

## Research Article

# Supplementation of a $\beta$ -Mannanase Enzyme Improves Feed Efficiency in Palm Kernel Expeller Rich Swine Diets

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$\beta$ -Mannans are strongly anti-nutritive polysaccharide fibres found in most vegetable feed ingredients. Estimated content of soluble  $\beta$ -mannans in common grow-finishing diets is only 0.15-0.35%, and *in vitro* studies have demonstrated that as little as 0.05% soluble  $\beta$ -mannan in feed can elicit a strong innate immune response. This innate response is referred to as a Feed Induced Immune Response (FIIR). Hemicell™ HT (Elanco) is a  $\beta$ -mannanase enzyme for animal feeds breaking down  $\beta$ -mannans, thereby preventing economic losses from this wasteful immune response to  $\beta$ -mannans. The objective was to investigate whether  $\beta$ -mannanase improves feed efficiency in grower/finisher pigs fed diets containing high amounts (5-15%) of Palm Kernel Expeller (PKE). A 3\*2 factorial design was applied consisting of 3 treatments (control, PKE, PKE+ $\beta$ -mannanase (PKE+)) and 2 sexes (barrows, gilts) and 2 subsequent batches. Groups were divided over 80 pens resulting in 13-14 repeats per group. Pigs were fed pelleted starter (0-28 d), grower (28-63 d), and finisher diets (63-99 d). Body Weight (BW) was recorded on days 0, 28, 63, and 99. Feed was offered *ad libitum* and recorded by pen. For each phase, Average Daily Gain (ADG), Average Daily Feed Intake (ADFI), and Feed Conversion Ratio (FCR) were calculated. Data analysis was performed in R. FCR improved significantly ( $P=0.004$ ) the first four weeks PKE+ (2.07) compared with Control (2.13). During grower/finisher phases, ADG and ADFI were significantly ( $P<0.05$ ) higher on PKE and PKE+ compared with Control. Overall, both PKE and PKE+ performed significantly better than Control, which PKE and PKE+ performed similarly. Feeding 10-15% PKE increased ADG and ADFI (potentially due to overestimated energy content of the PKE) both with and without  $\beta$ -mannanase, and resulted in similar FCR. Thus,  $\beta$ -mannanase may improve FCR on high PKE diets from 21 to 39 kg BW.

**Keywords:**  $\beta$ -Mannanase; Fattening pigs; Feed efficiency; Palm kernel expeller**Abbreviations**

ADFI: Average Daily Feed Intake; ADWG: Average Daily Weight Gain; FCR: Feed Conversion Rate; NSP: Non-Starch Polysaccharide; PKE: Palm Kernel Expeller; SBM: Soybean Meal

**Introduction**

Polysaccharides, polymers of monosaccharides linked by glycosidic bonds, are major components of all vegetable feed ingredients used in common swine diets. Starch, a polymer of glucose units linked by  $\alpha$ -(1-4) with a few  $\alpha$ -(1-6) bonds, is digested in the small intestine of pigs through endogenous enzyme activity. Non-Starch Polysaccharides (NSPs) are fibrous materials found in plant cell wall which include celluloses, hemicelluloses, pectins and oligosaccharides. Monogastric animals such as pigs do not produce endogenous enzymes needed to digest  $\beta$ -linked NSPs like  $\beta$ -mannans [23]. Beta-mannans in swine diets have been suggested to hinder the utilization of nutrients [36]. Positive effects of supplementing  $\beta$ -mannanase to maize-soybean meal (SBM)-based diets on nutrient digestibility and growth performance have been studied [34]. Palm Kernel Meal (PKM) and Palm Kernel Expellers (PKE), co-products from oil extraction of the African oil palm nuts, *Elaeis guineensis*

Jacq., are available in large quantities in many countries [33]. They are considered an inexpensive source of energy and nutrients and therefore, PKM and PKE have received increasing attention as an alternative protein source for swine [2,38] and poultry diets [1,3,24,29-32]. PKM and PKE contain moderate levels of protein and a high levels of fibre, which explains why, until now, they mainly have been used in feeds for ruminants [11,13,42] and rabbits [7]. A wide variation in crude fibre from 120 to 270 g per kg of PKM have been reported [12] and may be influenced by the variety of tree, growth conditions, and processing method used. Water-soluble dietary fibers, such as  $\beta$ -mannans, are very high in PKE [8], and several studies have reported that the utilization of glucose and protein is hampered by mannans in swine diets [36].

$\beta$ -Mannan is an antinutritive factor found in many common feed ingredients [9], which has received increasing attention in recent years.  $\beta$ -Mannans are linear polysaccharides composed of repeating units of  $\beta$ -1,4-mannose and  $\alpha$ -1,6-galactose and/or glucose units attached to the  $\beta$ -mannan backbone [14,19]. High concentrations of them are considered unsuitable in monogastric diets because of their antinutritive properties, mainly due to stimulation of the innate immune response. The innate immune cells identify pathogens using

distinct molecules, called Pathogen Associated Molecular Patterns (PAMP), expressed on the pathogen surface [10]. Binding of PAMP to Pathogen Recognition Receptors (PRR) present on innate immune cells, result in the release of innate defense molecules such as reactive oxygen and nitrogen species, bacteriolytic enzymes, antimicrobial peptides and complement proteins [39]. These PAMP include complex polysaccharides that resemble  $\beta$ -mannans [10]. Therefore,  $\beta$ -mannans from feed can create a false signal about the presence of pathogens in the gut that elicits an unwarranted immune activation [4,25], also known as a Feed-Induced Immune Response (FIIR) [5]. This recognition mistake leads to a futile immune response that causes energy and nutrients to be wasted [14]. Hydrolysis of these  $\beta$ -mannans through inclusion of exogenous  $\beta$ -mannanase enzymes can reduce and potentially eliminate their ability to induce FIIR.

In poultry, the inclusion of dietary  $\beta$ -mannanase has been shown to improve daily gain and feed efficiency, while decreasing digesta viscosity [6], and to upregulate a broad range of metabolic functions related to digestion, metabolism, and immunity [5]. Moreover, the beneficial effects of  $\beta$ -mannanase addition in chickens, challenged with *Eimeria* sp. and *Clostridium perfringens*, were observed with improved performance and reduced lesion scores in disease-challenged birds [18].

Recently, supplemental  $\beta$ -mannanase has been reported to improve the digestibility of dry matter and energy in PKE-containing diets fed to growing pigs [27]. Moreover, no interaction on apparent total tract digestibility of nutrients and energy has been observed for a combined supplementation of phytase and  $\beta$ -mannanase in a diet containing 100 g/kg PKE for growing pigs [27]. Supplementation of  $\beta$ -mannanase to low- and high-mannan diets has the potential to improve the performance of growing pigs [22]. Moreover, Palm Kernel Meal (PKM) may partially replace corn and SBM without reducing pig performance if  $\beta$ -mannanase is supplemented to the diet [22]. The improved overall performance following supplementation of  $\beta$ -mannanase to corn-SBM-PKM based swine diets might be due to increased adjusted ileal digestibility of different amino acids, which may most likely be due to the reduced innate immune activity [20,27,40]. Others concluded that  $\beta$ -mannanase improved growth performance in both weaning and growing-finishing pigs on corn-SBM diets [21,26,34] with minimal effects on nutrient digestibility [34].

Additionally,  $\beta$ -mannanase supplementation to corn-SBM diets reduced the population of fecal coliforms and tended to reduce the  $\text{NH}_3$  concentration of fecal slurry after 24 h fermentation [41]. The reduction of fecal coliforms might impact the environmental infection pressure from coliforms, related to clinical problems of Post Weaning Diarrhea (PWD). Another study demonstrated *in vivo* anti-inflammatory activity of mannanase-hydrolyzed copra meal in a porcine colitis model, with decreased expression of mRNA for ileal IL-1 $\beta$ , IL-6, IL-17 and TNF- $\alpha$  [17]. Innate immune activation is accompanied by down-regulation of anabolic functions [15], which translates into a reduced performance capacity. This may partially explain why addition of a combination of  $\beta$ -mannanase and phytase to PKE-containing diets fed to growing pigs has been shown to improve the digestibility of P and partially of amino acids [27]. Another study demonstrated no significant effect of increasing

inclusion levels from 20 to 40% of palm kernel cake on performance parameters in growing pigs [37], whereas carcass characteristics, such as dressing percentage, eye muscle area and weights of joints and cuts were significantly decreased with increasing PKC levels.

The objective of the current study was to evaluate the effects of  $\beta$ -mannanase supplementation of growing-finishing pig diets with increasing levels of palm kernel meal from 5 to 15% on performance under field conditions.

## Materials and Methods

### Description of Experimental Farm

The field trial was performed on a conventional fattening unit with 10 compartments of 8 pens each in the Netherlands. Compartments were ventilated through mechanical ventilation with a door air inlet. All pens had partially slatted plastic floors. Water was distributed through a nipple in the feeder. Each pen was equipped with a dry feeder. Pelleted feed was weighed upon distribution to each individual feeder.

### Experimental Design

**Treatment groups:** A 3 \* 2 factorial design was applied consisting of 3 treatments (Control, Palm Kernel Expeller (PKE) and palm kernel expeller + Hemicell HT (PKE+)) and 2 sexes (barrows and gilts). Groups were blinded to the farm personnel and only distinguished by color codes (red, green, and blue). The 3 groups were evenly allocated over the 10 compartments and 80 pens (containing each 8 to 9 pigs at a stocking density of 0.8 m<sup>2</sup>), resulting in 13 to 14 repeats per treatment group. In an individual pen, all animals were either barrows or gilts.

**Experimental diets:** The pigs were fed a pelleted 3-phase diet consisting of starter (0-28 d), grower (29-63 d) and finisher (64-99 d) diets in each treatment group. The main difference between the diets for the two PKE groups and Control was the substitution of wheat bran by PKE. In the PKE+ group, a  $\beta$ -mannanase enzyme (Hemicell HT; Elanco, Indianapolis; IN) was added at 300 g per tonne of feed, according to the manufacturer's instructions for use. All other enzymes (xylanase and phytase) in the diets remained at the same levels in the three treatment groups.

Details on diet composition and calculated nutrient values are given in Table 1 & 2, respectively. Nutritional composition and presence of the  $\beta$ -mannanase enzyme (Hemicell HT; Elanco, Indianapolis, IN) were confirmed for the specific diets through laboratory analysis (Eurofins, Barcelona, Spain).

**Experimental animals:** TopigsNorsvin 70 \* PIC408 barrows and gilts were obtained from a conventional commercial sow farm in the Netherlands. Piglets were vaccinated to protect against *Mycoplasma hyopneumoniae* and Porcine Circovirus type 2 (PCV-2) using a one-shot commercial vaccine (Ingelvac Combo-Flex; Boehringer Ingelheim). Two different groups of pigs were obtained (batch 1 and 2, respectively) for the feed trial.

### Performance Data Collection

Pig body weight (BW) at pen level was measured at 0, 28, 63 and 99 days after arrival on the finisher farm. Feed provision (*ad libitum*) was recorded daily at pen level. For each respective phase (starter, grower, finisher), Average Daily Weight Gain (ADWG; expressed as

g/d), Average Daily Feed Intake (ADFI; expressed as g/d) and Feed Conversion Rate (FCR; expressed as kg feed per kg of weight gain) were calculated.

### Veterinary Treatments

Individual antibiotic treatments were performed as needed due to the critical state of the piglet and in case of a broader health issue in the barn, group treatment could be performed. The same veterinary products and dosages (ml/kg) were used throughout the entire study period. Individual antibiotics treatments or group treatments were recorded daily by date, product, dose, ID number of treated piglets, presumed cause of treatment, and number of times the treatment was repeated.

### Data Management and Statistical Analysis

Data were hand-recorded by the farm personnel and stored in MS Excel on OneDrive at the end of each day. Following the end of the finisher phase, data were extracted from Excel into R 3.6.1 [28] and the blinded color-coded treatments were unblinded to reveal the respective treatment groups. Calculations, exploratory data analysis and quality review, and subsequent statistical analysis were all performed in R 3.6.1 [28]. Effect of sex, diet, and their interaction on parameters (BW, ADWG, ADFI, FCR) during each of the dietary phases (starter, grower, finisher, and overall) were estimated using a linear mixed model using pen nested within compartment as random effect. Batch (1, 2) was added to the model as a co variable to correct for effects of age group. If required, normalization of residuals was performed using a logarithmic transformation. All data are presented as (back-transformed) estimated marginal means with their respective pooled standard error of the mean (SEM). All means were tested for significant differences ( $P < 0.05$ ), using a post-hoc T-test with Tukey's adjustments to correct for multiple comparisons.

## Results

### Pig weight and Average Daily Weight Gain

Data on pig weight are given in (Table 3). The pigs arrived at the finisher facility at an average weight of 21.2 kg ( $\pm 0.9$ ). No significant differences ( $P > 0.05$ ) were present in the start weight (d0) between treatment groups or sex. At d28, pigs in PKE+ were slightly, but not significantly ( $P > 0.05$ ) heavier with 39.7 kg ( $\pm 1.4$  kg) as compared to PKE (37.9 kg) and Control (39.1 kg). At d63, the pigs in PKE+ were again slightly but not significantly ( $P > 0.05$ ) heavier with 73.8 kg ( $\pm 1.7$ ) comparison to PKE (72.2 kg) and Control (72.7 kg). At d99 at the end of the finisher phase, the bodyweight of the PKE+ pigs averaged 109.9 kg and tended ( $P = 0.07$ ) to be heavier than the pigs in PKE (108.4 kg) and Control (106.8 kg). Throughout the entire trial, no significant interaction ( $P > 0.05$ ) between treatment and sex could be observed. However, a significant difference ( $P < 0.001$ ) in body weight at d99 was observed between barrows and gilts, with the barrows between 1.4 and 7.5 kg heavier than the gilts in the respective treatment groups. The biggest difference (7.5 kg) was observed in the PKE group.

Data on ADWG are given in Table 4. In the starter phase, pigs in PKE+ had a significantly higher ( $P < 0.05$ ) ADWG (630 g/d  $\pm 12$ ) compared to PKE (595 g/d), while Control pigs gained intermediately to the two other treatments ( $P > 0.05$ ) (609 g/d). A significant interaction ( $P = 0.05$ ) between treatment and sex could be observed for ADWG in the starter phase. In the subsequent grower phase, both PKE and PKE+ had a higher ADWG compared to Control. The ADWG significantly differed ( $P = 0.04$ ) between PKE (949 g/d  $\pm 11$ ) and Control (913 g/d), and PKE+ (642 g/d) was intermediate and not significantly different from the two other treatments ( $P > 0.05$ ). Moreover, a significant effect ( $P < 0.001$ ) of sex on ADWG

**Table 1:** Composition of the trial diets.

Ingredient, %	Starter (0-28 d)			Grower (29-63 d)			Finisher (64-99 d)		
	Ctrl	PKE*	PKE+*	Ctrl	PKE	PKE+	Ctrl	PKE	PKE+
Wheat	27.4	27.1	27.1	29.4	28.6	28.6	31.6	30.1	30.1
Barley	25.0	25.0	25.0	20.0	20.0	20.0	15.0	15.0	15.0
Triticale	10.0	10.0	10.0	15.0	15.0	15.0	15.0	15.0	15.0
Soy bean meal	9.4	9.5	9.5	3.1	3.8	3.8	0.0	0.0	0.0
Corn	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Rapeseed meal	7.5	7.5	7.5	7.5	6.2	6.1	6.4	6.3	6.3
Wheat bran	6.2	1.7	1.6	9.1	1.0	1.0	15.0	3.4	3.3
Sunflower meal	2.5	2.5	2.5	5.0	5.0	5.0	5.0	5.0	5.0
Palm kernel expeller	0.0	5.0	5.0	0.0	10.0	10.0	0.0	15.0	15.0
Soy bean oil	1.6	1.3	1.3	1.0	0.5	0.5	1.5	0.5	0.5
Oat hulls	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0
Synthetic amino acids	1.08	1.10	1.10	1.01	1.06	1.07	0.91	0.97	0.97
Premix	1.90	1.87	1.88	1.38	1.36	1.36	1.41	1.22	1.22
Xylanase	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003
Phytase	0.011	0.011	0.011	0.009	0.011	0.011	0.004	0.004	0.004
Hemicell HT	0	0	0.03	0	0	0.03	0	0	0.03

\*Diet rich in palm kernel expeller (PKE).

\*PKE-rich diets supplemented with  $\beta$ -mannanase.

**Table 2:** Expected nutrient content of the trial diets.

Content, g/kg	Starter (0-28 d)			Grower(29-63d)			Finisher(64-99d)		
	Ctrl	PKE1	PKE+ 2	Ctrl	PKE	PKE +	Ctrl	PKE	PKE+
Moisture	114	112	112	115	110	110	115	109	109
Crude ash	48	47	47	42	40	40	42	39	39
Crude protein	171	172	172	156	157	156	142	146	146
Crude fat	35	35	35	30	31	31	35	35	35
Crude fiber	46	50	50	50	58	57	55	66	66
Starch	422	414	414	443	426	426	439	412	412
Energy(EW)	112	112	112	111	111	111	110	110	110
Lysine <sup>3</sup>	10.3	10.3	10.2	9.0	9.0	9.0	7.8	7.8	7.8
Methionine	3.5	3.5	3.5	3.0	3.1	3.1	2.6	2.6	2.6
Methionine+cysteine	6.2	6.2	6.2	5.5	5.5	5.5	5.0	4.8	4.8
Threonine	6.7	6.7	6.7	6.0	6.0	6.0	5.4	5.4	5.4
Tryptophan	1.9	1.9	1.9	1.7	1.7	1.7	1.5	1.5	1.5
Isoleucine	5.5	5.5	5.5	4.8	4.8	4.8	4.1	4.1	4.1
Arginine	9.0	9.2	9.2	7.9	8.3	8.3	7.0	7.7	7.7

<sup>1</sup>Diet rich in palm kernel expeller (PKE).

<sup>2</sup>PKE-rich diets supplemented with  $\beta$ -mannanase.

<sup>3</sup>All amino acids are given as standardized ileal digestible (SID) amino acids.

**Table 3:** Effects of treatment (Control, PKE and PKE+) on body weight by sex. Data are presented as estimated marginal means with pooled standard error of the means (SEM).

	Barrows			Gilts			SEM	Both sexes <sup>*</sup>			SEM	P-values <sup>*</sup>		
	Ctrl	PKE <sup>*</sup>	PKE+ <sup>*</sup>	Ctrl	PKE	PKE+		Ctrl	PKE	PKE+		Trt*sex	Trt	Sex
BW D0, kg	21.0	21.2	21.2	21.3	21.0	21.2	0.9	21.2	21.1	21.2	0.9	0.88	0.96	0.84
BW D28, kg	40.3	38.0	39.7	37.9	37.8	39.7	2	39.1	37.9	39.7	1.4	0.34	0.19	0.25
BW D63, kg	73.4	74.0	74.2	72.0	70.5	73.4	1.9	72.7	72.2	73.8	1.7	0.45	0.43	0.06
BW D99, kg	108.5	112.2	110.6	105.2	104.7	109.2	1.8	106.8	108.4	109.9	1.5	0.07	0.07	<0.001

<sup>\*</sup>Pooled performance for barrows and gilts, superscripts indicate differences between treatments solely.

<sup>\*</sup>Model-established *P*-values.

<sup>\*</sup>Palm kernel expeller (5–10–15 % in starter, grower, and finisher diets, respectively).

<sup>\*</sup>Addition of 0.03%  $\beta$ -mannanase (Hemicell HT; Elanco).

was observed. During the finisher phase, PKE+ pigs had (1009 g/d  $\pm$  12) significantly higher ( $P = 0.02$ ) ADWG compared to Control (968 g/d), with PKE (977 g/d) being intermediate. Both a significant effect of sex ( $P = 0.01$ ) and an interaction between treatment and sex ( $P = 0.05$ ) was observed in the finisher phase. Overall, ADWG was significantly higher ( $P < 0.001$ ) for PKE+ (871 g/d  $\pm$  7) compared to Control (833 g/d), with PKE without  $\beta$ -mannanase supplementation (858 g/d) being intermediate and only significantly different ( $P < 0.001$ ) from Control (833 g/d). A significant effect ( $P < 0.001$ ) of sex was observed on overall ADWG with gilts generally having a lower ADWG than the barrows.

### Average Daily Feed Intake and Feed Conversion Rate

Data on ADFI and FCR are given in Table 4. The ADFI did not significantly differ ( $P > 0.05$ ) between the treatment groups in the starter phase. Nevertheless, a significant effect ( $P = 0.002$ ) of sex and a significant interaction ( $P = 0.02$ ) between treatment and sex could be observed during the starter phase. In the grower phase, ADFI in PKE+ (2281 g/d  $\pm$  25) and PKE (2240 g/d) was significantly higher ( $P < 0.001$ ) ADFI than Control (2155 g/d). Gilts had significantly lower ( $P < 0.001$ ) ADFI than barrows in the grower phase, and ADFI in both

PKE+ (2818 g/d  $\pm$  32) and PKE (2777 g/d) was significantly higher ( $P < 0.001$ ) than in Control (2633 g/d). Again, a significant effect ( $P < 0.001$ ) of sex was observed. The overall ADFI in both PKE+ (2199 g/d  $\pm$  19) and PKE (2171 g/d) group was significantly higher ( $P < 0.001$ ) than in Control (2080 g/d). Overall, there also was a significant effect ( $P < 0.001$ ) of sex on ADFI, with ADFI ranging between 1974 and 2125 g/d for gilts and 2192 and 2300 g/d for barrows.

During the starter phase, pigs fed in PKE+ had significantly ( $P = 0.004$ ) better FCR (2.07  $\pm$  0.03) compared to PKE (2.19). FCR on Control pigs (2.13) was intermediate. During the grower phase, FCR did not differ significantly between treatments (2.36, 2.36 and 2.42 for Control, PKE and PKE+ group, respectively). However, gilts had a significantly better FCR (range: 2.27 – 2.37) than barrows (range: 2.42 – 2.47) in the grower phase. During the finisher phase, pigs in both PKE+ (2.84  $\pm$  0.02) and PKE (2.80) had significantly higher ( $P < 0.001$ ) FCR than Control pigs (2.72). A significant effect ( $P < 0.001$ ) of sex was also observed FCR, with gilts having lower (2.60 – 2.76) FCR compared to barrows (2.83 – 2.92). Overall, no significant difference ( $P > 0.05$ ) in FCR between treatment groups was observed. Nevertheless, a significant overall effect ( $P < 0.001$ ) of sex was



**Table 4:** Effects of treatments (Control, PKE and PKE+) on performance by sex. Data are presented as estimated marginal means with pooled standard error of the means (SEM). Superscript letters indicate significant differences ( $P < 0.05$ ) within a row.

		Barrows			Gilts			SEM	Both sexes <sup>1</sup>			SEM	P-values <sup>2</sup>		
		Ctrl	PKE <sup>3</sup>	PKE+ <sup>4</sup>	Ctrl	PKE	PKE+		Ctrl	PKE	PKE+		Trt*sex	Trt	Sex
Starter 0 - 28d	ADWG	642 <sup>b</sup>	602 <sup>ab</sup>	627 <sup>ab</sup>	578 <sup>a</sup>	588 <sup>ab</sup>	633 <sup>ab</sup>	16	609 <sup>xy</sup>	595 <sup>x</sup>	630 <sup>y</sup>	12	<b>0.05</b>	0.06	0.07
	ADFI	1,349 <sup>b</sup>	1,315 <sup>b</sup>	1,311 <sup>b</sup>	1,245 <sup>a</sup>	1,288 <sup>ab</sup>	1,294 <sup>ab</sup>	18	1,296	1,301	1,302	14	<b>0.02</b>	0.55	<b>0.002</b>
	FCR	2.10	2.19	2.09	2.15	2.19	2.04	0.04	2.13 <sup>ab</sup>	2.19 <sup>b</sup>	2.07 <sup>a</sup>	0.03	0.35	<b>0.004</b>	0.93
Grower 28 - 63d	ADWG	930	996	965	896	904	919	15	913 <sup>a</sup>	949 <sup>b</sup>	942 <sup>ab</sup>	11	0.15	<b>0.04</b>	<b>&lt;0.001</b>
	ADFI	2,278	2,406	2,384	2,039	2,085	2,182	33	2,155 <sup>a</sup>	2,240 <sup>b</sup>	2,281 <sup>b</sup>	25	0.14	<b>&lt;0.001</b>	<b>&lt;0.001</b>
	FCR	2.45	2.42	2.47	2.27	2.31	2.37	0.03	2.36	2.36	2.42	0.02	0.35	0.06	<b>&lt;0.001</b>
Finisher 63 - 99d	ADWG	979	1,014	1,009	958	942	1,008	16	968 <sup>a</sup>	977 <sup>ab</sup>	1,009 <sup>b</sup>	12	<b>0.05</b>	<b>0.02</b>	<b>0.01</b>
	ADFI	2,776	2,964	2,919	2,497	2,601	2,721	45	2,633 <sup>a</sup>	2,777 <sup>b</sup>	2,818 <sup>b</sup>	32	0.18	<b>&lt;0.001</b>	<b>&lt;0.001</b>
	FCR	2.83	2.92	2.90	2.60	2.76	2.70	0.03	2.72 <sup>a</sup>	2.80 <sup>b</sup>	2.84 <sup>b</sup>	0.02	0.47	<b>&lt;0.001</b>	<b>&lt;0.001</b>
Overall 0 - 99d	ADWG	854 <sup>yz</sup>	888 <sup>z</sup>	881 <sup>z</sup>	812 <sup>x</sup>	829 <sup>xy</sup>	861 <sup>yz</sup>	9	833 <sup>a</sup>	858 <sup>b</sup>	871 <sup>b</sup>	7	0.09	<b>&lt;0.001</b>	<b>&lt;0.001</b>
	ADFI	2,192	2,300	2,276	1,974	2,049	2,125	26	2,080 <sup>a</sup>	2,171 <sup>b</sup>	2,199 <sup>b</sup>	19	0.11	<b>&lt;0.001</b>	<b>&lt;0.001</b>
	FCR	2.57	2.59	2.58	2.43	2.47	2.47	0.03	2.50	2.52	2.53	0.02	0.89	0.19	<b>&lt;0.001</b>

<sup>1</sup>Pooled performance for barrows and gilts, superscripts indicate differences between treatments solely.

<sup>2</sup>Model-established P-values.

<sup>3</sup>Palm kernel expeller (5–10–15% instarter, grower, and finisher diets, respectively).

<sup>4</sup>Addition of 0.03%  $\beta$ -mannanase (Hemicell HT; Elanco).

observed for FCR, with gilts being more efficient (2.43 – 2.47) than barrows (2.57 – 2.59).

### Antimicrobial Treatment

During the starter phase (16–26 d), several pigs suffered from respiratory disease. Therefore, water medication with aspirin (4 days, 16–19 d) and subsequently with an antimicrobial (doxycycline, 6 days, 21–26 d) was administered to all pigs. The pigs recovered following the medication, so the study was continued as initially planned.

### Discussion

In the current study, a substantial amount of wheat bran was in starter diet and additionally an amount of rapeseed meal in grower and finisher diets was replaced by PKE, resulting in a step-wise increased PKE inclusion of 5 – 10 – 15% from starter over grower to finisher diet, respectively. Palm kernel expeller is known to contain a high amount of NSPs, such as  $\beta$ -mannans, a known antinutritive factor [3], which may stimulate an innate immune response through their resemblance with PAMPs [6]. This activation has been called FIIR (Feed Induced Immune Response; [10] and leads to an unnecessary immune activation, causing energy and nutrients to be wasted [4]. Therefore, 300 g/tonne of an exogenous  $\beta$ -mannanase enzyme (Hemicell HT; Elanco, Greenfield, IA) was added to hydrolyze these antinutritive  $\beta$ -mannans in the trial feed.

No effect of treatment (control vs. PKE and PKE+) on body weight of the pigs could be demonstrated throughout the entire trial. Only a marginal, non-significant effect of treatment ( $P = 0.07$ ) could be observed on the body weight of the pigs at d 99 of the trial. At d 99, pigs in the PKE+ group were heavier (109.9 kg  $\pm$  1.5) as compared to pigs in the PKE group (108.4 kg) and the control group (106.8 kg). At

the end of the trial, barrows were significantly heavier ( $P < 0.001$ ) as compared to gilts in all treatment groups.

Addition of  $\beta$ -mannanase (Hemicell HT) significantly improved ADWG in the grow-finisher phase and in the overall trial. Barrows grew significantly faster than gilts during grow-finisher phases and overall. Pigs fed the PKE supplemented diets consumed significantly more feed during grow-finisher phases and overall as compared to control pigs, resulting in a less favorable FCR, especially during the finisher phase ( $P < 0.001$ ). Moreover, the effect of sex on ADFI and FCR was significant ( $P < 0.001$ ) in grower and finisher phases and overall during the trial. Overall, increasing levels of PKE significantly increased ADWG and ADFI with no significant impact on FCR ( $P = 0.19$ ). No significant difference could be observed between the both PKE supplemented diets. However, in the starter phase, inclusion of PKE supplemented with  $\beta$ -mannanase resulted in a significantly ( $P = 0.004$ ) better FCR as compared to the PKE group.

The obtained overall results are in line with other studies [28] demonstrating no effect of  $\beta$ -mannanase supplementation on energy utilization in PKE fed to pigs through a standardized corn-soybean meal diet. Another study, with addition of PKE at 40% as the only protein source, concluded that obtained values for digestibility of crude protein and amino acids from PKE were sufficient to consider PKE as a useful ingredient in swine diet formulation [38]. Moreover, inclusion of PKE may help to reduce diet costs in swine production [22,38].

In other species, different strategies have been explored to improve PKE digestibility. The effect of fermentation of PKE by *Paenibacilluspolymyxa* ATCC 842 has been explored in broilers on digestibility, intestinal morphology and gut microbiome at inclusions

rates up to 15% [3]. Other physicochemical modifications of PKE, such as water-soaked, microwave-irradiated or a combination of both, have been explored on growth and feed utilization of Nile tilapia [23]. The best results on growth and FCR were obtained with the combination of both microwave-irradiated, water-soaked pre-treatment of PKE. In the current study, no additional pre-treatment of PKE was performed to further optimize the nutrient digestibility of this protein source.

## Conclusions

The current study results suggest that the use of an exogenous heat-tolerant  $\beta$ -mannanase allowed inclusion of at least 5% of PKE as a protein source to replace more expensive protein sources such as SBM with little to no effect on pig performance during the fattening period. Addition of a  $\beta$ -mannanase enzyme resulted in improved FCR during the first stage of fattening (d 0-28), whereas no significant effect on the overall fattening performance was present.

## Ethics Approval and Consent to Participate

Field trial with an EFSA approved feed supplement for use in swine. No additional ethical approval needed. Consent to participate was obtained following full information of farmer on the protocol to be carried out.

## Availability of Data and Material

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

## Competing Interests

The authors declare that they have no other competing interests.

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## Author's Contributions

FV was involved in study design, data collection, data analysis and manuscript preparation. OT was involved in data analysis and manuscript preparation. All authors read and approved the final manuscript.

## Author's Information

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## References

1. Abdollahi MR, Hosking B, Ravindran V. Nutrient analysis, metabolizable energy and ileal amino acid digestibility of palm kernel meal for broilers. *Anim. Feed Sci. Technol.* 2015; 206: 119-125.
2. Agunbiade JA, Wiseman J, Cole DJA. Energy and nutrient use of palm kernels, palm kernel meal and palm kernel oil in diets for growing pigs. *Anim. Feed Sci. Technol.* 1999; 80: 165-181.
3. Alshelmani MI, Loh TC, Foo HL, Sazili AQ, Lau WH. Effect of feeding different levels of palm kernel cake fermented by *Paenibacilluspolymyxa* ATCC 842 on nutrient digestibility, intestinal morphology, and gut microflora in broiler chickens. *Anim. Feed Sci. Technol.* 2016; 216: 216-224.
4. Anderson DM, Hsiao HY, Dale NM. Identification of an inflammatory compound for chicks in soybean meal. *Poult Sci.* 2008; 88: 153-157.
5. Arsenault RJ, Lee JT, Latham R, Carter B, Kogut MH. Changes in immune and metabolic gut response in broilers fed  $\beta$ -mannanase in  $\beta$ -mannan-containing diets. *Poultry Science.* 2017; 96: 4307-4316.
6. Balasubramanian B, Ingale SL, Park JH, Rathi PC, Shanmugam S, Kim IH. Inclusion of dietary  $\beta$ -mannanase improves performance and ileal digestibility and reduces ileal digesta viscosity of broilers fed corn-soybean meal-based diet. *Poult Sci.* 2018; 97: 3097-3101.
7. Carrion S, De Blas JC, Méndez J, Caídas A, García-Rebollar P. Nutritive value of palm kernel meal in diets for growing rabbits. *Anim. Feed Sci. Technol.* 2011; 165: 79-84.
8. Daud MJ, Jarvis MC. Mannan of oil palm kernel. *Phytochem.* 1992; 31: 463-464.
9. Ferrel J, Anderson, DM, Hsiao, H-Y. Content of soluble non-starch polysaccharides  $\beta$ -Mannan and xylan in legume meals, non-legume meals, and cereal grains or cereal grain by-products. *J. Anim. Sci.* 2014; 92: Abstract #328.
10. Forsberg NE, Wang Y. Nutrition and immunity in dairy cattle: implications to hemorrhagic bowel syndrome. *Proc. Mid-South Rum. Nutr Conf.* 2006: 11-20.
11. Freitas TB, Felix TL, Pedreira MS, Silva RR, Silva HGO, Moreira BS. Effects of increasing palm kernel cake inclusion in supplements fed to grazing lambs on growth performance, carcass characteristics, and fatty acid profile. *Anim. Feed Sci. Technol.* 2017; 226: 71-80.
12. Gohl B. Tropical feeds: Feed information summaries and nutritive values. *FAO Anim. Prod. Health Series, FAO, United Nations.* 1981; 12: 364-366.
13. Hindle VA, Steg A, van Vuuren AM, Vroons-de Bruin J. Rumen degradation and post-ruminal digestion of palm kernel by-products in dairy cows. *Anim Feed Sci Technol.* 1995; 51: 103-121.
14. Hsiao H-Y, Anderson DM, Dale NM. Levels of  $\beta$ -mannan in soybean meal. *Poult Sci.* 2006; 85: 1430-1432.
15. Humphrey BD, Klasing KC. The acute phase response alters cationic amino acid transporter expression in growing chickens (*Gallus gallusdomesticus*). *Comp Biochem Physiol A Mol Integr Physiol.* 2005; 142: 485-494.
16. Huntley NF, Nyachoti CM, Patience JF. Lipopolysaccharide immune stimulation but not  $\beta$ -mannanase supplementation affects maintenance energy requirements in young weaned pigs. *Journal of Animal Science and Biotechnology.* 2018; 9.
17. IBUKI M, FUKUI K, KANATANI H, MINE Y. Anti-Inflammatory Effects of Mannanase-Hydrolyzed Copra Meal in a Porcine Model of Colitis. *The Journal of Veterinary Medical Science.* 2014; 76: 645-651.
18. Jackson ME, Anderson DM, Hsiao HY, Mathis GF, Fodge DW. Beneficial Effect of  $\beta$ -Mannanase Feed Enzyme on Performance of Chicks Challenged with *Eimeria* sp. and *Clostridium perfringens*. *Bio One Complete.* 2003; 47: 759-763.
19. Jackson ME, Geronian K, Knox A, McNab J, McCartney E. A dose-response study with the feed enzyme  $\beta$ -mannanase in broilers provided with corn-soybean meal based diets in the absence of antibiotic growth promoters. *Poult Sci.* 2004; 83: 1992-1996.

20. Jeon SM, Hosseindoust A, Choi YH, Kim MJ, Kim KY, Lee JH, et al. Comparative standardized ileal amino acid digestibility and metabolizable energy contents of main feed ingredients for growing pigs when adding dietary  $\beta$ -mannanase. *Animal Nutrition*. 2019; 5: 359-365.
21. Jo JK, Ingale SL, Kim JS, Kim YW, Kim KH, Lohakare JD, et al. Effects of exogenous enzyme supplementation to corn- and soybean meal-based or complex diets on growth performance, nutrient digestibility, and blood metabolites in growing pigs. *Journal of animal science*. 2012; 90: 3041-3048.
22. Kim JS, Ingale SL, Hosseindoust AR, Lee SH, Lee JH, Chae BJ. Effects of mannan level and  $\beta$ -mannanase supplementation on growth performance, apparent total tract digestibility and blood metabolites of growing pigs. *Animal : an international journal of animal bioscience*. 2017; 11: 202-208.
23. Klein J, Williams M, Brown B, Rao S, Lee TJ. Effects of dietary inclusion of a cocktail NSPase and  $\beta$ -mannanase separately and in combination in low energy diets on broiler performance and processing parameters. *J Appl Poult Res*. 2015; 24: 489-501.
24. Li Y, Chen X, Chen Y, Li Z, Cao Y. Effects of  $\beta$ -mannanase expressed by *Pichia pastoris* in corn-soybean meal diets on broiler performance, nutrient digestibility, energy utilization and immunoglobulin levels. *Animal Feed Science and Technology*. 2010; 159: 59-67.
25. Li Y, Chen X, Chen Y, Li Z, Cao Y. Effects of  $\beta$ -mannanase expressed by *Pichia pastoris* in corn-soybean meal diets on broiler performance, nutrient digestibility, energy utilization and immunoglobulin levels. *Animal Feed Science and Technology*. 2010; 159: 59-67.
26. Lv JN, Chen YQ, Guo XJ, Piao XS, Cao YH, Dong B. Effects of Supplementation of  $\beta$ -Mannanase in Corn-soybean Meal Diets on Performance and Nutrient Digestibility in Growing Pigs. *Asian-Australasian Journal of Animal Sciences*. 2013; 26: 579-587.
27. Mok CH, Lee JH, Kim BG. Effects of exogenous phytase and  $\beta$ -mannanase on ileal and total tract digestibility of energy and nutrient in palm kernel expeller-containing diets fed to growing pigs. *Animal Feed Science and Technology*. 2013; 186: 209-213.
28. Mok CH, Kong C, Kim BG. Combination of phytase and  $\beta$ -mannanase supplementation on energy and nutrient digestibility in pig diets containing palm kernel expellers. *Anim Feed Sci Technol*. 2015; 205: 116-121.
29. Onwudike OC. Palm kernel meal as a feed for poultry. 1. Composition of palm kernel meal and availability of its amino acids to chicks. *Animal Feed Science and Technology*. 1986; 16: 179-186.
30. Onwudike OC. Palm kernel meal as a feed for poultry. 2. Diets containing palm kernel meal for starter and grower pullets. *Anim Feed Sci Technol*. 1986; 16: 187-194.
31. Onwudike OC. Palm kernel meal as a feed for poultry 3. replacement of groundnut cake by palm kernel meal in broiler diets. *Animal Feed Science and Technology*. 1986; 16: 195-202.
32. Onwudike OC. Palm kernel meal as a feed for poultry. 4. Use of palm kernel meal by laying birds. *Anim. Feed Sci Technol*. 1988; 20: 279-286.
33. Panigrahi S, Powell CJ. Effects of high rates of inclusion of palm kernel meal in broiler chick diets. *Animal Feed Science and Technology*. 1991; 34: 37-47.
34. Pettey LA, Carter SD, Senne BW, Shriver JA. Effects of beta-mannanase addition to corn-soybean meal diets on growth performance, carcass traits, and nutrient digestibility of weanling and growing-finishing pigs. *Journal of animal science*. 2002; 80: 1012-1019.
35. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2020.
36. Rainbird AL, Low AG, Zebrowska T. Effect of guar gum on glucose and water absorption from isolated loops of jejunum in conscious growing pigs. *British Journal of Nutrition*. 1984; 52: 489-498.
37. Ruhle SWA. Growth rate and carcass characteristics of pigs fed on diets containing palm kernel cake. *Anim Feed Sci Technol*. 1996; 61: 167-172.
38. Son AR, Hyun Y, Htoo JK, Kim BG. Amino acid digestibility in copra expellers and palm kernel expellers by growing pigs. *Anim Feed Sci Technol*. 2014; 187: 91-97.
39. Sukhithasri V, Nisha N, Biswas L, Kumar VA, Biswas R. Innate immune recognition of microbial cell wall components and microbial strategies to evade such recognitions. *Microbiological research*. 2013; 168: 396-406.
40. Upadhaya SD, Park JW, Lee JH, Kim IH. Ileal digestibility of nutrients and amino acids in low quality soybean meal sources treated with  $\beta$ -mannanase for growing pigs. *Animal : an international journal of animal bioscience*. 2016; 10: 1148-1154.
41. Upadhaya SD, Park JW, Lee JH, Kim IH. Efficacy of  $\beta$ -mannanase supplementation to corn-soya bean meal-based diets on growth performance, nutrient digestibility, blood urea nitrogen, faecal coliform and lactic acid bacteria and fecal noxious gas emission in growing pigs. *Arch Anim Nutr*. 2016; 70: 33-43.
42. Van Wyngaard JDV, Meeske R, Erasmus LJ. Effect of palm kernel expeller as supplementation on production performance of Jersey cows grazing kikuyu-ryegrass pasture. *Anim. Feed Sci Technol*. 2015; 199: 29-40.