JAST (Journalof Animal Science and Technology) TITLE PAGE

Upload this completed form to website with submission

ARTICLE INFORMATION	Fill in information in each box below
Article Type	Research article
Article Title (within 20 words without abbreviations)	Effects of emulsifier and β-mannanase supplementation on growth performance and nutrient utilization in grower pigs fed diets with different energy levels
Running Title (within 10 words)	Emulsifier and β-Mannanase Supplementation in Grower Pigs
Author	Seonah Park1, Abdolreza Hosseindoust1, Habeeb Tajudeen1, Sanghun Ha1, Junyoung Mun1, Priscilla Silvestre1, Sodam Choi1, Serin Park1, Sangsik Lee1, Youngin Kim2 and Jinsoo Kim1
Affiliation	1 Department of Animal Industry Convergence, Kangwon National University, Chucheon, Republic of Korea, 24341 2 CTC Bio, Inc., Seoul, 138-858, Republic of Korea, 18569
ORCID (for more information, please visit https://orcid.org)	Seonah Park (https://orcid.org/0009-0007-6584-2777) Abdolreza Hosseindoust (https://orcid.org/0000-0001-9191-0613) Habeeb Tajudeen (https://orcid.org/0000-0002-5623-3175) Sanghun Ha (https://orcid.org/0000-0003-3779-1144) Junyoung Mun (https://orcid.org/0000-0002-3075-7157) Priscilla Silvestre (https://orcid.org/0009-0007-0473-2603) Sodam Choi (https://orcid.org/0009-0009-2711-7106) Serin Park (https://orcid.org/0009-0007-1413-5853) Sangsik Lee (https://orcid.org/0009-0003-2171-8388) Youngin Kim (https://orcid.org/0000-0001-6821-6084) Jinsoo Kim (https://orcid.org/0000-0002-9518-7917)
Competing interests	No potential conflict of interest relevant to this article was reported.
Funding sources State funding sources (grants, funding sources, equipment, and supplies). Include name and number of grant if available.	Not applicable.
Acknowledgements	Not applicable.

Availability of data and material	Upon reasonable request, the datasets of this study can be available from the corresponding author.
Authors' contributions Please specify the authors' role using this form.	Conceptualization: Park SA, Kim JS. Data curation: Silvestre P, Choi SD. Formal analysis: Park SA, Lee SS, Mun JY. Methodology: Tajudeen H, Ha SH, Mun JY. Software: Tajudeen H, Silvestre P, Lee SS, Choi SD. Validation: Hosseindoust A, Kim YI, Kim JS. Investigation: Park SA. Park SR. Writing - original draft: Park SA, Hosseindoust A, Ha SH. Writing - review & editing: Park SA, Hosseindoust A, Tajudeen H, Ha SH, Mun JY, Silvestre P, Choi SD, Park SR, Lee SS, Kim YI, Kim JS.
Ethics approval and consent to participate	The animal care and experimental protocols used in the present study were approved by the Institution of Animal Care and Use Committee, Kangwon National University. (Ethical code: KW-240722-1).

CORRESPONDING AUTHOR CONTACT INFORMATION

For the corresponding author (responsible for correspondence, proofreading, and reprints)	Fill in information in each box below
First name, middle initial, last name	Jinsoo Kim
Email address – this is where your proofs will be sent	kjs896@kangwon.ac.kr
Secondary Email address	
Address	Department of Animal Industry Convergence, Kangwon National University, Chucheon, Republic of Korea, 24341
Cell phone number	+82-10-2566-5961
Office phone number	+82-33-250-8614
Fax number	+82-33-259-5572

Abstract

This study aimed to evaluate the effects of dietary energy level and supplementation with β-mannanase (βM) and an emulsifier (EM) on growth performance, nutrient digestibility, and fecal bacterial populations in grower pigs. A total of 192 pigs (Landrace × Yorkshire × Duroc) with an average initial body weight of 22.78±1.06 kg were randomly assigned to 8 treatments with 6 replicates (4 pigs per pen). The experiment lasted 35 days (phase 1, d 1–21; phase 2, d 22–35) and followed a 2×2×2 factorial design with metabolizable energy levels (3,350 and 3,250 kcal/kg), βM supplementation (- and +0.05%), and EM supplementation (- and +0.05%). There was a tendency toward an interaction between EM and βM supplementation during phase 1 on fecal bacterial populations, by an increase in *Bifidobacterium* abundance ($p = 0.083$) and a decrease in *Salmonella* counts ($p = 0.081$). The higher energy level increased ($p < 0.05$) final body weight, average daily gain, and feed efficiency. The dietary inclusion of EM tended to increase final body weight ($p = 0.081$) and average daily gain ($p = 0.082$). The supplementation of EM increased ($p < 0.05$) the dry matter, gross energy, and ether extract digestibility in phase 1, and enhanced ($p < 0.05$) dry matter, gross energy, organic matter, and ether extract digestibility in phase 2. The fecal *Escherichia coli* population tended to be lower while *Lactobacillus* increased ($p = 0.084$) in the EM-supplemented group ($p = 0.074$). In conclusion, EM supplementation improved growth performance and nutrient digestibility in grower pigs. βM supplementation showed limited effects but tended to interact with EM to help regulate intestinal *Salmonella* populations.

Keywords: β-mannanase, Emulsifier, Grower pigs, Growth performance, Nutrient digestibility.

INTRODUCTION

Corn and soybean meal are the predominant ingredients in swine diets, serving as the main energy and protein sources. Lipids are crucial energy source in swine diets, providing high caloric density in small amounts [1]. Increasing dietary lipid inclusion not only enhances the energy concentration of the diet but also reduces the need for low digestible fibrous plant-based components, thereby improving feed efficiency [2]. However, practical swine diets based on corn and soybean meal inevitably contain substantial amounts of plant-derived non-starch polysaccharides (NSP), which can influence nutrient utilization, including lipid digestion.

Plant-based diets are rich in NSP including β-mannans, glucomannans, and galactomannans, with approximately 150-370 g/kg of the total NSP content [3,4]. Among these, β-mannans are known to increase intestinal digesta viscosity [5], slowing the passage rate and impeding enzymatic digestion and nutrient absorption [6]. Young pigs lack endogenous enzymes capable of hydrolyzing β-1,4-mannosyl and α-1,6-galactosyl linkages, leading to incomplete degradation of β-mannans [7]. Although partial microbial fermentation occurs in the hindgut, the limited fermentative capacity of pigs often results in reduced nutrient digestibility and impaired growth performance [8]. Furthermore, undigested substrates can foster undesirable microbial fermentation, predisposing the gut environment to pathogenic bacterial proliferation [9,10]. To overcome these limitations, supplementation with exogenous β-mannanase (βM) has been proposed as a promising strategy. βM hydrolyzes β-mannans into manno-oligosaccharides (MOS) and mannose, which can be absorbed as additional energy sources [11]. Several studies have reported that βM supplementation reduces digesta viscosity, thereby enhancing nutrient digestibility [9,12] and growth performance in pigs [6,13].

An increased digesta viscosity not only hinders carbohydrate and protein digestion but also interferes with lipid digestion [14]. A viscous intestinal environment restricts lipase access and impairs fat emulsification [15]. Fat digestion depends largely on bile salts and pancreatic lipase, yet dietary fats exhibit inherently low digestibility due to their hydrophobic nature [16]. The use of exogenous emulsifiers (EM), which possess both hydrophilic and hydrophobic groups, has been shown to improve fat digestibility by enhancing emulsification and micelle formation [17-19]. Improved lipid utilization allows diets to be formulated with reduced energy density while maintaining overall nutrient balance and animal performance [20]. Moreover, enhanced fat digestion can promote the absorption of fat-soluble vitamins and improve feed efficiency [21,22]. Both βM and EM are recognized as effective feed additives for improving nutrient utilization and growth in pigs. Nevertheless, there is limited research exploring their combined or interactive effects, particularly under different dietary energy levels. Since βM may indirectly enhance lipid digestibility by reducing digesta viscosity, and EM directly promotes

lipid utilization, their concurrent use may yield synergistic effects, especially in low-energy diets in grower pig. Therefore, the objective of this study was to evaluate the effects of dietary energy level and supplementation with β-mannanase and an emulsifier, individually and in combination, on growth performance, nutrient digestibility, and fecal bacterial populations in grower pigs.

MATERIAL AND METHODS

The Institutional Animal Care and Use Committee of Kangwon National University approved the animal care and experimental techniques utilized in this study (Ethical code: KW-240722-1).

Additive information

The βM (800,000 U of βM/kg) was obtained from a commercial feed company (CTC Bio, Inc., Seoul, Republic of Korea) and it was produced by *Bacillus subtilis*. The EM produced by Molimen in Spain (Phospholipid + Lysophospholipid) was obtained from a commercial feed company (CTC Bio, Inc., Seoul, Republic of Korea).

Animals, experimental designs, and procedures

The study was conducted on a commercial farm in Haman, Gyeongsangnam-do, Republic of Korea. A total of 192 pigs (Landrace × Yorkshire × Duroc; approximately 9 weeks of age) with an initial body weight (BW) of 22.78 ± 1.06 kg were randomly assigned to 8 treatments with 6 replicates (2 barrows and 2 gilts per pen). Pigs were balanced across treatments according to initial body weight, age, and sex. The experiment was conducted for 35 days (phase 1, d 1-21; phase 2, d 22-35). The treatment groups were designed as a 2 × 2 × 2 factorial arrangement with energy levels (3,350 and 3,250 kcal/kg); βM supplementation (0 and 0.05%), and EM supplementation (0 and 0.05%). To ensure accurate inclusion of the low-level additives (0.05%), a pre-mix was prepared and thoroughly mixed with the basal diet using a precision batch mixer. Farm management, feeding, and animal health procedures followed the standard operating protocols established by the research facility, including twice-daily feeding, daily health checks, and strict biosecurity and sanitation measures. Diet formulations and chemical compositions are presented in Table 1, and all diets were formulated to meet or exceed NRC [23] nutrient recommendations. Experimental feeds were provided in mashed form and both feed and water were available *ad libitum*. No antibiotics, antimicrobial agents, or growth promoters were administered throughout the experimental period.

Experimental procedures and sample collection

The experimental pig's BW was measured at the beginning and end of every period for average daily gain (ADG) calculation. Feed intake data were obtained by documenting the amount of feed provided

and the residual feed remaining in the feeders on a daily basis, with phase totals used to determine average daily feed intake (ADFI). Feed efficiency (G:F) was calculated based on ADG and ADFI. Mortality was 0%, and no pigs were removed during the experimental period. Each treatment consisted of 6 replicate pens with 4 pigs per pen, which were maintained until the end of the study.

Nutrient digestibility

To measure nutrient digestibility, a chromic oxide (Cr_2O_3) indicator (0.25%) was added to all diets 7 days prior to sampling. On d 21 and 35, fecal samples were collected from at least one random pig per pen via rectal massage, ensuring uniform representation across all replicates. The collected feces and corresponding feed samples were analyzed for dry matter (DM), organic matter (OM), gross energy (GE), crude protein (CP), ether extract (EE), and crude fiber (CF). Samples were dried in a forced-air oven at 60°C for 72 h and ground through a 1 mm screen (Thomas Wiley Mill, Model 4). Digestibility markers in feed and feces were quantified spectrophotometrically using the method described by Jagger et al. (1992), and nutrient digestibility was calculated using the indicator method as follows:

Digestibility (%) = 100 − [100 × (marker in feed / marker in feces) × (nutrient in feces / nutrient in feed)].

Analytical procedures for DM, CP, EE, and CF followed AOAC International (2007) methods 930.15, 990.03, 960.39, and 978.10, respectively. OM was calculated as DM minus ash content. GE was determined using a bomb calorimeter (Model 1261, Parr Instrument Co., Moline, IL).

Blood vitamin

On d 21 and 35, blood samples were collected from all pigs in each pen to evaluate the retinol (Vitamin A), 25(OH)D_3 (vitamin D), α-tocopherol (vitamin E), and menadione (vitamin K) properties in blood. The collected blood from the pig vena cava vein was centrifuged and the plasma was separated. The separated plasma sample (1ml) was transferred to a 5ml tube, protein was denatured with 500μl of pure ethanol, mixed by vortexer for 15 seconds, and extracted twice with 500μl of n-hexane. The extracted sample was collected from the organic layer and dried using nitrogen. The dried sample was vortexed with 100μl of methanol and centrifuged at 1,380 x g for 30 seconds. The precipitate 35μl was analyzed using an HPLC (high-performance liquid chromatography, Agilent Technologies Inc, Santa Clara, CA, USA) device according to the method of Yang et al. [24].

Fecal bacterial populations

On d 21 and 35, fecal samples were collected from all experimental groups for microbial analysis. Two pigs per pen (1 barrow and 1 gilt) were selected based on their BW to ensure that pigs were similar condition. Feces were directly collected from the rectum using sterile gloves and placed into individually labeled sterile plastic tubes under aseptic conditions. Immediately after collection, samples were snap-frozen in liquid nitrogen, transported to the laboratory, and stored at –80°C until analysis.

For DNA extraction pretreatment, the QIAamp Fast DNA stool Mini Kit (cat. no. 51604/2016) was used, and the procedure is as follows: 1) After weighing 200 mg of fecal samples, they were placed in a 2 ml centrifuge tube and kept on ice. After adding 1 ml InhibitEX buffer to the centrifuge tube, it was vortexed for 1 minute to maximize the DNA concentration and ensure uniform mixing. The samples were centrifuged at 14,000 × g for 1 min to separate pellet particles. 2) 25 µl of proteinase K and 600 µl of the supernatant from step 1 were transferred into a new 2 ml centrifuge tube and vortexed. Then, 600 µl of ethanol was added to the centrifuge tube and vortexed again. The 600 µl supernatant was transferred into a QIAamp spin column and centrifuged for one minute at 14,000 rpm. The QIAamp spin column was placed in a new 2 ml centrifuge tube, and the existing tube was discarded. The QIAamp spin column was opened, 500 µl Buffer AW1 was added, and it was centrifuged under the same conditions. After transferring the QIAamp spin column to a new tube, 500 µl of Buffer AW2 was added and it was centrifuged for three minutes. Finally, DNA was extracted by treating the QIAamp spin column with the same process.

Real-time polymerase chain reaction (qPCR) was employed for quantifying *Lactobacillus* spp., *Bifidobacterium* spp., *Clostridium* spp., *Salmonella* spp., *Escherichia coli* (*E. coli*), and β-actin (reference gene). To quantify microorganisms, 1× universal SsoAdvancedTMR®Green Supermix, 2.5 ng/µl of primers, and 10 ng of DNA were added to a volume of 10 µl [25]. Primer sequences are shown in Table 2. After 40 cycles at 95°C for 15 seconds for enzyme activation, SYBR green fluorescent signals were recorded at 72°C, and PCR results were derived after diluting 10 times for microbial quantification. For microbial quantification, qPCR Rotor-Gene Qiagen 2 plex program (Serial Number 0312272, Corvette Research) was used by Tajudeen et al. [26].

Statistical analysis

Data generated in the present study were statistically analyzed using three-way factorial analysis of variance (ANOVA) based on the General Linear Model (GLM) procedure of SAS software (version 9.4; SAS Institute Inc., Cary, NC, USA) for growth performance, nutrient digestibility, blood vitamin and fecal bacterial populations parameters. The statistical model included dietary energy level, βM, and EM supplementation, and their interactions as fixed effects. The pen was considered the experimental unit for growth performance, whereas the individual pig was considered the experimental unit for nutrient digestibility, blood vitamin and fecal bacterial populations parameters. Significant differences among treatment means were determined using Tukey's honest significant difference (HSD) test. Data are presented as means ± standard error of the mean (SEM). Differences were considered statistically significant at $p < 0.05$, and considered a tendency when $0.05 \leq p < 0.10$.

RESULTS

Growth performance

Pigs fed the high-energy diet had higher ($p < 0.05$) final BW and showed a tendency ($p = 0.081$) for increased BW in the EM-supplemented group (Table 3). During phase 1, there were no significant differences among treatments in ADG, ADFI, or G:F. In phase 2, there was no change in ADG and ADFI, however, pigs fed the high-energy diet tended ($p = 0.091$). Overall (d 1–35), both ADG and G:F were increased ($p < 0.05$) by the high-energy diet, and ADG tended ($p = 0.082$) to be higher in pigs fed EM supplementation.

Nutrient digestibility

There were no significant interaction effects among treatments were observed for nutrient digestibility during the experimental period (Table 4). In phase 1, EM supplementation increased ($p < 0.05$) the digestibility of DM, GE, and EE, whereas CP and CF digestibility were unaffected. Neither dietary energy level nor βM supplementation influenced the digestibility of DM, OM, GE, CP, EE, or CF. In phase 2, EM supplementation increased ($p < 0.05$) the digestibility of DM, OM, GE, and EE, while dietary energy level and βM supplementation had no effects.

Blood vitamin concentration

There were no significant interaction effects among treatments were observed for blood vitamin concentrations in either phase (Figure 1). In phase 1, there was a tendency ($p = 0.087$) for higher blood retinol levels in pigs fed the high-energy diet, without any significant effects on 25(OH)D3, α-tocopherol, and menadione concentrations. The concentration of retinol, 25(OH)D3, α-tocopherol, and menadione in blood was not affected with supplementation of βM and EM. In phase 2, none of the vitamin concentrations differed among treatment groups (Figure 2). Additionally, no interaction effects among treatments were observed in eith.er phase.

Fecal bacterial populations

An interaction tendency between EM and βM supplementation was observed in phase 1, resulting in increased *Bifidobacterium* counts ($p = 0.083$) and decreased *Salmonella* counts ($p = 0.081$) (Table 5). In phase 2, a similar interaction tendency ($p = 0.084$) was observed, showing increased *Lactobacillus* abundance with the combined supplementation of βM and EM. Apart from these tendencies, no significant effects of dietary energy level, βM, or EM supplementation were detected on the populations of *Lactobacillus*, *Bifidobacterium*, *Clostridium*, *Salmonella*, or *E. coli* during either phase. However, EM supplementation tended to decrease *E. coli* counts ($p = 0.074$) in phase 2.

DISCUSSION

Energy sources account for approximately 70% of compound feed in swine production [27]. Consequently, strategies to improve energy utilization have received considerable attention [6,28]. In corn–soybean-based diets, NSP from plant cell walls are indigestible by endogenous enzymes, which can increase digesta viscosity, impair nutrient absorption, and impose metabolic burdens on pigs [29-31]. Therefore, exogenous enzymes such as βM have been widely studied for their ability to hydrolyze β-mannans, reduce viscosity, and enhance nutrient availability [4,32]. Likewise, the inclusion of exogenous EM in pig diets has been proposed to enhance lipid utilization. EM promotes emulsification by breaking down large fat globules into smaller micelles, thereby increasing lipase accessibility and improving energy digestibility [17-19].

In the present study, the high-energy diet improved growth performance, as reflected by a higher G:F despite no change in ADFI. This finding supports the established concept that increasing dietary energy density enhances feed efficiency. Similarly, EM supplementation tended to increase final body weight and ADG, which can be attributed to the observed increases in DM, GE, EE digestibility, reflecting improved lipid emulsification and energy utilization [16,17]. These findings align with the positive role of dietary energy and emulsifiers in grower pigs. In contrast, βM supplementation did not affect growth performance. This absence of effect is likely attributable to the low β-mannan concentration of the experimental diets (0.27–0.28%), which is lower than the β-mannan levels typically present in diets where consistent responses to βM supplementation have been reported in previous studies [31,33]. Mechanistically, βM is known to reduce intestinal viscosity and mitigate immune activation by degrading β-mannans that can mimic pathogen-associated molecular patterns [6]. Reduced immune stimulation may conserve metabolic energy for growth [13]. However, given the relatively low fiber and mannan levels in the current study, neither βM nor its interaction with EM produced measurable improvements in growth performance.

The EM supplementation increased the DM, OM, GE, and EE digestibility, consistent with previous studies demonstrating the positive effects of lysophospholipids and other emulsifiers on nutrient digestibility [19,34,35]. The tendency for higher growth performance in EM-supplemented treatments could be associated with enhanced digestibility. In our study, GE digestibility in phase 1 was higher in the low-energy diet when supplemented with EM compared to the high-energy diet. This suggests that EM can compensate for reduced dietary energy levels by improving fat emulsification and energy extraction efficiency. In contrast, neither dietary energy level nor βM supplementation significantly affected nutrient digestibility. While some studies have reported enhanced digestibility with βM supplementation in high energy diets [36,37], such responses are typically observed in diets with higher NSP or fiber levels [3,15]. The absence of effects on crude fiber digestibility in the present study likely

reflects the limited β-mannan content of the diet. Furthermore, no synergistic interaction was detected between βM and EM, suggesting that adequate substrate availability, either β-mannan or dietary fat, is essential to elicit a complementary effect.

The high-energy diet tended to increase the blood retinol concentration in grower pigs compared to the low-energy diet. This result may be explained by the higher fat content in the high-energy diet (6.55%) than in the 3,250 kcal/kg diet (4.59%). It is well known that a low-fat content in feed limits the absorption of fat-soluble vitamins [22]. However, other fat-soluble vitamins, such as 25(OH)D_3, α-tocopherol, and menadione, did not show significant differences. Currently, there is limited research on how the addition of EM and βM at different dietary energy levels affects blood vitamin levels in grower pigs, indicating a need for further investigation in this area.

Although statistical significance was not detected among treatment groups, there was a tendency for lower *E. coli* colonization with the supplementation of EM in phase 2. Furthermore, the interaction effects between the supplementation of EM and βM tended to increase *Bifidobacterium* and *Lactobacillus* levels while decreasing *Salmonella* levels. These tendencies may be associated with a reduction in undigested nutrients resulting from improved nutrient digestibility, rather than indicating a direct causal relationship. The fecal bacteria count serves as an indirect indicator of the intestinal microbiota, with the distribution of beneficial and harmful bacteria known to impact gut health, digestibility, and growth performance [38]. Factors influencing fecal microorganisms include nutritional elements such as feed composition and nutrients, as well as environmental factors like temperature and stress [39,40]. Some pathogenic microbes, such as *E. coli* and *Salmonella*, can utilize undigested nutrients for fermentation, including protein fractions, under certain dietary conditions and potentially produce harmful metabolites [9]. Among these, nutritional factors have the most significant impact on the gut microbiota, as undigested nutrients interact with gut microorganisms, affecting the microbial community [10,41,42].

CONCLUSION

In conclusion, regardless of dietary energy level, EM supplementation improved growth performance and nutrient digestibility in grower pigs and was reduced in fecal *Escherichia coli* populations. βM supplementation alone showed limited effects; however, a tendency for interaction between βM and EM was observed in fecal bacterial populations during the early growth phase. The reduction of dietary energy level to 3,250 kcal/kg is not recommended due to compromised growth performance, and further studies are warranted to optimize the combined use of βM and EM under practical feeding conditions.

REFERENCES

1. Kerr BJ, Curry SM, Lindblom SC. 2019. Digestibility of energy and caloric value in nursery pigs fed commercially available lipids. Appl Anim Sci. 35(3):291-297. https://doi.org/10.15232/aas.2018-01835

2. Ndou SP, Kiarie E, Walsh MC, Ames N, de Lange CFM, Nyachoti CM. 2019. Interactive effects of dietary fibre and lipid types modulate gastrointestinal flows and apparent digestibility of fatty acids in growing pigs. Br J Nutr. 121:469-480. https://doi.org/10.1017/S0007114518003434

3. Park S, Li W, St-Pierre B, Wang Q, Woyengo TA. 2020. Growth performance, nutrient digestibility and fecal microbial composition of weaned pigs fed multi-enzyme supplemented diets. J Anim Sci. 98(10):skaa306. https://doi.org/10.1093/jas/skaa306

4. Hosseindoust A, Lee SH, Nho WG, Song YH, Shin JS, Ingale SL, Rathi PC, Choi JW, Chae BJ, Kim JS. 2018. A dose–response study to evaluate the effects of pH-stable β-mannanase derived from *Trichoderma citrinoviride* on growth performance, nutrient retention, and intestine morphology in broiler chickens. Ital J Anim Sci. 18(1):147-154. https://doi.org/10.1080/1828051X.2018.1500872

5. Jang JC, Kim KH, Jang YD, Kim YY. 2020. Effects of dietary β-mannanase supplementation on growth performance, apparent total tract digestibility, intestinal integrity, and immune responses in weaning pigs. Animals. 10(4):703. https://doi.org/10.3390/ani10040703

6. Kiarie EG, Steelman S, Martinez M, Livingston K. 2021. Significance of single β-mannanase supplementation on performance and energy utilization in broiler chickens, laying hens, turkeys, sows, and nursery-finish pigs: a meta-analysis and systematic review. Transl Anim Sci. 5(4):txab160. https://doi.org/10.1093/tas/txab160

7. Aranda-Aguirre E, de Blas JC, Cervantes M, et al. 2021. A systematic review on the role of exogenous enzymes on non-starch polysaccharides in pig diets. Anim Feed Sci Technol. 274:114774. https://doi.org/10.1016/j.anifeedsci.2021.114774

8. Kim JS, Ingale SL, Hosseindoust AR, Lee SH, Lee JH, Chae BJ. 2017. Effects of mannan level and β-mannanase supplementation on growth performance, apparent total tract digestibility and blood metabolites of growing pigs. Anim. 11(2):202-208. https://doi.org/10.1017/S1751731116001385

9. Tiwari UP, Chen H, Kim SW, Jha R. 2018. Supplemental effect of xylanase and mannanase on nutrient digestibility and gut health of nursery pigs studied using both in vivo and in vitro models. Anim Feed Sci Technol. 245:77-90. https://doi.org/10.1016/j.anifeedsci.2018.07.002

10. Van Liefferinge E, Forte C, Degroote J, Ovyn A, Van Noten N, Mangelinckx S, Michiels J. 2022. In vitro and in vivo antimicrobial activity of cinnamaldehyde and derivatives towards the intestinal bacteria of the weaned piglet. Ital J Anim Sci. 21(1):493-506. https://doi.org/10.1080/1828051X.2022.2041113

11. Dhawan S, Kaur J. 2007. Microbial mannanases: an overview of production and applications. Crit Rev Biotechnol. 27(4):197-216. https://doi.org/10.1080/07388550701775919

12. Jang JC, Kim KH, Jang YD, Kim YY. 2020. Effects of dietary β-mannanase supplementation on growth performance, apparent total tract digestibility, intestinal integrity, and immune responses in weaning pigs. Animals. 10(4):703. https://doi.org/10.3390/ani10040703

13. Kim JS, Ingale SL, Lee SH, Kim KH, Kim JS, Lee JH, Chae BJ. 2013. Effects of energy levels of

diet and β-mannanase supplementation on growth performance, apparent total tract digestibility and blood metabolites in growing pigs. Anim Feed Sci Technol. 186(1-2):64-70. https://doi.org/10.1016/j.anifeedsci.2013.08.008

14. Lee GI, Hedemann MS, Jørgensen H, Bach Knudsen KE. 2022. Influence of dietary fibre on nutrient digestibility and energy utilisation in growing pigs fed diets varying in soluble and insoluble fibres from co-products. Anim. 16:100511. https://doi.org/10.1016/j.animal.2022.100511

15. Paternostre L, De Boever J, Millet S. 2021. Interaction between fat and fiber level on nutrient digestibility of pig feed. Anim Feed Sci Technol. 281:115126. https://doi.org/10.1016/j.anifeedsci.2021.115126

16. Bontempo V, Comi M, Jiang XR. 2016. The effects of a novel synthetic emulsifier product on growth performance of chickens for fattening and weaned piglets. Anim. 10(4):592-597. https://doi.org/10.1017/S1751731115002189

17. Zhao PY, Li HL, Hossain MM, Kim IH. 2015. Effect of emulsifier (lysophospholipids) on growth performance, nutrient digestibility and blood profile in weanling pigs. Anim Feed Sci Technol. 207:190-195. https://doi.org/10.1016/j.anifeedsci.2015.06

18. Li TS, Liu WC, Zhao PY, Kim IH. 2017. Evaluation of essential oil or/and emulsifier in low energy density diets on growth performance, nutrient digestibility, blood cholesterol and meat quality in finishing pigs. Ital J Anim Sci. 16(4):624-630. https://doi.org/10.1080/1828051X.2017.1325718

19. Kim MJ, Hosseindoust AR, Choi YH, Kumar A, Jeon SM, Lee SH, Jung BY, Kil DY, Chae BJ. 2018. An evaluation of metabolizable energy content of main feed ingredients for growing pigs when adding dietary lysophospholipids. Livest Sci. 210:99-103. https://doi.org/10.1016/j.livsci.2018.01.014

20. Sun HY, Kim IH. 2019. Evaluation of an emulsifier blend on growth performance, nutrient digestibility, blood lipid profiles, and fecal microbial in growing pigs fed low energy density diet. Livest Sci. 227:55-59. https://doi.org/10.1016/j.livsci.2019.06.016

21. Bruno RS, Leonard SW, Park SI, Zhao Y, Traber MG. 2006. Human vitamin E requirements assessed with the use of apples fortified with deuterium-labeled α-tocopheryl acetate. Am J Clin Nutr. 83(2):299-304. https://doi.org/10.1093/ajcn/83.2.299

22. Yang B, Huang S, Yang N, Cao A, Zhao L, Zhang J, Zhao G, Ma Q. 2022. Porcine bile acids promote the utilization of fat and vitamin A under low-fat diets. Front Nutr. 9:1005195. https://doi.org/10.3389/fnut.2022.1005195

23. National Research Council. 2012. Nutrient Requirements of Swine, 11th ed. National Academies Press, Washington DC

24. Yang P, Zhao J, Wang H, Li L, Ma Y. 2020. Effects of vitamin forms and levels on vitamin bioavailability and growth performance in piglets. Appl Sci. 10(14):4903. https://doi.org/10.3390/app10144903

25. Stringer CB, Finlayson JC, Barton RNE, Fernández-Jalvo Y, Cáceres I, Sabin RC, Riquelme-Cantal JA. 2008. Neanderthal exploitation of marine mammals in Gibraltar. Proc Natl Acad Sci USA. 105(38):14319-14324. https://doi.org/10.1073/pnas.0805474105

26. Tajudeen H, Ha SH, Hosseindoust A, Mun JY, Park SR, Park SI, Choi PS, Hermes RG, Taechavasonyoo A, Rodriguez R, Kim JS. 2024. Effect of dietary inclusion of *Bacillus*-based probiotics

on performance, egg quality, and the fecal microbiota of laying hen. Anim Biosci. 37(4):689-696. https://doi.org/10.5713/ab.23.0299

27. Noblet J, Wu SB, Choct M. 2022. Methodologies for energy evaluation of pig and poultry feeds: a review. Anim Nutr. 8:185-203. https://doi.org/10.1016/j.aninu.2021.06.015

28. Tan FP, Beltranena E, Zijlstra RT. 2021. Resistant starch: implications of dietary inclusion on gut health and growth in pigs: a review. J Anim Sci Biotechnol. 12:124. https://doi.org/10.1186/s40104-021-00644-5

29. Kim HJ, Nam SO, Jeong JH, Fang LH, Yoo HB, Yoo SH, Son SW, Ha SH, Kim YY. 2017. Various levels of copra meal supplementation with β-mannanase on growth performance, blood profile, nutrient digestibility, pork quality and economical analysis in growing-finishing pigs. J Anim Sci Technol. 59:19. https://doi.org/10.1186/s40781-017-0144-6

30. Yang P, Zhao J, Wang H, Li L, Ma Y. 2020. Effects of vitamin forms and levels on vitamin bioavailability and growth performance in piglets. Appl Sci. 10(14):4903. https://doi.org/10.3390/app10144903

31. Jang M, Park M, Kim YY. 2024. Effects of varying levels of β-mannanase on growth performance, nutrient digestibility, blood metabolites and diarrhea incidence in weaning pigs. J Anim Sci Technol. https://doi.org/10.5187/jast.2024.e100

32. Kim JS, Hosseindoust A, Ju IK, Yang X, Lee SH, Noh HS, Lee JH, Chae BJ. 2018. Effects of dietary energy levels and β-mannanase supplementation in a high mannan-based diet during lactation on reproductive performance, apparent total tract digestibility and milk composition in multiparous sows. Ital J Anim Sci. 17(1):128-134. https://doi.org/10.1080/1828051X.2017.1345663

33. Huntley NF, Nyachoti CM, Patience JF. 2018. Lipopolysaccharide immune stimulation but not β-mannanase supplementation affects maintenance energy requirements in young weaned pigs. J Anim Sci Biotechnol. 9(47):1-16. https://doi.org/10.1186/s40104-018-0264-y

34. Bai G, He W, Yang Z, Fu H, Qiu S, Gao F, Shi B. 2019. Effects of different emulsifiers on growth performance, nutrient digestibility and digestive enzyme activity in weanling pigs. J Anim Sci. 97(10):4235-4241. https://doi.org/10.1093/jas/skz276

35. Sun HY, Yoon SB, Kim IH. 2019. Growth performance, nutrient digestibility, blood lipid profile and fecal *Escherichia coli* and *Lactobacillus* counts on growing pigs fed with de-oiled lecithin emulsifier. Ital J Anim Sci. 18(1):1111-1116. https://doi.org/10.1080/1828051X.2019.1620140

36. Lv JN, Chen YQ, Guo XJ, Piao XS, Cao YH, Dong B. 2013. Effects of supplementation of β-mannanase in corn-soybean meal diets on performance and nutrient digestibility in growing pigs. Asian-Australas J Anim Sci. 26(4):579-587. https://doi.org/10.5713/ajas.2012.12612

37. An JS, Yun W, Lee JH, Oh HJ, Kim TH, Cho EA, Kim GM, Kim KH, Lee SD, Cho JH. 2020. Effects of exogenous emulsifier supplementation on growth performance, energy digestibility, and meat quality in broilers. J Anim Sci Technol. 62(1):43-51. https://doi.org/10.5187/jast.2020.62.1.43

38. Martin R, Miquel S, Ulmer J, Kechaou N, Langella P, Bermudez-Humaran LG. 2013. Role of commensal and probiotic bacteria in human health: a focus on inflammatory bowel disease. Microb Cell Fact. 12(71):1-11. https://doi.org/10.1186/1475-2859-12-71

39. Isaacson R, Kim HB. 2012. The intestinal microbiome of the pig. Anim Health Res Rev. 13(1):100-

109. https://doi.org/10.1017/S1466252312000084

40. Abubakar RH, Madoroba E, Adenubi OT, Morar-Leather D, Fasina FO. 2017. Bacterial pathogens of pigs with particular reference to *Escherichia coli*: a systematic review and meta-analysis. J Vet Med Anim Health. 9(7):159-185. https://doi.org/10.5897/JVMAH2017.0594

41. Beaumont M, Blachier F. 2020. Amino acids in intestinal physiology and health. Adv Exp Med Biol. 1265:1-20. https://doi.org/10.1007/978-3-030-45328-2_1

42. Blachier F, Andriamihaja M, Kong XF. 2022. Fate of undigested proteins in the pig large intestine: What impact on the colon epithelium? Anim Nutr. 9:110-118. https://doi.org/10.1016/j.aninu.2021.08.001

Tables

Table 1. Experimental basal diet

Metabolizable energy, kcal/kg	3,350	3,250
Ingredients, %		
Corn	59.46	61.62
Soybean meal	16.31	15.90
Dried distiller's grains with soluble	10.00	10.00
Wheat bran	4.00	4.00
Animal fat	4.03	2.00
Molasses	3.00	3.00
$_{L}$-Lysine (78%)	0.56	0.57
$_{DL}$-Methionine (99%)	0.13	0.13
$_{L}$-Threonine (99%)	0.15	0.15
$_{L}$-Tryptophan (100%)	0.31	0.58
Limestone	1.01	1.02
Dicalcium phosphate	0.34	0.33
Salt	0.30	0.30
Vitamin premix[1]	0.15	0.15
Mineral premix[2]	0.15	0.15
Choline chloride	0.05	0.05
Phytase[3]	0.05	0.05
Total	100.00	100.00
Chemical composition, %		
ME, kcal/kg	3,350	3,250
Crude Protein	16.00	16.00
Crude fat	6.55	4.59
Crude fiber	3.57	3.59
Ca	0.66	0.66
Na	0.10	0.10
STTD P	0.31	0.31
SID Lysine	0.98	0.98
SID Threonine	0.59	0.59
SID Met + Cys	0.55	0.55

[1]Supplied per kg of diet: 16,000 IU vitamin A (palmitate), 2.00mg vitamin B_1 (thiamin), 5.00mg vitamin B_2 (riboflavin), 2.00mg vitamin B_6 (pyridoxine), 0.03 mg vitamin B_{12} (cyanocobalamin), 25.00 mg niacin, 0.40 mg folic acid, 0.05 mg biotin, 5.00 mg ethoxyquin, 2,000 IU vitamin D_3 (cholecalciferol), 75.00 mg vitamin E (dl-α-tocopheryl acetate), 2.00 mg vitamin K_3 (menadione).

[2]Supplied per kg of diet: 100 mg Fe, 6 mg Cu, 4 mg Mn, 0.3 mg Se, 0.14 mg I, 0.25 mg Co.

[3]6-phytase from *E. coli* was added at 500 FTU/kg. Assigned nutrient release: 0.10% digestible P, 0.02% Ca.

Table 2. Primer sequence

Item	Primer sequence	Reference
Lactobacillus spp.	F: AGCAGTAGGGAATCTTCCA R: CACCGCTACACATGGAG	Walter et al., 2001
Bifidobacterium spp.	F: TCGCGTCYGGTGTGAAAG R: CCACATCCAGCRTCCAC	Rinttila et al., 2004
Clostridium spp.	F: GGCGGCYTRCTGGGCTTT R: CCAGGTGGATWACTTATTGTGTTAA	Omar et al., 2013
Salmonella spp.	F: TCGTCATTCCATTACCTACC R: AAACGTTGAAAAACTGAGGA	Idrus et al., 2021
Escherichia coli	F: AAAACGGCAAGAAAAAGCAG R: GCGTGGTTACAGTCTTGCG	Amofa et al., 2022
β-Actin	F: CTCCTTCTTGGGCATGGA R: CGCACTTCATGATCGAGTTGA	Leng et al., 2007

Table 3. The effects of β-mannanase (βM) and emulsifier (EM) supplementation in different dietary energy levels (EN) on growth performance of grower pigs.

EN, kcal/kg	3,350				3,250					*p*-value						
βM[1]	-		+		-		+		SEM	EN	EM	βM	EN × EM	EN × βM	EM × βM	EN× EM× βM
EM[2]	-	+	-	+	-	+	-	+								
BW, kg																
Initial	22.78	22.76	22.79	22.79	22.76	22.78	22.77	22.80	0.002	0.470	0.885	0.750	0.750	0.543	0.543	0.885
Final	44.01	44.38	44.05	44.36	43.03	43.83	43.46	43.88	0.174	0.019	0.081	0.646	0.611	0.667	0.681	0.768
Phase 1 (d 0-21)																
ADG, g/d	751	762	751	758	712	746	734	745	9.537	0.269	0.405	0.825	0.716	0.744	0.716	0.824
ADFI, g/d	1,343	1,340	1,335	1,343	1,346	1,350	1,350	1,348	5.837	0.488	0.852	0.956	0.954	0.907	0.923	0.730
G:F, g/g	0.56	0.57	0.56	0.56	0.53	0.55	0.55	0.55	0.007	0.156	0.451	0.789	0.729	0.747	0.635	0.891
Phase 2 (d 22-35)																
ADG, g/d	765	780	767	783	734	757	743	762	8.152	0.185	0.334	0.802	0.879	0.909	0.970	0.939
ADFI, g/d	1,510	1,517	1,500	1,514	1,551	1,522	1,531	1,522	8.775	0.235	0.826	0.633	0.403	0.935	0.707	0.854
G:F, g/g	0.51	0.52	0.51	0.52	0.47	0.50	0.48	0.50	0.007	0.091	0.336	0.735	0.679	0.878	0.867	0.954
Overall (d 0-35)																
ADG, g/d	607	617	608	617	579	602	591	603	3.802	0.020	0.082	0.644	0.609	0.662	0.688	0.767
ADFI, g/d	1,410	1,411	1,401	1,412	1,428	1,419	1,422	1,418	5.071	0.195	0.981	0.713	0.535	0.981	0.744	0.911
G:F, g/g	0.43	0.44	0.43	0.44	0.41	0.42	0.42	0.43	0.003	0.009	0.134	0.579	0.478	0.723	0.598	0.877

[1, 2] -, without supplementation; +, 0.05% supplemented.
Abbreviation: SEM, standard error of means; BW, body weight; ADG, average daily weight gain; ADFI, average daily feed intake; G:F, feed efficiency.
Each value represents the mean of 6 replicate pens (2 barrow and 2 gilts per pen).

Table 4. The effects of β-mannanase (βM) and emulsifier (EM) supplementation in different dietary energy levels (EN) on nutrient digestibility of grower pigs.

EN, kcal/kg	3,350				3,250				SEM	*p*-value[3]						
βM[1]	-		+		-		+			EN	EM	βM	EN × EM	EN × βM	EM × βM	EN× EM× βM
EM[2]	-	+	-	+	-	+	-	+								
Phase 1 (d 21)																
DM, %	78.09	78.19	78.81	79.46	78.88	78.95	78.75	79.53	0.121	0.117	0.016	0.104	0.121	0.917	0.197	0.874
OM, %	79.10	79.20	79.55	80.08	79.51	79.64	79.69	80.14	0.190	0.494	0.197	0.430	0.670	0.967	0.625	0.941
GE, %	82.95	83.34	83.36	83.83	83.07	83.52	83.27	83.64	0.097	0.989	0.037	0.122	0.957	0.460	0.997	0.845
CP, %	79.20	79.38	79.55	79.56	79.28	79.37	79.41	79.99	0.198	0.820	0.427	0.589	0.888	0.764	0.832	0.678
EE, %	70.19	70.76	74.53	75.43	69.59	70.75	74.33	74.89	0.253	0.507	<0.001	0.123	0.947	0.907	0.893	0.646
CF, %	41.59	42.41	42.27	42.70	42.95	42.02	41.55	42.83	0.193	0.813	0.311	0.809	0.558	0.318	0.246	0.100
Phase 2 (d 35)																
DM, %	77.18	77.35	77.76	78.52	77.90	77.94	78.15	78.56	0.130	0.100	0.015	0.191	0.407	0.652	0.364	0.835
OM, %	78.61	78.79	79.07	79.19	78.94	79.00	79.39	79.46	0.110	0.204	0.049	0.621	0.957	0.855	0.948	0.939
GE, %	81.96	82.61	82.28	82.87	82.06	82.67	82.16	82.85	0.120	0.976	0.012	0.380	0.944	0.761	0.978	0.885
CP, %	78.34	78.43	78.55	78.61	78.35	78.38	78.45	78.88	0.196	0.932	0.533	0.698	0.905	0.845	0.813	0.787
EE, %	69.84	70.17	74.02	74.85	69.03	70.39	73.53	74.38	0.253	0.453	<0.001	0.104	0.854	0.608	0.994	0.625
CF, %	42.30	42.57	42.89	42.85	42.35	42.45	42.67	42.75	0.173	0.775	0.773	0.284	0.974	0.859	0.818	0.839

[1, 2] -, without supplementation; +, 0.05% supplemented.

Abbreviation: SEM, standard error of means; DM, dry matter; OM, organic matter; GE, gross energy; CP, crude protein; EE, ether extract.

Each value represents the mean of 6 replicate pens (2 barrow and 2 gilts per pen).

Table 5. The effects of β-mannanase (βM) and emulsifier (EM) supplementation in different dietary energy levels (EN) on fecal bacterial populations of grower pigs

EN, kcal/kg	3,350				3,250				SEM	*p*-value[3]						
βM[1]	-		+		-		+			EN	EM	βM	EN × EM	EN × βM	EM × βM	EN× EM× βM
EM[2]	-	+	-	+	-	+	-	+								
Phase 1 (d 21)																
Lactobacillus	1.42	1.56	1.54	1.58	1.49	1.48	1.46	1.40	0.100	0.394	0.687	0.925	0.418	0.431	0.615	0.873
Bifidobacterium	1.83	2.00	1.77	1.78	1.92	1.99	2.10	1.71	0.130	0.321	0.684	0.278	0.155	0.630	0.083	0.388
Clostridium	0.54	0.52	0.53	0.43	0.57	0.48	0.60	0.73	0.120	0.259	0.799	0.565	0.608	0.243	0.637	0.366
Salmonella	0.79	0.56	0.55	0.63	0.94	0.78	0.56	0.86	0.120	0.192	0.983	0.156	0.332	0.740	0.081	0.839
E. coli	0.61	0.51	0.56	0.63	0.59	0.48	0.45	0.57	0.090	0.421	0.933	0.885	0.875	0.664	0.156	0.847
Phase 2 (d 35)																
Lactobacillus	1.51	1.40	1.44	1.54	1.65	1.57	1.41	1.65	0.110	0.211	0.603	0.773	0.543	0.453	0.084	0.698
Bifidobacterium	1.86	1.96	1.87	1.90	2.03	1.91	1.82	1.86	0.110	0.912	0.888	0.367	0.495	0.520	0.770	0.476
Clostridium	0.68	0.47	0.54	0.44	0.58	0.47	0.57	0.51	0.100	0.923	0.100	0.593	0.626	0.501	0.593	0.834
Salmonella	0.63	0.76	0.56	0.74	0.68	0.67	0.56	0.69	0.130	0.782	0.243	0.612	0.599	0.972	0.612	0.803
E. coli	0.64	0.45	0.51	0.53	0.75	0.45	0.59	0.51	0.100	0.586	0.074	0.625	0.484	0.867	0.166	0.991

[1, 2] -, without supplementation; +, 0.05% supplemented.
Abbreviation: SEM, standard error of means.

Each value represents the mean of 6 replicate pens (2 barrow and 2 gilts per pen).

Figure legends

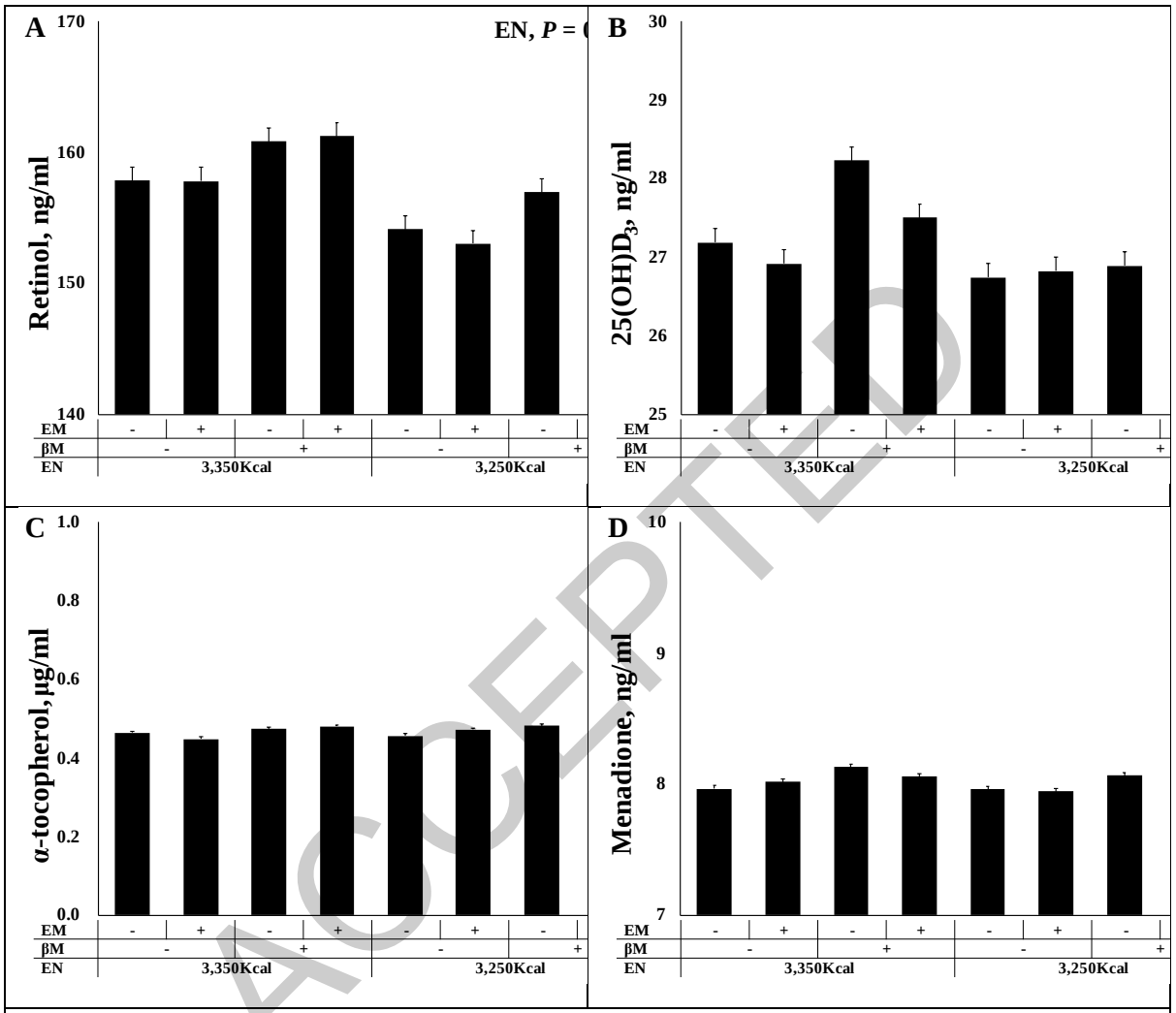

Figure 1. The effects of β-mannanase (βM) and emulsifier (EM) supplementation in different dietary energy levels (EN) on blood vitamin profile of grower pigs (phase 1).

Figure 1. The effects of β-mannanase (βM) and emulsifier (EM) supplementation in different dietary energy levels (EN) on blood vitamin profile of grower pigs (phase 1).

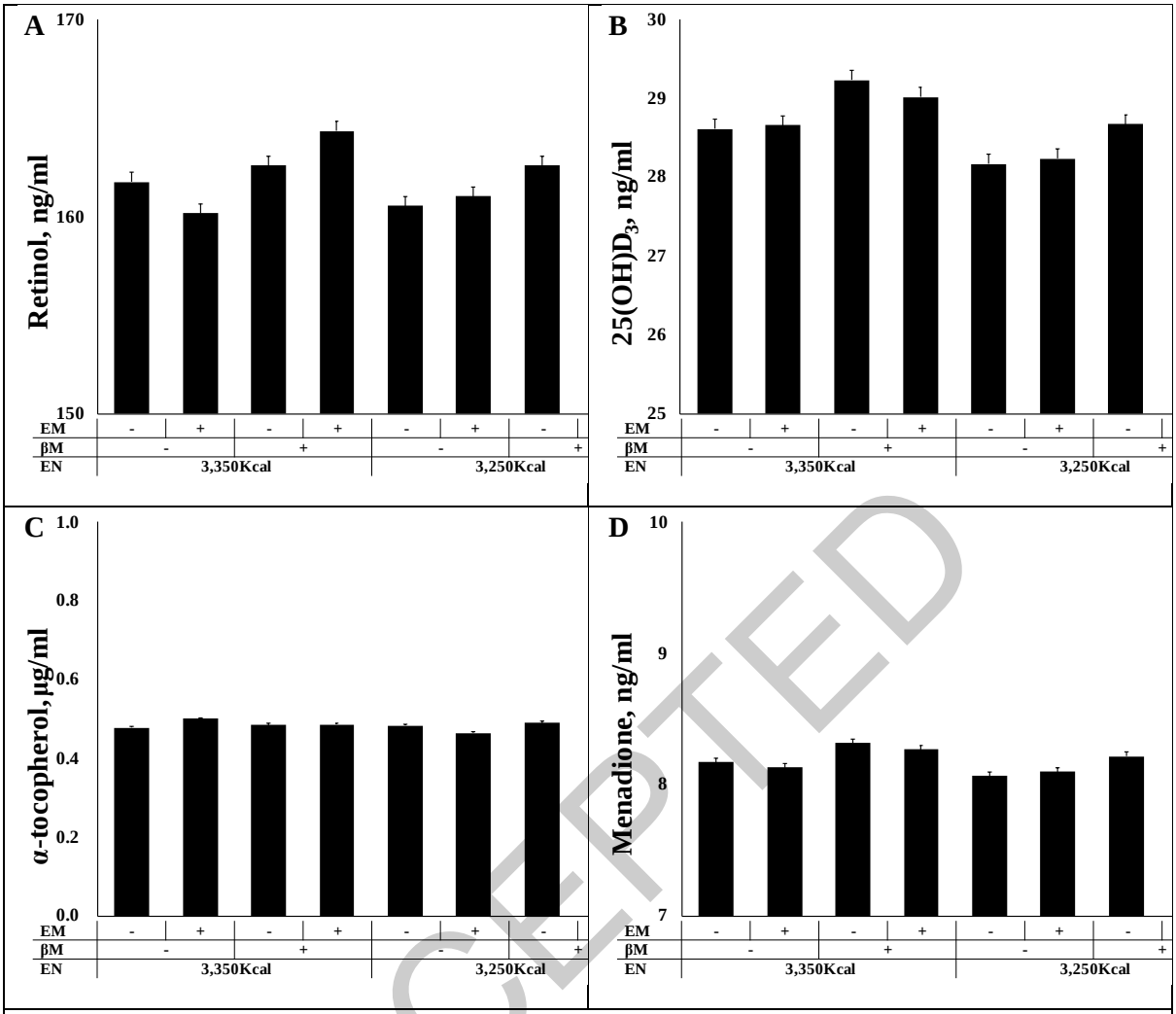

Figure 2. The effects of β-mannanase (βM) and emulsifier (EM) supplementation in different dietary energy levels (EN) on blood vitamin profile of grower pigs (phase 2).

Figure 2. The effects of β-mannanase (βM) and emulsifier (EM) supplementation in different dietary energy levels (EN) on blood vitamin profile of grower pigs (phase 2).