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

Filed by

BASF Enzymes LLC

3550 John Hopkins Court

San Diego, CA 92121

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List of Figures

Note: Figures 1-5, 7, 8, 10, and 11 are the same as in AGRN 55.

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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

Jonathan McDonough
Senior Regulatory Affairs Specialist
BASF Enzymes LLC
3550 John Hopkins Court
San Diego, CA 92121
(b)(6)

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 that is expressed by *P. fluorescens* strain BD50104.
Phytase 50104 protein	The specific phytase protein that is 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 swine

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 swine 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:

Jonathan McDonough
Senior Regulatory Affairs Specialist
BASF Enzymes LLC
3350 John Hopkins Court
San Diego, CA 92121

H. Statement of Exemption from FOIA Disclosure

Business confidential information was presented and indicated as such in AGRN 55.

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 swine diets).

Signed,

(b)(6) Digitally signed by FieldiR
Date: 2022.04.27 10:51:03 -07'00'

Roderick Fielding Date:

Enzyme Production and Supply Chain Manager

BASF Enzymes LLC

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

A. Scientific Data that Identifies the Notified Substance

Detailed information regarding the enzyme identity, amino acid sequence, enzyme substrate, characteristic properties, and source organism was provided in the GRAS Notification for the use of Cibenza® Phytaverse® L10 and G10, a 6-phytase preparation produced by a *Pseudomonas fluorescens* strain expressing a synthetic gene coding for a 6-phytase from *E.Coli* for use in poultry nutrition, AGRN 55.

B. Method of Manufacture

Detailed information regarding the method of manufacture was provided in the GRAS Notification for the use of Cibenza® Phytaverse® L10 and G10, a 6-phytase preparation produced by a *Pseudomonas fluorescens* strain expressing a synthetic gene coding for a 6-phytase from *E.Coli* for use in poultry nutrition, AGRN 55.

C. Composition and Specifications

Detailed information regarding the composition and specifications was provided in the GRAS Notification for the use of Cibenza® Phytaverse® L10 and G10, a 6-phytase preparation produced by a *Pseudomonas fluorescens* strain expressing a synthetic gene coding for a 6-phytase from *E.Coli* for use in poultry nutrition, AGRN 55.

D. Physical or Technical Effect

The purpose of using phytase as an ingredient in swine 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 swine 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 those listed in the 2022 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), 2021a; Association of American Feed Control Officals (AAFCO), 2021b; FDA Center for Veterinary Medicine, 2017; FDA Center for Veterinary Medicine, 2019a; 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 swine diets.

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 feed is 250 to 500 U/kg of feed. 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.

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). Additionally, to demonstrate the utility of the phytase 50104 enzyme preparation to increase the availability of phytin-bound phosphorus in swine diets, CIBENZA® PHYTAVERSE® G10 Phytase Enzyme was used in two different feeding experiments. The diets used in the studies were representative of U.S. corn and soybean meal diets for swine. The swine feeding experiments were published providing pivotal evidence for the utility of phytase 50104 enzyme preparation (Ren, P. *et al.*, 2020). One of the swine feeding experiments is described in the section below.

Please note that corn and soybean contain endogenous phytases at very low levels (Nys, Y. *et al.*, 1996). Since the diets for the studies described below are in mash form, the endogenous phytases were not inactivated as they would have been in a pelleted feed. Therefore, the lowest dose used in the utility studies discussed below had slightly higher recoveries than expected (ranged between 17 and 22% higher than the expected dose of 250 U/kg of feed). A homogeneity

study on the diets dosed with CIBENZA® PHYTAVERSE® G10 Phytase Enzyme for the poultry utility study described below, shows that the CIBENZA® PHYTAVERSE® G10 Phytase Enzyme is homogenously mixed into the diets (see Appendix 1). 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 the 250 U/kg dose diet had above 15% the expected phytase activity; all other subsamples, including the remaining 250 U/kg dose subsamples and all of the 500 U/kg doses subsamples, were well within ±15% of the expected dose. Therefore, it was concluded, even though there were slightly higher phytase recoveries at the lowest dose for both utility studies, the utility studies described below support the use of CIBENZA® PHYTAVERSE® G10 Phytase Enzyme at the 250 U/kg and the 500 U/kg of feed doses.

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1. Published utility data

The swine feeding experiments are published in Ren, P. *et al.* (2020). Experiment 1 is described below. The published paper is provided in Appendix 2.

a) CIBENZA® PHYTAVERSE® G10 phytase enzyme dose response on growth performance and metacarpal bone ash in weanling pigs

To demonstrate the utility of CIBENZA® PHYTAVERSE® Phytase Enzyme, a swine study was conducted at the (b) (4), (b)(6) The swine study evaluated the utility of adding CIBENZA® PHYTAVERSE® G10 Phytase Enzyme at two doses (250 and 500 U/kg diet) in corn-soybean diets containing sub-optimal levels of non-phytate phosphorus by assessing metacarpal bone ash in weanling pigs, as an indicator of phosphorus availability.

1. Experimental Design

One hundred and sixty (160) pigs (approximate age of 35 days; 80 males, 80 females) were randomized to 40 pens of 4 pigs each (2 males, 2 females) and were randomly assigned to one of the four treatment groups (10 pens per treatment group). The treatment groups for phase I and phase II diets consisted of the following:

- Positive control (PC) – The diet met or exceeded the NRC 2012 nutritional requirements.
- Negative control (NC) – The diet met or exceeded the NRC 2012 nutritional requirements with the exception that digestible phosphorus levels were reduced by 0.15% and 0.14% in phase I and II, respectively.
- 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 of phosphate from phytate per minute at 37°C and a pH 5.5 in accordance to the assay.

Phase I and phase II diets were fed in mash form and were comprised primarily of corn and soybean meal. The phase I diet was feed from days 0 to 14 of the study, and the phase II diet was feed from days 14 to 28. Feed and water were provided *ad libitum* throughout the study.

The test facility, pens, and pigs were observed at least twice daily for general drove conditions, lighting, water, feed, ventilation, and unanticipated events. All animals were observed regularly, and any adverse effects were recorded. Mortality was observed for daily. The pigs were weighed at placement (day 0), day 14, and day 28. Feed offered was weighed per pen and feed removed was weighed per pen on days 0 to 14, 0 to 28, and 14 to 28. Average pig weight gain and average feed intake were calculated for days 0 to 14, 0 to 28, and 14 to 28. The feed conversion ratio (FCR) (average and adjusted FCR) was also calculated.

Percent metacarpal bone ash is a direct indicator of the bioavailability of phosphorus in swine and the efficacy of CIBENZA® PHYTAVERSE® Phytase Enzyme in phosphorus deficient diets. At the conclusion of the study, the 2 pigs (1 barrow and 1 gilt) selected from each pen were euthanized via captive bolt stunning and the right and left front feet were removed. The third and fourth metacarpals of the right foot were used for bone ash analysis. The left foot served as the backup sample.

Results

No differences among dietary treatments were observed for initial body weight (BW) of pigs. Pigs fed the positive control diet had greater ($P < 0.05$) final BW during phase 2, greater ($P<0.05$) average daily gain, average feed intake, and less ($P<0.05$) feed-to-gain ratio during phase I, phase II, and the entire experimental period than pigs fed the negative control diet. These

observations were expected because pigs fed the negative control diet did not receive the quantity of P needed to support maximum gain. However, as CIBENZA® PHYTAVERSE® G10 was added to the diets in increasing quantities, average daily gain and average daily feed intake were linearly ($P<0.05$) increased and the feed-to-gain ratio were linearly ($P<0.05$) decreased during phase II and the entire experimental period. This was likely due to the increasing amount of P and other nutrients released from the feed matrix. However, pigs receiving 500 U/kg of CIBENZA® PHYTAVERSE® G10 still has less ($P<0.05$) final body weight and average daily gain and greater ($P<0.05$) feed-to-gain ratio compared with the positive control diet. No difference was observed between the diets supplemented with 250 and 500 U/kg CIBENZA® PHYTAVERSE® G10 in the final body weight, average daily gain and average daily feed intake during phase I, phase II, and overall period. However, supplementation of 250 or 500 U/kg CIBENZA® PHYTAVERSE® G10 reduced ($P<0.05$) feed-to-gain of pigs during phase II and the entire experimental period, compared with negative control.

Pigs fed the positive control diet had greater ($P<0.05$) fat-free bone weight, metacarpal bone ash weight, bone ash percentage, bone P weight, and bone P percentage compared to pigs fed the negative control diet. Inclusion of graded levels of CIBENZA® PHYTAVERSE® G10 linearly increased ($P<0.05$) fat-free bone weight, bone ash weight, bone ash percentage, and bone P weight. No differences were observed in all bone measurements between 250 and 500 U/kg CIBENZA® PHYTAVERSE® G10. Supplementation of 500 U/kg CIBENZA® PHYTAVERSE® G10 increased ($P<0.05$) bone ash percentage compared to the negative control diet. However, pigs fed the diets with CIBENZA® PHYTAVERSE® G10 still had less ($P<0.05$) fat-free dried bone weight, bone ash weight, bone ash percentage, and bone P weight, compared with those fed positive control diets. These results indicate that the inclusion of CIBENZA® PHYTAVERSE® G10 improves mineralization in weaned pigs compared with the negative diet, even though the highest dose (500 U/kg) of CIBENZA® PHYTAVERSE® G10 did not reach the same bone mineralization as the positive control diet. However, it was not expected that these doses of CIBENZA® PHYTAVERSE® G10 would reach the positive control response due to the greater deficiency of digestible P in the negative control diet. Although, supplementation of 250 U/kg CIBENZA® PHYTAVERSE® G10 did not differ from the negative control in terms of ash percentage and P percentage, it increased fat-free dried bone weight, bone ash weight, and bone P weight. The reason for this observation is most likely that bone percentage is less responsive to change in P intake

than bone ash weight because the size of the bone is usually changed if P is limiting maximum bone tissue deposition. Consequently, bone ash weight is reduced due to reduced bone size, whereas the composition of bone ash is not changed, which is the reason bone ash percentage remains constant for a wider range of P intake levels. Inclusion of 250 U/kg CIBENZA® PHYTAVERSE® G10 was as effective as inclusion of 500 U/kg CIBENZA® PHYTAVERSE® G10 in terms of fat-free dried bone, bone ash weight, bone ash percentage, bone P weight, and bone P percentage.

1. Conclusion

In this swine study, the inclusion of 250 or 500 U of CIBENZA® PHYTAVERSE® G10 Phytase Enzyme per kg of phosphorus deficient diet based on corn and soybean meal increases phosphorus utilization by weaned pigs, improving performance, bone ash weight, and bone phosphorus weight. Please see Appendix 2 for the complete study report.

Therefore, the results of this swine study indicate and support the efficacy of CIBENZA® PHYTAVERSE® Phytase Enzyme in swine at either 250 or 500 u/kg diet containing sub-optimal levels of non-phytate phosphorus.

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 swine feed. The recommended use rate is 250 to 2000 U/kg feed.

Calculations are provided below in Table 2 for target animal consumption and exposure. Table 2 below for poultry is applicable to swine evaluations as broiler chickens are considered a worst-case scenario 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 2. 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 those listed in the 2022 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 (FDA Center for Veterinary Medicine, 2019b; 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 swine 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.

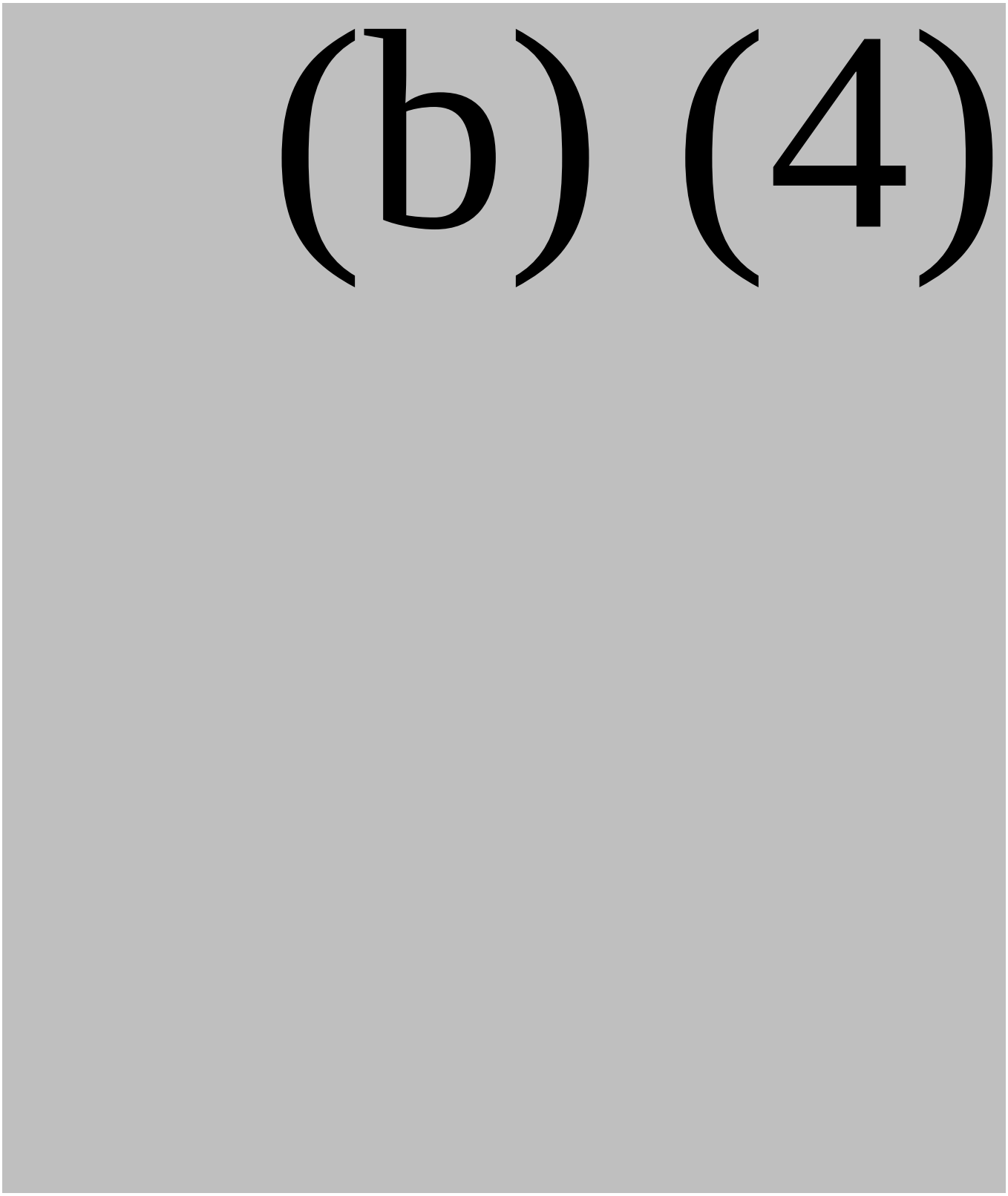
3. Amount of other substance that is present with the notified substance either naturally or due to its manufacture

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)

The first step of the recovery process (b) (4)

Therefore, (b) (4) .



To determine the worst-case maximum dietary exposure in swine 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) as 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 3.

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 swine, 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 3. Potential (b) (4) intake estimate and safety margin in swine

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

Information in this section is the same as information provided in AGRN 55.

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

Information in this section is the same as information provided in AGRN 55.

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

(b) (4) is used during the manufacturing process during fermentation to induce the production of phytase 50104 enzyme. It is expected that (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

Information in this section is the same as information provided in AGRN 55.

2. Assessment of allergenic potential

Information in this section is the same as information provided in AGRN 55.

1. History of safe use

Information in this section is the same as information provided in AGRN 55.

2. Absence of pathogenicity and toxicity

Information in this section is the same as information provided in AGRN 55.

3. Safe strain lineage

Information in this section is the same as information provided in AGRN 55.

C. Safety of the Donor Organism

1. Introduction

Information in this section is the same as information provided in AGRN 55.

2. Taxonomy

Information in this section is the same as information provided in AGRN 55.

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

Information in this section is the same as information provided in AGRN 55.

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

Information in this section is the same as information provided in AGRN 55.

5. Summary

Information in this section is the same as information provided in AGRN 55

D. Safety of the Inserted Genetic Material

Information in this section is the same as information provided in AGRN 55.

E. Safety of the Manufacturing Process

Information in this section is the same as information provided in AGRN 55.

F. Safety Studies

Information in this section is the same as information provided in AGRN 55.

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

Information in this section is the same as information provided in AGRN 55.

2. Genotoxicity studies

Information in this section is the same as information provided in AGRN 55.

3. Oral toxicity studies

Information in this section is the same as information provided in AGRN 55.

4. Worker safety studies

Information in this section is the same as information provided in AGRN 55.

5. Safety margin calculation

The safety margin calculation for swine is discussed and provided in Part 3 Section A.1. Briefly, the safety margin calculations for swine 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.

G. 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), 2020).

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 swine 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 swine 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 (swine) resulting from the use of the formulated enzyme products, CIBENZA® PHYTAVERSE® L10 Phytase Enzyme and CIBENZA® PHYTAVERSE® G10 Phytase Enzyme.

2. To humans

Information in this section is the same as information provided in AGRN 55.

H. 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, swine 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 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, 2003; FDA Center for Food Safety and Applied Nutrition, 2013; FDA Center for Food Safety and Applied Nutrition, 2015).

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 study 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 swine (Ren, P. *et al.*, 2020). 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 swine(Ren, P. *et al.*, 2020).

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 (Ping, R. *et al*., 2020) 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 (b) (4)_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 3). 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

A. List of Appendices

Appendix 1	Homogeneity of CIBENZA® PHYTAVERSE® G10 Phytase Enzyme in Broiler Starter Feed
Appendix 2	Effects of a novel *E.coli* phytase expressed in *Pseudomonas fluorescens* on growth, bone mineralization, and nutrient digestibility in pigs fed cord-soybean meal diets.
Appendix 3	External Expert Opinion Letter from Dr. Michael Pariza

B. List of References

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

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Appendix 1: 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".

Homogeneity of CIBENZA® PHYTAVERSE® in Broiler Starter Feed Report

Gloria Ramírez
Sr. Team Leader

April 13, 2017

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

BASF
We create chemistry

Homogeneity of CIBENZA® PHYTAVERSE® in Broiler Starter Feed Report

SIGNATURE PAGE

Quality Control:

(b)(6)

Gloria Ramírez
Sr. Team Leader

13 Apr 2017
Date

Quality Assurance:

(b)(6)

Mark Burcin
Sr. Manager, QA/QC

13 Apr 2017
Date

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

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) section 8.8. 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 (b) (4) , *Evaluation of CIBENZA® PHYTAVERSE® G10 Phytase Enzyme Homogeneity in Broiler Starter Feed.*
2. Phytase activity was determined using (b) (4) . 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 2: Effects of a novel *E.coli* phytase expressed in *Pseudomonas fluorescens* on growth, bone mineralization, and nutrient digestibility in pigs fed cord-soybean meal diets.

Appendix 3: External Expert Opinion Letter from Dr. Michael Pariza

Michael W. Pariza Consulting LLC
7102 Valhalla Trail
Madison, WI 53719
(608) 271-5169
mwpariza@gmail.com

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,

(b) (6)

Michael W. Pariza, Ph. D.
Member, Michael W. Pariza Consulting, LLC
Professor Emeritus, Food Science
Director Emeritus, Food Research Institute
University of Wisconsin-Madison

BASF
We create chemistry

31 January 2023

Wasima Wahid, M.S.
Staff Fellow-CSO
Center for Veterinary Medicine
Office of Surveillance and Compliance
Division of Animal Food Ingredients
Wasima.Wahid@fda.hhs.gov

To Whom It May Concern,

The information contained in this letter is to address CVMs questions provided to BASF Enzymes LLC via email on January 13, 2023, pertaining to GRAS Notice for Phytase enzyme produced by *Pseudomonas fluorescens* strain BD50104 for Swine.

CVM Request:

"The intended use level for this notice as submitted is 250 - 2,000 U/kg. CVM notes that for a prior GRAS notice submission for poultry (AGRN 39), CVM questioned the lower use rate of 250 U/kg feed. In a subsequent GRAS notice for poultry (AGRN 55), the lower use rate was increased to 500 U/kg feed. Please clarify the intended use rate and provide any justification for the lower use rate, if that is the use rate that is intended for in this notice."

BASF Enzyme LLC Response:

BASF Enzymes LLC has no objections to raising the minimum does from 250U/kg to 500 U/kg. Therefore, the submission shall be amended to read as 500 U/kg in the following subsections:

Part 1.D: "Intended Conditions of Use"; page 7
Part 3.A.1: "Target Animal Consumption"; page 16
Part 6.H.1: "Safety of the Cibenza Phytaverse L10 Phytase Enzyme and the Cibenza Phytaverse G10 Phytase Enzyme To Animals"; page 26

Furthermore, to align with the AGRN 55 language amendments (11 July 2022) the following paragraphs (in *italics*) shall be amended to what is in yellow.

Part 1.D: INTENDED CONDITIONS OF USE; the following paragraphs:

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 swine.

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San Diego, CA 92121
www.basf.us

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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 swine diets.

Should be amended to read:

CIBENZA® PHYTAVERSE® L10 Phytase Enzyme and CIBENZA® PHYTAVERSE® G10 Phytase Enzyme will be used to increase the availability of phytin-bound phosphorus in swine diets.

Supplementation of complete swine feed using CIBENZA® PHYTAVERSE® L10 Phytase Enzyme and CIBENZA® PHYTAVERSE® G10 Phytase Enzyme has a minimum recommended dose of 500 U/kg of feed and a maximum recommended dose of 2,000 U/kg of feed.

In Part 3.A.1: TARGET ANIMAL CONSUMPTION, the following paragraph:

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 swine feed. The recommended use rate is 250 to 2000 U/kg feed.

Should be amended to read:

The phytase 50104 enzyme preparation (i.e., CIBENZA® PHYTAVERSE® L10 Phytase Enzyme and CIBENZA® PHYTAVERSE® G10 Phytase Enzyme) is intended for use in swine feed. The minimum recommended dose is 500 U/kg of feed and the maximum recommended dose is 2,000 U/kg of feed.

In Part 6.H: RESULTS AND CONCLUSIONS, the following paragraph:

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, swine feed is 250 to 2000 U/kg of feed.

Should be amended to read:

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, swine feed is a minimum recommended dose of 500 U/kg to a maximum recommended dose of 2000 U/kg of feed.

These proposed minor amendments should address CVM's concerns regarding the dose rates and language surrounding the dose rates

CVM Request:

"Your submission refers to information provided in AGRN 55 with regard to chemistry, manufacturing and controls, utility, target animal safety and human food safety. CVM requests that section/part numbers and page numbers from the submission referenced be provided for this information to be considered. Please note that an amendment was also provided for AGRN 55 when referencing."

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3550 John Hopkins Court
San Diego, CA 92121
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BASF Enzyme LLC Response:

Upon review of the cross-references to AGRN 55, a discrepancy was observed in PART 6 regarding the Safety of the Production Organism. In this case, on Page 22, following PART 6.B.2, it should read PART 6.C: Safety of the Production Organism. Subsequently, Sections C-H would now become Sections D-J.

The Table below is a more explicit correlation of the data from AGRN 55 applied to this notice. Additionally, the amendments made to AGRN 55 in July 2022 are associated with the appropriate sections and subsections in the table below.

Should you have any additional questions, comments, or concerns, please feel free to contact me.

Best Regards,

Jonathan P. McDonough
Senior Regulatory Affairs Specialist, Project Manager
(b) (4), (b)(6)

BASF Enzymes LLC
3550 John Hopkins Court
San Diego, CA 92121
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Part 2.A Scientific Data that Identifies the Notified Substance	Page 8-13
Part 2.B Method of Manufacture	Page 13-30; Part 2.B.1.h: Absence of Production Organism (Page 23) is further expanded upon in a filed amendment. Part 2.B.2.a: Raw Materials (page 24) references Appendix 11. Appendix 11 was corrected with a filed amendment. Part 2.B.2.f (Packaging materials) was added into AGRN 55 by way of the 11 July 2022 Amendment.
Part 2.C Composition and Specifications	Page 30-38
Part 3.B.1.a Residues of the Notified Substance	Page 57-98
Part 3.B.1.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	Page 57-98
Part 6.B.1 Safety of Phytase: History of Safe Use	Page 60-61
Part 6.B.2 Assessment of allergenic potential	Page 60-62
Part 6.C Safety of Production Organism	Page 62 (Note: this assumes Section Heading C)
Part 6.C.1 History of Safe Use	Page 62-63
Part 6.C.2 Absence of pathogenicity and toxicity	Page 63-66
Part 6.C.3 Safe Strain Lineage	Page 66-68
Part 6.D Safety of Donor Organism; subsections 1-5	Pages 68-71 (NOTE: This was changed from Section heading C)
Part 6.E Safety of Inserted Genetic Material	Page 72-74 (Note: The section heading was changed from D to E)
Part 6.F Safety of the Manufacturing Process	Page 75 (Note: the section heading was changed from E to F)
Part 6.G Safety Studies; subsections 1-4	Page 75-82 (Note: the section heading was changed from F to G)

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