



Animal Food GRAS Notice

**Beta-mannanase Enzyme Preparation from a
Thermothelomyces thermophilus Strain in Swine Diets**

Filed by BASF Corporation

June 17, 2025

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PART 1: SIGNED STATEMENTS AND CERTIFICATION (21 CFR §570.225)

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 Corporation (Corp.) hereby notifies the FDA of the determination by BASF Corp. that mannanase 19287 enzyme preparations, produced by *Thermothelomyces thermophilus* strain DSM 33149, encoding the mannanase 19287 enzyme (an altered man1 gene from *Trichoderma reesei*) and marketed as Natupulse® TS and Natupulse® TS L, are generally recognized as safe (GRAS) to increase the digestibility of β -mannans when used as intended in complete and balanced swine diets, based on scientific procedures.

B. Name and Address of Notifier

BASF Corporation
100 Park Avenue
Florham Park, NJ 07932
USA

Contact for this dossier:

Andreas Kern, PhD
Sr. Reg. Affairs Manager Animal Nutrition
BASF Corporation
3350 John Hopkins Court
San Diego, CA 92121

C. Name of Notified Substance

The notified substance being addressed in this submission is the mannanase 19287 enzyme preparation which is marketed as the final dry product Natupulse® TS and the final liquid product Natupulse® TS L.

D. Intended Conditions of Use

- a. Natupulse® TS L will be added in a post-pelleting application to complete and balanced pelleted feeds; Natupulse® TS will be added to animal feeds in non-pelleted compound feed, pelleted compound feed, and for use in premix.
- b. Proposed levels of use: The target level of supplementation in a complete and balanced feed is 800 TMU / kg of feed. One Thermostable Mannanase Unit (TMU) per kg of feed (TMU) is defined as the amount of enzyme that produces reducing carbohydrates having a reducing power corresponding to one μmol mannose from locust bean gum (0.3 g / 100 ml, final concentration in the reaction solution: 0.27 g / 100 ml) in one minute under the assay conditions of 50.0 ± 0.1 °C and pH 3.5).
- c. Animal species intended: Natupulse® TS and Natupulse® TS L will be added to complete and balanced swine feed.
- d. The intended use for Natupulse® TS and Natupulse® TS L is to increase the digestibility of β -mannans when used in complete and balanced swine diets.

E. Basis for Conclusion of GRAS Status

The statutory basis for the conclusion of GRAS status for mannanase 19287 enzyme preparation, which is marketed as Natupulse® TS and Natupulse® TS L, 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 mannanase 19287 enzyme products, which are marketed as Natupulse[®] TS and Natupulse[®] TS L, are GRAS under the conditions of intended use to increase the digestibility of β -mannans when used in complete and balanced swine diets.

G. Statement of Availability of Data and Information

The data and information that are the basis for BASF Corporation's conclusion of GRAS status are available for FDA's review, and copies will be sent to FDA upon request, in either electronic format or by paper copy. Requests for copies and arrangements for review of materials cited may be directed to the contact for this dossier listed above, Andreas Kern, PhD, Sr. Regulatory Affairs Manager, Animal Nutrition.

We are incorporating by reference (as permitted by 21 CFR 570.215, Incorporation into a GRAS notice) the information as previously submitted by BASF Corp. and reviewed by the agency in AGRN 67, Beta-mannanase from a *Thermothelomyces thermophilus* strain expressing an altered man1 gene from *Trichoderma reesei*. All the data required for each section can be found in the notice as described.

H. Statement of Exemption from FOIA Disclosure

The following information is exempt from FOIA Disclosure (5 USC §582(b)(4)):

The Annexes contained in the Appendix to the clarifying amendment to AGRN 67 have been incorporated by reference in the current Animal GRAS Notice. BASF, Corp. considers these Annexes to contain confidential business information (CBI); therefore, the referenced Annexes are exempt from FOIA Disclosure (5 USC §582(b)(4)).

Certification

On behalf of BASF Corporation, 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 Corporation, and pertinent to the evaluation of safety and GRAS status of mannanase 19287 enzyme, which is marketed as Natupulse[®] TS and Natupulse[®] TS L, under the conditions of intended use in complete and balanced swine diets.

Signed,

(b) (6)

May 16th, 2025

Gisele Santos Bin
Global Head of Sales, Nutrition Ingredients
BASF Corporation

Date

Please Note: The following definitions and dossier terms are used throughout to the current Animal Food GRAS Notice (**Table 1**).

Table 1: Naming Convention

Definition	Dossier Term
Dry final product	Natupulse® TS
Liquid final product	Natupulse® TS L
Production strain name	<i>Thermothelomyces (T.) thermophilus</i> DSM 33149
Mannanase enzymes in general	endo-β-1,4-D-mannanase, β-mannanase
Synthetic mannanase gene expressed in <i>T. thermophilus</i> DSM 33149	(b) (4) gene (an altered man1 gene from <i>Trichoderma reesei</i>)

Table 2: List of Acronyms

Acronym	Definition
AAFCO	Association of American Feed Control Officials
AGRN	Animal GRAS Notice
APO	Absence of Production Organism
BLAST	Basic Local Alignment Search Tool
bw	Body weight
CFR	Code of Federal Regulations
CFSAN	Center for Food Safety and Applied Nutrition
CFU	Colony-forming units
cGMPs	Current Good Manufacturing Practices
CONF	Confidential
CV	Coefficient of Variation
DSM	German Collection of Microorganisms and Cell Cultures
DSP	Downstream Processing
EDI	Estimated Daily Intake
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FCC	Food Chemicals Codex
FDA	Food and Drug Administration
GRAS	Generally Recognized as Safe
IUBMB	International Union of Biochemistry and Molecular Biology
MOS	Mannan Oligosaccharide
MPV	Mean Platelet Volume
NOAEL	No Observed Adverse Effect Levels
NSP	Non-Starch Polysaccharide
PBL	Peripheral Blood Lymphocytes
PO	Production Organism
ppm	parts per million
RDW	Red cell Distribution Width
SAS	Statistical Analysis System
SOP	Standard Operating Procedure
TOS	Total Organic Solids
VICH	Veterinary International Cooperation on Harmonization
WHO	World Health Organization

PART 2: IDENTITY, MANUFACTURE, SPECIFICATIONS, TECHNICAL EFFECT

A. Scientific Data that Identifies the Notified Substance

Detailed information was provided in GRAS Notice AGRN 67 – Beta-mannanase from a *Thermothelomyces thermophilus* Strain Expressing an Altered man1 Gene from *Trichoderma reesei*. Additional data were provided in a clarifying amendment “Notifier’s Response Letter AGRN 67” and its Annexes contained in the Appendix. As is noted in **Part 1.H.** of this Notice, the cited Annexes in the Appendix to the amendment incorporated by reference are confidential business information (CBI) and are exempt from FOIA provisions.

	<i>Part/Section</i>	<i>Pages</i>	<i>Appendix to Amendment</i>
<i>AGRN 67</i>	<i>Part 2.A.1</i>	<i>9-11</i>	
	<i>Part 2.A.2</i>	<i>14-15</i>	
<i>Response Letter</i>	<i>Question 1</i>	<i>1</i>	
	<i>Question 9</i>	<i>3</i>	<i>Annex Q9 - CBI</i>
	<i>Question 10</i>	<i>3-4</i>	<i>Annex Q10 - CBI</i>
	<i>Question 14</i>	<i>5</i>	<i>Annex Q14 - CBI</i>
	<i>Question 16</i>	<i>6</i>	<i>Annex Q16 - CBI</i>

B. Method of Manufacture

Detailed information was provided in GRAS Notice AGRN 67, a clarifying amendment and its Annexes. As is noted in **Part 1.H.** of this Notice, the cited Annexes in the Appendix to the amendment incorporated by reference are confidential business information (CBI) and are exempt from FOIA provisions.

	<i>Part/Section</i>	<i>Pages</i>	<i>Appendix to Amendment</i>
<i>AGRN 67</i>	<i>Part 2.B.1</i>	<i>17-24</i>	
	<i>Part 2.B.2</i>	<i>24-30</i>	
<i>Response Letter</i>	<i>Question 11</i>	<i>4</i>	<i>Annex Q11 - CBI</i>
	<i>Question 15</i>	<i>6</i>	<i>Annex Q15 - CBI</i>
	<i>Question 16</i>	<i>6</i>	<i>Annex Q16 - CBI</i>
	<i>Question 17</i>	<i>7</i>	<i>Annex Q17 - CBI</i>
	<i>Question 21</i>	<i>10</i>	

C. Composition and Specifications

Detailed information was provided in GRAS Notice AGRN 67, a clarifying amendment and its Annexes. As is noted in **Part 1.H.** of this Notice, the cited Annexes in the Appendix to the amendment incorporated by reference are confidential business information (CBI) and are exempt from FOIA provisions.

	<i>Part/Section</i>	<i>Pages</i>	<i>Appendix to Amendment</i>
AGRN 67	<i>Part 2.A.1.e</i>	<i>11</i>	
	<i>Part 2.C.1</i>	<i>30</i>	
	<i>Part 2.C.2</i>	<i>31</i>	
	<i>Part 2.C.3</i>	<i>31</i>	
	<i>Part 2.C.4</i>	<i>32</i>	
	<i>Part 2.C.5</i>	<i>33-37</i>	
Response Letter	<i>Question 1</i>	<i>1</i>	
	<i>Question 2</i>	<i>1-2</i>	
	<i>Question 3</i>	<i>2</i>	<i>Annex Q3 - CBI</i>
	<i>Question 5</i>	<i>2</i>	
	<i>Question 6</i>	<i>2</i>	
	<i>Question 7</i>	<i>2-3</i>	
	<i>Question 8</i>	<i>3</i>	<i>Annex Q8 - CBI</i>
	<i>Question 9</i>	<i>3</i>	<i>Annex Q9 - CBI</i>
	<i>Question 10</i>	<i>4</i>	<i>Annex Q10 - CBI</i>
	<i>Question 11</i>	<i>4</i>	<i>Annex Q11 - CBI</i>
	<i>Question 12</i>	<i>4</i>	
	<i>Question 13</i>	<i>5</i>	<i>Annex Q13 - CBI</i>

1. Compositions of Market Formulations

	<i>Part/Section</i>	<i>Page</i>
AGRN 67	<i>Part 2.A.1.e</i>	<i>11</i>

2. Specifications for BASF market formulations:

	<i>Part/Section</i>	<i>Page</i>
Response Letter	<i>Question 2</i>	<i>1</i>

D. Technical Effect

Detailed information was provided in GRAS Notice AGRN 67 and a clarifying amendment.

	<i>Part/Section</i>	<i>Pages</i>
<i>AGRN 67</i>	<i>Part 2.D.1</i>	40
	<i>Part 2.D.2</i>	41-42
<i>Response Letter</i>	<i>Question 18</i>	7-9
	<i>Question 19</i>	9

1. Technical effect Natupulse[®] TS/TS L in swine feed

The main ingredients of animal feeds, e.g. grains (corn, others) and seeds (soybeans, others), contain cellulose, hemicellulose and lignin as structural plant components, with xylans and β -mannans making up the bulk of these substances. While grain sources are generally low in Non-Starch-Polysaccharides (NSPs), many protein sources used in animal feed, including soybean and other seed meals, contain varying levels of soluble and insoluble β -mannans. Non-ruminant animal species, including swine, are not able to metabolize NSPs in the stomach and the small intestine due to the lack of adequate endogenous enzymes (Hedemann, M.S. and Jensen, B.B., 2004; Hsiao, H.Y. *et al.*, 2006; Jensen, M.S. *et al.*, 1998; Kiarie, E.G. *et al.*, 2021).

β -Mannans are composed of linear polysaccharides of D-mannose or D-mannose/D-glucose units (mannan and glucomannan, respectively); galactomannans also contain D-galactose monosaccharides covalently attached to the linear backbone via α -1,6-bonds. Endo- β -1,4-D-mannanases catalyze the hydrolysis 1,4- β -mannan bonds randomly in the mannan backbone, generating mannose oligosaccharides, or MOS (Jackson, M.E., 2010; Kiarie, E.G. *et al.*, 2021). These oligosaccharides can be further degraded and assimilated by the animal's digestive system and microbiome.

As formalized in 21 CFR 570.230, use levels and technical effect are required to address safety of a notified substance:

“In Part 2 of your GRAS notice, you must include: (...)

(d) When necessary to demonstrate safety, relevant data and information bearing on the physical or other technical effect the notified substance is intended to produce, including the quantity of the notified substance required to produce such effect.”

In the GRAS Final Rule (“Substances Generally Recognized as Safe” *Federal*

Register 81, (August 17, 2016): 54960) FDA/CVM stated in response to comment 144: “We agree that data and information bearing on the physical or other technical effect the notified substance is intended to produce are only necessary when they bear on safety.”

In the case of endo- β -1,4-D-mannanase, the enzyme preparation is provided to catalyze the hydrolysis of β -mannans to mannan oligosaccharides which are known to have an increased digestibility through the naturally present enzymes, microbiome, and acids in the animal’s digestive system. However, there is no intention to add β -mannanase in a diet that is not formulated to meet the swine nutritional requirements. Hence use of β -mannans will increase the nutritional value from the complete feeds, but a possible failure will not impact safety. The formulated diet contains the necessary nutrients.

PART 3: TARGET ANIMALS AND HUMAN EXPOSURE

A. Target Animal Exposure

1. Amount of the notified substance during intended use in swine

The Natupulse® TS/TS L final commercial products are formulated to contain (b) (4) TMU/g at an approximate Total Organic Solids (TOS) content of 23.6 % (or approx. 2.3 µg TOS/TMU). The mannanase 19287 enzyme preparation is recommended to deliver a dose of the product at 800 TMU/kg feed (0.8 TMU/g feed). This corresponds to (b) (4) Natupulse® TS per (b) (4). One thermostable mannanase unit (TMU) is defined as the amount of enzyme that produces reducing carbohydrates having a reducing power corresponding to one µmol mannose from locust bean gum (0.3 g / 100 ml, final concentration in the reaction solution: 0.27 g / 100 ml) in one minute under the assay conditions of 50.0 ± 0.1 °C and pH 3.5).

Swine feed is formulated at the target rate of 800 TMU/kg feed (or 0.8 TMU/g feed) by mixing (b) (4) of Natupulse TS (NP TS, (b) (4) with (b) (4) (b) (4) or 800,000 TMU/1000 kg feed). The estimated daily intake in pigs can be calculated using information published by the study animal provider (Pig Improvement Company, 2016; Pig Improvement Company, 2019). Based on these nutrition guidelines, the intake of the notified substance can be estimated for the commercial swine production process.

For wean-to-finish pigs, the average pig's bw is 144.8 kg at 25 weeks/175 days after weaning (or 28 weeks/196 days since birth); the average diet consumption is expected to be 356.6 kg over that period (Pig Improvement Company, 2019). This calculates to an EDI_{avg} of 14.1 g feed/kg bw/day ($356,600 \text{ g} \div 175 \text{ d} = 2,038 \text{ g/d}$; $2,038 \text{ g/d} \div 144.8 \text{ kg bw} = 14.1 \text{ g feed/kg bw/d}$). At 0.8 TMU/g feed, the finishing pigs have consumed 11.3 TMU/kg bw/d Natupulse® or 25.9 µg TOS/kg bw/day ($\text{EDI}_{\text{avg}} = 14.1 \text{ g/kg bw/d} \times 0.8 \text{ TMU/g} = 11.3 \text{ TMU/kg bw/d}$; $\text{EDI}_{\text{avg}} = 11.3 \text{ TMU/kg bw/d} \times 2.3 \text{ µg TOS/TMU} = 26.0 \text{ µg TOS/kg bw}$).

Similarly, reproducing sows are estimated to weigh 180 kg at breeding and will gain 9 kg net maternal weight during a 112-day gestation period. Sows are fed 2.1 kg/d gestating feed over 112 days. Therefore, during gestation sows consume 235 kg feed in 112 days and have a pre-farrowing net weight of 189 kg; this calculates to an EDI_{avg} of 11.1 g feed/kg bw/d and 8.9 TMU/kg bw/d or 20.4 µg TOS/kg bw/day from Natupulse® TS/TS L. Post-farrowing, sows weigh

an estimated 175 kg bw and lose 10 kg weight during a 21-day lactating period (Pig Improvement Company, 2016). Over the 21 lactating days the animals take in an estimated 126 kg (6 kg lactating diet/d). We therefore can calculate an EDI_{avg} of 36.4 g feed/kg bw/d (6,000 g feed/d ÷ 165 kg bw = 36.4 g/kg bw/d) and 29.1 TMU/kg bw/d or 67 µg TOS/kg bw/day from feed containing Natupulse® TS/TS L.

To summarize current commercial production processes, wean-to-finish pigs consume an EDI_{avg} of 26.0 µg TOS/kg bw/day whereas sows consume on average 87.4 µg TOS/kg bw/day ((20.4 + 67) µg TOS/kg bw/day) in each reproductive cycle.

2. Amount of other substances formed in feed because of notified substance

Mannan oligosaccharides (MOS) are generated by the activity of the notified endo-β-1,4-mannanase enzyme preparation. Please see AGRN 67 for details.

	<i>Part/Section</i>	<i>Pages</i>	
<i>AGRN 67 (broiler)</i>	<i>3.A.2</i>	<i>43-44</i>	

3. Amount of other substances present in swine feed from the notified substance

The previously established safety of the endo-β-1,4-mannanase and its production strain as well as the use of feed grade production raw materials and excipients during manufacture of the commercial products ensure that no components of the enzyme preparation raise safety concerns for the notified substance. See information provided in **Part 2.C.** on **page 11** of this notification.

4. Data for notified substance and other substances consumed by target animal

The mannanase 19287 enzyme preparation is manufactured by fermentation of the production organism *T. thermophilus* DSM 33149 and subsequent DSP steps using raw materials suitable for food and feed production. The resulting commercial products will contain very low level of products as found in a typical animal diet. Please see AGRN 67 for details.

	<i>Part/Section</i>	<i>Page</i>
<i>AGRN 67</i>	<i>Part 3.A.4.</i>	<i>44</i>

B. Human Exposure

Mannanase 19287 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. As such, its use in the target animal will not result in residues in human food related to the use of the mannanase product. Please see AGRN 67 for details.

	<i>Part/Section</i>	<i>Pages</i>
<i>AGRN 67</i>	<i>3.B</i>	<i>45-46</i>

PART 4: SELF- LIMITING LEVELS OF USE

This part is not applicable. There are no self-limiting levels of use associated with Natupulse[®] TS or Natupulse[®] TS L products 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 ON THE CONCLUSION OF GRAS STATUS

A. Introduction

Please see AGRN 67 for details.

	<i>Part/Section</i>	<i>Page</i>
AGRN 67	6.A	49

The following subsections discuss the safety aspects of the notified substance of this AGRN, the endo- β -1,4-mannanase enzyme preparation produced with *T. thermophilus* DSM 33149, for the intended use to increase the digestibility of β -mannans when used in complete and balanced swine diets, based on scientific evidence. Otherwise, they refer to relevant sections in the previous notification by BASF Corp., namely AGRN 67 (“Beta-mannanase from a *Thermothelomyces thermophilus* Strain Expressing an Altered man1 gene from *Trichoderma reesei*”), dated March 11, 2024, filed by FDA/CVM on April 18, 2024 and amended by BASF for additional clarification on September 16, 2024. FDA/CVM responded to AGRN 67 with a “No questions” letter on January 10, 2025 (FDA Center for Veterinary Medicine, 2025).

It is BASF’s determination that these results support the submitters conclusion of GRAS for the endo- β -mannanase (E.C. 3.2.1.78) enzyme preparation produced with *T. thermophilus* DSM 33149 when used as intended use to increase the digestibility of β -mannans in complete and balanced swine diets.

B. Safety of Endo- β -1,4-D-Mannanase

Please see AGRN 67 for a detailed safety narrative of endo- β -1,4-D-mannanases, including their uses in poultry feeds.

	<i>Part/Section</i>	<i>Pages</i>
<i>AGRN 67</i>	<i>2.A.1.e</i>	<i>11</i>
<i>AGRN 67</i>	<i>2.B.3.</i>	<i>24-29</i>
<i>AGRN 67</i>	<i>6.B</i>	<i>50-52</i>

The following section is focused on the use of endo- β -1,4-D-mannanases in complete and balanced swine feeds and includes several relevant references.

1. History of safe use in pig

For applications in swine diets, a β -mannanase isolated from *Bacillus subtilis* is listed in Table 30.1 of the AAFCO Official Publication to hydrolyze beta-mannans in swine diets (FDA Center for Veterinary Medicine, 2024). Also in Table 30.1, several β -mannanases from *B. lentus*, *Paenibacillus lentus*, *Aspergillus niger* or *Trichoderma longibrachiatum* (also known as *T. reesei*) are included to hydrolyze beta-mannans in poultry diets.

Numerous studies that test the inclusion of β -mannanases in swine diets have been published over the last 20+ years; these publications have been comprehensively reviewed recently (Kiarie, E.G. *et al.*, 2021). Overall, weaned piglets or growing/finishing pigs did not show significant negative impacts on growth performance or safety-related outcomes when fed diets in the presence of β -mannanase enzyme preparations (Jang, K.B. *et al.*, 2024; Kim, J.S. *et al.*, 2017; Pettey, L.A. *et al.*, 2002; Tajudeen, H. *et al.*, 2025; Yoon, S.Y. *et al.*, 2010). Similarly, sows fed gestation and/or lactation diets in the presence of β -mannanase enzyme preparations or carbohydrase mixtures containing β -mannanase enzyme activity did not demonstrate negative impacts on the reproductive performance or on the safety of the target animals (Ji, F. and Kim, S.W., 2004; Kim, J.S. *et al.*, 2018).

Currently, a β -mannanase produced by *P. lentus* has been authorized in the EU for both poultry and pigs (EU Reg 2023/2645), following the publication of the EFSA Scientific Opinion for this β -mannanase (EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) *et al.*, 2018).

Additional support for the safety of the notified substance comes from AGRN 67, BASF's endo- β -1,4-D-mannanase enzyme preparation produced with the production strain *T. thermophilus*

DSM 33149 for its intended use to increase the digestibility of β -mannans in complete and balanced poultry diets. On January 10, 2025, FDA/CVM responded with a “No questions” letter to AGRN 67 (FDA Center for Veterinary Medicine, 2025).

Furthermore, the safety of the notified substance is supported by the outcome of a tolerance study in weaned piglets using the commercial enzyme preparation Natupulse® TS at 0, 800 and 100,000 TMU/kg feed, respectively (Acosta, J.P. et al., 2025). At the end of the nursery pig feeding phase (6 weeks/42 days after weaning, or at the age of 9 weeks/63 days), the average piglets’ bw were 24.6 kg (0 TMU/kg), 24.7 kg (800 TMU/kg) or 25.3 kg (100,000 TMU/kg), respectively. Based on the reported average daily feed intake (ADFI) of 659 g/d (0), 660 g/d (800) and 653 g/d (100k), respectively, we calculate EDI_{avg} values of 26.8, 26.6 or 25.8 g feed/kg bw/day, respectively (e.g., 659 g/d ÷ 24.6 kg bw = 26.8 g feed/kg bw/d). Please see **Appendix 1, Table 4** for the results.

At the target rate of 800 TMU/kg, the study animals consumed 21.3 TMU/kg bw/d or 49 μ g TOS/kg bw/d by the end of the study (EDI_{avg} = 26.6 g/kg bw/d x 0.8 TMU/g = 21.3 TMU/kg bw/d; EDI_{avg} = 21.3 TMU/kg bw/d x 2.3 μ g TOS/TMU = 49 μ g TOS/kg bw). Weaned piglets fed the tolerance diet (100,000 TMU/kg feed) consumed 2,580 TMU/kg bw/d or 5.9 mg TOS/kg bw/day during the tolerance study.

No negative impact on growth performance or safety outcomes was observed in the piglet study (Acosta, J.P. et al., 2025). The absence of negative impacts on growth performance or target animal safety is corroborated by an equivalent tolerance study in broiler (Fickler, A. et al., 2024). Furthermore, please note that Wistar rats consumed 1100 – 1300 mg TOS/kg bw/d at the highest dose during the 90-day subchronic toxicity feeding study; the study animals did not show significant adverse effects (Kern, A. et al., 2020).

While there are significant differences in the digestive systems of diverse target animals (e.g., rats, pigs and poultry), these comparisons still supports our finding that there are no safety concerns related to feeding the mannanase 19287 enzyme preparation produced with *T. thermophilus* DSM 33149 to animals produced for human consumption at rates of 800 and 100,000 TMU/kg feed, respectively.

It is the submitters evaluation that endo- β -1,4-D-mannanases have been used safely in pig diets for decades and support the safety of these enzymes, as cited above and reviewed recently (Kiarie, E.G. et al., 2021). The notified substance of this AGRN, the mannanase 19287 enzyme

preparation produced by *T. thermophilus* DSM 33149, is an endo- β -1,4-D-mannanase (E.C. 3.2.1.78). The final products Natupulse® TS and TS L are formulated with safe and suitable ingredients to contain mannanase 19287 enzyme activities (b) (4) product (please see **Part 2.A.1.e, page 11 in AGRN 67** and **Part 2.C. 2., page 12** in this notification).

The intended use for mannanase 19287 (endo- β -mannanase) is to increase the digestibility of β -mannans when used in complete and balanced swine diets. A nutritionally adequate diet, even without addition of the proposed enzyme will provide the required nutrients for the animals to grow, sustain, and elicit production performance.

There is no intention or recommendation to add the β -mannanase enzyme preparation produced with *T. thermophilus* DSM 33149 in a diet that is not formulated to meet the swine nutritional requirements nor in a diet that is intentionally formulated with lower nutrient content. Thus, β -mannanase enzyme preparations are added to an otherwise nutritionally balanced diet, which may further increase the nutritional value of that complete feed. It should also be noted that the industry practice does not include formulating diets lower in nutrients, especially energy content, when mannanase or other non-starch polysaccharide hydrolyzing enzymes are included in the feed formulation. Therefore, with or without the addition of the proposed β -mannanase enzyme, or even if the added β -mannanase enzyme does not have functionality, the nutrient availabilities from a complete and balanced diet, formulated to meet the nutrient requirements of the target animal, are adequate to meet the nutrient requirements for maintenance, production, and wellbeing of the target animal, in this case swine. Therefore, it should be noted that lack of functionality of the notified substance does not impact the safety of the target animal. Thus, the notifier does not include empirical data to support enzyme functionality in this GRAS notice.

In summary, these results support our determination that the endo- β -mannanase enzyme preparation, commercialized as Natupulse® TS and TS L, is GRAS for its intended use to increase the digestibility of β -mannans in complete and balanced swine diets.

C. Safety of the Production Organism *Thermothelomyces thermophilus*

Please see AGRN 67, the clarifying amendment and its Appendix for details. As is noted in **Part 1.H.** of this Notice, the cited Annexes in the Appendix to the clarifying amendment to AGRN 67, incorporated by reference, are confidential business information (CBI) and are exempt from FOIA provisions.

	<i>Part/Section</i>	<i>Pages</i>	<i>Appendix to Amendment</i>
<i>AGRN 67</i>	<i>2.B.2.c</i>	<i>21</i>	
<i>AGRN 67</i>	<i>Part 6.C.</i>	<i>53-64</i>	
<i>Response Letter</i>	<i>Question 11</i>	<i>4</i>	<i>Annex Q11 - CBI</i>
	<i>Question 15</i>	<i>6</i>	<i>Annex Q15 - CBI</i>
	<i>Question 16</i>	<i>6</i>	<i>Annex Q16 - CBI</i>
	<i>Question 17</i>	<i>7</i>	<i>Annex Q17 - CBI</i>
	<i>Question 20</i>	<i>9</i>	
	<i>Question 21</i>	<i>10</i>	

To summarize the safety evaluation in AGRN 67, the wild-type C1 strain of *T. thermophilus* was isolated and developed into a family of host and production strains using mutational and recombinant methods. Enzyme preparations generated by descendants of *T. thermophilus* C1 have been used for decades in industrial, food and feed applications and have undergone Regulatory evaluations in different jurisdictions.

Mycotoxins of regulatory importance are tested for according to the commercial product specifications. The β -mannanase production strain *T. thermophilus* DSM 33149 has been developed from a derivate *T. thermophilus* (b) (5) strain. All genetic modifications in the development of *T. thermophilus* DSM 33149 were analyzed for the unintended generation of protein sequences of fungal toxins and virulence factors. No novel proteins with high homologies to known fungal proteins of concern were identified (EFSA (European Food Safety Authority), 2024).

Six fungal toxins with very low homologies were identified in the genome of the production organism as well as the fully sequenced standard strain *T. thermophilus* ATCC42464 (Berka, R.M. *et al.*, 2011). These data demonstrate that the use of recombinant technologies in the development of *T. thermophilus* DSM 33149 did not result in the unintended production of proteins that negatively impact target animal safety during the fermentation of the organism. We have concluded that the likelihood of the unintended production of proteins detrimental to the safety of the target animal species is considered to be very low. Even if unintended proteins without regulatory importance were synthesized, they would be digested in the animals digestive system.

This evaluation also applies to secondary metabolites (tenuazonic acid (TeA) and myceliothermophins A-F) that *T. thermophilus* DSM 33149 as well as standard strain *T. thermophilus* ATCC42464 have the potential to produce (Yang, Y.L. *et al.*, 2007; Yun, C. *et al.*, 2017). While TeA has demonstrated adverse effects in broiler (Giambrone, J.J. *et al.*, 1978), the toxicities were observed at much higher concentrations than what was found in two Mannanase CC concentrates and three endproduct batches. Taken together with the use rate of the final products for animal diets (b) (4) we consider the risk for target animal safety to be minimal.

Lastly, the production strain was analyzed for the presence of new and unintended open reading frames (ORFs) for potential novel proteins generated during the development of *T. thermophilus* DSM 33149 when compared to the genome of the standard strain *T. thermophilus* ATCC 42464. No significant homologies to these proteins were identified at the integration sites.

Our safety evaluation of *T. thermophilus* DSM 33149 is further supported by the outcome of two tolerance studies in target animals. The consumption of the mannanase 19287 enzyme preparation at 100,000 TMU/kg feed in complete and balanced poultry and pig diets demonstrated the absence of negative impacts on production parameters or triggered any adverse events (Acosta, J.P. *et al.*, 2025; Fickler, A. *et al.*, 2024), respectively.

In summary, these results support our determination that the mannanase 19287 enzyme preparation, produced with the production organism *T. thermophilus* DSM 33149, is GRAS for its intended use to increase the digestibility of β -mannans in complete and balanced swine diets.

D. Safety of the Donor Organism

Please see **AGRN 67** for the safety evaluation.

	<i>Part/Section</i>	<i>Page(s)</i>
<i>AGRN 67</i>	<i>Part 6.D</i>	64

The synthetic (b) (4) sequence used for the expression of mannanase 19287 is a novel recombinant variant of the native *T. reesei* man1 gene (GenBank ID L25310). No genetic material was isolated from *T. reesei* for the generation of the expression cassette.

(b) (4)

E. Safety of the Manufacturing Process

Please see **AGRN 67** for further details.

	<i>Part/Section</i>	<i>Page(s)</i>
<i>AGRN 67</i>	<i>Part 2.A.1.e</i>	11
	<i>Part 6.E</i>	64-66
<i>Response Letter</i>	<i>Question 2</i>	1

The raw materials are of a purity and quality suitable for their intended use. In addition, the results of a 42-day piglet tolerance study (Acosta, J.P. *et al.*, 2025) support the safety of process ingredients; please see Part 6.F below. A similar conclusion was identified in AGRN 67, based on the published tolerance study in broiler (Fickler, A. *et al.*, 2024).

F. Safety Studies

Please see **AGRN 67** for further details.

	Part/Section	Pages
AGRN 67	<i>Part 6.F.</i>	66-76
Response Letter	<i>Question 20</i>	9
	<i>Question 21</i>	10

We have determined that the scientific literature clearly demonstrates that the intended use of endo- β -mannanases (E.C. 3.2.1.78) to increase the digestibility of β -mannans in complete and balanced animal diets has no bearing on target animal safety; therefore, no utility data or information are required for the determination of safety of the endo- β -1,4-D-mannanase enzyme preparation produced by *T. thermophilus* DSM 33149 (please see **Part 2.D.1., pages 12-13** of this notification for further discussion).

1. Target animal study

The safety of the endo- β -1,4-D-mannanase enzyme preparation produced by *T. thermophilus* strain DSM 33149 in swine diets can be demonstrated by comparing a control diet without enzyme supplementation with diets at the target or tolerance doses (containing a significant excess of the notified substance). The tolerance of weaned piglets to the mannanase 19287 preparation was tested using the marketed Natupulse® TS at 100,000 TMU/kg feed and compared to the target rate of 800 TMU/kg feed and a control diet in the absence of the notified substance (Acosta, J.P. et al., 2025).

Weaned piglets were fed for 42 days following weaning with standard corn-soybean-based diets in two phases (please see **Appendix 1, Tables 1 and 2**). All diets were formulated on a digestible amino acid basis to meet or exceed all nutrient estimates (National Research Council (U.S.) and Subcommittee on Swine Nutrition, 2012). Phase I diets were provided from day 1 to day 21 after weaning; these diets contained dried blood plasma and whey proteins in addition to the soybean meal (SBM) protein source. Phase II diets contained only SBM, no animal proteins were present and were provided for the remainder of the study (days 22-42).

A total of 150 newly weaned pigs (75 castrated male and 75 female pigs; initial body weight: 6.20 ± 0.68 kg; weaned on day 21) were allotted to pens with 5 pigs per pen for a total of 10 replicate pens per treatment. Animals were allotted to three experimental diets, i.e., control, control plus 800 thermostable mannanase units (TMU)/kg, or control plus 100,000 TMU/kg (please see **Appendix 1, Table 3**. Pigs had unlimited access to Phase I diets from d 1 to 21 and to Phase II diets from d

22 to 42 post-weaning.

Blood samples from two pigs per pen (one male and one female pig, assigned at the start of the study) were collected on days 1, 21, and 42 and analyzed for serum chemistry and hematology. One pig per pen from the control treatment (n = 10) and two pigs per pen from each of the β -mannanase treatments (n = 20, respectively), were euthanized at the end of the experiment on d 42. Organs were weighed and examined for gross pathology. Data were analyzed using the MIXED procedure of SAS with pen as the experimental unit. Differences were considered as significant ($P < 0.05$) or as trending ($P < 0.10$).

There were no negative impacts on growth performance in the study (**Appendix 1, Table 4**). The absence of significant differences in mortality rates (**Appendix 1, Table 4**), serum chemistry (**Appendix 1, Table 4**), hematology (**Appendix 1, Table 6**), or organ weights (**Appendix 1, Table 7**) and the lack of pathological observations between the three groups demonstrated the safety of the notified substance in swine. Observed significant differences in serum phosphorus, blood red cell distribution width (RDW), and mean platelet volume (MPV), respectively, were considered within the expected biological variability for the parameter (please see **Appendices 2 and 3**), as described (Cooper, C.A. et al., 2014; (b) (4)) and were not affected by inclusion of up to 100,000 TMU/kg feed by the notified β -mannanase enzyme preparation in the diet.

The lack of negative impacts on growth performance and the absence of significant differences in safety-related outcomes support our determination that the mannanase 19287 enzyme preparations (marketed as Natupulse® TS and Natupulse® TS L) are GRAS, when used as intended to increase the digestibility of β -mannans in complete and balanced swine diets.

In summary, the results of the published swine tolerance study (Acosta, J.P. et al., 2025) support our conclusion that the mannanase 19287 enzyme preparation, the notified substance of this dossier, is GRAS for its intended use to increase the digestibility of β -mannans when used in complete and balanced swine diets.

G. Results and Conclusion

BASF Corporation has reviewed all available data and information and is not aware of any data and information that are, or may appear to be, inconsistent with our conclusion of GRAS status for the mannanase 19287 enzyme preparation by *T. thermophilus* DSM 33149 and its intended use to increase the digestibility of β -mannans when used in complete and balanced swine diets.

1. Safety evaluation regarding target animal consumption of the notified substance

The data included and discussed in this Animal GRAS Notification and BASF's previous notification AGRN67 (regarding the notified substance in poultry feeds) support the conclusion by BASF Corporation that the mannanase 19287 enzyme preparation in Natupulse® TS and Natupulse® TS L products is GRAS for its intended use to increase the digestibility of β -mannans when used in complete and balanced swine diets.

The notified substance mannanase 19287 is an enzyme preparation and enzymes are generally considered to be non-toxic (Olempska-Beer, Z.S. et al., 2006). Therefore, the safety evaluation of enzyme preparations for animal diets should focus on the safety of the production organism. The mannanase 19287 enzyme, like all endo- β -mannanase enzymes, catalyzes the hydrolysis of β -mannan components of hemicellulose (FDA Center for Veterinary Medicine, 2024). Therefore, the mannanase 19287 enzyme will liberate mannan oligomers from the hemicellulose polymer constituents. The resulting β -(1,4)-mannan oligosaccharides can be hydrolyzed further by endogenous glycosidases (van Zyl, W.H. et al., 2010). The oligo- and monosaccharides generated by these enzymatic activities will be metabolized as nutrients. The processing aids used in the manufacture of the mannanase 19287 enzyme preparation are suitable for their intended use and are not expected to affect target animal safety.

The safety of the mannanase 19287 enzyme preparation and its production organism *T. thermophilus* DSM 33149 is supported by the outcome of its evaluation according to the well-established decision tree for feed enzymes (Pariza, M.W. and Cook, M., 2010). Using this evaluation approach, the notified substance (the mannanase 19287 enzyme preparation) is not considered toxic (see **AGRN 67 Part 6.C.4, pages 60-63** and **Table 10**, therein).

The genome of the *T. thermophilus* DSM 33149 production organism as well as the published genome sequence of the standard *T. thermophilus* ATCC 42464 strain were probed against known fungal toxin and virulence factors identified in the UniProt database

(<https://www.uniprot.org/>) using the BLAST P algorithm. The custom database contained 1265 protein sequences (as of 2024). Using a standard definition of homology to genes of concern by EFSA, no homology to such genes were found (EFSA (European Food Safety Authority), 2024). Both genomes contained homologous protein sequences with low identity scores to six toxins in the custom database. Since the identical toxin homologs were found in both the production strain and the previously sequenced standard strain of *T. thermophilus* (ATCC 42464), we determined that the recombinant methods used during the DSM 33149 development did not generate the observed low identity homologies.

This evaluation also applies to secondary metabolites tenuazonic acid (TeA) and myceliothermophins that may be produced by *T. thermophilus* (please see our response to FDA's **comment 20, page 9** in the **clarifying amendment**). We have evaluated the safety of the enzyme preparations regarding the highly unlikely presence of toxins and secondary metabolites in combination with the use rate of the commercial products for animal diets (b) (4). We have determined that the risk for target animal safety is minimal.

Hence it is very unlikely that the identified toxins and mycotoxin proteins are of concern at the above mentioned level in target animal feed. Even at a 125-fold excess of the recommended use rate of 800 TMU/kg feed, the enzyme preparation did not adversely impact the production parameters or trigger any adverse events (Acosta, J.P. et al., 2025). Therefore, the safety of the notified substance is established and any potential toxins in the notified enzyme substance, even if present, is not a concern.

As noted in **Part 2.B.2.c, page 21 of AGRN 67**, as well as our response to FDA's comment # 21, **page 10 of BASF's clarifying amendment**, the production strain was analyzed for the presence of new and unintended open reading frames (ORFs) for potential novel proteins generated during the development of *T. thermophilus* DSM 33149 when compared to the genome of the standard strain *T. thermophilus* ATCC 42464. These modifications included the (b) (4)

(b) (4)

(b) (4); (b) (5)

No significant homologies to these proteins were identified at the integration sites.

Even if other potential proteins without toxicological concerns were present in the endo-

1,4- β -D-mannanase preparation produced by *T. thermophilus* DSM 33149 (Natupulse® TS/TS L), they would be digested like other feed proteins. Based on the results of peer-reviewed published tolerance studies, animals fed at a 125-fold excess rate of the recommended enzyme preparation inclusion did not show negative impacts on growth performance or significant safety-related adverse effects in the target animals (Acosta, J.P. *et al.*, 2025; Fickler, A. *et al.*, 2024).

The β -mannanase enzyme preparation produced with *T. thermophilus* DSM 33149 is added to an otherwise nutritionally balanced diet and may further increase the nutritional value of that complete feed. Therefore, with or without the addition of the proposed β -mannanase enzyme, or even if the added β -mannanase enzyme does not have functionality, the nutrient availabilities from a complete and balanced diet, formulated to meet the nutrient requirements of pigs, are adequate to meet the nutrient requirements for maintenance, production, and wellbeing of the target animal. Therefore, it should be noted that lack of functionality of the notified substance would not impact the safety of the target animal.

Based on all the available information in this GRAS Notification and in AGRN 67, BASF Corp. concludes that the mannanase 19287 enzyme preparation produced by *T. thermophilus* DSM 33149 is safe for its intended use to increase the digestibility of β -mannans in complete and balanced swine diets.

2. Safety assessment regarding human consumption of target animal products

Enzyme preparations are used in small quantities during feed production, and it is expected that the target animals will digest any residual salts, carbohydrates and proteins to use their constituents as nutrients during metabolism. Mannanase 19287 is a protein and will be digested into amino acids in the animal's digestive tract. The Enzyme Marketing Coordination document of the AAFCO OP confirms in its Safety section that enzymes as proteins are expected to be digested by the target animals (FDA Center for Veterinary Medicine, 2024). Enzymes evaluated by the Pariza safety assessment process updated for microbial enzyme preparations in animal feed (Pariza, M.W. and Cook, M., 2010) should raise no human safety concerns.

In summary, based on all the available information in this GRAS Notification, BASF Corporation concludes, through scientific procedures, that the mannanase 19287 enzyme preparation produced by *T. thermophilus* DSM 33149 is GRAS for its intended use to increase the digestibility of β -mannans in complete and balanced swine diets and is safe for humans consuming edible products derived from these animals.

PART 7: LIST OF SUPPORTING DATA AND INFORMATION IN GRAS NOTICE

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Appendices

Appendix 1. Data Tables in Acosta et al 2025

Appendix 2. Hematologic Clinical Reference Intervals

Appendix 3. Iowa State Veterinary Pathology_Reference Intervals

Appendix 4. PIC® 2016 Tables P3 and P4_Gestating and Lactating Diets

Appendix 5. PIC® 2019 Wean to Finish_Reference of Growth and Feed Intake Curve

Appendix 1:
Data Tables Acosta et al.,
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9, txaf061 (2025).

Acosta et al., 2025 Data Tables

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Table 1. Nutrient composition of ingredients, as-is basis

Item	Corn	Soybean meal	Whey powder	Protein plasma
Gross energy, kcal/kg	3,921	4,289	3,637	4,947
Dry matter, %	87.09	89.26	90.74	91.97
Ash, %	1.26	6.06	7.96	6.91
Crude protein, %	6.48	44.45	10.08	80.59
Acid hydrolyzed ether extract, %	3.85	2.26	0.84	0.21
Total dietary fiber, %	9.00	17.35	-	-
Insoluble dietary fiber, %	8.60	15.75	-	-
Soluble dietary fiber, %	0.40	1.60	-	-
Mannose, %	-	0.28	-	-

Table 2. Ingredient composition of experimental diets

Feedstuff, %	Phase 1			Phase 2		
	Control	Control + 800 TMU/kg β -Mannanase ¹	Control + 100,000 TMU/kg β -Mannanase	Control	Control + 800 TMU/kg β -Mannanase	Control + 100,000 TMU/kg β -Mannanase
Ground corn	41.90	41.892	40.65	62.63	62.622	61.38
Soybean meal, dehulled	28.00	28.00	28.00	32.00	32.00	32.00
Protein plasma, spray dried	2.00	2.00	2.00	-	-	-
Choice white grease	1.80	1.80	2.05	2.00	2.00	2.25
Whey powder	23.00	23.00	23.00	-	-	-
L-Lys HCl	0.35	0.35	0.35	0.35	0.35	0.35
DL-Met	0.17	0.17	0.17	0.12	0.12	0.12
L-Thr	0.08	0.08	0.08	0.10	0.10	0.10
Dicalcium phosphate	0.95	0.95	0.95	1.15	1.15	1.15
Limestone	0.85	0.85	0.85	0.75	0.75	0.75
Vitamin-mineral premix ²	0.50	0.50	0.50	0.50	0.50	0.50
Salt	0.40	0.40	0.40	0.40	0.40	0.40
Natupulse TS ³	0.00	0.008	1.00	0.00	0.008	1.00
Total	100.00	100.00	100.00	100.00	100.00	100.00

¹ β -Mannanase = Natupulse® TS; BASF SE, Ludwigshafen, Germany. The β -mannanase unit TMU is defined as the amount of enzyme that produces reducing carbohydrates having a reducing power corresponding to one μ mol mannose from locust bean gum (0.3 g/100 ml buffer solution) in one minute under the assay conditions of 50.0 $\pm$ 0.1 °C and pH 3.5.

²Provided the following quantities of vitamins and micro-minerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,136 IU; vitamin D₃ as cholecalciferol, 2,208 IU; vitamin E as DL-alpha tocopheryl acetate, 66 IU; vitamin K as menadione dimethylprimidinol bisulfite, 1.42 mg; thiamin as thiamine mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride, 0.24 mg; vitamin B₁₂, 0.03 mg; D-pantothenic acid as D-calcium pantothenate, 23.5 mg; niacin, 44.1 mg; folic acid, 1.59 mg; biotin, 0.44 mg; Cu, 20 mg as copper chloride; Fe, 126 mg as ferrous sulfate; I, 1.26 mg as ethylenediamine dihydriodide; Mn, 60.2 mg as manganese hydroxychloride; Se, 0.3 mg as sodium selenite and selenium yeast; and Zn, 125.1 mg as zinc hydroxychloride.

³The 0, 0.008, and 1.0 inclusion levels are equivalent to 0, 100, and 12,500 mg of β -mannanase per kg of complete diet, respectively.

Table 3. Nutrient composition of experimental diets, as-is basis

Item	Phase 1			Phase 2		
	Control	Control + 800 TMU/kg β -Mannanase ¹	Control + 100,000 TMU/kg β -Mannanase	Control	Control + 800 TMU/kg β -Mannanase	Control + 100,000 TMU/kg β -Mannanase
Gross energy, kcal/kg	3,952	3,986	3,999	4,017	4,029	4,045
Dry matter, %	89.01	89.03	89.07	90.74	90.63	90.76
Ash, %	6.02	6.02	5.48	5.09	5.30	4.93
Crude protein, %	18.39	18.59	18.37	18.70	19.14	19.19
Acid hydrolyzed ether extract, %	4.34	4.00	4.68	5.40	5.45	5.32
Total dietary fiber, %	10.05	9.70	10.00	13.45	13.80	13.80
Insoluble dietary fiber, %	8.50	8.40	8.60	12.50	12.50	12.50
Soluble dietary fiber, %	1.55	1.30	1.40	0.95	1.30	1.30
β -mannanase activity, TMU/kg	< 100	961	116,926	< 100	842	115,013
Indispensable AA, %						
Arg	1.18	1.19	1.17	1.27	1.30	1.32
His	0.51	0.51	0.50	0.51	0.53	0.54
Ile	0.95	0.95	0.92	0.87	0.90	0.92
Leu	1.78	1.79	1.75	1.71	1.73	1.73
Lys	1.48	1.49	1.46	1.38	1.39	1.39
Met	0.45	0.43	0.43	0.38	0.39	0.37
Phe	0.98	0.99	0.96	1.00	1.02	1.05
Thr	0.91	0.92	0.95	0.82	0.83	0.82
Trp	0.27	0.28	0.27	0.23	0.23	0.23
Val	1.05	1.05	1.03	0.98	0.99	1.01
Dispensable AA, %						
Ala	1.05	0.99	0.97	0.99	0.99	1.01
Asp	2.24	2.07	1.97	1.99	1.95	2.04
Cys	0.38	0.37	0.32	0.31	0.30	0.31
Glu	3.80	3.57	3.43	3.55	3.54	3.64
Gly	0.82	0.76	0.74	0.81	0.80	0.83
Pro	1.18	1.11	1.10	1.13	1.13	1.13

Ser	0.92	0.88	0.86	0.85	0.85	0.87
Tyr	0.68	0.65	0.61	0.64	0.61	0.65

¹β-Mannanase = Natupulse® TS; BASF SE, Ludwigshafen, Germany. The β-mannanase unit TMU is defined as the amount of enzyme that produces reducing carbohydrates having a reducing power corresponding to one μmol mannose from locust bean gum (0.3 g/100 ml buffer solution) in one minute under the assay conditions of 50.0 +/-0.1 °C and pH 3.5.

Table 4. Growth performance for pigs fed experimental diets¹

Item	Control	Control + 800 TMU/kg β -Mannanase ²	Control + 100,000 TMU/kg β -Mannanase	SEM	<i>P</i> -value
Phase 1 (d 1 to 21)					
Initial body weight, kg	6.19	6.21	6.19	0.22	0.197
ADG ³ , g	200.94	214.10	220.5	9.53	0.349
ADFI ³ , g	290.04	305.58	299.73	12.86	0.641
G:F ³	0.70	0.70	0.74	0.02	0.197
Body weight on d 21, kg	10.41	10.71	10.82	0.33	0.346
Phase 2 (d 22 to 42)					
ADG, g	676.94	663.77	687.61	16.03	0.405
ADFI, g	1,026.17	1,014.28	1,007.11	27.31	0.816
G:F	0.66 ^b	0.66 ^b	0.68 ^a	0.01	0.039
Final body weight, kg	24.63	24.65	25.26	0.62	0.427
Overall Phase (d 1 to 42)					
ADG, g	438.94	438.93	454.05	10.97	0.422
ADFI, g	658.11	659.93	653.42	18.73	0.952
G:F	0.67 ^b	0.67 ^b	0.70 ^a	0.01	0.015

^{a-b}Values within a row lacking a common superscript letter are different ($P < 0.05$).

¹Data are least square means of 10 observations for all treatments.

² β -Mannanase = Natupulse® TS; BASF SE, Ludwigshafen, Germany. The β -mannanase unit TMU is defined as the amount of enzyme that produces reducing carbohydrates having a reducing power corresponding to one μ mol mannose from locust bean gum (0.3 g/100 ml buffer solution) in one minute under the assay conditions of 50.0 $\pm$ 0.1 °C and pH 3.5.

³ADG = average daily gain; ADFI= average daily feed intake; G:F = gain-to-feed ratio.

Table 5. Serum chemistry markers of pigs fed experimental diets¹

Item	Reference value ²	Control	Control + 800 TMU/kg β -Mannanase ³	Control + 100,000 TMU/kg β -Mannanase	SEM	P-value
Sodium, mEq/L	135 – 150	136.37	135.93	135.97	0.28	0.386
Potassium, mEq/L	4 – 7	5.06	4.96	5.11	0.11	0.362
Chloride, mEq/L	95 – 110	100.97	100.53	100.75	0.37	0.627
Bicarbonate, mEq/L	19 - 31	26.72	27.15	26.83	0.48	0.791
Calcium, mg/dl	8 – 12	10.98	10.82	10.81	0.06	0.103
Phosphorus, mg/dl	4.5 – 11.5	10.40 ^b	10.42 ^b	11.10 ^a	0.18	0.017
Magnesium, mg/dl	1.82 – 3.65	2.06	2.04	2.10	0.03	0.133
BUN ⁴ , mg/dl	6 – 30	4.80	4.70	4.97	0.28	0.768
Creatinine, mg/dl	0.5 – 2.7	0.91	0.92	0.94	0.02	0.651
Glucose, mg/dl	65 – 150	119.83	116.32	117.27	1.55	0.367
Total protein, gm/dl	7.0 – 8.9	4.92	4.88	4.91	0.05	0.866
Albumin, gm/dl	3.0 – 4.5	3.22	3.15	3.16	0.04	0.352
AST ⁴ , IU/L	10 – 300	46.95	49.80	47.02	3.16	0.766
Creatine kinase, IU/L	100 – 2500	898.47	1,029.55	1,018.38	161.56	0.695
ALKP ⁴ , IU/L	25 – 130	570.48	499.87	555.98	31.44	0.352
GGT ⁴ , IU/L	10 – 100	41.62	37.08	34.18	1.57	0.110
Total bilirubin, mg/dl	0 – 1	0.42	0.41	0.42	0.03	0.992
Anion gap	14 - 29	13.76	13.13	13.68	0.47	0.408

^{a-b}Values within a row lacking a common superscript letter are different ($P < 0.05$).

¹Data are least square means of 10 observations for all treatments.

²Reference Intervals were reported by Iowa State University's Clinical Pathology Laboratory (2011) and Cooper et al. (2014).

³ β -Mannanase = Natupulse® TS; BASF SE, Ludwigshafen, Germany. The β -mannanase unit TMU is defined as the amount of enzyme that produces reducing carbohydrates having a reducing power corresponding to one μ mol mannose from locust bean gum (0.3 g/100 ml buffer solution) in one minute under the assay conditions of 50.0 $\pm$ 0.1 °C and pH 3.5.

⁴ BUN = blood urea nitrogen; AST = aspartate transaminase; ALKP = alkaline phosphatase; GGT = gamma-glutamyl transferase.

Table 6. Hematological characters of pigs fed experimental diets¹

Item	Reference value ²	Control	Control + 800 TMU/kg β -Mannanase ³	Control + 100,000 TMU/kg β -Mannanase	SEM	P-value
WBC ⁴ , $\times 10^3/\text{ul}$	11.35 – 28.9	15.05	16.50	17.04	0.65	0.105
RBC ⁴ , $\times 10^6/\text{ul}$	5.88 – 8.19	6.17	6.19	6.22	0.08	0.929
Hemoglobin, g/dl	11.2 – 14.7	11.13	11.50	11.43	0.15	0.181
Hematocrit, %	32.3 – 42.6	37.14	38.11	38.02	0.45	0.251
MCV ⁴ , fl	47.5 – 59.2	60.26	61.56	61.36	0.74	0.383
MCH ⁴ , pg	16.3 – 20.6	18.06	18.58	18.44	0.26	0.221
MCHC ⁴ , g/dl	33.3 – 35.8	29.96	30.18	30.08	0.12	0.389
RDW ⁴ , %	16.4 – 32.3	20.85 ^a	19.15 ^b	19.47 ^{ab}	0.47	0.031
Platelet, $\times 10^3/\text{ul}$	118.9 – 522.9	388.88	402.46	411.08	22.50	0.780
MPV ⁴ , fl	6.8 – 10.8	10.61 ^a	9.67 ^b	9.94 ^{ab}	0.23	0.028
Neutrophil, $\times 10^3/\text{ul}$	2.0 – 10.4	6.92	8.02	8.04	0.59	0.315
Lymphocyte, $\times 10^3/\text{ul}$	5.30 – 17.9	7.07	7.39	7.75	0.27	0.169
Monocyte, $\times 10^3/\text{ul}$	0.0 – 3.7	0.55	0.55	0.62	0.03	0.170
Eosinophil, $\times 10^3/\text{ul}$	0.0 – 1.3	0.31	0.33	0.36	0.04	0.574
Basophils, $\times 10^3/\text{ul}$	0.0 – 0.4	0.05	0.06	0.06	0.01	0.625
Absolute LUC ⁴ , $\times 10^3/\text{ul}$	-	0.13	0.16	0.16	0.01	0.269

^{a-b}Values within a row lacking a common superscript letter are different ($P < 0.05$).

¹Data are least square means of at least 8 observations for all treatments.

²Reference Intervals were reported by Iowa State University's Clinical Pathology Laboratory (2011) and Cooper et al. (2014).

³ β -Mannanase = Natupulse® TS; BASF SE, Ludwigshafen, Germany. The β -mannanase unit TMU is defined as the amount of enzyme that produces reducing carbohydrates having a reducing power corresponding to one μmol mannose from locust bean gum (0.3 g/100 ml buffer solution) in one minute under the assay conditions of 50.0 $\pm$ 0.1 °C and pH 3.5.

⁴WBC = total white blood cells count; RBC = red blood cell count; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; RDW = red cell distribution width; MPV = mean platelet volume; LUC = absolute large unstained cells.

Table 7. Weights of organs of pigs at necropsy on d 42 post-weaning^{1, 2}

Item	Control	Control + 800 TMU/kg β -Mannanase ³	Control + 100,000 TMU/kg β -Mannanase	SEM	P-value
Liver, g	895	865	875	35	0.794
Kidney, g	148	150	143	6	0.445
Spleen, g	58	54	54	3	0.658
Heart, g	153	155	147	6	0.297
Stomach, g	705	673	683	41	0.869
Small intestine, g	1,725	1,650	1,695	53	0.555

¹Data are least squares means for each dependent variable represent 10 observations for the control treatment and 20 observations for the control + 800 TMU/kg and control + 100,000 TMU/kg treatments.

²All organs were inspected for pathological changes, but no abnormalities were detected.

³ β -Mannanase = Natupulse® TS; BASF SE, Ludwigshafen, Germany. The β -mannanase unit TMU is defined as the amount of enzyme that produces reducing carbohydrates having a reducing power corresponding to one μ mol mannose from locust bean gum (0.3 g/100 ml buffer solution) in one minute under the assay conditions of 50.0 $\pm$ 0.1 °C and pH 3.5.

Appendix 2:
Data Table Cooper et al.,
Journal of Animal Science and
Biotechnology 2014, 5:5.

Table 2 Hematologic and clinical biochemical reference intervals for 6-week-old Hampshire-Yorkshire pigs between 10–20 kg

Analyte	Unit	N	Mean	Median	Min	Max	Reference interval	Data distribution
Hematology analytes								
RBC	m/uL	66	7.31	7.33	5.84	9.04	5.52 - 9.11	Non-Gaussian
HGB	gm/dL	65	10.7	10.8	8.6	12.8	8.8 - 12.7	Gaussian
HTC	%	66	35.5	35.7	25.4	43.8	28.3 - 42.7	Gaussian
MCV	fL	66	48.9	49.8	37.7	59.1	38.4 - 59.3	Gaussian
MCH	pgm	66	14.8	15.3	11.1	18.3	11.1 - 18.4	Non-Gaussian
MCHC	gm/dL	66	30.2	30.3	27.3	32.4	27.9 - 32.4	Gaussian
RDW	%	54	24.4	24.5	16.1	33.3	16.4 - 32.3	Gaussian
WBC	/μL	66	15,315	14,735	5,650	26,470	5,443 - 25,186	Gaussian
Neutrophils	/μL	66	5,668	5,266.5	1,192	15,745	810 - 13,397	Gaussian with square root transformation
Lymphocytes	/μL	66	8,677	8,239	4,045	15,856	3,810 - 14,919	Gaussian with square root transformation
Monocytes	/μL	66	692	619	237	1,460	219 - 1,705	Gaussian with logarithmic transformation
Eosinophils	/μL	64	219	201.5	58	574	45 - 481	Gaussian with square root transformation
Basophils	/μL	64	53	43	11	151	14 - 146	Gaussian with logarithmic transformation
Platelets	/μL	66	540,773	545,500	138,000	909,000	208,588 - 872,957	Gaussian
Biochemical analytes								
Anion gap	mmol/L	63	21	20	13	31	14 - 29	Gaussian with logarithmic transformation
Sodium	mmol/L	63	141	140	125	159	131 - 151	Non-Gaussian
Potassium	mmol/L	62	4.9	4.8	3.7	6.3	3.7 - 6.1	Gaussian with square root transformation
Chloride	mmol/L	63	100	100	90	112	93 - 108	Non-Gaussian
Bicarbonate	mmol/L	63	25	25	17	31	19 - 31	Gaussian
Phosphorus	mg/dL	63	8.9	8.8	6.1	12.3	6.3 - 11.5	Gaussian
Calcium	mg/dL	63	11.2	11.2	9.7	12.8	9.9 - 12.5	Gaussian
BUN	mg/dL	63	10	10	4	21	4 - 18	Gaussian with square root transformation
Creatinine	mg/dL	63	0.8	0.8	0.5	1.1	0.5 - 1.1	Non-Gaussian
Glucose	mg/dL	61	106	105	70	139	75 - 136	Gaussian
Total protein	g/dL	63	4.9	4.9	4.1	5.9	4.0 - 5.8	Gaussian
Albumin	g/dL	63	3.9	3.9	3.2	4.7	3.1 - 4.8	Non-Gaussian
Globulin	g/dL	63	1.0	0.9	0.2	1.8	0.3 - 1.7	Gaussian
AST	IU/L	63	44	36	13	141	13 - 111	Gaussian with logarithmic transformation
Creatine kinase	IU/L	62	1,358	921	170	6,823	153 - 5,427	Gaussian with logarithmic transformation
Alkaline phosphatase	IU/L	63	297	280	135	603	130 - 513	Gaussian with square root transformation
GGT	IU/L	63	57	55	34	112	33 - 94	Gaussian with logarithmic transformation
Total bilirubin	mg/dL	63	0.1	0.1	0	0.4	0 - 0.2	Non-Gaussian
SDH-37	IU/L	63	0.5	0	0	4	0 - 1.7	Non-Gaussian

RBC, red blood cells; HGB, hemoglobin; HTC, hematocrit; MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration; RDW, erythrocyte distribution width; WBC, white blood cells; BUN, blood urea nitrogen; AST, aspartate aminotransferase; GGT, γ-glutamyltransferase; SDH-37, sorbitol dehydrogenase.

Appendix 3:
Iowa State Veterinary Pathology
Reference Intervals
Hematology
Chemistry

Reference Intervals were established at Iowa State University's Clinical Pathology Laboratory.

1. **Home** < <https://vetmed.iastate.edu/vpath>>
2. **Services** < <https://vetmed.iastate.edu/vpath/services/>>
»
3. **Diagnostic Services** < <https://vetmed.iastate.edu/vpath/services/diagnostic-services/>>
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4. **Clinical Pathology Diagnostic and Research Services** < <https://vetmed.iastate.edu/vpath/services/diagnostic-services/clinical-pathology-diagnostic-and-research-services/>>
»
5. **Testing and Fees** < <https://vetmed.iastate.edu/vpath/services/diagnostic-services/clinical-pathology-diagnostic-and-research-services/testing-and-fees/>>
»
6. **Reference Intervals**

Reference Intervals

Reference Intervals were established at Iowa State University's Clinical Pathology Laboratory.

Reference Intervals from Clinical Pathology Laboratory February 2011
– *Department of Veterinary Pathology, Iowa State University*

Pathology Type Reset:

Pathology Type	Test	Unit	Canine	Feline	Bovine	Equine	Porcine
Hematology	Band neutrophils	$\times 10^3/\text{ul}$	0.0 – 0.3	0.0 – 0.3	0.00-0.12	0.0 – 0.2	
Hematology	Basophils	$\times 10^3/\text{ul}$	0.0 – 0.1	0.0 – 0.1	0.0 – 0.2	0.0 – 0.2	(0.0-1.6)**, (0.0-0.4)***
Hematology	Eosinophils	$\times 10^3/\text{ul}$	0.1-0.75	0.0-0.75	0.0-2.4	0.0-0.5	(0.0-0.5)**; (0.0-1.3)***
Hematology	Fibrinogen	mg/dl	100 – 400	100 – 400	100 – 500	100 – 400	100 – 500
Hematology	Hb	g/dl	12-18 (8-12)*	10-15 (8-11)*	8-15	11.5-16.0	(8.08 – 11.9)**; (11.2 – 14.7)***

Pathology Type	Test	Unit	Canine	Feline	Bovine	Equine	Porcine
Hematology	Hct	%	37-55 (25-37)*	30-45 (24-34)*	24 – 46	34 – 45	(28.22 – 39.8)**; (32.3 – 42.6)***
Hematology	Lymphocytes	x10 ³ /ul	1.0 – 4.8	1.5 – 7.0	2.5 – 7.5	1.3 – 4.5	(4.02 – 12.50)**; (5.30 – 17.9)***
Hematology	MCH	pg	19.5-30	12.5-17.5	11-17	12.7-17.5	(12.4 – 19.3)**; (16.3 – 20.6)***
Hematology	MCHC	g/dl	32 – 36	30 – 36	30 – 36	34 – 36	(27.39 – 31.4)**; (33.3 – 35.8)***

Pathology Type	Test	Unit	Canine	Feline	Bovine	Equine	Porcine
Hematology	MCV	fL	60 – 77	39 – 55	40 – 60	36 – 49	(43.4 – 64.5)**; (47.5 – 59.2)***
Hematology	Monocytes	$\times 10^3/\text{ul}$	0.15-1.35	0.0-0.85	0.03-0.85	0.0-0.5	(0.05-2.30)** : (0.0-3.7)***
Hematology	Plasma Protein	g/dl	5.3 – 7.2	6.3 – 8.2	6.9 – 7.7	6.0 – 8.2	
Hematology	Platelets	$\times 10^3/\text{ul}$	200 – 500	300 – 800	300 – 800	130 – 300	(374.3-1080.8)**; (118.9-522.9)***
Hematology	RBC	$\times 10^6/\text{ul}$	5.5 – 8.5	5 – 10	5 – 10	7 – 11	(4.87 – 7.88)** : (5.88 – 8.19)***

Pathology Type	Test	Unit	Canine	Feline	Bovine	Equine	Porcine
Hematology	Segmented neutrophils	$\times 10^3/\text{ul}$	3.0-11.4	2.5-12.5	0.6-4.0	2.1-6.7	(2.35-11.9)** (2.00-10.4)***
Hematology	WBC	$\times 10^3/\text{ul}$	6.0-17.0	5.5-19.5	4.0-12.0	5.0-11.0	(9.62-25.2)**; (11.35-28.9)***

Showing 1 to 16 of 16 entries (filtered from 55 total entries)

*Lowest in dogs and cats 5-6 weeks old, gradual increase to adult values by 5 months of age.

**Pigs 0-6 weeks old

***Pigs 6 weeks to 2 years-old

Reference Intervals were established at Iowa State University's Clinical Pathology Laboratory.

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4. **Clinical Pathology Diagnostic and Research Services** < <https://vetmed.iastate.edu/vpath/services/diagnostic-services/clinical-pathology-diagnostic-and-research-services/>>
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»
6. **Reference Intervals**

Reference Intervals

Reference Intervals were established at Iowa State University's Clinical Pathology Laboratory.

Reference Intervals from Clinical Pathology Laboratory February 2011
– *Department of Veterinary Pathology, Iowa State University*

Pathology Type Reset:

Pathology Type	Test	Unit	Canine	Feline	Bovine	Equine	Porcine
Chemistry	Albumin	g/dl	2.7 – 4.0	2.8 – 4.0	3.2 – 3.9	2.5 – 3.9	3.0 – 4.5
Chemistry	Alk Phos	IU/L	20 – 150	25 – 70	29 – 136	50 – 220	25 – 130
Chemistry	ALT/SGPT	IU/L	24 – 90	20 – 120	n/a	n/a	n/a
Chemistry	Anion Gap	mEq/L	8 – 17	13 – 22	14 – 21	7 – 14	n/a
Chemistry	AST/SGOT	IU/L	5 – 50	20 – 60	68 – 156	100 – 465	10 – 300
Chemistry	Bilirubin – Total	mg/dl	0.10-0.60	0.10-0.50	0.00-0.18	0.70-2.40	0.0-1.0
Chemistry	BUN	mg/dl	10 – 30	15 – 29	7 – 32	14 – 21	6 – 30

Pathology Type	Test	Unit	Canine	Feline	Bovine	Equine	Porcine
Chemistry	Ca	mg/dl	9.7-11.3	8.5-11.2	8.2-10.1	10.6-12.8	8.0-12.0
Chemistry	Cholesterol	mg/dl	132 – 300	75 – 260	78 – 120	77 – 125	n/a
Chemistry	CK	IU/L	55 – 200	50 – 450	1 – 350	100 – 575	100 – 2500
Chemistry	Cl	mEq/L	112 – 121	118 – 126	94 – 109	102 – 114	95 – 110
Chemistry-Special	Cortisol-post ACTH		5.5-2.0	4.5-15.0			
Chemistry-Special	Cortisol-post Dex		see report	see report			
Chemistry-Special	Cortisol-resting	ug/dl	1.0 – 5.0	1.0 – 5.0			

Pathology Type	Test	Unit	Canine	Feline	Bovine	Equine	Porcine
Chemistry	Creat	mg/dl	0.5 – 1.5	0.8 – 2.1	0.1 – 1.9	1.0 – 2.1	0.5 – 2.7
Chemistry	GGT	IU/L	5 – 16	1 -10	1 – 50	7 – 26	10 – 100
Chemistry	Glucose	mg/dl	68 – 115	70 – 120	40 – 100	80 – 113	65 – 150
Chemistry	iCa	mg/dl	5.0 – 5.8	4.0 – 5.6		6.32-7.60	n/a
Chemistry	K	mEq/L	3.9 – 5.3	3.8 – 5.4	3.7 – 5.3	2.6 – 4.5	4.0 – 7.0
Chemistry	LDH	IU/L	50 – 520	60 – 490	280 – 410	150 – 425	n/a
Chemistry	Lipase	IU/L	100 – 700	0 – 175	n/a	n/a	n/a

Pathology Type	Test	Unit	Canine	Feline	Bovine	Equine	Porcine
Chemistry	Mg	mg/dl	1.70-2.50	1.97-2.50	2.10-2.90	1.43-2.68	1.82-3.65
Chemistry	Na	mEq/L	141 – 151	152 – 159	133 – 147	137 – 145	135 – 150
Chemistry	Phos	mg/dl	3.2 – 6.0	3.0 – 6.9	3.8 – 7.9	2.6 – 5.0	4.5 – 9.0
Chemistry	SDH	IU/L	0 – 8	0 – 8	0 – 50	0 – 6	n/a
Chemistry-Special	T4	ug/dl	1.0 – 3.0	1.0 – 4.0		0.9 – 2.8	
Chemistry	TCO2	mEq/L	19 – 26	15 – 23	21 – 31	24.5-33.5	n/a
Chemistry	Total Protein	g/dl	5.2 – 7.1	6.1 – 8.0	6.7 – 8.7	5.8 – 8.0	7.0 – 8.9

Pathology Type	Test	Unit	Canine	Feline	Bovine	Equine	Porcine
Chemistry	Triglyceride	mg/dl	24- 115	21 – 120	25 – 120	n/a	n/a

Showing 1 to 29 of 29 entries (filtered from 55 total entries)

*Lowest in dogs and cats 5-6 weeks old, gradual increase to adult values by 5 months of age.

**Pigs 0-6 weeks old

***Pigs 6 weeks to 2 years-old

Appendix 5:
Pig Improvement Company
PIC® 2019
WEAN TO FINISH
GUIDELINES

Appendix J: Reference of
Growth and Feed Intake
Curve for PIC Sired Pigs

PIC NUTRIENT SPECIFICATIONS FOR GESTATING GILTS AND SOWS IN IDEAL BODY CONDITION (AS-FED).

This tables are presented in percentage for practical reasons. Nutrients should be considered in grams of intake per day based on farm-specific feed intake.

ITEM ^{a,b}	UNIT	GILTS	SOWS	HERD
NRC Net energy (NE) diet ^c	kcal/kg	2390	2390	2390
NRC NE, d 0-28	Mcal/d	4.3	5.4	---
NRC NE, d 28-90	Mcal/d	4.3	4.3	---
NRC NE, d 90-112	Mcal/d	6.5	4.3	---
NRC Metabolizable energy (ME) diet	kcal/kg	3230	3230	3230
NRC ME, d 0-28	Mcal/d	5.9	7.3	---
NRC ME, d 28-90	Mcal/d	5.9	5.9	---
NRC ME, d 90-112	Mcal/d	8.8	5.9	---
Estimated feed intake + 5% wastage ^{d,e}	kg/day	2.18	2.09	2.13
Standardized Ileal Digestible amino acids				
Lysine	%	0.60	0.60	0.60
Methionine + cysteine:Lysine	Ratio	70	70	70
Threonine:Lysine	Ratio	76	76	76
Tryptophan:Lysine	Ratio	19	19	19
Valine:Lysine	Ratio	71	71	71
Isoleucine:Lysine	Ratio	58	58	58
Leucine:Lysine	Ratio	92	92	92
Histidine:Lysine	Ratio	35	35	35
Phenylalanine + tyrosine:Lysine	Ratio	96	96	96
L-Lysine-HCl, max. ^f	%	0.25	0.25	0.25
Minerals ^g				
Total calcium	%	0.85	0.85	0.85
Av. phosphorus	%	0.40	0.40	0.40
STTD phosphorus ^h	%	0.44	0.44	0.44
Sodium ⁱ	%	0.24	0.24	0.24
Chloride	%	0.24	0.24	0.24
Added trace minerals				
Zinc	PPM	125	125	125
Iron	PPM	100	100	100
Manganese	PPM	50	50	50
Copper	PPM	15	15	15
Iodine	PPM	0.35	0.35	0.35
Selenium	PPM	0.3	0.3	0.3
Added vitamins ^{j,k}	per kg diet			
Vitamin A	IU/kg	9920	9920	9920
Vitamin D	IU/kg	1985	1985	1985
Vitamin E ^l	IU/kg	66	66	66
Vitamin K (menadione)	mg/kg	4.4	4.4	4.4
Choline ^m	mg/kg	660	660	660
Niacin	mg/kg	44	44	44
Riboflavin	mg/kg	10	10	10
Pantothenic acid	mg/kg	33	33	33
Vitamin B12	mcg/kg	37	37	37
Folic Acid	mcg/kg	1325	1325	1325
Biotin	mcg/kg	220	220	220
Thiamin	mg/kg	2.2	2.2	2.2
Vitamin B6 (Pyridoxine)	mg/kg	3.3	3.3	3.3

^aThese feed specifications should be used as a guide. They require adjustment for feed intake, local conditions, and markets.

^bAssumption: Gilt – 135 kg body weight (BW) at breeding and 34 kg net maternal gain, Sow – 180 kg BW at breeding and 9 kg net maternal gain.

^cNet energy was estimated using a conversion factor of 0.74 from metabolizable energy. For different diet compositions this may vary (i.e., 0.73 to 0.76) depending on the ingredients used.

^dFor thin sows, provide 10.2 Mcal ME or 7.6 Mcal NE per day until recovery. For fat sows, provide 5.2 Mcal ME or 3.8 Mcal NE per day until return to ideal condition.

^eIf gestating gilts or sows are fed less than the recommended amount of feed per day, the percentage levels need to be adjusted to achieve the minimum amount in grams per day of each nutrient.

^fL-Lysine-HCl maximum inclusions are recommended based on corn and soybean meal based diets and are to be used as a guideline.

^gCalcium and phosphorus values are considering release due to phytase; however, release values need to be based on suppliers' recommendation established from peer-reviewed scientific research.

^hStandardized total tract digestible phosphorus.

ⁱSodium: if sodium levels are not known in major ingredients use at least 80% of sodium coming from sodium chloride.

^jGilts and sows vitamins tend to be 2.5 x NRC in general.

^kPelleting and (or) expanding decreases vitamin stability by 10-12% and 15-20% respectively. Consult vitamin manufacturer to verify their specific vitamin stability under pelleting conditions so additional fortification can be made as required.

^lAdd 5.1 IU of Vitamin E/kg of complete diet for each 1% fat above 3% total dietary fat.

^mCholine content is based on corn and soybean meal based diets. For other diet compositions, a total level of 1325 mg of

PIC NUTRIENT SPECIFICATIONS FOR LACTATING GILTS AND SOWS (AS-FED).

This tables are presented in percentage for practical reasons. Nutrients should be considered in grams of intake per day based on farm-specific feed intake.

ITEM ^{a,b}	UNIT	GILTS	SOWS	HERD
Net Weight Body Loss ^b	%	<10	<10	<10
Fat Loss, Max ^b	mm	0-2	0-2	0-2
Litter Growth ^b	kg/d	2.50	2.72	2.61
NRC NE ^c	kcal/kg	2489	2489	2489
NRC NE	Mcal/d	13.0	15.4	14.9
NRC ME	kcal/kg	3362	3362	3362
NRC ME	Mcal/d	17.5	20.7	20.1
Average Feed Intake (21-d lactation)	kg/d	5,22	6,01	5,99
Average Feed Intake (28-d lactation)	kg/d	5,49	6,44	6,26
Standardized Ileal Digestible amino acids				
Lysine	g/d	63	63	63
Lysine (21-d lactation)	%	1.21	1.02	1.05
Lysine (28-d lactation)	%	1.15	0.98	1.01
Methionine + cysteine:Lysine	Ratio	53	53	53
Threonine:Lysine	Ratio	64	64	64
Tryptophan:Lysine	Ratio	19	19	19
Valine:Lysine	Ratio	64	64	64
Isoleucine:Lysine	Ratio	56	56	56
Leucine:Lysine	Ratio	114	113	114
Histidine:Lysine	Ratio	40	40	40
Phenylalanine + tyrosine:Lysine	Ratio	113	112	113
L-Lysine-HCl, max. ^d	%	0.45	0.45	0.45
Minerals ^e				
Total calcium	%	0.85	0.85	0.85
Av. phosphorus	%	0.40	0.40	0.40
STTD phosphorus ^f	%	0.44	0.44	0.44
Sodium ^g	%	0.24	0.24	0.24
Chloride	%	0.24	0.24	0.24
Added trace minerals				
Zinc	PPM	125	125	125
Iron	PPM	100	100	100
Manganese	PPM	50	50	50
Copper	PPM	15	15	15
Iodine	PPM	0.35	0.35	0.35
Selenium	PPM	0.3	0.3	0.3
Added vitamins ^{h,i}	per kg diet			
Vitamin A	IU/kg	9920	9920	9920
Vitamin D	IU/kg	1985	1985	1985
Vitamin E ^j	IU/kg	66	66	66
Vitamin K (menadione)	mg/kg	4.4	4.4	4.4
Choline ^k	mg/kg	660	660	660
Niacin	mg/kg	44	44	44
Riboflavin	mg/kg	10	10	10
Pantothenic acid	mg/kg	33	33	33
Vitamin B12	mcg/kg	37	37	37
Folic Acid	mcg/kg	1325	1325	1325
Biotin	mcg/kg	220	220	220
Thiamin	mg/kg	2.2	2.2	2.2
Vitamin B6 (Pyridoxine)	mg/kg	3.3	3.3	3.3

^aThese feed specifications should be used as a guide. They require adjustment for feed intake, local conditions, and markets.

^bAssumption: Gilt – 135 kg body weight (BW) at breeding and 34 kg net maternal gain, sow – 180 kg BW at breeding and 9 kg net maternal gain. Assuming 175 kg BW post farrowing, 10 kg weight loss and 2500-2800 g/d litter growth.

^cNet energy was estimated using a conversion factor of 0.74 from metabolizable energy. For different diet compositions this may vary (i.e., 0.73 to 0.76) depending on the ingredients used.

^dL-Lysine-HCl maximum inclusions are recommended based on corn and soybean meal based diets and are to be used as a guideline.

^eCalcium and phosphorus values are considering release due to phytase; however, release values need to be based on suppliers' recommendation established from peer-reviewed scientific research.

^fStandardized total tract digestible phosphorus.

^gSodium: if sodium levels are not known in major ingredients use at least 80% of sodium coming from sodium chloride.

^hSows tend to be 2.5 x NRC for vitamins in general.

ⁱPelleting and (or) expanding decreases vitamin stability by 10-12% and 15-20% respectively. Consult vitamin manufacturer to verify their specific vitamin stability under pelleting conditions so additional fortification can be made as required.

^jAdd 5.1 IU of Vitamin E/kg of complete diet for each 1% fat above 3% total dietary fat.

^kCholine content is based on corn and soybean meal based diets. For other diet compositions, a total level of 1325 mg of choline per kg should be achieved.

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**Appendix J: Reference of
Growth and Feed Intake
Curve for PIC Sired Pigs**

Appendix J

Reference of Growth and Feed Intake Curve for PIC Sired Pigs From Weaning to 28 Weeks of Age

Age days/weeks	Weight lb (kg)	Average daily gain lb (g)	Weekly feed intake lb (kg)	Cumulative average daily gain lb (g)/day	Cumulative feed intake lb (kg)	Cumulative feed conversion
21/3	13.1(5.9)					
28/4	16.1(7.3)	0.42(190)	3.1(1.4)	0.42(190)	3(1.4)	1.05
35/5	20.9(9.5)	0.69(313)	5.8(2.6)	0.56(254)	9(4.0)	1.14
42/6	27.0(12.2)	0.87(394)	7.9(3.6)	0.66(299)	17(7.6)	1.21
49/7	34.0(15.4)	1.01(458)	9.9(4.5)	0.75(340)	27(12.1)	1.27
56/8	43.7(19.8)	1.37(621)	14.2(6.4)	0.87(394)	41(18.5)	1.33
63/9	54.5(24.7)	1.54(698)	17.1(7.8)	0.98(444)	58(26.3)	1.4
70/10	65.9(29.9)	1.63(738)	19.2(8.7)	1.08(489)	77(35.0)	1.46
77/11	78.2(35.4)	1.75(793)	22.4(10.1)	1.16(525)	100(45.1)	1.53
84/12	91.2(41.3)	1.86(843)	25.5(11.6)	1.24(562)	125(56.7)	1.6
91/13	104.9(47.5)	1.96(888)	28.6(12.9)	1.31(593)	154(69.6)	1.68
98/14	119.1(54.0)	2.02(915)	31.4(14.2)	1.37(621)	185(83.8)	1.75
105/15	133.6(60.5)	2.08(942)	33.9(15.4)	1.43(648)	219(99.2)	1.82
112/16	148.5(67.3)	2.13(965)	36.2(16.4)	1.49(675)	255(115.6)	1.88
119/17	163.6(74.1)	2.15(974)	38.2(17.3)	1.54(698)	293(132.9)	1.95
126/18	178.7(81.0)	2.16(978)	39.9(18.1)	1.58(716)	333(151.0)	2.01
133/19	193.8(87.8)	2.16(978)	41.4(18.8)	1.62(734)	375(169.8)	2.08
140/20	208.9(94.6)	2.15(974)	42.7(19.4)	1.65(747)	417(189.1)	2.13
147/21	223.8(101.4)	2.12(960)	43.8(19.8)	1.67(757)	461(208.9)	2.19
154/22	238.4(108.0)	2.10(951)	44.7(20.3)	1.70(770)	506(229.2)	2.25
161/23	252.8(114.5)	2.05(929)	45.5(20.6)	1.71(775)	551(249.8)	2.3
168/24	267.0(121.0)	2.02(915)	46.2(20.9)	1.73(784)	598(270.7)	2.35
175/25	280.7(127.2)	1.96(888)	46.7(21.2)	1.74(788)	644(291.9)	2.41
182/26	294.1(133.2)	1.91(865)	47.2(21.4)	1.74(788)	692(313.3)	2.46
189/27	307.0(139.1)	1.85(838)	47.6(21.6)	1.75(793)	739(334.8)	2.51
196/28	319.6(144.8)	1.80(815)	48.0(21.7)	1.75(793)	787(356.6)	2.57