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Effects of glycozyme addition on fatty acid and meat quality characteristics of growing pigs

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Abstract

Synbiotics help to improve gut health by promoting the growth of beneficial bacteria while glyconutrients provide a source of energy for the gut bacteria and may also have immune-modulating effects. The aim of the present study was to assess the effect of this combination on fatty acid and meat quality characteristics of growing pigs. In a five-week experiment, 804 growing three-way crossbred ([Landrace × Yorkshire] × Duroc) pigs with an initial body weight of 31.90 ± 2.6 kg on average were assigned to two treatments: 1) CON (basal diet) and 2) TRT1 (basal diet + 0.3% glycozyme [synbiotics and glyconutrient]), each consisting of 402 pigs. The TRT1 groups showed significantly higher values of palmitoleic acid (C16:1), capric acid (C10:0), myristic acid (C14:0), lauric acid (C12:0), elaidic acid (C18:1, t), pentadecylic acid (C15:0), gondoic acid (C20:1), lignoceric acid (C24:0), and omega-6 : omega-3 in fat than the CON groups. Moreover, in the lean tissues of the pig, the levels of C12:0, C14:0, C17:0, and C20:1 were significantly higher in TRT1 than in CON. However, significant differences were not observed after glycozyme addition in pH, water holding capacity, cooking loss, longissimus muscle area, drip loss, meat color, and sensory evaluation parameters. To conclude, the positive results of the fatty acid composition indicate that glycozyme may be an effective pig feed additive.

Key words: fatty acids, glyconutrient, pork quality, prebiotic, probiotic

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Introduction

Every pig production stage requires a healthy gastrointestinal tract (GIT) to ensure optimal nutritional uptake (Munezero and Kim, 2022). Having a stable GIT allows pigs to use dietary nutrients efficiently and effectively, which leads to high productivity (Liao, 2021; Munezero et al., 2022). In recent years, there has been an escalating interest in improving dietary nutrient utilization to enhance gut health. Glycozyme is becoming increasingly popular as a feed additive, owing to its positive effects on gut health and performance (Valencia et al., 2017; Castro-Pérez et al., 2021).

Glycozyme is a feed additive that is composed of synbiotics and glyconutrients. Synbiotics are a combination of probiotics and prebiotics (Markowiak and Śliżewska, 2018). Probiotics are live

microorganisms that are commonly known for improving or restoring gut microbiota, which in turn contributes to a host's health and general well-being when taken in sufficient amounts (Reid et al., 2003). A few examples of probiotics that have been mostly used as feed additives are *Lactobacillus plantarum, Bacillus subtilis,* and *Saccharomyces cerevisiae. L. plantarum* is known as a safe bacteria and possesses beneficial effects on gut health and immunity, whereas *B. subtilis* is another probiotic that has been shown to improve digestion and reduce gut disorders (Cebeci and Gürakan, 2003; Siezen and van Hylckama Vlieg, 2011; Hu et al., 2014). *S. cerevisiae* is a yeast commonly used in animal feeds because of its ability to improve nutrient utilization (Sun et al., 2022). Prebiotics are fermentable fibers such as oligosaccharides and polysaccharides, which are used in the diet of pigs to calibrate the gut environment by supplying nutrients to the gut microbiota to enable the pigs to achieve optimum performance (Gibson et al., 2004; Han et al., 2022). Therefore, the combination of these two additives will produce a synergistic effect to improve the gut health and performance of animals. Glyconutrients, on the other hand, refer to plant monosaccharides such as galactose, glucose, arabinose, glucosamine, mannose, xylose, rhamnose, and fucose which are thought to be beneficial to the body by nourishing the host's cells and promoting the gastrointestinal health (Mannatech Science, 2023).

Synbiotics and glyconutrients have been proven to improve animal health and performance. Several studies have been conducted to evaluate glycozyme as a feed additive in livestock (Valencia et al., 2017; Castro-Pérez et al., 2021; Núñez-Benítez et al., 2021), but all of them focused on ruminants, and none evaluated the effectiveness of glycozyme in non-ruminants, such as pigs. Therefore, this study aimed to determine how glycozyme affects the meat quality and fatty acid composition in growing pigs. We expect that the treatment containing glycozyme will improve meat quality, as well as produce pork with a more favorable fatty acid composition.

Materials and Methods

Animals and ethics

An approval (DK-2-2136) of our experimental protocol has been received from Dankook University's IACUC after a careful review of our protocol.

Experimental animals, diets, and design

The experiment involved 804 growing pigs of three-way crossbreed ([Landrace × Yorkshire] × Duroc) which started with 31.90 ± 2.6 kg initial body weight (BW) and lasted for 5 weeks. They were selected based on BW and sex. Two dietary treatments used to determine the effect of glycozyme on meat quality and fatty acid composition were 1) CON (basal diet) and 2) TRT1 (basal diet + 0.3% glycozyme), each consisting of 402 pigs. NRC (2012) Requirements for swine were followed when formulating a corn-soybean meal based feed (Table 1). A feed mixer at the research farm was used to mix basal diet with glycozyme consisting of probiotic (*L. plantarum, B. subtilis, S. cerevisiae)*, prebiotic (yeast cell wall ß-glucans), and glyconutrients (N-acetylglucosamine, D-xylose, and Fucose) that was procured from Maxcell Global Co., LTD. (Seoul, Korea). The mixed feeds were allowed to be freely consumed by experimental pigs which were confined in a controlled environment. Water was also adjusted to ensure that it could be freely consumed by the pigs.

Table 1. Experimental diet ingredient composition (as-fed basis).

Raw material	0 - 14 days (%)	15 - 35 days (%)
Corn	63.71	68.91
Soybean meal	19.84	11.90
Rapeseed meal	3.00	4.00
DDGS (corn)	5.00	7.00
Tallow	3.40	3.10
Molasses	2.00	2.00
Limestone	1.24	1.27
MDCP	0.53	0.37
Salt	0.30	0.30
DL-methionine	0.04	-
L-lysine H2SO4	0.41	0.45
L-threonine	0.06	0.07
L-tryptophan (10%)	0.17	0.33
Vit/min premix[z]	0.20	0.20
Phytase	0.05	0.05
Carbohydrase	0.05	0.05
Total	100.00	100.00
Analyzed values		
Moisture	12.90	12.98
CP	16.74	14.41
EE	5.71	5.64
Fiber	2.95	2.89
Ash	5.07	4.72
NSP	120.55	116.40
NDF	10.17	10.80
ADF	2.98	3.09
Ca	0.69	0.66
P	0.42	0.38
Na	0.15	0.16
Cl	0.28	0.28
K	0.83	0.71
Lysine	1.0164	0.8560
Methionine	0.3241	0.2629
Threonine	0.6729	0.5864
Tryptophan	0.1961	0.1771
Met + Cys	0.6204	0.5329

DDGS, dried distillers grains solubles; MDCP, monodicalcium phosphate; CP, crude protein; EE, ether extract; NSP, non-starch polysaccharides; NDF, neutral-detergent fiber; ADF, acid-detergent fiber; Met, methionine; Cys, cystine.

[z] Provided per kg diet: Fe, 100 mg as ferrous sulfate; Cu, 17 mg as copper sulfate; Mn, 17 mg as manganese oxide; Zn, 100 mg as zinc oxide; I, 0.5 mg as potassium iodide; Se, 0.3 mg as sodium selenite; vitamin A, 10,800 IU; vitamin D3, 4,000 IU; vitamin E, 40 IU; vitamin K3, 4 mg; vitamin B1, 6 mg; vitamin B2, 12 mg; vitamin B6, 6 mg; vitamin B12, 0.05 mg; biotin, 0.2 mg; folic acid, 2 mg; niacin, 50 mg; D-calcium pantothenate, 25 mg.

Meat quality evaluation and fatty acids measurement

Fatty acids measurement process

The fatty acid characteristics were analyzed by collecting samples at the end of the test (5 weeks). After the sample was collected using a crude fat extractor, it was put in a cellulose cup and mixed in 5 mL of n-Hexane. Then, 3 mL of BF-3Methanol and 1ml of the extracted sample were added and homogenized, and the solution was reacted at 100℃ for 1 hour. When the reaction was completed, 2 mL of saturated saline and 2 mL of Hexane were mixed, homogenized, and then purified. After 30 minutes, the hexane layer (upper layer) was extracted and analyzed by GC (Gas Chromatography)-FID (Agilent, Santa Clara, USA).

Evaluation of meat quality

After slaughter, the longissimus dorsi muscle was divided and stored at 4℃ for 24 hours before being used for meat quality analysis. The meat color was measured twice for each sample by a device called a chromameter, its Model is CR-410 from Minolta Co. (Osaka, Japan) and the average value was calculated. At this time, the standard color plate was set to 89.2 for L* (lightness), 0.921 for a* (redness), and 0.783 for b* (yellowness). The method of Kauffman et al. (1986) was followed to measure the water holding capacity and the area was obtained with a planimeter (Ushikata 360 d, X-plan, Tokyo, Japan), and the surface area of the meat was expressed as a value divided by the area of moisture. The pH value of meat was measured using a pH meter (Model 77p, Istek, Seoul, Korea) for all samples after slaughter. The area of the longissimus muscle was measured using a planimeter. A cooking loss measurement was performed by measuring the weight of the sample after it had been shaped into a certain shape, placed into a polyethylene bag, heated for 30 minutes at a constant temperature of 75℃, cooled for 30 minutes at room temperature, and then weighed. For sensory evaluation, following the grading criteria based on intramuscular fat and meat color, the meat color (color: 1 - 5), intramuscular fat degree (marbling: 1 - 5), and firmness (firmness: 1 - 5) of the fresh meat were examined. Drip loss was measured after 1, 3, 5, and 7 days after slicing the sample into a uniform shape and packaging it in a polyethylene bag, then storing it in a refrigerator at 4℃ for 7 days.

Statistical analysis

Duncan's multiple range test was carried out with SAS software (SAS Inst. Inc., Cary, NC, USA) to test whether there were significant differences between means for all data.

Results and Discussion

Fatty acid composition in fat and muscle

Tables 2 and 3 show the effects of glycozyme supplementation in diets for growing pigs on the fatty acid profiles of fat and lean tissues in finishing pigs, respectively. The myristic acid (C14:0) and palmitoleic acid (C16:1) as one of the main essential fatty acids in pork (Zhang et al., 2019), were significantly higher in the TRT1 group. The presence of palmitoleic acid in pig meat is believed to be responsible for its pleasant flavor (Cameron et al., 2000). Because fatty acid composition has a significant impact on pork quality (Aboagye et al., 2020; Zhu et al., 2022), meat from these pigs is likely to have an adequate nutritional value which will lead to attractive eating quality. The elaidic acid (C18:1, t) in fat was significantly lower in the

TRT1 group compared to the CON group. This implies that glycozyme supplementation can reduce trans-fatty acid levels, including elaidic acid, which are normally linked to heart disease, diabetes, and other health problems (Islam et al., 2019). The TRT1 group had higher values of lauric acid (C12:0), pentadecylic acid (C15:0), gondoic acid (C20:1), lignoceric acid (C24:0), capric acid (C10:0), and omega 6 : omega 3 fatty acids in fat than the CON group. At the end of the test, the levels of C12:0, C14:0, C17:0, and C20:1 in the muscle for the TRT1 treatment group were improved than those in the CON treatment group. Some research has revealed that probiotics mixed with pig diets can to improve the fatty acid profile of pork (Ross et al., 2012). There is a need to balance certain fatty acid ratios in the diet. This is because people with fatty acid imbalance can have a greater risk of developing cardiovascular disease, inflammation, diabetes, and autoimmune diseases (Simopoulos, 2011). As of today, there is no literature available that discusses the impact of glyconutrients on fatty acid composition that we could use in comparison with the results obtained from our study. However, a combination of probiotics, prebiotics, and glyconutrients may have accounted for the positive results observed.

Meat quality attributes

Table 4 shows the effects of glycozyme supplementation in feeds for growing pigs on the meat quality characteristics of finishing pigs. The meat quality parameters (pH, water holding capacity, cooking loss, longissimus muscle area, drip loss, meat color, and sensory evaluation) did not reveal any significant differences between treatment groups. Some authors have evaluated the effects of probiotics on meat quality for pigs and they found to have positive effects (Alexopoulos et al., 2004; Jukna and Šimkus, 2005), however, other researchers observed contradictory results (Quadros et al., 2001). A study conducted on chicken found that the supply of dietary probiotics improved water-holding capacity, tenderness, and sensory properties (Yang et al., 2010). In the research conducted by Pelícia et al. (2004) and Zhang et al. (2012) failed to demonstrate the synergistic effect between probiotics and prebiotics on the meat quality of chicken. The impact of glyconutrients on meat quality has not been investigated in literature as of today. Thus, we believe that the variable results of our current study with the previous studies may be caused by several factors, including bacteria strains, supplementation level, diet composition, and interactions with other additives.

Conclusion

Based on the result of this study, glycozyme feeding influenced the levels of lignoceric acid (C24:0), gondoic acid (C20:1), elaidic acid (C18:1), margaric acid (C17:0), palmitoleic acid (C16:1), pentadecylic acid (C15:0), myristic acid (C14:0), lauric acid (C12:0), capric acid (C10:0) as well as the ratio of omega 6 : omega 3 fatty acids in pig meat. However, it has been found that the inclusion of glycozyme did not affect the meat quality parameters in any way. The exact mechanisms of action for synbiotics and glyconutrients are not fully understood. Therefore, further research is needed to draw a meaningful conclusion about the relative benefits of glycozyme to the gut health and immune function of swine.

Table 2. The effect of dietary glycozyme supplementation on fatty acid profile in growing pig's fat.

Item (%)	CON	TRT1	SEM
C4:0	0.00	0.00	0.01
C6:0	0.04	0.04	0.01
C8:0	0.04	0.00	0.01
C10:0	0.00b	0.03a	0.00
C11:0	0.00	0.00	0.01
C12:0	0.00b	0.13a	0.00
C13:0	0.00	0.00	0.03
C14:0	0.86b	1.15a	0.00
C14:1	0.00	0.00	0.01
C15:0	0.00b	0.03a	0.00
C15:1	0.00	0.00	0.39
C16:0	20.73	22.17	0.09
C16:1	1.77b	2.34a	0.02
C17:0	0.44	0.44	0.00
C17:1	0.00	0.00	0.64
C18:0	11.45	10.28	0.01
C18:1,t	0.30b	0.22a	0.62
C18:1,c	46.55	45.79	0.00
C18:2n6t	0.00	0.00	0.41
C18:2n6c, LA	14.91	14.56	0.00
C18:3n6	0.00	0.01	0.03
C18:3n3, ALA	0.88	0.83	0.05
C20:0	0.98	0.88	0.01
C20:1	0.00b	0.05a	0.02
C20:2	0.55	0.49	0.04
C20:3n6	0.06	0.17	0.03
C21:0	0.17	0.13	0.01
C20:3n3	0.08	0.08	0.00
C20:4n6	0.00	0.00	0.00
C20:5n3, EPA	0.00	0.00	0.02
C22:0	0.02	0.01	0.03
C22:1n9	0.03	0.00	0.00
C22:2	0.00	0.00	0.01
C23:0	0.10	0.11	0.00
C24:0	0.01b	0.06a	0.00
C22:6n3, DHA	0.00	0.00	0.02
C24:1n9	0.02	0.00	0.03
ω-3 fatty acid	0.88	0.83	0.43
ω-6 fatty acid	14.97	14.73	0.16
ω-6 : ω-3	16.99b	17.62a	1.04
ΣSFA (saturated fatty acids)	34.84	35.46	1.04
ΣUSFA (unsaturated fatty acids)	65.16	64.54	0.65
ΣMUFA (monounsaturated fatty acids)	48.68	48.40	0.47
ΣPUFA (polyunsaturated fatty acids)	16.48	16.15	0.06
MUPA/SFA	1.40	1.37	0.03
PUFA/SFA	0.48	0.46	0.00
Unknown	0.00	0.00	0.00
Total FA	100.00	100.00	0.00

CON, basal diet; TRT1, basal diet + 0.3% glycozyme; SEM, standard error of means; LA, linoleic acid; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

a, b: Means in the same row with different superscripts differ.

Table 3. The effect of dietary glycozyme supplementation on fatty acid profile in growing pig's lean.

Item (%)	CON	TRT1	SEM
Crude fat	18.24	14.14	2.03
C4:0	0.00	0.00	0.00
C6:0	0.04	0.05	0.005
C8:0	0.01	0.01	0.001
C10:0	0.04	0.05	0.006
C11:0	0.00	0.00	0.00
C12:0	0.05b	0.10a	0.005
C13:0	0.00	0.00	0.00
C14:0	0.90b	1.03a	0.013
C14:1	0.02	0.02	0.006
C15:0	0.05	0.07	0.007
C15:1	0.01	0.04	0.03
C16:0	22.30	22.71	0.23
C16:1	2.24	2.56	0.10
C17:0	0.35b	0.40a	0.010
C17:1	0.00	0.00	0.00
C18:0	11.87	11.24	0.55
C18:1,t	0.26	0.25	0.01
C18:1,c	47.71	47.11	0.86
C18:2n6t	0.00	0.00	0.00
C18:2n6c, LA	11.60	11.19	0.41
C18:3n6	0.00	0.01	0.006
C18:3n3, ALA	0.66	0.61	0.014
C20:0	0.88	0.96	0.02
C20:1	0.12b	0.15a	0.006
C20:2	0.39	0.46	0.02
C20:3n6	0.31	0.50	0.16
C21:0	0.00	0.13	0.06
C20:3n3	0.06	0.06	0.002
C20:4n6	0.00	0.00	0.00
C20:5n3, EPA	0.00	0.00	0.00
C22:0	0.01	0.02	0.009
C22:1n9	0.01	0.00	0.007
C22:2	0.00	0.00	0.00
C23:0	0.08	0.16	0.02
C24:0	0.03	0.07	0.01
C22:6n3, DHA	0.00	0.04	0.02
C24:1n9	0.00	0.00	0.00
ω-3 fatty acid	0.66	0.65	0.01
ω-6 fatty acid	11.91	11.70	0.54
ω-6 : ω-3	17.91	18.43	1.12
ΣSFA (saturated fatty acids)	36.60	36.99	0.63
ΣUSFA (unsaturated fatty acids)	63.40	63.01	0.63
ΣMUFA (monounsaturated fatty acids)	50.38	50.14	0.85
ΣPUFA (polyunsaturated fatty acids)	13.02	12.87	0.55
MUPA/SFA	1.38	1.36	0.04
PUFA/SFA	0.36	0.35	0.02
Unknown	0.00	0.00	0.00
Total FA	TRT1	100.00	0.00

CON, basal diet; TRT1, basal diet + 0.3% glycozyme; SEM, standard error of means; LA, linoleic acid; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

a, b: Means in the same row with different superscripts differ.

Table 4. The effect of dietary glycozyme supplementation on meat quality in growing pig.

Item	CON	TRT1	SEM
pH	5.64	5.60	0.05
Longissimus muscle area (mm^2)	8,447.25	9,220.18	536.00
Water holding capacity (%)	59.67	61.94	2.30
Meat color			
L*	47.79	48.22	1.62
a*	10.04	10.99	0.57
b*	7.56	7.73	0.19
Cooking loss (%)	20.04	19.08	2.23
Sensory evaluation			
Color	3.53	3.44	0.09
Firmness	2.90	2.85	0.08
Marbling	2.56	2.66	0.09
Drip loss (%)			
d1	0.47	0.47	0.03
d3	1.20	1.18	0.13
d5	1.94	1.90	0.22
d7	2.55	2.37	0.16

CON, basal diet; TRT1, basal diet + 0.3% glycozyme; SEM, standard error of means; L*, lightness; a*, redness; b*, yellowness; d1, day one; d3, day three; d5, day five; d7, day seven.

Conflict of Interests

No potential conflict of interest relevant to this article was reported.

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