129	7486	43.65	2.1510	0.9390
2	M	129	7487	45.89	2.1378	0.9810
2	M	129	7488	44.88	2.0546	0.9221
2	M	129	7489	45.30	1.8861	0.8554
Total/Averages				43.60	2.0779	0.9046
Standard Deviations				3.10	0.11	0.06
CVs				7.11%	5.48%	7.09%
4	M	130	1918	52.86	2.6387	1.3948
4	M	130	1919	52.91	2.4180	1.2793
4	M	130	1920	53.61	3.1046	1.6643
4	M	130	1921	50.57	2.9318	1.4826
4	M	130	1922	50.39	2.4207	1.2196
Total/Averages				52.07	2.7028	1.4082
Standard Deviations				1.48	0.31	0.18
CVs				2.84%	11.33%	12.47%

Table 16. Tibia Ash Weights of Cobb 500 Broilers Summarized by Pen

(b) (4) Project No. NV-13-2

BLDG 7

Treatment	Sex	Pen No.	Bone No.	% Ash (600°C)	Dry bone Wt (105°C) (grams)	Ash Wt (600°C) (grams)
1	M	131	7505	49.06	2.4196	1.1870
1	M	131	7506	53.02	2.7621	1.4645
1	M	131	7507	53.20	2.5525	1.3580
1	M	131	7508	52.65	2.5094	1.3212
1	M	131	7509	52.58	2.5579	1.3607
Total/Averages				52.10	2.5883	1.3583
Standard Deviations				1.72	0.13	0.10
CVs				3.30%	4.92%	7.47%
4	M	132	1938	52.22	2.6916	1.4056
4	M	132	1939	52.68	2.5754	1.3567
4	M	132	1940	53.65	3.0904	1.6582
4	M	132	1941	53.04	3.2625	1.7304
4	M	132	1942	53.25	2.5786	1.3730
Total/Averages				52.97	2.8397	1.5048
Standard Deviations				0.55	0.32	0.15
CVs				1.03%	11.18%	11.05%
2	M	133	7525	41.72	2.3217	0.9685
2	M	133	7526	36.91	1.5875	0.5860
2	M	133	7527	50.72	2.3540	1.1940
2	M	133	7528	41.77	1.7222	0.7193
2	M	133	7529	41.36	1.9816	0.8200
Total/Averages				42.50	1.9934	0.8579
Standard Deviations				5.03	0.34	0.20
CVs				11.84%	17.21%	27.33%
3	M	134	1952	49.02	2.4651	1.2283
3	M	134	1958	58.89	2.6251	1.5464
3	M	134	1959	54.17	3.0068	1.6287
3	M	134	1960	46.96	2.4059	1.1298
3	M	134	1961	52.81	2.3210	1.2257
Total/Averages				52.37	2.5648	1.3478
Standard Deviations				4.66	0.27	0.22
CVs				8.87%	10.57%	16.60%
2	M	135	7545	51.82	2.4479	1.2684
2	M	135	7546	47.17	2.4230	1.1429
2	M	135	7547	43.05	1.8997	0.8181
2	M	135	7548	42.61	1.7684	0.7532
2	M	135	7551	43.64	2.4130	1.0530
Total/Averages				45.66	2.1884	1.0068
Standard Deviations				3.83	0.33	0.22
CVs				8.39%	15.08%	21.82%

Table 16. Tibia Ash Weights of Cobb 500 Broilers Summarized by Pen
(b) (4) Project No. NV-13-2
BLDG 7

Treatment	Sex	Pen No.	Bone No.	% Ash (600°C)	Dry Bone Wt (105°C) (grams)	Ash Wt (600°C) (grams)
4	M	136	1978	52.93	2.3127	1.2241
4	M	136	1979	54.89	2.4515	1.3511
4	M	136	1980	53.09	2.7504	1.4603
4	M	136	1981	54.83	2.6798	1.4693
4	M	136	1982	51.89	2.4517	1.2773
Total/Averages				53.43	2.5392	1.3564
Standard Deviations				1.20	0.16	0.11
CVs				2.45%	7.05%	8.02%
1	M	137	7562	51.35	2.7549	1.4198
1	M	137	7565	51.23	2.2010	1.1276
1	M	137	7566	54.00	2.4420	1.3187
1	M	137	7568	53.74	2.5153	1.3518
1	M	137	7569	53.99	2.2324	1.2053
Total/Averages				52.86	2.4311	1.2846
Standard Deviations				1.44	0.23	0.12
CVs				2.72%	9.45%	9.12%
3	M	138	1998	50.89	2.7911	1.4203
3	M	138	1999	47.12	2.2756	1.0722
3	M	138	2000	52.08	2.5685	1.3377
3	M	138	2001	52.78	2.2805	1.2035
3	M	138	2002	41.52	1.8957	0.7883
Total/Averages				48.88	2.3823	1.1844
Standard Deviations				4.88	0.34	0.25
CVs				8.63%	14.28%	21.04%

Table 17 Tibia Ash Weights of Cobb 500 Broilers Summarized by Treatment
(b) (4) Project No. NV-13-2
BLDG 7

Treatment	Sex	Pen No.	Bone No.	% Ash (600°C)	Dry Bone Wt (105°C) (grams)	Ash Wt (600°C) (grams)
1	M	83	2800	56.31	2.7031	1.4852
1	M	83	2801	53.85	2.7256	1.4678
1	M	83	2802	54.97	2.8442	1.5634
1	M	83	2803	53.05	2.1170	1.1231
1	M	83	2804	53.14	2.8586	1.5349
1	M	89	2860	55.22	1.9941	1.0957
1	M	89	2861	50.92	2.4571	1.2563
1	M	89	2862	54.32	2.1947	1.1922
1	M	89	2863	52.22	2.7223	1.4215
1	M	89	2864	52.71	2.5655	1.3470
1	M	93	2900	54.72	3.0426	1.6650
1	M	93	2901	53.61	2.0720	1.1109
1	M	93	2902	52.62	2.5027	1.3169
1	M	93	2903	52.96	2.3984	1.2706
1	M	93	2904	54.52	2.3139	1.2615
1	M	95	2920	53.13	2.8997	1.5405
1	M	95	2921	54.04	2.6528	1.4335
1	M	95	2922	56.52	2.4489	1.3842
1	M	95	2923	52.26	2.6701	1.3955
1	M	95	2924	50.18	2.2150	1.1114
1	M	106	6971	53.25	2.4253	1.2920
1	M	106	6972	53.83	2.8475	1.5329
1	M	106	6973	53.97	2.6380	1.4237
1	M	106	6975	52.53	2.5865	1.3587
1	M	106	6976	55.46	2.2796	1.2643
1	M	110	7011	55.14	2.4732	1.3638
1	M	110	7012	51.33	2.3624	1.2230
1	M	110	7013	53.25	2.5512	1.3586
1	M	110	7014	54.39	2.7910	1.5180
1	M	110	7015	53.80	2.6621	1.4321
1	M	114	1798	53.16	2.3633	1.2564
1	M	114	1799	54.49	2.6053	1.4196
1	M	114	1800	56.48	2.4767	1.4000
1	M	114	1801	54.26	2.2127	1.2006
1	M	114	1802	53.77	2.5361	1.3636
1	M	116	1818	55.15	2.6299	1.4503
1	M	116	1819	54.19	2.6190	1.4193
1	M	116	1820	53.74	2.3650	1.2665
1	M	116	1821	53.83	2.5886	1.3934
1	M	116	1822	52.17	2.5331	1.3214
1	M	126	1878	52.23	2.3777	1.2418
1	M	126	1879	50.89	2.6017	1.3241
1	M	126	1880	56.05	3.1047	1.7092
1	M	126	1881	53.71	2.3856	1.2813
1	M	126	1882	55.64	2.7734	1.5430
1	M	127	7465	53.18	2.4496	1.3027
1	M	127	7466	54.37	2.2373	1.2164

Table 17. Tibia Ash Weights of Cobb 500 Broilers Summarized by Treatment

(b) (4) Project No NV-13-2

BLDG 7

Treatment	Sex	Pen No.	Bone No.	% Ash (600°C)	Dry bone Wt (105°C) (grams)	Ash Wt (600°C) (grams)
1	M	127	7467	54.12	2.7291	1.4771
1	M	127	7468	54.20	2.1346	1.1569
1	M	127	7469	53.44	3.0581	1.6342
1	M	131	7505	49.06	2.4196	1.1870
1	M	131	7506	53.02	2.7621	1.4645
1	M	131	7507	53.20	2.5626	1.3580
1	M	131	7508	52.65	2.5094	1.3212
1	M	131	7509	52.58	2.5879	1.3607
1	M	137	7562	51.36	2.7549	1.4196
1	M	137	7565	51.23	2.2010	1.1276
1	M	137	7566	54.00	2.4420	1.3187
1	M	137	7568	53.74	2.5153	1.3518
1	M	137	7569	53.99	2.2324	1.2053
Tots/Averages				53.00	2.63	1.39
Standard Deviations				1.44	0.26	0.14
C/a				2.69%	9.70%	10.37%
2	M	85	2820	50.76	2.7269	1.3843
2	M	85	2821	47.68	2.1667	1.0330
2	M	85	2822	41.27	2.0233	0.8350
2	M	85	2823	47.97	2.0609	0.9700
2	M	85	2825	44.63	2.2301	0.9952
2	M	87	2840	45.47	1.9931	0.9063
2	M	87	2841	46.77	1.6986	0.7944
2	M	87	2842	52.08	2.0838	1.0848
2	M	87	2843	44.58	1.9225	0.8590
2	M	87	2844	45.21	2.0052	0.9066
2	M	92	6871	51.73	1.9374	1.0022
2	M	92	6872	43.32	1.9062	0.8257
2	M	92	6873	40.23	2.3142	0.9310
2	M	92	6874	45.22	1.8082	0.8176
2	M	92	6875	47.56	2.1572	1.0307
2	M	96	6911	45.17	2.0661	0.9332
2	M	96	6912	47.23	1.6976	0.8962
2	M	96	6913	40.10	1.6575	0.6586
2	M	96	6914	48.40	1.3734	0.6647
2	M	96	6915	38.20	1.9247	0.7352
2	M	103	2960	38.83	2.4272	0.9424
2	M	103	2961	45.20	1.8557	0.8386
2	M	103	2962	37.23	2.7594	1.0311
2	M	103	2963	40.45	1.7301	0.6998
2	M	103	2964	39.77	1.7453	0.6941
2	M	108	6991	45.06	2.1518	0.9696
2	M	108	6992	51.97	2.4363	1.2661
2	M	108	6993	41.36	2.3412	0.9684
2	M	108	6996	47.94	2.1261	1.0193
2	M	108	6997	43.19	1.9543	0.8440

Table 17. Tibia Ash Weights of Cobb 500 Broilers Summarized by Treatment
(b) (4) Project No. NV-13-2
BLDG 7

Treatment	Sex	Pen No.	Bone No.	% Ash (600°C)	Dry Bone Wt (105°C) (grams)	Ash Wt (600°C) (grams)
2	M	111	7346	44.04	1.8710	0.8240
2	M	111	7347	44.31	1.9219	0.8515
2	M	111	7348	43.32	2.4051	1.0420
2	M	111	7349	47.75	2.2203	1.0603
2	M	111	7350	40.41	1.9283	0.7792
2	M	115	7385	50.82	2.3196	1.1787
2	M	115	7386	46.66	2.0190	0.9420
2	M	115	7387	43.71	1.8281	0.7991
2	M	115	7388	47.01	2.1811	1.0253
2	M	115	7389	43.71	2.0795	0.9090
2	M	123	7425	41.33	1.8743	0.7747
2	M	123	7426	46.54	2.3251	1.0820
2	M	123	7427	49.35	2.8882	1.4254
2	M	123	7428	53.96	2.6073	1.4066
2	M	123	7429	39.55	1.6735	0.6618
2	M	129	7485	38.25	2.1580	0.8254
2	M	129	7486	43.65	2.1519	0.9390
2	M	129	7487	45.89	2.1378	0.9810
2	M	129	7488	44.89	2.0548	0.9221
2	M	129	7489	45.30	1.8581	0.8554
2	M	133	7525	41.72	2.3217	0.9685
2	M	133	7526	36.91	1.5878	0.5860
2	M	133	7527	50.72	2.3540	1.1940
2	M	133	7528	41.77	1.7222	0.7193
2	M	133	7529	41.38	1.9816	0.8200
2	M	135	7545	51.82	2.4479	1.2684
2	M	135	7546	47.17	2.4230	1.1429
2	M	135	7547	43.06	1.8997	0.8181
2	M	135	7548	42.61	1.7654	0.7522
2	M	135	7551	43.64	2.4130	1.0530
Tests / Averages				44.76	2.08	0.94
Standard Deviations				4.03	0.35	0.19
CVs				9.00%	14.24%	19.81%
3	M	84	6791	48.05	2.5369	1.2189
3	M	84	6792	49.86	2.4174	1.2053
3	M	84	6793	48.67	2.3645	1.1509
3	M	84	6794	54.26	2.0342	1.1037
3	M	84	6795	49.72	2.4974	1.2418
3	M	88	6831	38.72	3.9010	1.5106
3	M	88	6832	49.52	2.5565	1.2656
3	M	88	6833	49.66	2.7142	1.3478
3	M	88	6834	50.60	2.8025	1.4182
3	M	88	6835	52.90	2.0756	1.0979
3	M	91	3580	52.77	2.3927	1.2626
3	M	91	2581	51.88	2.7104	1.4061
3	M	91	3582	51.83	2.3595	1.2236

Table 17. Tibia Ash Weights of Cobb 500 Broilers Summarized by Treatment

(b) (4) Project No. NV-13-2

BLDG 7

Treatment	Sex	Pen No.	Bone No.	% Ash (600°C)	Dry bone Wt (105°C) (grams)	Ash Wt (600°C) (grams)
3	M	91	2883	52.50	2.2596	1.1863
3	M	91	2884	54.59	2.5544	1.3945
3	M	97	2940	51.40	2.6523	1.3633
3	M	97	2941	51.37	2.3575	1.2111
3	M	97	2942	53.63	2.9441	1.5789
3	M	97	2943	53.86	2.6184	1.4103
3	M	97	2944	46.39	1.9567	0.9077
3	M	105	2980	53.06	2.5069	1.3301
3	M	105	2981	52.85	2.6124	1.4864
3	M	105	2982	52.62	2.2427	1.1802
3	M	105	2983	51.99	2.7456	1.4275
3	M	105	2984	54.07	2.5123	1.3583
3	M	109	7325	53.16	2.3036	1.2246
3	M	109	7326	52.90	2.6656	1.4100
3	M	109	7327	50.41	2.5636	1.2923
3	M	109	7328	52.00	2.7632	1.4369
3	M	109	7329	48.23	2.5129	1.2119
3	M	113	7365	53.29	2.3355	1.2447
3	M	113	7366	50.93	2.3880	1.2162
3	M	113	7367	54.56	2.3034	1.2568
3	M	113	7368	52.95	2.4369	1.2904
3	M	113	7369	51.38	2.4756	1.2720
3	M	118	1838	47.55	2.2941	1.0909
3	M	118	1839	52.76	2.1341	1.1260
3	M	118	1840	49.35	2.8552	1.4091
3	M	118	1841	52.92	2.7271	1.4431
3	M	118	1842	51.18	2.3260	1.1906
3	M	124	1858	52.44	2.6520	1.4956
3	M	124	1859	49.57	2.3485	1.1642
3	M	124	1860	49.84	3.1403	1.5652
3	M	124	1861	50.61	2.7085	1.3706
3	M	124	1862	54.00	2.4691	1.3334
3	M	128	1898	51.08	2.4974	1.2756
3	M	128	1899	50.66	2.4723	1.2525
3	M	128	1900	52.49	2.3981	1.2587
3	M	128	1901	53.51	2.8552	1.5278
3	M	128	1902	53.84	2.6962	1.4516
3	M	134	1952	49.02	2.4651	1.2083
3	M	134	1958	58.89	2.6261	1.5464
3	M	134	1959	54.17	3.0066	1.6287
3	M	134	1960	46.96	2.4059	1.1296
3	M	134	1961	52.81	2.3210	1.2257
3	M	138	1998	50.89	2.7911	1.4203
3	M	138	1999	47.12	2.2756	1.0722
3	M	138	2000	52.08	2.5685	1.3377
3	M	138	2001	52.78	2.2806	1.2036
3	M	138	2002	41.52	1.8987	0.7883

Table 17. Tibia Ash Weights of Cobb 500 Broilers Summarized by Treatment
(b) (4) Project No. NV-13-2
BLDG 7

Treatment	Sex	Pen No.	Bone No.	% Ash (600°C)	Dry bone Wt (105°C) (grams)	Ash Wt (600°C) (grams)
Total/Averages				51.24	2.69	1.28
Standard Deviations				3.07	0.81	0.16
CVs				5.99%	12.23%	12.28%
4	M	86	6811	53.46	2.2779	1.2178
4	M	86	6812	55.09	2.5335	1.3958
4	M	86	6813	52.74	3.0109	1.5879
4	M	86	6814	52.88	2.7855	1.4730
4	M	86	6815	54.07	3.0836	1.6673
4	M	90	6851	54.39	2.7901	1.5176
4	M	90	6852	51.76	2.6543	1.3738
4	M	90	6853	55.36	2.7086	1.4996
4	M	90	6854	52.55	2.4406	1.2825
4	M	90	6855	53.08	2.6300	1.3959
4	M	94	6891	52.74	2.6373	1.3909
4	M	94	6892	51.91	2.5421	1.3197
4	M	94	6893	52.47	2.1056	1.1048
4	M	94	6894	50.97	2.8143	1.4345
4	M	94	6895	52.70	2.3393	1.2329
4	M	98	6931	51.81	2.6858	1.3916
4	M	98	6932	52.22	2.3326	1.2182
4	M	98	6933	54.01	2.9648	1.6012
4	M	98	6934	51.79	3.2365	1.6762
4	M	98	6935	53.70	2.6372	1.4162
4	M	104	6951	52.56	2.8390	1.4922
4	M	104	6952	52.41	2.6857	1.4076
4	M	104	6953	51.53	2.8527	1.4855
4	M	104	6954	51.25	2.0469	1.0491
4	M	104	6955	52.92	2.3989	1.2694
4	M	107	3000	53.21	2.3582	1.2601
4	M	107	3001	53.62	2.7344	1.4634
4	M	107	3002	54.81	2.7585	1.5120
4	M	107	3003	51.01	2.8361	1.4467
4	M	107	3004	53.86	2.5535	1.3752
4	M	112	7031	52.53	2.5180	1.3227
4	M	112	7032	53.58	2.6913	1.4419
4	M	112	7033	50.96	2.1788	1.1103
4	M	112	7034	53.57	2.6806	1.4361
4	M	112	7035	53.23	3.0142	1.6045
4	M	117	7405	52.83	2.2453	1.1861
4	M	117	7406	55.45	2.5279	1.4016
4	M	117	7407	51.16	2.4302	1.2432
4	M	117	7408	54.62	2.3652	1.2225
4	M	117	7409	50.57	2.7499	1.3906
4	M	125	7445	54.11	2.4645	1.3335
4	M	125	7446	53.00	2.8532	1.5123
4	M	125	7447	52.74	2.8858	1.5219

Table 17. Tibia Ash Weights of Cobb 500 Broilers Summarized by Treatment

(b) (4) Project No. NV-13-2

BLDG 7

Treatment	Sex	Pen No.	Bone No.	% Ash (600°C)	Dry bone Wt (105°C) (grams)	Ash Wt (600°C) (grams)
4	M	125	7448	53.15	2.4753	1.3171
4	M	125	7449	50.78	3.0749	1.5615
4	M	130	1918	52.86	2.6367	1.3948
4	M	130	1919	52.91	2.4150	1.2793
4	M	130	1920	53.61	3.1046	1.6643
4	M	130	1921	50.57	2.9318	1.4826
4	M	130	1922	50.39	2.4207	1.2196
4	M	132	1938	52.22	2.6916	1.4056
4	M	132	1939	52.68	2.5754	1.3567
4	M	132	1940	53.66	3.0904	1.6582
4	M	132	1941	53.04	3.2525	1.7304
4	M	132	1942	53.25	2.5786	1.3730
4	M	136	1978	52.93	2.3127	1.2241
4	M	136	1979	54.89	2.4615	1.3511
4	M	136	1980	53.09	2.7504	1.4603
4	M	136	1981	54.83	2.6798	1.4693
4	M	136	1982	51.89	2.4617	1.2773
Total/Averages				52.88	2.86	1.49
Standard Deviations				1.24	0.27	0.16
CVs				2.34%	10.31%	10.67%

Graph 4. Tibia Ash Weights of Cobb 500 Broilers
(b) Project No. NV-13-2
BLDG 7

Treatment	% Ash (600°C)	Treatment Description
1	53.50	Positive Control (PC)
2	44.75	Negative Control (NC)
3	51.24	NC with 250 U CIBENZA® PHYTAVERSE™ G10 per kg diet
4	52.56	NC with 500 U CIBENZA® PHYTAVERSE™ G10 per kg diet

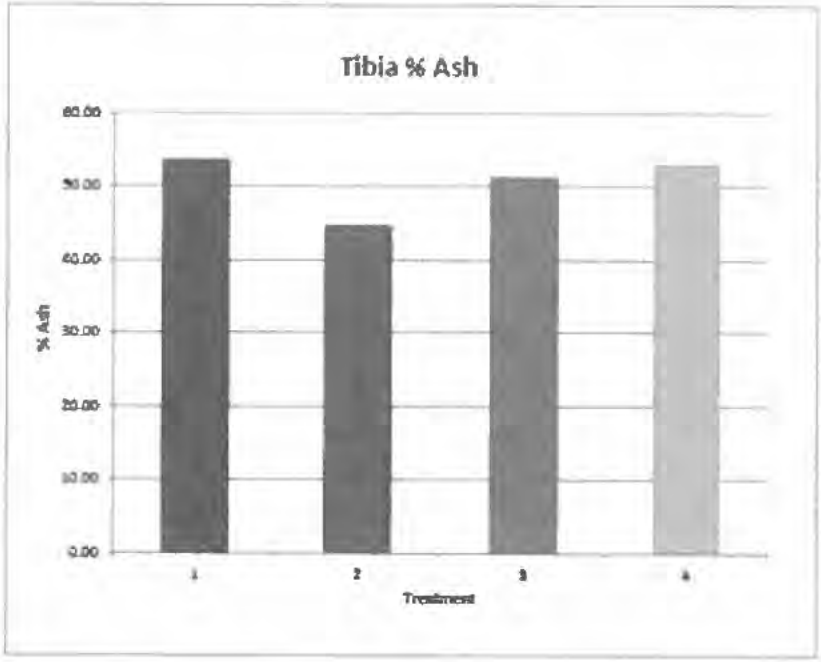


Table 18. Tibia Ash Calcium, Phosphorus, and Magnesium
(b) (4) Project No. NV-13-2
BLOG 7

Treatment	Pen No.	Calcium W/W%	Phosphorus W/W%	Magnesium W/W%
1	83	36.10	17.06	0.78
3	84	36.39	16.82	0.69
2	85	36.20	16.28	0.63
4	86	36.48	17.33	0.72
2	87	36.12	16.46	0.62
3	88	36.26	16.32	0.69
1	89	37.36	17.56	0.75
4	90	37.49	17.33	0.74
3	91	37.42	17.33	0.71
2	92	37.89	17.00	0.63
1	93	38.39	18.13	0.79
4	94	38.70	17.99	0.76
1	95	38.25	17.91	0.77
2	96	38.03	17.51	0.68
3	97	36.70	17.18	0.69
4	98	39.93	18.26	0.76
2	103	38.93	17.52	0.63
4	104	37.45	17.78	0.73
3	105	38.84	18.26	0.72
1	106	36.53	17.59	0.76
4	107	38.78	18.49	0.77
2	108	39.26	17.68	0.65
3	109	38.89	18.35	0.78
1	110	39.52	19.14	0.67
2	111	38.81	17.19	0.60
4	112	40.11	18.26	0.76
3	113	35.79	16.49	0.70
1	114	39.45	18.47	0.81
2	115	37.80	16.86	0.62
1	116	37.85	18.04	0.77
4	117	37.57	17.63	0.78
3	118	36.95	16.64	0.67
2	123	38.65	17.35	0.71
3	124	38.58	17.53	0.68
4	125	38.38	17.82	0.73
1	126	37.51	17.81	0.81
1	127	37.43	17.36	0.78
3	128	38.67	17.60	0.73
2	129	35.91	16.30	0.59
4	130	38.57	17.59	0.75
1	131	37.07	18.16	0.82
4	132	36.86	17.11	0.75
2	133	38.42	16.88	0.63
3	134	38.09	17.59	0.71
2	135	37.37	16.76	0.66
4	136	38.32	17.55	0.73
1	137	38.19	17.81	0.79
3	138	37.71	17.39	0.70

Table 19. Tibia Ash Calcium, Phosphorus, and Magnesiu

(b) (4) Project No. NV-13-2

BLDG 7

Treatment	Pen no.	Calcium W/W%	Phosphorus W/W%	Magnesium W/W%
1	83	36.10	17.05	0.78
1	89	37.36	17.55	0.75
1	93	36.39	16.13	0.79
1	95	38.25	17.91	0.77
1	106	36.53	17.59	0.76
1	110	39.52	19.14	0.87
1	114	39.45	18.47	0.81
1	116	37.85	16.04	0.77
1	125	37.51	17.81	0.81
1	127	37.43	17.36	0.78
1	131	37.07	18.16	0.82
1	137	38.19	17.81	0.79
Total/Averages		37.80	17.82	0.79
Standard Deviations		1.04	0.64	0.03
CVs		2.74%	3.59%	4.10%
2	85	36.20	16.28	0.63
2	87	36.12	16.46	0.62
2	92	37.89	17.00	0.63
2	96	39.03	17.51	0.68
2	103	38.93	17.52	0.63
2	108	39.26	17.68	0.65
2	111	38.81	17.19	0.60
2	115	37.80	16.86	0.62
2	123	38.85	17.35	0.71
2	129	35.91	16.30	0.59
2	133	38.42	16.88	0.63
2	135	37.97	16.76	0.66
Total/Averages		37.89	16.99	0.64
Standard Deviations		1.91	0.48	0.03
CVs		5.29%	2.83%	5.29%
3	84	36.39	16.82	0.69
3	88	35.26	16.32	0.69
3	91	37.42	17.33	0.71
3	97	36.70	17.18	0.69
3	105	36.84	16.25	0.72
3	109	36.89	16.35	0.78
3	113	35.79	16.49	0.70
3	118	36.96	16.84	0.67
3	124	38.68	17.53	0.68
3	128	39.67	17.60	0.73
3	134	38.09	17.59	0.71
3	138	37.71	17.39	0.70
Total/Averages		37.65	17.01	0.71
Standard Deviations		1.08	0.63	0.03
CVs		2.88%	3.69%	4.07%

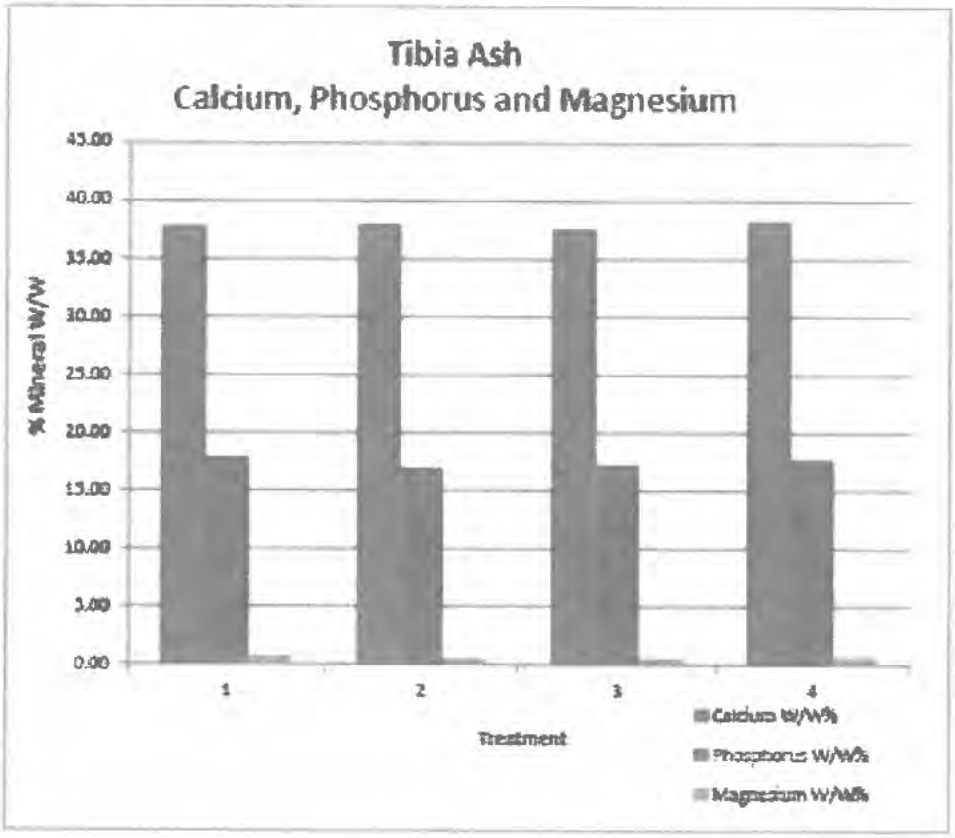
Table 19. Tibia Ash Calcium, Phosphorus, and Magnesiu
(b) (4) Project No. NV-13-2

BLDG 7

Treatment	Pen No.	Calcium W/W%	Phosphorus W/W%	Magnesium W/W%
4	88	36.48	17.33	0.72
4	90	37.49	17.33	0.74
4	94	38.70	17.99	0.76
4	98	39.93	18.25	0.75
4	104	37.45	17.78	0.73
4	107	38.78	18.49	0.77
4	112	40.11	18.25	0.78
4	117	37.57	17.83	0.78
4	125	38.38	17.62	0.73
4	130	38.57	17.89	0.75
4	132	38.06	17.11	0.75
4	136	38.32	17.55	0.73
Trt# 4 Average		38.26	17.78	0.75
Standard Deviations		1.12	0.42	0.02
CVs		2.94%	2.39%	2.70%

Graph 5. Tibia Ash Calcium, Phosphorus, and Magnesium Results
(b) (4) Project No. NV-13-2
BLDG 7

Treatment	Calcium w/w%	Phosphorus w/w%	Magnesium w/w%	Treatment Description
1	37.20	17.92	0.79	Positive Control (PC)
2	37.93	16.98	0.84	Negative Control (NC)
3	37.35	17.31	0.71	NC with 250 U CIBENZA® PHYTASE™ G10/per kg diet
4	38.23	17.76	0.75	NC with 500 U CIBENZA® PHYTASE™ G10/per kg diet



Appendix 25: Homogeneity of CIBENZA® PHYTAVERSE® in Broiler Starter Feed Report

Please note we do not consider this appendix as confidential. This report was inadvertently marked as "Proprietary and Confidential".

BASF
We create chemistry

Homogeneity of CIBENZA® PHYTAVERSE® in Broiler Starter Feed Report

Gloria Ramírez
Sr. Team Leader

April 13, 2017

BASF Enzymes LLC
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Homogeneity of CIBENZA® PHYTAVERSE® in Broiler Starter Feed Report

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Homogeneity of CIBENZA® PHYTAVERSE® in Broiler Starter Feed Report

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Homogeneity of CIBENZA® PHYTAVERSE® in Broiler Starter Feed Report

Purpose

The study *Evaluation of CIBENZA® PHYTAVERSE® G10 Phytase Enzyme Homogeneity in Broiler Starter Feed* conducted at (b) (4) protocol (b) (4) evaluates the homogeneity of CIBENZA® PHYTAVERSE® G10 Phytase Enzyme in broiler starter feed manufactured for the U.S. utility trial.

Summary

The distribution of CIBENZA® PHYTAVERSE® G10 Phytase Enzyme in broiler starter feed diets used for the U.S. utility trial was analyzed by the measurement of phytase activity in 10 samples collected throughout manufacturing of the diets. CIBENZA® PHYTAVERSE® G10 Phytase Enzyme was determined to be homogeneously distributed in the diets, manufactured to contain 250 U/kg and 500 U/kg of the enzyme, based upon the coefficient of variation (CV) of the measured phytase activity. The calculated CV was 10% for the 250 U/kg diet and 7% for the 500 U/kg diet. The positive and negative controls that do not contain CIBENZA® PHYTAVERSE® G10 Phytase Enzyme were tested for information only and were not used to determine the homogeneity of the dosed enzyme in the feed.

Materials

Phytase

CIBENZA® PHYTAVERSE® G10 Phytase Enzyme, Lot P26641

Feed

Mash starter poultry feed diets were compromised of primarily corn and soybean meal with macro- and micro- minerals and vitamin supplementation to meet or exceed the NRC (1994) and industry broiler nutrient requirements. Diets were formulated and manufactured per instructions in (b) (4). The treatment number, study blinding code, diet, and the amount of CIBENZA® PHYTAVERSE® G10 Phytase Enzyme dosed in each treatment can be found in Table 1.

Table 1: CIBENZA® PHYTAVERSE® G10 Phytase Enzyme Homogeneity Broiler Starter Feed Diets

Treatment	Treatment Blinding Code	Diet	Enzyme
1	D	Positive Control	0
2	A	Negative Control	0
3	B	Negative Control	250 U CIBENZA® PHYTAVERSE® G10 /kg diet
4	C	Negative Control	500 U CIBENZA® PHYTAVERSE® G10 /kg diet

During the feed manufacturing, ten samples were collected after completion of mixing and during the transfer of the batch from the mixer to the packaging hopper. For each of the four treatment diets, approximately 500 grams of sample were collected at regular intervals from the first to the last of the transfer ensuring "across the batch" sampling. Each sample was individually packed and labeled with the study number, treatment blinding code, sample number in sequential order of collection, and the sampling date.

Methods

1. Feed preparation and sample collection were performed per CQR Project Number NV-13-2FD, *Evaluation of CIBENZA® PHYTAVERSE® G10 Phytase Enzyme Homogeneity in Broiler Starter Feed.*
2. Phytase activity was determined using [redacted] (b) (4) [redacted]. Phytase activity is determined by the release of inorganic phosphate from phytate. The inorganic phosphate forms a colored complex with a molybdate/vanadate reagent, which is measured using a fixed wavelength spectrophotometer at 415 nm. Activity is calculated as U/kg, where one unit is defined as the amount of enzyme that releases 1 µmol of inorganic phosphate from phytate per minute under the standard assay conditions.
3. Homogeneity was determined using the CV of the phytase activity results for samples containing CIBENZA® PHYTAVERSE® G10 Phytase Enzyme. The positive and negative controls that do not contain CIBENZA® PHYTAVERSE® G10 Phytase Enzyme were tested for information only and were not used to determine the homogeneity of the dosed enzyme in the feed.

Results

Phytase activity was determined in 10 independent samples of mash broiler starter feed diets dosed with 250 U/kg and 500 U/kg of CIBENZA® PHYTAVERSE® G10 Phytase Enzyme, see Table 2. The average phytase activity in the diet dosed with 250 U/kg of CIBENZA® PHYTAVERSE® G10 Phytase Enzyme was 271 U/kg with a CV of 10%. The average activity in the diet dosed with 500 U/kg of CIBENZA® PHYTAVERSE® G10 Phytase Enzyme was 509 U/kg with a CV of 7%.

Table 2: Homogeneity Analysis of CIBENZA® PHYTAVERSE® G10 Phytase Enzyme in Broiler Starter Feed

Treatment Blinding Code (Treatment)	Dose (U/kg)	Sample Number										Average	Standard Deviation	CV (%)
		1	2	3	4	5	6	7	8	9	10			
B (3)	250	(b) (4)										271	28	10
C (4)	500											509	34	7

Conclusion

Homogeneity was evaluated in broiler starter feed diets containing CIBENZA® PHYTAVERSE® G10 Phytase Enzyme. Phytase activity was determined in 10 samples collected throughout the manufacturing of each broiler starter feed diet. The positive and negative controls that do not contain CIBENZA® PHYTAVERSE® G10 Phytase Enzyme were tested for information only and were not used to determine the homogeneity of the dosed enzyme in the feed. The starter feed diets were dosed correctly during manufacturing, as the average phytase activity value of 271 U/kg for the 250 U/kg dose represents a recovery of 108% and the average phytase activity value of 509 U/kg in the 500 U/kg dose represents a recovery of 102%. CIBENZA® PHYTAVERSE® G10 Phytase Enzyme was determined to be homogeneously distributed throughout the broiler starter feed diets with a CV of 10% in the 250 U/kg dose and 7% in the 500 U/kg dose.

Appendix 26: Safety Evaluation of Phytase 50104 Enzyme Preparation (Also Known asVR003), Expressed in Pseudomonas fluorescens, Intended for Increasing Digestibility of Phytase in Monogastrics

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Appendix 27: External Expert Opinion Letter from Dr. Michael Pariza

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Michael W. Pariza, Member

October 17, 2018

Roxanna Van Dorn
Senior Regulatory Affairs Specialist
BASF Enzymes LLC
3550 John Hopkins Court
San Diego, CA 92121

RE: GRAS opinion on the intended uses of BASF Enzyme's Phytase 50104 enzyme preparation from *Escherichia coli* that is expressed in a non-pathogenic, non-toxigenic strain of *Pseudomonas fluorescens*

Dear Mrs. Van Dorn,

I have reviewed the information you provided on BASF Enzyme's Phytase 50104 enzyme preparation from *Escherichia coli* K-12 that is expressed in a non-pathogenic, non-toxigenic strain of *Pseudomonas fluorescens* (*P. fluorescens* BD50104), intended to increase the digestibility of phytin-bound phosphorous in poultry and swine diets. BASF Enzyme's Phytase 50104 enzyme preparation will be marketed in two forms under the names CIBENZA® PHYTAVERSE® L10 phytase enzyme and CIBENZA® PHYTAVERSE® G10 phytase enzyme.

In evaluating Phytase 50104, I considered the biology of *P. fluorescens* and *E. coli* K-12 and their history of safe use in food-grade enzyme manufacture; the history of safe use in animal foods of phytase enzyme preparations from other microbial species; information that you provided in the published document entitled, "Use of Phytase 50104 Enzyme Preparation to Increase the Digestibility of Phytin-Bound Phosphorous in Poultry and Swine Diets," which includes the safe lineage of the production strain *P. fluorescens* BD50104; the cloning methodology which included removal of antibiotic resistance markers; safety evaluation studies on the Phytase 50104 enzyme preparation; manufacturing methods and materials; product specifications; and other information that is publicly available in the peer-reviewed scientific literature.

By way of background, *P. fluorescens* has not been associated with food poisoning or illness in humans or animals, other than occasional reports of opportunistic pathogenicity in immunocompromised individuals. The species is commonly isolated from plant surfaces, decaying vegetation, soil, and water, indicating that *P. fluorescens* is widely consumed by humans and domesticated herbivores. Strains derived from *P. fluorescens* MB101, the parental strain of *P. fluorescens* BD50104, have a history of safe use as production organisms for food grade enzymes. Safety studies have been conducted on numerous different enzyme preparations produced by strains within the safe lineage of *P.* fluorescens MB101. The results of these studies indicate the test materials did not contain toxic or genotoxic substances. An example is GRN 126, for which FDA issued a 'no questions' letter.

Escherichia coli K-12 has a long history of safe use in both food and pharmaceutical applications, both as a production organism and gene donor. The phytase gene (50104) that is expressed by *P. fluorescens* BD50104 is a derivative of the native *Escherichia coli* K-12 *appA* gene, which has been cloned and sequenced. To produce the phytase 50104 gene, the native *appA* gene from *E. coli* K-12 strain MG1655 was modified for thermotolerance to withstand the high temperatures encountered during the production of pelleted feeds. The phytase 50104 protein product was sequenced and studied for potential safety issues, specifically amino acid sequences that might elicit allergenicity or toxicity concerns. No such sequences were found.

The phytase 50104 enzyme preparation was evaluated for safety using a battery of genotoxicity assays and toxicological studies in experimental animals, which included an acute oral toxicity test in rats, a 14-day dose range-finding oral toxicity study in rats, a 90 day oral toxicity study in rats, an acute inhalation test in rats, a primary eye irritation study in rabbits, a primary dermal irritation study in rabbits, and a delayed contact hypersensitivity test in guinea pigs. Based on the findings of the 90-day oral toxicity study in rats, the No Observed Adverse Effect Level (NOAEL) was determined to be the highest dose tested, 2000 mg/kg. Using this value and the estimated phytase 50104 consumption levels for the target animal species poultry and swine, respectively, the margins of safety were determined to be 6233 and 7169, respectively.

The *P. fluorescens* BD50104 production strain and its product phytase 50104 were formally evaluated using the Pariza-Johnson decision tree as adapted for animal feed by Pariza and Cook (Regulatory Toxicol. Pharmacol. 56: 332-342, 2010). The conclusion of this analysis was that the production strain and enzyme preparation were accepted.

The cloning techniques and methodologies employed to construct *P. fluorescens* BD50104 are appropriate for use in the genetic modification of production strains for food ingredient manufacture. The manufacturing process, including the ingredients used for fermentation, extraction and concentration, and the specifications for the phytase 50104 enzyme preparation, are appropriate for a food ingredient.

Based on the foregoing, I concur with the evaluation made by BASF Enzymes LLC that its *P. fluorescens* BD50104 production strain is safe and appropriate to use for the manufacture of food-grade phytase. I further concur with the conclusion of BASF Enzymes LLC that the phytase

50104 enzyme preparation, manufactured in a manner that is consistent with current Good Manufacturing Practice (cGMP) and meeting appropriate food-grade specifications, is *GRAS* (Generally Recognized As Safe) based on scientific procedures for use in poultry and swine feed to increase the digestibility of phytin-bound phosphorous.

It is my professional opinion that other qualified experts would also concur with these conclusions.

Sincerely,

Michael W. Pariza

Michael W. Pariza, Ph. D.
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