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Non Ruminant Nutrition

Tolerance of weanling pigs and effects on growth performance of supplementing corn-soybean meal-based diets with graded levels of a novel exogenous β-mannanase

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ABSTRACT

The hypothesis that a novel endo-β-mannanase can be used in diets for weanling pigs without negatively impacting growth performance, serum chemistry, hematological characters, or organ weights was tested. A total of 150 newly weaned pigs (75 castrated male and 75 female pigs; initial body weight: 6.20 ± 0.68 kg) were used. Pigs were allotted to three experimental diets (i.e., control, control plus 800 thermostable mannanase units (**TMU**)/kg, or control plus 100,000 TMU/kg). Pigs were allotted to pens with 5 pigs per pen for a total of 10 replicate pens per treatment. Pigs were fed phase 1 diets from d 1 to 21, and phase 2 diets from d 22 to 42 post-weaning. Average daily gain (**ADG**), average daily feed intake (**ADFI**), and gain:feed (**G:F**) were calculated. Blood samples from two pigs per pen (one male and one female pig) were collected on d 1, 21, and 42. One pig per pen from the control treatment and two pigs per pen from each of the β-mannanase treatments were euthanized at the end of the experiment and organs were collected. Data were analyzed using the Proc MIXED procedure of SAS with pen as the experimental unit. Results indicated that for the overall experiment, there were no differences in ADG, ADFI, or final body weight among treatments. However, pigs fed the diet with 100,000 TMU/kg of β-mannanase had greater ($P < 0.05$) G:F from d 22 to 42 and for the overall experimental period compared with pigs fed the control diet or the diet with 800 TMU/kg of β-mannanase. Most serum chemistry markers and blood hematological characters were not different among pigs fed experimental diets and concentrations were within the normal biological range for pigs. However, serum phosphorus was greater ($P < 0.05$) in pigs fed the diet with 100,000 TMU/kg of β-mannanase compared with pigs fed the other diets, but red cell distribution width and mean platelet volume were greater ($P < 0.05$) in pigs fed the control diet compared with pigs fed the control diet + 800 TMU/kg of β-mannanase. Abnormalities in liver, kidney, spleen, heart, stomach, or the small intestine were not observed, and the weight of these organs was not affected by dietary treatments. In conclusion, pigs fed diets containing 100,000 TMU/kg of β-mannanase had greater G:F from d 1 to 42 post-weaning compared with pigs fed control diets or the diets with 800 TMU/kg, and β-mannanase did not negatively impact general health and growth of the pigs even if included at a very high dose.

Lay summary

An experiment was conducted to test the hypothesis that pigs tolerate high doses of β-mannanase without negative impact on growth and health. Newly weaned pigs were allotted to a control diet without mannanase or two diets containing 800 units/kg of β-mannanase, which is the recommended inclusion rate, or 100,000 units/kg of β-mannanase. Average daily gain (ADG), average daily feed intake (ADFI) and gain to feed ratio (G:F) were not different among treatments during the initial 21 d post-weaning, but G:F was greater ($P < 0.05$) in pigs fed the diet containing 100,000 units/kg of β-mannanase from day 22 to 42 and also from day 1 to 42. However, inclusion of 100,000 units/kg of β-mannanase had no negative impact on serum chemistry markers or hematological characters. Likewise, the diet containing 100,000 units/kg of mannanase did not result in any abnormalities in kidney, liver, heart, spleen, stomach, or small intestine and dietary treatments did not impact the weight of any organs. Overall, results confirmed the hypothesis for the experiment and the β-mannanase used in this experiment is safe when added to diets for weanling pigs even if the inclusion rate is higher than the recommended dose.

Key words: growth performance, tolerance, weanling pigs, β-mannanase

INTRODUCTION

β-mannans are plant cell wall polysaccharides of D-mannose units linked by β-(1-4) glycosidic bonds (Lee and Brown, 2022). β-mannans are a complex carbohydrate and may consist of a long chain of only mannose units or a chain of mannose units with side chains of α-1,6-linked galactose or glucose residues, resulting in galactomannans or galactoglucomannans, respectively (Chen et al., 2018). Soybean meal is a common plant protein in diets for pigs due to its well-balanced amino acid profile and digestibility. However, soybean meal also contains anti-nutritional factors such as allergenic proteins and non-starch polysaccharides, which limits its use in diets for weanling pigs (Koepke et al., 2017). Soybean meal contains 17% to 27% non-starch

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polysaccharides, including 0.7% to 2.1% β-mannans (Bach Knudsen, 1997; Hsiao et al., 2006; Kiarie et al., 2021). β-mannans in diets for pigs cannot be digested in the small intestine because pigs lack the endogenous enzymes that target the β-1-4-mannosyl bonds. As a result, β-mannans pass through the small intestine undigested, but they may reduce water absorption by increasing digesta viscosity due to their high water-holding capacity, which may cause impaired diffusion of digestive enzymes, resulting in reduced digestibility of nutrients (Jang et al., 2020; Kiarie et al., 2021). Pigs also experience high stress at weaning, resulting in significant physiological and immunological changes, including reduced feed intake, impaired intestinal function, and increased susceptibility to diseases (Campbell et al., 2013). However, supplementation of an exogenous β-mannanase to diets for weanling pigs may mitigate the negative impacts of β-mannans on pig growth and immune response (Lee and Brown, 2022; Baker et al., 2024). Recently a novel endo-β-mannanase, Natupulse® TS, was developed, but there are no data demonstrating the efficiency, the safety, or the tolerance to an overdose of this enzyme when included in diets for weanling pigs. Safety of feed enzymes need to be determined to avoid undesirable effects on the target animal, such as allergies and irritations (Pariza and Cook, 2010). Therefore, it was hypothesized that if pigs can tolerate a very high dose of the enzyme (i.e., > 100 times the recommended inclusion level), the β-mannanase can be considered safe and will not cause undesirable effects if included in diets for pigs. Therefore, an experiment was conducted to test the hypothesis that the novel β-mannanase can be added to corn-soybean meal diets fed to weanling pigs without negative effects on growth performance or health, even if included at a very high dose.

MATERIALS AND METHODS

The protocol for the experiment was submitted to and approved by the Institutional Animal Care and Use Committee at the University of Illinois prior to initiation of the experiment. Pigs were the offspring of Line 800 males mated to Camborough females (Pig Improvement Company, Henderson, TN, USA).

Animals, Housing, and Experimental Design

A total of 150 newly weaned pigs (75 castrated male and 75 female pigs; initial body weight: 6.20 ± 0.68 kg) were allotted to one of three experimental diets using a randomized complete block design, with weaning weight as the blocking factor. Gender was balanced within each pen and across treatments. Thus, within each treatment, there were 5 pens with 3 barrows and 2 gilts, and 5 pens with 2 barrows and 3 gilts for a total of 10 replicate pens per treatment.

A 2-phase feeding program was used with d 1 to 21 as phase 1, and d 22 to 42 as phase 2. In each phase, three diets based on corn and soybean meal were formulated to contain 0 thermostable mannanase units (**TMU**)/kg (control diet), 800 TMU/kg, or 100,000 TMU/kg, respectively (Tables 1, 2, and 3). The mannanase unit TMU is defined as the amount of enzyme that produces reducing carbohydrates that have 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. The β-mannanase (*Natupulse® TS*) was supplied by BASF SE, Ludwigshafen, Germany. The 0, 800, and 100,000 TMU/kg feed activity levels were equivalent to 0, 100, and 12,500 mg of β-mannanase per kg of diet, respectively, and the β-mannanase was included in the diets at the expense of corn. The recommended inclusion in corn-soybean meal diets of this β-mannanase in 800 TMU/kg and the 100,000 TMU/kg inclusion was tested to determine if an overdose of β-mannanase has negative impacts on pig growth or health. All dietary nutrients were included in the diets to meet or exceed current requirement estimates (NRC, 2012). All diets were fed in mash form. Throughout the experiment, pigs had free access to feed and water.

Pigs were housed in pens (1.2 × 1.4 m) in an environmentally controlled barn. Floors were fully slatted with plastic coating. A 4-hole feeder and a nipple drinker were installed in each pen. Temperature, humidity, lighting, feeder and water space were identical for all experimental groups. Barns had a negative pressure ventilation system and had lights turned on at all times. Barn temperatures were 30 °C in week 1 post-weaning, 28 °C in week 2, 26 °C in week 3, 24 °C in week 4, and 22 °C in weeks 5 and 6 post-weaning.

Pigs received routine vaccinations before the start of the experiment, but were not vaccinated during the experiment. There was no routine application of medications during the experiment. General health status, morbidity, and mortality were recorded twice daily.

Blood Sample Collection and Chemical Analyses

Two samples of blood were collected on d 1, 21, and 42 from the jugular vein of two pigs per pen (one male and one female pig). Within each pen, the same pig was bled on the three sampling days. For the first sample, approximately 6 mL of whole

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 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.
[2]Provided the following quantities of vitamins and micro-minerals per kilogram of complete diet: Vitamin A as retinyl acetate, 11,136 IU; vitamin D_3 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 thiamin mononitrate, 0.24 mg; riboflavin, 6.59 mg; pyridoxine as pyridoxine hydrochloride,0.24 mg; vitamin B_{12}, 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.
[3]The 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.

blood was collected into a serum separation vacutainer. Blood was allowed to clot for 15 to 30 minutes before centrifuging at 769 ×*g* per 10 min to yield blood serum, which was transferred into sterile microtubes. For the second sample, approximately 5 mL of whole blood was collected into a vacutainer containing ethylenediaminetetraacetic acid (**EDTA**). Immediately after collection, tubes were gently inverted several times to ensure thorough mixing of the blood and anticoagulant. Serum and blood EDTA tubes were shipped on ice packs right after collection to the Clinical Pathology Laboratory at Iowa State University, Ames, IA, USA, for analysis. Serum samples were analyzed for chemistry markers, including sodium, potassium, chloride, bicarbonate, calcium, phosphorus, magnesium, blood urea nitrogen (**BUN**), creatinine, glucose, total protein, albumin, aspartate transaminase (**AST**), creatine kinase, alkaline phosphatase (**ALKP**), gamma-glutamyl transferase (**GGT**), total bilirubin, and anion gap. Blood EDTA samples were analyzed for hematology profile including total white blood cells count (**WBC**), WBC differential (i.e., neutrophil, lymphocyte, monocyte, eosinophil, basophil), absolute large unstained cells (**LUC**), red blood cell count (**RBC**), hemoglobin, hematocrit, mean corpuscular volume (**MCV**), mean corpuscular hemoglobin (**MCH**), mean corpuscular hemoglobin concentration (**MCHC**), red cell distribution width (**RDW**), platelet count, and mean platelet volume (**MPV**).

Ingredients and diets were analyzed for dry matter (Method 930.15; AOAC Int., 2019) and nitrogen was analyzed using the combustion procedure (Method 990.03; AOAC Int., 2019) on a LECO FP628 (LECO Corp., Saint Joseph, MI, USA). Crude protein was calculated as nitrogen × 6.25. Diets and ingredients were also analyzed for ash (Method 942.05; AOAC Int., 2019) and insoluble dietary fiber (**IDF**) and soluble dietary fiber (**SDF**) were analyzed according to method 991.43 (AOAC Int., 2019) using the Ankom TDF Dietary Fiber Analyzer (Ankom Technology, Macedon, NY, USA). Total dietary fiber (**TDF**) was calculated as the sum of IDF and SDF. Gross energy in diets and ingredients was measured using an isoperibol bomb calorimeter (Model 6400, Parr Instruments, Moline, IL, USA). Diets and ingredients were analyzed for acid hydrolyzed ether extract by acid hydrolysis using 3*N* HCl (Ankom HCl Hydrolysis System, Ankom Technology, Macedon, NY, USA) followed by fat extraction (Method 2003.06; AOAC Int., 2019] using petroleum ether (Ankom XT-15 Extractor, Ankom Technology, Macedon, NY, USA). Diets were analyzed for amino acids [Method 982.30 E (a, b, c); AOAC Int., 2019] on a Hitachi Amino Acid Analyzer (Model No. L8800; Hitachi High Technologies America, Inc., Pleasanton, CA, USA) using ninhydrin for post column derivatization and norleucine as the internal standard. Prior to analysis, samples were hydrolyzed with 6*N* HCl for 24 h at 110 °C [Method 982.30 E(a); AOAC Int., 2019]. Methionine and Cys were determined as Met sulfone and cysteic acid after cold performic acid oxidation overnight before hydrolysis [Method 982.30 E(b); AOAC Int.,

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 amino acids, %						
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 amino acids, %						
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

[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 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.

2019]. Tryptophan was determined after NaOH hydrolysis for 22 h at 110 °C [method 982.30 E(c); AOAC Int., 2019]. Diets were analyzed for β-mannanase activity using procedure FEN/0005/01 (BASF SE, Ludwigshafen, Germany). Soybean meal was also analyzed for mannose using gas-liquid chromatography based on the individual sugar constituent as alditol acetates (Oxley et al., 2004).

Necropsy and Histopathology Examinations

One pig per pen from the control treatment and two pigs per pen from the β-mannanase treatments were euthanized at the end of the experiment on d 42. The liver, kidney, spleen, heart, stomach, and small intestine from these pigs were examined by a board-certified pathologist for visual abnormalities or indications of toxicity. The weights of these organs were also recorded.

Calculations and Statistical Analyses

Individual pig weights were recorded at the beginning of the experiment and at the conclusion of each phase. Daily feed allotments were recorded, and feed left in the feeders was weighed at the end of each phase to calculate feed disappearance. If a pig was removed from a pen during the experiment, the feed left in the feeder and individual weights of

Table 4. Growth performance for pigs fed experimental diets[1]

Item	Control	Control + 800 TMU/kg β-Mannanase[2]	Control + 100,000 TMU/kg β-Mannanase	SEM	*P*-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
Mortality, %	2.00	4.00	2.00	2.24	0.387

[a-b]Values within a row lacking a common superscript letter are different ($P < 0.05$).
[1]Data 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 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.
[3]ADG = average daily gain; ADFI = average daily feed intake; G:F = gain-to-feed ratio.

the remaining pigs in the pen were recorded on the day the pig was removed. Feed intake for the remaining pigs in the pen was adjusted for the feed consumed by the pig that was removed based on weight gain (Lindemann and Kim, 2007; Lee et al., 2016). Data were summarized to calculate average daily gain (**ADG**), average daily feed intake (**ADFI**) and average gain to feed ratio (**G:F**) with the pen as the experimental unit. Data were calculated for phase 1 (d 1 to 21), phase 2 (d 22 to 42) and for the overall experiment (d 1 to 42).

No outliers were removed from the dataset. Growth performance data were analyzed using the PROC MIXED procedure of SAS (SAS Stats Inc. Cary, NC, USA). Because blood samples were collected on day 1, 21, and 42 from the same pigs, an additional analysis was conducted to analyze the effect of time on serum chemistry and hematology markers. These data were analyzed as repeated measures with unstructured variance using the MIXED and REPEATED procedures of SAS. For serum chemistry and hematology markers, the average of the two pigs represented the pen, and the pen was the experimental unit for all analysis. The statistical model included diet as fixed effect and replicate as random effect. Mean values were calculated using the LSMeans statement and if significant differences were identified, means were separated using the PDIFF procedure with Tukey adjustment (Tukey, 1977). Statistical significance was considered at $P < 0.05$.

RESULTS

Growth Performance

There were no differences in initial body weight, ADG, ADFI, or G:F of pigs from d 1 to 21, or for the body weight of pigs on d 21 (Table 4). There were also no differences in ADG and ADFI of pigs from d 22 to 42, but pigs fed the control diet + 100,000 TMU/kg of β-mannanase had greater ($P < 0.05$) G:F compared with pigs fed the control diet or the control diet + 800 TMU/kg of β-mannanase from d 22 to 42. For the entire experimental period (d 1 to 42) no differences in ADG, ADFI, or final body weight were observed among treatments, but G:F was greater for pigs fed the control diet + 100,000 TMU/kg of β-mannanase compared with pigs fed the control diet or the control diet + 800 TMU/kg of β-mannanase. The mortality was 2% (n = 1) for pigs fed the control diet or the control diet + 100,000 TMU/kg of β-mannanase, and 4% (n = 2) for pigs fed the control diet + 800 TMU/kg of β-mannanase, but these values were not different.

Serum Chemistry Markers

There were no differences in sodium, potassium, chloride, bicarbonate, calcium, magnesium, BUN, creatinine, glucose, total protein, albumin, AST, creatine kinase, ALKP, GGT, or total bilirubin among experimental diets (Table 5). However, phosphorus was greater ($P < 0.05$) in pigs fed the control diet + 100,000 TMU/kg of β-mannanase compared with pigs fed the control diet or the control diet + 800 TMU/kg of β-mannanase.

Hematological Characters

There were no differences in WBC, RBC, hemoglobin, hematocrit, MCV, MHC, MCHC, platelet, neutrophil, lymphocyte, monocyte, eosinophil, and basophils among experimental diets (Table 6). However, RDW and MPV were greater ($P < 0.05$) in pigs fed the control diet compared with pigs fed the control diet + 800 TMU/kg of β-mannanase, but pigs fed the diets with 100,000 TMU/kg of β-mannanase were not different from pigs fed the control diets.

Necropsies and Organ Weights

Necropsies conducted at the end of the experiment revealed no lesions in organs of pigs fed the control diet + 800 TMU/

Table 5. Serum chemistry markers of pigs fed experimental diets[1]

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	1029.55	1018.38	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 ($P < 0.05$).
[1]Data are least square means of 10 observations for all treatments.
[2]Reference 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 +/-0.1 °C and pH 3.5.
[4]BUN = blood urea nitrogen; AST = aspartate transaminase; ALKP = alkaline phosphatase; GGT = gamma-glutamyl transferase.

kg of β-mannanase or in organs of pigs fed the control diet + 100,000 TMU/kg of β-mannanase. There were also no differences on d 42 post-weaning in the weights of organs among pigs fed the three experimental diets (Table 7).

DISCUSSION

Concentrations of dry matter, gross energy, ash, acid-hydrolyzed ether extract, IDF, SDF, and TDF, and crude protein in ingredients were in agreement with reported values (NRC, 2012). Likewise, the analyzed nutrient composition of the diets and the β-mannanase activity in the diets used in the experiment were in agreement with calculated values.

β-mannans are linear polysaccharides formed from repeating β-(1-4) mannose units and are part of the cell wall in leguminous plants (Jackson et al., 2004; Lee and Brown, 2022). The β-mannan backbone may have sidechains containing galactose or glucose monomers that are linked to the backbone via α-(1-6) glycosidic bonds. Soybean meal contains between 0.7% and 2.1% β-mannans, which are mostly associated with the hull fraction (Kiarie et al., 2021); therefore, it was expected that the soybean meal used in this experiment provided the substrate for the enzyme tested. To estimate the content of β-mannan in soybean meal, the monosaccharide mannose was analyzed. However, the analyzed mannose in the soybean meal used in this experiment was low compared with reported values (Hsiao et al., 2006), indicating that the content of β-mannans in the soybean meal used in this experiment is also low, possibly due to the removal of the hulls during soybean processing because dehulled soybean meal contains less β-mannan than soybean meal with hulls (Hsiao et al., 2006).

The lack of an effect of β-mannanase on ADG was in agreement with data from experiments using β-mannanase in diets for weanling pigs (Huntley et al., 2018; Jang et al., 2020, 2024). Likewise, the improved G:F in response to the addition of β-mannanase to the diets is in agreement with previous data (Kiarie et al., 2021; Baker et al., 2024; Tajudeen et al., 2025). Addition of β-mannanase to diets for weanling pigs may increase hydrolysis of the backbone of galactomannans resulting in generation of manno-oligosaccharides, which may be fermented by microbial enzymes in the hindgut (Pettey et al., 2002). Therefore, β-mannanase may increase fermentability of IDF, increases the digestible energy of the diet, and results in greater G:F (Pettey et al., 2002; Kiarie et al., 2013). Likewise, β-mannanase may decrease the water holding capacity of β-mannans and decrease digesta viscosity in the small intestine, as has been demonstrated in poultry (Jackson et al., 2004; Chegeni et al., 2011; Fickler et al., 2023) and weanling pigs (Baker et al., 2024; Jang et al., 2024). A reduced intestinal viscosity may result in an increase in the activity and efficiency of digestive enzymes to reach their substrates and consequently improve nutrient and energy digestibility. Differences in effects of β-mannanase among recent experiments may be due to differences in the amount of β-mannans in the diets, indicating that if a relatively small amount of mannan-oligosaccarides is released from β-mannan hydrolysis by the β-mannanase enzyme, a low or non-detectable energy contribution for the animals may be the result, which is the reason for a lack of an effect on ADG.

Table 6. Hematological characters of pigs fed experimental diets[1]

Item	Reference value[2]	Control	Control + 800 TMU/kg β-Mannanase[3]	Control + 100,000 TMU/kg β-Mannanase	SEM	*P*-value
WBC[4], × 10^3/ul	11.35–28.9	15.05	16.50	17.04	0.65	0.105
RBC[4], × 10^6/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, × 10^3/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, × 10^3/ul	2.0–10.4	6.92	8.02	8.04	0.59	0.315
Lymphocyte, × 10^3/ul	5.30–17.9	7.07	7.39	7.75	0.27	0.169
Monocyte, × 10^3/ul	0.0–3.7	0.55	0.55	0.62	0.03	0.170
Eosinophil, × 10^3/ul	0.0–1.3	0.31	0.33	0.36	0.04	0.574
Basophils, × 10^3/ul	0.0–0.4	0.05	0.06	0.06	0.01	0.625
Absolute LUC[4], × 10^3/ul	-	0.13	0.16	0.16	0.01	0.269

[a-b]Values within a row lacking a common superscript letter are different ($P < 0.05$).
[1]Data are least square means of at least 8 observations for all treatments.
[2]Reference 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 +/-0.1 °C and pH 3.5.
[4]WBC = 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

[1]Data 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.
[2]All 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 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.

However, the β-mannanase effect on G:F may be a result of increased growth rate and not increased feed intake (Kiarie et al., 2021), which would indicate a greater nutrient and energy digestibility of the diet. Because nutrient digestibility and digesta viscosity were not determined in this experiment, this hypothesis cannot be verified, but warrants further research.

Serum chemistry markers provide insight into potential disruptions in organ activity. For example, BUN, AST, and creatinine indicate dysregulation of skeletal or cardiac muscle, whereas bilirubin, glucose, calcium, phosphorus, ALKP, albumin, total protein, GGT, and AST provide information about changes in liver function (Wilson et al., 1972; Rymut et al., 2021). Likewise, glucose and triglycerides provide information about energy balance, and chlorine, sodium, potassium, and bicarbonate are indicators of disorders in the metabolic system and digestive function, whereas anion gap indicates the difference between positively and negatively charged electrolytes (Kaneko et al., 1997; Rymut et al., 2021). The observation that with the exception of the concentration of phosphorus none of the serum chemistry markers were different among treatments indicates that regardless of treatment, pigs were healthy throughout the experiment, and concentrations of all serum markers were within the normal biological range for pigs (Iowa State University Clinical Pathology Laboratory, 2011; Cooper et al., 2014). This was also true for pigs fed the diets containing 100,000 TMU of

β-mannanase, demonstrating that even when the dose of the enzyme is higher than the recommended inclusion rate, no negative impact on pig health was observed.

Exogenous enzymes are generally not considered toxic when added to diets for pigs due to their substrate specificity and catalytic activity (Lessard et al., 2021). The observation that the β-mannanase used in this experiment did not result in increased mortality of pigs is in agreement with other experiments using β-mannanase fed to pigs (Sánchez-Uribe et al., 2022; Tajudeen et al., 2025), indicating that the enzyme is safe. Likewise, the observation that the majority of hematology characters and blood chemistry markers, which are sensitive to nutritional malabsorption, disease, and other physiological disorders, were not influenced by supplementation of β-mannanase in the diets, indicates that the physiology of the animals was not altered by consuming the enzyme even at a high inclusion level. However, the observation that supplementation of β-mannanase did not influence the majority of serum chemistry markers 42-d post-weaning, is in contrast with data indicating increased blood glucose concentrations when β-mannanase was included in diets for growing pigs (Kim et al., 2013, 2017). The increased glucose in serum may be explained by greater glucose absorption after the hydrolysis of glucomannans. However, the β-mannans in the diets used in this experiment are expected to be galactomannans, because they are present in legumes, such as soybeans (de Vries and Visser, 2001), which may be the reason that glucose absorption was not increased in this experiment. Serum concentrations differences of phosphorus and blood RDW and WBC did not indicate a negative impact of the enzyme. The lack of differences in serum and blood parameters has been previously documented in porcine animals fed diets containing exogenous enzymes (Schliffka et al., 2019; Lessard et al., 2021).

The observation that supplementation with β-mannanase to diets for weanling pigs did not influence the weight of the organs or generate pathological lesions is in agreement with results of research demonstrating that there are no changes in the weight of organs of pigs fed a multienzyme supplement compared with pigs fed a control diet (Agyekum et al., 2012). The weights of liver, heart, and kidney were in good agreement with weights previously reported for weanling pigs (Choi et al., 2021). Organ weights may be increased by high concentrations of fiber in diets (Pond et al., 1989), but because the diets used in this experiment contained a relatively small amount of dietary fiber, no impact of dietary treatments on organ weights were expected.

CONCLUSIONS

The endo-β-mannanase, Natupulse® TS, added to corn-soybean meal diets, improved the G:F of weanling pigs and can be used in diets for pigs without negatively impacting the general health of the pigs. The enzyme had no detrimental effects on serum biochemical and hematological parameters, pathological lesions, organ weight, or growth performance when fed to weanling pigs at the recommended level of 800 TMU/kg or at a tolerance level of 100,000 TMU/kg in diets for pigs during the initial 42 d post-weaning. It is, therefore, concluded that the tested enzyme is safe to use in pigs.

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Conflict of Interest

A. Fickler is an employee of BASF SE, Ludwigshafen, Germany, which is the company that manufactured the β-mannanase used in the experiment and provided financial support. The other authors have no conflict of interest.

Author Contributions

Anna Fickler (Conceptualization, Funding acquisition, Writing - review & editing), Hans Stein (Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Writing - review & editing), Jessica P. Acosta (Data curation, Investigation, Methodology, Writing - original draft, Writing - review & editing), and Su A Lee (Data curation, Formal analysis, Investigation, Project administration, Writing - review & editing)

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