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Effect of multi-enzyme supplementation on growth performance, digestibility, blood profile, intestinal villus height, and faecal gas emission in weaning pigs

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ABSTRACT. The objective of this study was to evaluate the effects of a multi-enzyme complex containing xylanase, amylase, and protease (XAP) on growth performance, apparent digestibility, blood profile, intestinal villus height, and faecal gas emission in weaning pigs. One hundred and twenty-eight piglets [(Yorkshire × Landrace) × Duroc] were used in a two-phase feeding trial: phase I (days 1 to 14) and phase II (days 15 to 42). Piglets were randomly allotted to two treatments, one 100 mg/kg XAP (xylanases 2000 U/kg diet, amylases 200 U/kg diet, proteases 2000 U/kg diet) and one without, with 16 replicates (two barrows and two gilts) per pen. In phase II, the XAP group showed increased average daily gain (ADG) ($P < 0.05$) compared to the control group. At the end of the feeding trial, piglets supplemented with XAP showed enhanced ADG and gain-to-feed ratio ($P < 0.05$). Moreover, pigs fed the XAP diet had a higher ($P < 0.05$) duodenal and jejunal villus height compared to the control group. There were no significant differences in other parameters. The inclusion of 100 mg/kg XAP in maize-soybean meal-based diets improved growth performance and intestinal villus height in piglets, suggesting its potential benefits for the growth and gut health of these animals.

Introduction

Xylan, a type of haemicellulose present in feedstuff for pigs (Knudsen, 1997; Huisman et al., 1998), can slow down digesta flow rate and increase viscosity in the gut, However, the pig's digestive tract lacks the enzymes, such as xylanases to resolve these issues (Kiarie et al., 2013). Additionally, piglets have immature small intestine villi and lack endogenous enzymes like amylases and proteases (Scheller et al., 2010; Zhang et al., 2014), which can lead to reduced growth and digestibility and increased faecal noxious gas emissions (Pluske et al., 1997; Sterk et al., 2007). High emissions of harmful faecal gases on farms can also cause lung lesions and hinder piglet growth. Inclusion of a multi-enzyme complex containing xylanases, amylases, and proteases (XAP) in the feed can help to hydrolyse dietary xylan, starch and protein, improve growth performance and apparent digestibility (Kiarie et al., 2012; Payling et al., 2017), as well as reduce blood urea nitrogen (BUN) levels in piglets during weaning stress (Diebold et al., 2004). However, it was reported that diets supplemented with xylanases decreased the activity of gut endogenous enzymes, including amylases (Fan et al., 2009).

Other report showed that high-dosage supplementation of XAP (xylanases 2000 U/kg diet, amylases 200 U/kg diet, proteases 2000 U/kg diet) in piglet diets did not lead to an improvement in growth performance (Olukosi et al., 2007).

These previous studies served as guides for preparing the dosage and composition of XAP supplementation in this study. It was also found that limited research had been conducted on the effect of a single- or multi-enzyme supplementation on gut morphology and faecal ammonia, hydrogen sulphide, and total mercaptan gas emissions in piglets, i.e. factors intrinsically linked to gut health. Therefore, the current study was conducted to evaluate the effect of XAP on growth, digestibility, blood creatinine and urea nitrogen levels gut morphology, and noxious gas release from faeces in weaning pigs.

Material and methods

This experiment and protocols (DK-1-1335) involving animals were approved and reviewed by the Animal Care & Use Committee of the Dankook University (South Korea).

XAP preparation

XAP was prepared following the procedure outlined in a previous study (Amerah et al., 2017). The XAP product used in this study contained xylanases, amylases and proteases at doses of 2000, 200, and 2000 U/kg diet, respectively.

Animals, diets, housing and sampling

One hundred and twenty eight piglets [(Yorkshire × Landrace) × Duroc], weaned at day 24 after birth, weighing 6.75 ± 0.16 kg were utilised in the current experiment. These piglets were randomly allocated to 32 pens according to their gender and weaning body weight (BW). There were two treatments studied and each pen (two barrows and two gilts) served as a replicate, amounting to a total of 16 replicates per treatment. The two treatments were as follows: a basal diet (control), and a control diet + 100 mg/kg XAP (xylanases 2000 U/kg diet, amylases 200 U/kg diet, proteases 2000 U/kg diet). To ensure thorough mixing of XAP with the diets, XAP product mixed with light brown powder was added to 5 kg of diet, and subsequently mixed with the remaining feed (Liu and Kim, 2016). The basal diet was pelleted (2.4 mm in diameter) and formulated according to the nutrient requirements outlined by the NRC (2012) for two phases of piglet growth: phase I (days 1 to 14) for piglets weighing 7 to 11 kg and phase II (days 15 to 42) for piglets weighing 11 to 25 kg (Table 1) (NRC, 2012). All piglets were housed in an environmentally controlled room equipped with a mechanical ventilation system. From days 1 to 7, the house temperature was maintained at 30 °C, and then gradually reduced by 1 °C per week over the following 5 weeks.

Table 1. Composition of the basal diets, as-fed basis

Item	Phase I (days 1 to 14)	Phase II (days 15 to 42)
Ingredient, %		
maize	39.25	58.88
soybean meal	25.70	23.40
fish meal	3.00	2.00
whey powder	10.00	8.00
lactose	13.00	-
sprayed dried plasma protein	1.78	-
fermented soybean meal	5.00	5.00
L-lysine HCl	0.30	0.35
DL-methionine	0.15	0.12
L-threonine	0.02	0.01
L-tryptophan	0.01	0.06
limestone	1.06	1.13
dicalcium phosphate	0.29	0.20
vitamin-mineral premix[1]	0.30	0.30
salt	0.09	0.50
phytase	0.05	0.05
Composition, %		
gross energy, kcal/kg	4 122.0	4 046.0
crude protein	20.80	19.20
crude fibre	1.940	2.190
crude fat	6.170	6.400

[1] vitamin premix provided per kg of diet: IU: retinyl acetate 10 000, cholecalciferol 1 300, DL-α-tocopheryl acetate 40; mg: menadion (menadione bisulphate complex) 3.0, riboflavin 5.2, pyridoxine 2.6, niacin 32, d-pantothenic acid (as d-calcium pantothenate) 20; µg: cobalamin 26; mineral premix provided per kg of diet: mg: Cu (as $CuSO_4 \cdot 5H_2O$) 19, Fe (as $FeSO_4 \cdot 7H_2O$) 70, Zn (as $ZnSO_4$) 50, Mn (as MnO_2) 50, I (as KI) 0.5, Co (as $CoSO_4 \cdot 7H_2O$) 0.3, Se (as $Na_2SeO_3 \cdot 5H_2O$) 0.2. The premix did not contain additional copper, zinc, antibiotics or probiotics.

Feedstuff consumption and BW were recorded to calculate average daily gain (ADG), average daily feed intake (ADFI) and gain-to-feed ratio (G:F). An indigestible marker (0.2% chromic oxide) was used to determine the coefficient of apparent total tract nutrient digestibility (CATTD) of gross energy, dry matter (DM) and nitrogen (N). Piglets were fed diets supplemented with chromic oxide from day 7 and day 35. On day 14 and day 42, fresh faeces collected by rectal massage from each piglet in the pen were thoroughly mixed to obtain representative samples in duplicate for each pen. One set of samples was stored in a refrigerator at −20 °C until digestibility analysis, and the other was used directly for faecal gas emission analysis.

On the last day of phases I and II, 12 piglets were randomly selected from different pens within each treatment group. Blood (10 ml) was sampled from the anterior vena cava, half of which was collected into heparinised tubes for BUN analysis, and the rest was transferred into tubes without heparin for blood creatinine analysis. The blood samples were allowed to rest at 24 °C for 30 min and centrifuged (1500 × g, 4 °C) for 20 min to collect serum, which was subsequently stored at −20 °C. On day 42, 12 piglets were euthanized by bloodletting, after which segments measuring 1 cm from the duodenum, jejunum, and ileum were promptly collected (Cai et al., 2015). The intestinal segment samples were rinsed with ice-cold saline and then fixed in 10% formalin for 48 h. Following fixation, the intestinal segment samples were stored at 24 °C until wax embedding.

Laboratory analysis

Faecal and feed samples were thawed before chemical analysis and dried at 70 °C for 72 h. Afterwards, they were ground to a size that could pass through a 1mm screen. The analysis of DM (method 934.01; AOAC International, 2000) and N (method 968.06; AOAC International, 2000) contents in feed and faecal samples was performed according to the methods outlined by AOAC International (2000). The nitrogen content was determined using a 2300 Kjeltec Analyzer (Foss Tecator AB, Hoeganaes, Sweden), while gross energy was measured by an Oxygen Bomb Calorimeter (Parr Instrument Co., Moline, IL). The analysis of chromium content was conducted using UV absorption spectrophotometry (Shimadzu, UV-1201, Shimadzu, Kyoto, Japan). The calculation of digestibility followed the methodology described in our previous study (Cai et al., 2015).

Fresh faecal samples were collected in duplicate and stored in 2.6 l-sealed plastic boxes in weighed amounts of 300 g. A small hole cut in the wall of each box was sealed using adhesive plaster. The samples in the boxes were fermented at 24 °C for 24 h. A GV-100 gas sampling pump (Gastec Corp., Kanagawa, Japan) was utilised for gas detection. The faecal samples were homogenised by shaking for 30 sec. After 5 days, a 100 ml sample of headspace air, located approximately 2 cm above the faecal surface, was obtained after puncturing adhesive plaster. Gas detection and calculations, as well as BUN and blood creatinine level analyses were performed according to the procedures outlined in our previous study (Cai et al., 2015).

Formalin-preserved intestinal segments were embedded in paraffin blocks, which were subsequently cut into 6 µm sections using a microtome and stained with eosin and azur A. The stained villus length was determined using a light microscope equipped with a calibrated ocular micrometre (CH30; Olympus, Tokyo, Japan) at a magnification of 100×.

Statistical analysis

Student's t-test implemented in a SAS software (SAS Institute, Cary, NC, USA) was used to analyse the data in this study. CATTD and growth performance data depended on pens, while other were analysed at the individual pig level. Differences in the range of $0.05 \leq P \leq 0.10$ were considered a trend, and a statistically significant difference was set at $P < 0.05$.

Results

Growth performance and nutrient digestibility

In phase II (days 15 to 42), piglets fed diets with XAP had higher ($P < 0.05$) ADG than the control group (Table 2). Overall, ADG and G:F ratio were increased ($P < 0.05$) in the XAP group compared to the control group. The data in Table 3 show that on day 14, piglets supplemented with XAP showed an increased apparent digestibility of DM compared to the control group ($P < 0.05$), and a tendency towards increased digestibility of gross energy ($P = 0.08$). Throughout the feeding trial, BW, ADFI, and CATTD of N were not affected by XAP supplementation.

Table 2. Effect of multi-enzyme (XAP) on growth performance in piglets

Item	CON	CON + XAP	SEM	*P*-value
Body weight, kg				
initial	6.75	6.75	0.159	0.99
day 14	11.22	11.35	0.190	0.64
day 42	24.36	24.80	0.245	0.22
Days 1 to 14 (phase I)				
ADG, g	320	328	3.8	0.10
ADFI, g	398	396	4.6	0.77
G:F	0.80	0.83	0.014	0.18
Days 15 to 42 (phase II)				
ADG, g	469	480	3.6	0.04
ADFI, g	716	718	6.1	0.80
G:F	0.65	0.66	0.006	0.12
Days 1 to 42 (overall)				
ADG, g	419	430	2.9	0.02
ADFI, g	610	611	4.2	0.88
G:F	0.68	0.70	0.004	0.02

XAP contained: U/kg diet: xylanases 2000, amylases 200, proteases 2000; CON – control, ADG – average daily gain, ADFI – average daily feed intake, G:F – gain-to-feed ratio; SEM – standard error of the mean. $P < 0.05$ indicates that data are significantly different

Table 3. Effect of multi-enzyme (XAP) on apparent total tract nutrient digestibility in piglets

Item	CON	CON + XAP	SEM	*P*-value
Day 14				
dry matter	0.794	0.816	0.008	0.05
nitrogen	0.783	0.796	0.008	0.27
gross energy	0.791	0.810	0.007	0.08
Day 42				
dry matter	0.726	0.742	0.007	0.13
nitrogen	0.697	0.715	0.009	0.16
gross energy	0.734	0.746	0.006	0.18

XAP contained: U/kg diet: xylanases 2000, amylases 200, proteases 2000. CON – control, SEM – standard error of the mean, $P < 0.05$ indicates that data are significantly different

Blood profiles

The data from Table 4 demonstrate that the inclusion of XAP in the piglet diet did not affect BUN and blood creatinine levels.

Table 4. Effect of multi-enzyme (XAP) on blood creatinine and blood urea nitrogen levels in piglets

Item, mg/dl	CON	CON + XAP	SEM	*P*-value
Day 14				
BUN	15.04	12.68	0.852	0.07
BC	1.27	1.35	0.100	0.56
Day 42				
BUN	15.06	14.41	1.066	0.68
BC	1.57	1.53	0.136	0.83

XAP contained: U/kg diet: xylanases 2000, amylases 200, proteases 2000. CON – control, BUN – blood urea nitrogen, BC – blood creatinine, SEM – standard error of the mean, $P < 0.05$ indicates that data are significantly different

Table 5. Effect of multi-enzyme (XAP) on intestinal villus height and faecal ammonia, hydrogen sulphide and total mercaptan emissions in piglets

Item	CON	CON + XAP	SEM	*P*-value
Villus height, µm				
Day 42				
duodenum	462.6	522.6	15.33	0.01
jejunum	455.5	502.2	13.47	0.02
ileum	471.8	506.0	20.01	0.25
Faecal gas emission, ppm				
Day 14				
ammonia	17.8	15.4	1.25	0.20
H_2S	11.6	8.8	1.05	0.10
total mercaptan	6.1	5.4	0.90	0.61
Day 42				
ammonia	10.3	8.8	2.65	0.70
H_2S	8.3	8.6	0.74	0.75
total mercaptan	6.7	6.1	0.60	0.50

XAP contained: U/kg diet: xylanases 2000, amylases 200, proteases 2000. CON – control, H_2S – hydrogen sulphide, SEM – standard error of the mean. $P < 0.05$ indicates that data are significantly different

Intestinal villus height and faecal gas emission

Piglets supplemented with XAP had a higher ($P < 0.01$) villus height in the duodenum and jejunum than the control group at the end of the feeding trial (Table 5). The concentration of ammonia, hydrogen sulphide and total mercaptan was not influenced by the exogenous enzyme.

Discussion

According to the findings of the current study, a positive effect of XAP on ADG, and G:F ratio was observed in piglets. However, BW and ADFI were not affected throughout the feeding trial, which was consistent with previous studies (Owusu-Asiedu et al., 2010; Kiarie and Petracek, 2015; Sangwoo et al., 2020). In addition, there was a trend towards improved apparent digestibility in the XAP group in this study. Earlier works reported that exogenous enzymes (e.g., xylanases, amylases or proteases) did not affect the growth and digestibility of piglets (Yin et al., 2001; Olukosi et al., 2007), which contrasts with the findings of the present study. The positive results recorded in our study may be attributed to several factors. Enhanced nutrient utilisation can contribute to better overall health, growth and performance, as reflected in parameters such as ADG and feed conversion ratio. The observed improvement in intestinal morphological features, particularly an increase in villus height, could be associated with enhanced nutrient absorption. Longer villi provide a larger surface area for nutrient absorption, potentially leading to improved digestibility and nutrient absorption. Nevertheless, differences in research outcomes could be attributed to variances in genetic makeup, age, diet composition, environmental conditions, methodological differences, and the health status of populations studied by individual authors.

BUN is a metabolic waste product that can be used to measure N absorption and excretion rates in a variety of animal species, and its concentration reflects the digestibility of dietary protein (Puchal et al., 1962; Kohn et al., 2005). In our study, we did not observed any significant impact of XAP supplementation on BUN concentration in piglet blood, but a previous study found that piglets supplemented with XAP had a lower BUN level on day 10 of the feeding trial (Yin et al., 2001). Dietary protease supplementation was shown to increase N retention in weaned pigs (Munezero et al., 2022). It seems that the addition of exogenous enzymes in the early post-weaning period may improve the quality

of protein or the balance of amino acid composition in the gut (Amerah et al., 2017). However, another report discovered that the concentration of BUN decreased or tended to decrease in piglets fed diets with xylanases or proteases at the end of a 6-week feeding experiment (Tapingkae et al., 2008; Payling et al., 2017), which is not consistent with the results of the present study. Improved digestibility, as indicated by favourable blood profiles (e.g., lower BUN levels) can suggest that weaning pigs are utilising dietary nutrients more efficiently. The discrepancies among different studies may be due to the composition of exogenous enzymes and nutrient density. On the other hand, exogenous enzymes did not affect blood creatinine levels of piglets in the present and previous studies (Payling et al., 2017).

In this study, the height of duodenal and jejunal villi was increased in XAP-supplemented piglets, but the height of ileal villi was not affected. The increased heights of the villi in the duodenum and jejunum could be the reason for the improved growth performance. On the other hand, non-starch polysaccharide-degrading enzyme supplements in cereal-soybean meal-based diets were previously demonstrated to increase the height of the ileal villi in growing pigs (Willamil et al., 2012). The differences in the basal diet, age, and dietary enzymes may contribute to the variations in villus heights in different segments of the gastrointestinal tract. The improved villus height of the duodenum and jejunum may be due to the fact that proteases may increase the gut content of arginine or other amino acids that are beneficial for intestinal histomorphology (Yao et al., 2011).

Odorous gases such ammonia and hydrogen sulphide are released from pig production facilities and can pollute the environment, potentially affecting pig performance. Emissions of hydrogen sulphide on a farm are mainly derived from faecal undigested organic compounds broken down by anaerobes. In this study, no effect on the faecal hydrogen sulphide content was observed. Generally, faecal gas emissions are related to dietary digestibility; however, we did not recorded improved nutrition digestibility at the end of the feeding trial. To date, there have been only several similar studies published that can be compared with the present work. Previous studies reported that ammonia and mercaptan emissions from the ileal digesta remained unchanged in piglets supplemented with xylanases (Diebold et al., 2005; Kiarie et al., 2012; Tactacan et al., 2016); however, the dietary addition of xylanase was shown to reduce total volatile fatty acid (VFA) concentration in manure (O'Shea et al., 2014). Faecal gas emissions are influenced by the microbial fermentation of undigested nutrients in the lower gastrointestinal tract. Improved nutrient decomposition may result in less undigested substrates reaching the hindgut, potentially leading to reduced gas emissions. It is well-known that total VFAs are associated with faecal odour. Therefore, in future studies, we would like to investigate the effects of XAP on total VFA concentrations.

Conclusions

In conclusion, piglets supplemented with 100 mg/kg XAP (xylanases 2000 U/kg diet, amylases 200 U/kg diet, proteases 2000 U/kg diet) exhibited improved growth performance and intestinal histomorphology in this study. Future research should aim to elucidate the molecular mechanisms underlying the beneficial effects of this blend of xylanases, amylases, and proteases in weaning pigs.

Conflict of interest

The Authors declare that there is no conflict of interest.

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