December 9, 2024

Center for Regulatory Services, Inc.
Attention: Kristi Smedley, Ph.D.
5200 Wolf Run Shoals Rd.
Woodbridge, VA 22192

Re: GRAS Notice AGRN 68 – Dried Fat Encapsulated *Ruminococcus bovis* Strain ASCUSDY10 (NRRL B-67764)

Dear Dr. Smedley:

The Food and Drug Administration's (FDA, the Agency) Center for Veterinary Medicine (CVM or we) refers to a generally recognized as safe (GRAS) notice, dated March 29, 2024, submitted on behalf of your client, Native Microbials, Inc. (Native Microbials or the notifier). The subject of the notice is dried fat encapsulated *Ruminococcus bovis* (*R. bovis*) strain ASCUSDY10 (NRRL B-67764) to be used as a supplemental source of viable microorganisms for dairy cattle when included at a recommended level of $1x10^8$ colony forming unit (CFU)/cow/day. The submission informs us of the notifier's conclusion that the subject of the submission is GRAS through scientific procedures. You were notified in a letter dated April 26, 2024 that the GRAS notice, as amended, was acceptable for filing, and the notice was designated as animal GRAS notice number (AGRN) 68. On September 10, 2024, CVM received an amendment from the notifier containing additional genome, microbial, and target animal safety information. We have completed our evaluation of AGRN 68 and have no questions at this time.

The notifier provides information for the manufacture and process controls, analytical methods used to enumerate viable cell count, and analytical methods for potential microbial contaminants and heavy metals. The notified substance, dried fat encapsulated *R. bovis* strain ASCUSDY10 (NRRL B-67764), is produced through an anaerobic maltose batch fermentation. After the fermentation process, the biomass is harvested by centrifugation, mixed with cryoprotectants, and freeze-dried. The freeze-dried cell concentrate is encapsulated with hydrogenated glycerides or other fat to produce the dried fat encapsulated *R. bovis* strain ASCUSDY10 (NRRL B-67764). The notifier provides specifications for the finished product, which include viable cell count no less than $2x10^7$ colony forming unit/grams (CFU/g), coliforms less than 10 CFU/g, *Escherichia coli* less than 10 CFU/g, *Salmonella* negative/25g, and *Listeria* negative/25g. The notifier also provides the stability and packaging information.

The notifier concludes that information demonstrating the physical or other technical effect of the notified substance is not necessary based on the notifier's conclusion that utility of the notified substance does not impact its safety in target animals as the animals would still consume nutritionally adequate diets.

To address the target animal safety of the intended use of the notified substance, the notifier provides a narrative based on scientific data and literature that addresses aspects of safe use of the notified bacterial strain for the intended use in dairy cattle. The notifier used whole genome

sequence analysis, in silico analyses, and publicly available information in the scientific literature to support: a) the identification of the microbial strain as *R. bovis*, b) that *R. bovis* occurs in a variety of ruminant species, c) the genome of the notified strain lacks potential plasmids and antimicrobial resistance genes, and d) the virulence related genes present in the genome do not pose a safety concern to the intended target animals. The notifier also conducted an exposure assessment of the target species to the components of the notified substance to support its conclusion that the notified strain is safe for the intended use as a supplemental source of viable microorganisms in dairy cattle feed, at the intended use rate of $1x10^8$ CFU/cow/day.

To address the human food safety of the notified substance, the notifier notes that *R. bovis* is a ubiquitous and prevalent member of the rumen microbiome and naturally present in the rumen of a variety of ruminant species across the globe. The notifier adds that whole genome sequence analysis indicates absence of active genes involved in toxin production or other virulence factors known to be associated with pathogenicity. The notifier corroborates this analysis with in vitro testing and multiple feed studies. The notifier states that no transfer of viable *R. bovis* from the rumen to milk or other edible tissues is anticipated under the conditions of intended use, nor would such transfer if it did occur, be pathogenic to humans. The notifier concludes that these data indicate that the notified substance should not be associated with any safety concerns for dairy cattle or any human food safety under the intended conditions of use as a direct fed microbial in dairy cattle feed. The notifier further states a conclusion of safety is scientifically justified based on a preponderance of evidence.

To support the genome safety, the notice includes a description of the whole genome sequence analysis conducted for the notified substance. The notifier used both Illumina Miseq (short reads sequencing) and Oxford Nanopore (long reads sequencing) platforms to sequence whole genome of *R. bovis* ASCUSDY10. Subsequently the sequenced genome was processed for downstream analyses. The taxonomic identification was carried out using 16S ribosomal ribonucleic acid (rRNA) analysis and whole genome Average Nucleotide Identity analysis. Subsequently a series of bioinformatics tools and online databases were used to analyze the coding sequence of the *R. bovis* ASCUSDY10 genome for the presence of potential plasmids, antimicrobial resistance genes, and pathogenicity and virulence related genes.

To support identity and microbial safety, the notifier provides a narrative based on scientific data and literature that addresses different aspects, including genomic analysis, the presence of the microbial species in animals, pathogenicity, toxin production, and published literature, to support its conclusion that fried fat encapsulated *R. bovis* strain ASCUSDY10 (NRRL B-67764) is safe for the intended use as a source of viable microorganisms for dairy cattle.

The Association of American Feed Control Officials publishes in their Official Publication a list of names and definitions for animal feed ingredients. FDA recognizes the name “Dried *Ruminococcus bovis* Fermentation Product” as the common or usual name for the notified substance.

Section 403(a) of the Federal Food, Drug, and Cosmetic Act (FD&C Act)

Under section 403(a) of the FD&C Act, a food is misbranded if its labeling is false or misleading in any particular. The notifier did not provide any information to determine that the notified substance, dried fat encapsulated *Ruminococcus bovis* strain ASCUSDY10 (NRRL B-67764), functions as intended because the notifier concluded that the intended use would not be expected to impact safety. Therefore, FDA did not evaluate whether the notified substance

would achieve the effect claimed. However, please note that if products containing dried fat encapsulated *Ruminococcus bovis* strain ASCUSDY10 (NRRL B-67764) bear any claims on the label or in labeling regarding the function of the notified substance, these claims should be supported by appropriate data and information. FDA may take enforcement action if any claims on labels or labeling are found to be false or misleading.

Section 301(ll) of the Federal Food, Drug, and Cosmetic Act

Section 301(ll) of the FD&C Act prohibits the introduction or delivery for introduction into interstate commerce of any food that contains a drug approved under section 505 of the FD&C Act, a biological product licensed under section 351 of the Public Health Service Act, or a drug or a biological product for which substantial clinical investigations have been instituted and their existence made public, unless one of the exemptions in section 301(ll) (1)-(4) applies. In our evaluation of Native Microbial's notice, as amended, concluding that dried fat encapsulated *Ruminococcus bovis* strain ASCUSDY10 (NRRL B-67764) as a supplemental source of viable microorganisms for dairy cattle when included at a recommended level of $1x10^8$ CFU/cow/day of complete feed is GRAS under its intended conditions of use, we did not consider whether section 301(ll) or any of its exemptions apply to foods containing dried fat encapsulated *Ruminococcus bovis* strain ASCUSDY10 (NRRL B-67764). Accordingly, our response should not be construed to be a statement that foods containing dried fat encapsulated *Ruminococcus bovis* strain ASCUSDY10 (NRRL B-67764), if introduced or delivered for introduction into interstate commerce, would not violate section 301(ll).

Conclusion

Based on the information contained in the notice, as amended, submitted on behalf of Native Microbials, Inc., and other information available to the FDA, we have no questions at this time regarding the notifier's conclusion that dried fat encapsulated *Ruminococcus bovis* strain ASCUSDY10 (NRRL B-67764) is GRAS under the intended conditions of use. The Agency has not, however, made its own determination regarding the GRAS status of the intended use of the notified substance in animal food under 21 CFR 570.35. Unless noted above, our evaluation did not address other provisions of the FD&C Act. As always, it is the continuing responsibility of Native Microbials, Inc. to ensure that animal food ingredients that the notifier markets are safe and are otherwise in compliance with all applicable legal and regulatory requirements.

In accordance with 21 CFR 570.275(b)(2), the text of this letter responding to AGRN 68 is accessible to the public on our website for the Current Animal Food GRAS Notices Inventory at https://www.fda.gov/animal-veterinary/generally-recognized-safe-gras-notificationprogram/current-animal-food-gras-notices-inventory.

If you have any questions or comments, please contact Ms. Megan Hall at 301-796-3801 or at megan.hall@fda.hhs.gov.

Sincerely,

/s/
Timothy Schell, Ph.D.
Director
Office of Surveillance and Compliance
Center for Veterinary Medicine