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Effects of Brewer Grain Meal with Enzyme Combination on Growth Performance, Nutrient Digestibility, Intestinal Morphology, Immunity, and Oxidative Status in Growing Pigs

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Abstract: This study investigated the effects of supplementing feed with various levels of brewer grain meal (BGM) and enzymes (amylase, xylanase, β-glucanase, lipase, cellulase, β-mannanase, phytase, and pectinase) on growth performance, nutrient digestibility, intestinal morphology, immunity, and oxidative status in growing pigs. Eighty growing pigs were subjected to four feed treatments (five replicates per treatment), based on a corn-soybean basal diet: feeds with 0.1% enzyme combination supplementation (PC), no enzyme supplementation (NC), 20% BGM with 0.1% enzyme combination (BGM20), and 40% BGM with 0.1% enzyme combination (BGM40). Supplementing the feed with both BGM-supplemented diets significantly increased final body weight, average daily gain, the digestibility of crude protein and ash, serum concentration of total proteins, superoxide dismutase activity, villus height in the duodenum and jejunum, and duodenal villus height to crypt depth ratio; however, it did not significantly increase blood urea nitrogen, tumor necrosis factor-alpha, malondialdehyde levels, and duodenal crypt depth compared to the NC diet ($p < 0.05$). Furthermore, a lower hindgut pH in the middle of the colon was detected following the BGM-supplemented diet compared to PC treatment ($p = 0.005$). Increased levels of triglycerides and albumin were detected in BGM20-fed pigs, whereas increased levels of glucose, total antioxidant capacity, and glutathione peroxidase but decreased interleukine-6 levels were observed in the BGM40 compared with the NC group ($p = 0.05$). No differences were observed in the average daily feed intake and gain to feed ratio, in the serum levels of aspartate aminotransferase or immunoglobulins ($p > 0.05$). The addition of up to 40% BGM combined with 0.1% enzyme supplementation positively promotes the growth performance, nutrient utilization, and intestinal health of growing pigs.

Keywords: brewer grain meal; combined enzymes; growing pigs; nutrient utilization; gut integrity

Citation: Boontiam, W.; Hong, J.; Jaikan, W. Effects of Brewer Grain Meal with Enzyme Combination on Growth Performance, Nutrient Digestibility, Intestinal Morphology, Immunity, and Oxidative Status in Growing Pigs. *Fermentation* **2022**, *8*, 172. https://doi.org/10.3390/fermentation8040172

Academic Editor: Spiros Paramithiotis

Received: 4 March 2022
Accepted: 5 April 2022
Published: 8 April 2022

1. Introduction

The gradual increase in global feed price has a major effect on the net return of swine production. Therefore, the discovery of alternative or unconventional feed ingredients to achieve cost-effective swine production is necessary. Dried brewer's grain meal (BGM), a byproduct of the brewing industry, can be a low-cost replacement for conventional feedstuffs, as it contains a substantial amount of feed residue following the beer manufacturing process [1]. Grain meal contains crude protein (20%), lysine (0.9%), methionine (0.4%), and ether extract (6%) [2]. Additionally, the BGM derived from solid state fermentation has an increased nutritional composition, antioxidant capacity, and reduced phytic acid content [3]. However, the addition of BGM to a swine diet has certain limitations due to a higher non-starch polysaccharides (NSPs) content [4]. It consists of a large quantity of a lignocellulosic material composed of 25% hemicellulose (mainly arabinoxylan) and cellulose, including 28% lignin [4]. This limitation results in an impairment of growth

performance, feed efficiency, and carcass weight in growing pigs fed a 35% BGM diet [5]. Furthermore, substituting sow and weaner meals with greater than 25% BGM significantly decreased average daily gain, daily feed consumption, and feed efficiency in growing pigs [6].

The use of enzyme combinations to degrade the structure and antinutritional factors of NSPs reportedly increases the digestibility and availability of nutrients in a BGM-supplemented diet [7]. An in vitro study showed that the addition of combined enzymes containing peptidase and carbohydrase effectively hydrolyzed BGM into carbohydrate- and protein-derived fractions [8]. In addition, Al-Khalaifah et al. [9] demonstrated that birds fed 10% BGM containing 1 g/kg of fiber-degrading enzymes experienced improved growth performance, meat yield, and gene expression profiles related to digestion and nutrient transport along the avian gastrointestinal tract. However, little is known about the effect of incorporating combined enzymes (amylase, xylanase, β-glucanase, lipase, cellulase, β-mannanase, phytase, and pectinase) in a BGM diet for growing pigs. Therefore, we evaluated the effects of supplementing various levels of BGM with combined enzymes on growth performance, nutrient digestibility, intestinal structure, immunity, and oxidative status in growing pigs.

2. Materials and Methods

All animal protocols were reviewed and approved by the Institutional Animal Care and Use Committee of Khon Kaen University (Khon Kaen, Thailand; authorization no. IACUC-KKU 123/64, approved on 18 November 2021).

2.1. Brewer Grain Meal

The dried BGM was the residue of barley malt in combination with rice from the brewery industry. It contained 91.24% of dry matter (DM), 27.58% of crude protein (CP), 4.61% of ether extract (EE), and 41.57% of crude fiber.

2.2. Animals and Housing

Eighty 10-week-old pigs ((Landrace × Large White) × Duroc), with an average initial body weight (BW) of 25.38 ± 0.28 kg, were used during a 42-day period. All the pigs were grouped in 20 pens (two barrows and two gilts per pen; five replicates per treatment) with a stocking density of 1.08 m^2/pig (pen size, 2.03 m width × 2.13 m length), in a randomized complete block design with BW as the blocking factor. Each pen had concrete flooring, metal spindle walls (1.3 m high), and was provided with a polyvinyl feeder and a nipple drinker under semi-controlled housing. The temperature and relative humidity ranged from 26 °C to 31 °C and 55 % to 65%, respectively. Hygiene and sanitizing practices were performed daily to eliminate ammonia. All pigs were vaccinated for Aujeszky's disease and mycoplasma.

2.3. Experimental Diet

Four experimental diets were devised: (i) a corn-soybean meal (SBM)-based diet with 0.1% enzyme combination (positive control, PC), (ii) corn SBM-based diet without enzyme combination (negative control, NC), and (iii–iv) corn SBM-based diet with 20% (BGM20) and 40% (BGM40) BGM plus 0.1% enzyme combination, respectively. The enzyme product was derived from *Aspergillus niger* and *Bacillus licheniformis* and contained 20,000,000 U/kg amylase, 20,000,000 U/kg xylanase, 10,000,000 U/kg β-glucanase, 9,000,000 U/kg lipase, 7,000,000 U/kg cellulase, 5,000,000 U/kg β-mannanase, and 1,000,000 U/kg phytase and pectinase. The mash diets were calculated to have consistent quantities of CP (19.5%), metabolizable energy (ME, 3265 kcal/kg), Ca (0.67%), P (0.59%), lysine (0.12%), methionine + cysteine (0.68%), and threonine (0.78%), as recommended by the National Research Council [10] (Table 1) for pigs with a body weight from 25 to 50 kg. Mash feed and fresh water were provided ad libitum throughout the entire period.

Table 1. Ingredients and composition values of the experimental diets (% as-fed basis) [1].

Ingredient	PC	NC	BGM20	BGM40
Corn	54.91	55.01	40.24	23.45
Soybean meal (40.38% CP)	29.81	29.81	20.11	10.11
Rice bran	10.00	10.00	10.00	10.00
Rice bran oil	2.03	2.03	6.40	12.00
Brewer grain meal (26.5% CP)	0.00	0.00	20.00	40.00
L-lysine (78% CP)	0.52	0.52	0.52	1.01
DL-methionine (99% CP)	0.32	0.32	0.32	0.48
Threonine (99% CP)	0.20	0.20	0.20	0.46
Dicalcium phosphate	0.25	0.25	0.25	1.03
Limestone	1.26	1.26	1.26	0.76
NaCl	0.35	0.35	0.35	0.35
Combined enzymes complex	0.10	-	0.10	0.10
Vitamin–mineral premix [2]	0.25	0.25	0.25	0.25
Total	100	100	100	100
Calculated value (%)				
Metabolizable energy (kcal/kg)	3265	3265	3265	3265
Crude protein	19.5	19.5	19.5	19.5
Lysine	1.12	1.12	1.11	1.11
Methionine + cysteine	0.68	0.68	0.66	0.68
Threonine	0.78	0.78	0.74	0.78
Ca	0.67	0.67	0.67	0.67
Total phosphorus	0.59	0.59	0.59	0.59
Crude fiber	4.78	4.78	12.05	19.23
EE	5.73	5.73	9.79	14.84

[1] PC, positive control with combined enzymes in the corn-soybean basal diet; NC, negative control without combined enzymes inclusion; BGM20, a basal diet with 20% brewer grain meal + 0.1% combined enzymes inclusion; BGM40, a basal diet with 40% brewer grain meal + 0.1% combined enzymes inclusion. [2] Supplied (per kilogram of complete diet): vitamin A, 4,000,000 IU; vitamin D3, 600,000 IU; vitamin E, 8 g; vitamin K_3, 0.4 g; vitamin B_1, 0.3 g; vitamin B_2, 1 g; vitamin B_6, 0.5 g; vitamin B_{12}, 4 mg; niacin, 4 g; choline chloride, 30 g; calcium pantothenate, 3 g; biotin, 10 mg; folic acid, 0.1 g; cobalt, 0.2 g; copper, 40 g; ferrous, 36 g; manganese, 16 g; zinc, 20 g; iodine, 0.2 g; selenium, 0.02 g; and ethoxyquin, 10 g.

2.4. Growth Performance

At the beginning and end of the 42-day feeding period, each pig's body weight (BW) was measured. Feed intake was recorded by measuring the amount of feed provided and the residual feed, to calculate the average daily gain (ADG), average daily feed intake (ADFI), and gain-to-feed ratio (G/F).

2.5. Nutrient Digestibility

A total of 20 barrows (average BW of 29.38 ± 0.24 kg) were allotted to 4 treatments in 5 replicates. During a 5-day fecal collection, chromic oxide and ferric oxide (2 g/100 g feed) were homogeneously mixed in the diet as an indigestible marker at both the first and last meals, respectively. The experimental diet was offered at 12 h intervals (at 07:00 and 19:00). The fecal collection was initiated when the first marker appeared in the feces and terminated when the final marker was observed in the feces, as previously described by Adeola [11]. Fresh feces were kept in a sealed plastic container and subsequently dried in a drying oven at 65 °C for 72 h. Diets and feces were subsequently ground in a grinding mill (Wiley Mill, Arthur H. Thomas Co., Philadelphia, PA, USA) using a 0.88 mm sieve particle size, and then sampled as 300 g quantities in sealed plastic bags for nutrient assays. The official method for feed and fecal samples, as detailed in Association of Official Analytical Chemists [12], was followed for the analysis of DM (#930.15 using an oven at 135 °C for 2 h), CP (#984.13, N × 6.25 using Kjeldahl method), ash (#942.15 using a muffle furnace at 600 °C for 2 h), and EE (#920.39 using a Soxhlet apparatus). All analyses were performed in triplicate for the calculation of apparent total tract digestibility (ATTP) as the following Equation (1):

$$ATTP(\%) = \frac{X_{ingested} - X_{excreted}}{X_{ingested}} \times 100 \quad (1)$$

where $X_{ingested}$ indicates total intake of DM, CP, ash, and EE in the feed (% DM), and $X_{excreted}$ indicates total fecal excretion of DM, CP, ash, and EE (% DM).

2.6. Blood Collection and Chemical Analyses

At the end of the experiment (42 days), 20 pigs (five pigs per treatment, one pig per pen), with BW close to the average BW of the pen, were chosen and slaughtered by captive bolt stunning after 12 h fasting. Upon euthanasia, blood was collected from the 20 pigs via jugular vein puncture using a disposable syringe and needle (Nipro Corporation, Ayutthaya, Thailand). The sample was divided into three portions in serum vacutainer tubes coated with silica (Greiner Bio-one, Chonburi, Thailand, 4 mL/tube, n = 60), and immediately centrifuged at 1872× *g* at 4 °C for 15 min to harvest the serum. The first portion (20 samples) was assayed for blood metabolites: aspartate aminotransferase (AST, as 0.1 M glutamate standard), glucose, triglycerides (1 mM triglyceride standard), total cholesterol (2 μg/μL cholesterol standard), total protein (TP), albumin, and blood urea nitrogen (BUN, 100 mL urea standard). All metabolites were assayed using colorimetric kits (Abcam Assay Kits, Cambridge, UK), and the values were detected by measuring absorbance at 532 nm except for AST, which was detected at 450 nm as per the manufacturer's instructions. The second portion (20 samples) of blood samples was used to assay immunoglobulins (IgA), interleukine-1β (IL-1β), IL-6, and tumor necrosis factor-alpha (TNFα) using ELISA porcine immunoassay kits (Bethyl Laboratories, Montgomery, LA, USA) at an absorbance of 450 nm. The third blood (20 samples) portion was used to quantify the serum antioxidant status by measuring total antioxidant capacity (TAC) and levels of superoxide dismutase (SOD), glutathione peroxidase (GPx), and malondialdehyde (MDA) using specific commercial kits (Sigma-Aldrich, St. Louis, MO, USA). All assays were completed in triplicate to minimize variation.

2.7. Intestinal Morphology

After blood collection, the digestive tract was removed, and sections of the duodenum (about 50 cm caudal from the pylorus of the stomach), jejunum (5 cm between stomach sphincter and the ileocecal junction), and ileum (15 cm above the ileocecal orifice) were excised and opened longitudinally at the mesenteric attachment. Each section was flushed with phosphate-buffered solution and kept in a neutral buffer (pH 7.4) with formalin (10% vol/vol) in an automatic shaker for 48 h. Then, each section was dehydrated with ethanol and xylene, and embedded in paraffin wax at 60 °C. A rotary microtome (Leica RM2235, Wetzlar, Germany) was used to transversely cut each paraffin section into 5 μm thick slices. Each section was then placed on a glass slide, stained with hematoxylin, and counter-stained with eosin (H&E staining, Sigma-Aldrich, St. Louis, MO, USA) for an oculometry study of intestinal morphology at a magnification of 40× using a compound microscope (Olympus Biological Model CX31, Shinjuku, Tokyo, Japan). Ten well-oriented villi per stain section were randomly chosen, and villus height (VH, from the villus tip to the muscularis mucosa), crypt depth (CD, between brush border membrane), and villus-to-crypt depth ratio (VH/CD, by calculation) were measured.

2.8. Hindgut pH

At the end of the experiment, the cecum and colon were immediately collected from the euthanized pigs (20 samples). Sections of colonic digesta were cut into three equal segments (proximal, middle, and distal). The pH was measured using a portable pH meter (AP 110, Fisher Scientific, Pittsburgh, PA, USA) after a 2-point calibration with standard solutions (pH 4 and 7).

2.9. Statistical Analysis

All data were analyzed using the SAS package (version 9.4, SAS Institute, Inc., Cary, NC, USA) according to a randomized, complete block design using the GLM procedure, considering the diets as the main effect and the replication as the block. Each pen (n = 20)

was an experimental unit for growth performance, whereas an individual pig was the experimental unit for nutrient digestibility, blood metabolites, immunity, oxidative status, intestinal morphology, and hindgut pH. Duncan's new multiple range test was used to compare significant differences among dietary treatments. Statistical significance was defined as $p < 0.05$ for all measurements. The represented values were reported as the mean plus standard error of mean (SEM).

3. Results

3.1. Growth Performance

The inclusion of PC, BGM20, and BGM40 significantly increased the final BW ($p = 0.005$) and ADG ($p = 0.019$) compared to the NC diet, but no differences between the PC and the BGM-supplemented diets were observed (Table 2). No differences were observed for ADFI and G/F between dietary treatments.

Table 2. Effect of dietary BGM with combined enzymes inclusion on growth performance of growing pigs [1].

Item	Treatment				SEM	*p*-Value
	PC	NC	BGM20	BGM40		
Initial BW (kg)	25.34	25.41	25.11	25.66	0.283	0.601
Final BW (kg)	50.11 [a]	48.34 [b]	51.26 [a]	50.33 [a]	0.458	0.005
ADG (g)	590 [a]	546 [b]	610 [a]	600 [a]	12.7	0.019
ADFI (g)	1546	1504	1518	1536	33.3	0.822
G/F	0.382	0.364	0.402	0.391	0.0112	0.121

Data are shown as group mean plus SEM (5 replicates per treatment). [1] BW, body weight; ADG, average daily gain; ADFI, average daily feed intake; G/F, gain-to-feed ratio; PC, positive control with combined enzymes in the corn-soybean basal diet; NC, negative control without combined enzymes inclusion; BGM20, a basal diet with 20% brewer grain meal + 0.1% combined enzymes inclusion; BGM40, a basal diet with 40% brewer grain meal + 0.1% combined enzymes inclusion. [a,b] Values in the same rows with different superscript letters differ significantly ($p < 0.05$).

3.2. Nutrient Digestibility

As shown in Table 3, an improvement in CP and ash digestibility was observed in pigs fed the BGM with enzyme-supplemented diets compared to the NC diet ($p = 0.048$ and $p = 0.039$, respectively) but not compared to PC diet ($p > 0.05$). However, treatments did not affect the digestibility of DM and EE ($p > 0.05$).

Table 3. Effect of dietary BGM with combined enzymes inclusion on nutrient digestibility of growing pigs [1].

Item	Treatment				SEM	*p*-Value
	PC	NC	BGM20	BGM40		
Apparent total tract digestibility (%)						
DM	90.66	87.06	92.79	91.61	1.320	0.062
CP	86.99 [a,b]	82.11 [b]	89.68 [a]	88.09 [a]	1.648	0.048
Ash	51.93 [a,b]	44.93 [b]	70.52 [a]	69.09 [a]	6.145	0.039
EE	74.58	66.47	81.16	75.77	3.596	0.098

Data are shown as group mean plus SEM (n = 5 pigs per treatment). [1] DM, dry matter; CP, crude protein; EE, ether extract; PC, positive control with combined enzymes in the corn-soybean basal diet; NC, negative control without combined enzymes inclusion; BGM20, a basal diet with 20% brewer grain meal + 0.1% combined enzymes inclusion; BGM40, a basal diet with 40% brewer grain meal + 0.1% combined enzymes inclusion. [a,b] Values in the same rows with different superscript letters differ significantly ($p < 0.05$).

3.3. Blood Metabolites

The concentrations of glucose ($p = 0.024$) and TP ($p = 0.025$) were significantly higher in pigs fed the BGM40 diet than those fed the NC diet (Table 4). Moreover, the BGM20

diet showed positive improvements in triglyceride (p = 0.045) and albumin (p = 0.036) concentrations when compared to the NC diet. A lower concentration of BUN was also found after the BGM-supplemented treatments compared to after the NC diet (p = 0.028). However, there was no change in AST and total cholesterol concentrations after BGM or enzyme supplementation.

Table 4. Effect of dietary BGM with combined enzymes inclusion on blood metabolites of growing pigs [1].

Item	Treatment				SEM	*p*-Value
	PC	NC	BGM20	BGM40		
AST (U/L)	84.24	77.63	76.04	82.90	4.969	0.603
Glucose (mg/dL)	150.98 [a,b]	141.02 [b]	155.63 [a,b]	164.46 [a]	4.605	0.024
Triglyceride (mg/dL)	68.53 [a]	58.37 [b]	66.91 [a]	65.37 [a,b]	2.345	0.045
Total cholesterol (mg/dL)	90.16	87.68	87.77	91.76	4.672	0.907
TP (g/dL)	6.32 [a,b]	5.61 [b]	6.92 [a]	6.56 [a]	0.261	0.025
Albumin (g/dL)	4.66 [a,b]	3.88 [b]	5.21 [a]	4.59 [a,b]	0.274	0.036
BUN (mg/dL)	20.62 [a,b]	22.19 [a]	18.11 [b]	17.76 [b]	1.014	0.028

Data are shown as group mean plus SEM (n = 5 pigs per treatment). [1] AST, aspartate aminotransferase; TP, total protein; BUN, blood urea nitrogen; PC, positive control with combined enzymes in the corn-soybean basal diet; NC, negative control without combined enzymes inclusion; BGM20, basal diet with 20% brewer grain meal + 0.1% combined enzymes inclusion; BGM40, a basal diet with 40% brewer grain meal + 0.1% combined enzymes inclusion. [a,b] Values in the same rows having different superscript letters differ significantly (p < 0.05).

3.4. Immunity and Pro-Inflammatory Cytokines

The supplementation of BGM and PC diets showed a reduction in the pro-inflammatory cytokine secretion of IL-6 (p = 0.024) and TNFα (p = 0.003) over those of the NC pigs. BGM20 and NC showed no significant difference for IL-6 (Table 5). No significant effects on IgA and IL-1β in the growing pigs were found among the dietary treatments.

Table 5. Effect of dietary BGM with combined enzymes inclusion on the immunity of growing pigs [1].

Item	Treatment				SEM	*p*-Value
	PC	NC	BGM20	BGM40		
IgA (mg/dL)	0.97	0.84	1.04	0.99	0.148	0.805
IL-1β (pg/mL)	306.53	337.33	286.37	309.13	36.452	0.803
IL-6 (pg/mL)	116.85 [b]	135.69 [a]	122.05 [a,b]	109.76 [b]	5.169	0.024
TNFα (pg/mL)	53.72 [b]	77.08 [a]	58.57 [b]	55.16 [b]	3.787	0.003

Data are shown as group mean plus SEM (n = 5 pigs per treatment). [1] IgA, immunoglobulins A; IL-1β, interleukin-1β; IL-6, interleukine-6; TNFα, tumor necrosis factor-alpha; PC, positive control with combined enzymes in the corn-soybean basal diet; NC, negative control without combined enzymes inclusion; BGM20, a basal diet with 20% brewer grain meal + 0.1% combined enzymes inclusion; BGM40, a basal diet with 40% brewer grain meal + 0.1% combined enzymes inclusion. [a,b] Values in the same rows with different superscript letters differ significantly (p < 0.05).

3.5. Oxidative Stress

Compared with control diets, BGM40 significantly increased the activities of TAC, SOD, and GPx (p < 0.05); however, the activities of TAC and GPx in BGM20 were not significantly different from those in NC (p > 0.05). In addition, a decreased concentration of MDA was observed in the BGM-supplemented diet compared to the PC diet (p < 0.014; Table 6).

Table 6. Effect of dietary BGM with combined enzymes inclusion on oxidative stress of growing pigs [1].

Item	Treatment				SEM	*p*-Value
	PC	NC	BGM20	BGM40		
TAC (U/mL)	6.62 [c]	8.13 [b,c]	9.08 [b]	11.14 [a]	0.644	0.003
SOD (U/mL)	86.47 [b]	92.58 [b]	112.33 [a]	123.06 [a]	5.571	0.002
GPx (U/mL)	774.24 [c]	881.45 [b,c]	941.20 [a,b]	1006.69 [a]	38.435	0.007
MDA (nmol/mL)	7.94 [a]	6.46 [a,b]	5.78 [b]	4.46 [b]	0.623	0.014

Data are shown as group mean plus SEM (n = 5 pigs per treatment). [1] TAC, total antioxidant capacity; SOD, superoxide dismutase; GPx, glutathione peroxidase; MDA, malondialdehyde; PC, positive control with combined enzymes in the corn-soybean basal diet; NC, negative control without combined enzymes inclusion; BGM20, a basal diet with 20% brewer grain meal + 0.1% combined enzymes inclusion; BGM40, a basal diet with 40% brewer grain meal + 0.1% combined enzymes inclusion. [a,b,c] Values in the same rows having different superscript letters differ significantly ($p < 0.05$).

3.6. Intestinal Morphology

Longer VHs in the duodenum and jejunum, including a greater VH/CD ratio and shorter CD in the duodenum, were observed in pigs fed BGM and PC-supplemented diets compared to the pigs in the NC group, except for duodenal CD ($p < 0.05$) (Table 7). However, none of the treatments affected the intestinal morphology of the ileum segment.

Table 7. Effect of dietary BGM with combined enzymes inclusion on intestinal morphology of growing pigs [1].

Item	Treatment				SEM	*p*-Value
	PC	NC	BGM20	BGM40		
Duodenum						
VH (μm)	629.96 [a]	530.64 [b]	653.73 [a]	623.78 [a]	27.098	0.035
CD (μm)	344.61 [a,b]	361.33 [a]	309.78 [c]	312.72 [b,c]	10.775	0.014
VH/CD	1.84 [a]	1.47 [b]	2.12 [a]	2.02 [a]	0.106	0.005
Jejunum						
VH (μm)	509.85 [a,b]	466.11 [b]	548.67 [a]	517.95 [a]	16.033	0.024
CD (μm)	254.53	262.76	249.71	246.01	18.150	0.922
VH/CD	2.12	1.78	2.22	2.13	0.183	0.371
Ileum						
VH (μm)	443.78	406.87	433.94	452.94	24.104	0.580
CD (μm)	207.21	217.84	180.69	196.61	13.654	0.306
VH/CD	2.23	1.88	2.47	2.35	0.227	0.323

Data are shown as group mean plus SEM (n = 5 pigs per treatment). [1] VH, villus height; CD, crypt depth; VH/CD, villus-to-crypt-depth ratio; PC, positive control with combined enzymes in the corn-soybean basal diet; NC, negative control without combined enzymes inclusion; BGM20, basal diet with 20% brewer grain meal + 0.1% combined enzymes inclusion; BGM40, a basal diet with 40% brewer grain meal + 0.1% combined enzymes inclusion. [a,b,c] Values in the same rows with different superscript letters differ significantly ($p < 0.05$).

3.7. Hindgut pH

As shown in Table 8, lower hindgut pH in the middle colon was observed in pigs who consumed BGM-supplemented diets and the NC diet than in those fed the PC ($p = 0.005$). However, the hindgut pH in the cecum and proximal and distal colon was unaffected among dietary treatments.

Table 8. Effect of dietary BGM with combined enzymes inclusion on hindgut pH of growing pigs [1].

Item	Treatment				SEM	*p*-Value
	PC	NC	BGM20	BGM40		
Cecum	6.43	5.89	5.28	5.11	0.326	0.054
Colon						
Proximal colon	6.77	5.42	5.08	5.31	0.485	0.112
Middle colon	6.89 [a]	5.32 [b]	5.16 [b]	4.91 [b]	0.334	0.005
Distal colon	6.54	5.29	5.56	5.51	0.388	0.157

Data are shown as group mean plus SEM (n = 5 pigs per treatment). [1] DM, dry matter; CP, crude protein; EE, ether extract; PC, positive control with combined enzymes in the corn-soybean basal diet; NC, negative control without combined enzymes inclusion; BGM20, a basal diet with 20% brewer grain meal + 0.1% combined enzymes inclusion; BGM40, a basal diet with 40% brewer grain meal + 0.1% combined enzymes inclusion. [a,b] Values in the same rows with different superscript letters differ significantly ($p < 0.05$).

4. Discussion

The contents of crude fiber and EE in the PC diet were lower than the those in the BGM-supplemented treatment; however, the growth performance in cases of both supplemented treatments were not different. There was no clear evidence to establish the benefit of enzyme combination in promoting the growth performance of pigs that were fed a corn-soybean basal diet, which was similar to the BGM-supplemented diets. A plausible explanation for this observation may be the presence of bioactive compounds (isoflavones (2096 mg/kg) and saponins (30 to 100 μ/mL)) in corn SBM-based diets, which have potent antibacterial and anti-inflammatory properties [13]. These properties may prevent the secretion of pro-inflammatory cytokines, such as IL-6 and TNFα, thereby enhancing immunity, [13] which in turn would promote the performance of the animals, as observed in our study. It was also observed in this study that the serum IgA levels did not increase, suggesting the need for further research to explore the production of secretory IgA rather than serum IgA. The inclusion of 0.1% of a multi-enzyme combination in BGM-supplemented diets significantly increased BW and ADG, which was in line with the previous finding of Ndou et al. [14]. A possible explanation for this is that the combined enzyme can hydrolyze plant cell wall components (particularly mannan, xylan, and beta-glucan), allowing the release of protein, lipid, starch, and other minerals that are trapped within the cell wall matrix [15–17], ultimately promoting nutrient utilization and growth performance in growing pigs. In this study, the diet contained high levels of NSPs due to the inclusion of BGM (12.05% to 19.23% crude fiber) and included an energy ingredient from rice bran oil (9.79% to 14.84% EE) that may provide better substrates for enzyme function (xylanase, β-glucanase, cellulase, β-mannanase, and lipase). Taken together, it may enable the breakdown of several nutrients to a greater extent in a BGM-supplemented diet. Indeed, the supplemented diet contains microorganisms such as yeast and bacteria that produce enzymes and may reduce hindgut pH, thereby affecting the digestibility and availability of nutrients. In addition, yeast also has an effective prebiotic function. Therefore, the BGM-supplemented diet positively promoted the growth of pigs, facilitated by increased nutrient utilization, similar to pigs fed the PC diet.

The lower digestibility of CP and ash in the NC diet may be related to the presence of the digestible carbohydrate complex, antigen proteins, such as β-conglycinin and glycinin, and antinutritional factors, which are poorly degraded by endogenous enzymes [18]. Therefore, the combined enzymes play a role in plant cell wall degradation, thereby increasing cell wall permeability and allowing the rapid diffusion of amylolytic and fibrolytic enzymes that aid in releasing hard-to-release nutrients from the cereal grain [19]. This diffusion also increased protein degradation by neutral proteases that interacted with NSPs and starch to break these down into small peptides and amino acids [20,21]. These will be further absorbed in the small intestine for better nutrient utilization, consistent with results published by O'Connell et al. [22], who demonstrated that a combination of this enzyme potentially degraded the viscous NSPs in finishing pigs. The improvements yielded by the enzyme blends containing xylanase and amylase could be attributed to the enzyme-supplemented

BGM diet containing more substrates (both acid detergent fiber and neutral detergent fiber), as well as energy, rather than the corn-based diet [23–25]. This suggests that the addition of BGM diets up to 40% should be coupled with 0.1% multi-enzyme formulations to overcome detrimental effects on nutrient digestibility in growing pigs. In our findings, the digestibility of CP and ash did not differ between the PC and the BGM-supplemented diets, which could be related to the ability of the growing pigs to better utilize nutrients more efficiently as they aged, as well as their health status. This consequently increased nutrient digestion and absorption, which is in accordance with the longer VH observed in this study.

A metabolic profile is an important indicator of nutritional status and pig health. In this study, dietary BGM–enzyme inclusion increased glucose and triglyceride concentrations, resulting in greater energy availability. This is consistent with the study of Ao et al. [26], who observed that 0.1% multi-enzyme supplementation remarkably increased glucose concentration in growing pigs fed a corn SBM-based diet. Moreover, serum TP is normally used to determine the amount of albumin and globulin, which could affect the metabolic status of protein in the blood circulation, tissue dysfunction, as well as cell and tissue growth. In this study, pigs fed a BGM–enzyme-supplemented diet exhibited an increase in TP, according to the increased CP digestibility. Furthermore, a lower BUN concentration was considered favorable in growing pigs fed a BGM–enzyme diet, as it indicated highly efficient protein utilization [27]. This is consistent with our previous findings, which demonstrated that increasing BGM supplementation by up to 20%, plus 0.1% multi-enzyme combinations in the diet, decreased BUN concentration in weaning pigs [28], supported by the enhanced growth performance and nutrient digestibility in pigs fed BGM with a combination of enzymes.

Our previous study in weaning pigs demonstrated that the BGM-supplemented diets with enzyme supplementation significantly decreased serum TNFα concentration [28], indicating the potential anti-inflammation effect of the experimental diet. The diet may contribute to an increased capacity to release phenolic compounds capable of highly specific binding with cellulolytic compounds. Kim et al. [29] demonstrated that the release of hydroxycinnamic acid derivatives (i.e., ferulic acid and *p*-coumaric acid) could alter nitric oxide synthase (NO) in macrophages and suppress lipopolysaccharide-triggered NO secretion. The mannan oligosaccharides derived from the partial degradation of NSPs could also alleviate inflammatory responses via a reduction in pro-inflammatory cytokine secretion (IL1β, IL6, TNFα, and toll-like receptor 4) and the activation of anti-inflammatory cytokines (IL10) [30,31]. This activation could alter the Type-1 fimbriae-mediated adherence to attach to the binding site of Gram-negative bacteria, subsequently inducing mucosal inflammation clearance [32]. Our finding of lower IL6 and TNFα concentrations might contribute to the synergistic effects of the higher release of these active compounds contributing to maintaining gut health, as confirmed by the diminished CD and increased VH-to-CD ratio in the BGM-supplemented diet.

Oxidative stress originates from the excessive production of free radicals that can damage DNA, membrane lipid bilayers, and proteins in tissues [33]. However, the adverse effects can be alleviated by antioxidants, including non-enzymatic and enzymatic system components. The defense mechanism of oxidative stress through non-enzymatic antioxidants is often evaluated as the TAC [34], while the enzymatic antioxidant systems, SOD, and GPx collaborate in detoxifying superoxide anions and hydrogen peroxide in cells resulting in lower MDA production as an end product of lipid peroxidation [33]. Although the corn SBM-basal diet is proven to contain some phenolic compounds, this might be present in a relatively lower amounts compared to those found in the BGM-supplemented diets [35] as a consequence of a lower antioxidant capacity via reduced antioxidant enzyme capacity and higher susceptibility to cell membrane damage. Our study found that the inclusion of a BGM-supplemented diet positively affected antioxidant capacity with the increased productions of TAC, SOD, GPx, and decreased MDA concentrations with BGM content. Although little is known about the combined effect of BGM supplemented with

complex enzymes, the positive effect may be associated with major phenolic components, mainly ferulic acid (FA) and p-coumaric acid, which presented at 1984 and 794 mg/g DM, respectively [35]. In addition, a greater TAC might underly these favorable effects. Nevertheless, further research is warranted to explore the main component of phenolic compounds in the BGM prior to in-feed supplementation. The structure of FA is a covalently bound form of lignocellulosic biomass [36]; therefore, combined enzymes may cleave the lignin/phenolic-carbohydrate complex structure, more efficiently yielding phenolic compounds. The hydroxyl groups of these compounds are highly efficient at trapping free radicals, consequently scavenging hydrogen peroxide free radicals before causing lipid peroxidation and protein oxidation [35]. This suggests that the inclusion of combined enzymes in a BGM diet could potentially protect the cells from oxidative damage by improving whole-body antioxidant capacity and ameliorating MDA content in growing pigs.

The intestinal morphology of VH, CD, and VH/CD is an important indication of gut integrity in growing pigs. A longer VH length indicates a greater capacity for digesting and absorbing nutrients via the activation of the mRNA expression of mucosal enzyme functions and nutrient transporters [37]. Increased VH and VH/CD are directly associated with a rapid enterocyte turnover rate, whereas a shorter CD is indicative of lower epithelial cell destruction, an inflammatory response, and intestinal sloughing from pathogen infection [38]. In this study, the increase in duodenum VH may be a positive influence on active nutrient digestion, whereas increased jejunal VH may result in greater amino acid absorption as a result of an increased absorptive area for membrane-bound peptidase activity [39]. Additionally, the mature enterocyte of BGM pigs may play a functional role in inhibiting enterocyte migration, normal shedding, and providing available nutrients for their growth performance. The mode of action of an enzyme combination (amylase, xylanase, β-glucanase, lipase, cellulase, β-mannanase phytase, and pectinase) may drive substrate availability for bacterial growth changes [40]. This combination of enzymes may result in a reduction in gut viscosity and nutrient caging with long fiber chains, as well as large and nonadherent layers, resulting in increased nutrient uptake across the gut barrier [41]. This is in line with observations made by Kim et al. [42], who observed increased VH and VH/CD and decreased CD in pigs fed an SBM-based diet supplemented with a 0.1% enzyme combination containing amylase, proteinase, phytase, mannanase, and xylanase. Accordingly, Moeser and Kempem [43] demonstrated that the inclusion of fiber-degrading enzymes in a high-fiber diet (22.9% of neutral detergent fiber) tended to increase nitrogen digestibility by 2%. It is, therefore, possible that the BGM-supplemented diet used in this study provided actual substrates (12.05% and 19.23% in BGM20 and BGM40%, respectively) from various ingredient sources in the diet, promoting mucosal development, in turn, yielding increased nutrient digestibility and growth performance in pigs. However, no significant difference in the intestinal morphology of pigs that were fed PC and BGM-supplemented diets was observed in our study. Even though the crude fiber content in PC was lower than in the BGM-supplemented diets, it represented a higher amount of β-mannan (derived from soybean meal, 1.67% DM vs. 0.80% DM), which could cause a detrimental effect on digesta viscosity [44,45]. This phenomenon may diminish VH or elongate CD by activating enterocyte proliferation and tissue renewal [45]. The supplementation of β-mannanase in the corn SBM-based diet might have decreased the digesta viscosity of the growing pigs in the current study. Furthermore, the addition of β-mannanase in the diet containing SBM lead to the increased release of mannan-oligosaccharides, which could have produced butyrate [45]. Previous studies have reported butyrate as the activator of glucagon-like peptide 2 (GLP-2), which is a hormone secreted by entero-endocrine L cells in the small intestine and plays a vital role in multiple downstream mediators [46]. This phenomenon showed positively longer VH [47]. The increase in VH was incomparable to the greater nutrient digestibility observed in this study.

The change in hindgut pH can determine microbial activity and is considered integral to the intestinal health of pigs in controlling pathogenic invasion [48]. It is established that pH changes in the large intestine are mainly influenced by the quantity of fermentable

carbohydrates (e.g., cellulose, arabinoxylan, pectins, and β-glucan) that enter the large intestine. This process causes a shift in the fermentation profile to produce short-chain fatty acids, thereby supplying nutrients for lactic-acid-producing bacteria [49,50]. A study conducted by Metzler-Zebeli et al. [51] demonstrated that the inclusion of 15% dietary fiber reduced middle colonic pH. However, the lack of a significant difference in proximal colonic pH may be affected by the experimental period, pig age, and dietary fiber quantity. Interestingly, our findings demonstrated that pigs that were fed the BGM and NC diets showed a greater reduction in middle colonic pH than those that were fed the PC diet. However, why the middle colonic pH was reduced by dietary NC remains unclear. It appears that factors other than crude fiber content might influence the ability to reduce hindgut pH of the growing pigs. The reduction in middle colonic pH in this study may increase the production of short-chain fatty acids and the weights of cecum and large intestine. Nonetheless, further research is needed to explore this positive effect.

5. Conclusions

The inclusion of 40% BGM with the supplementation of enzyme combinations positively affects growth performance, CP digestibility, and gut health in growing pigs, through increases in VH, VH/CD, and antioxidant status, as well as decreased IL-6 and TNFα levels.

Author Contributions: Conceptualization, W.B. and J.H.; methodology, W.B. and J.H.; data curation, W.B.; formal analysis, W.B., W.J. and J.H.; investigation, W.B.; data curation, W.B.; project administration, W.B.; funding acquisition, W.B.; writing—original draft preparation, W.B. and J.H.; writing—review and editing, W.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Fundamental Fund of Khon Kaen University, which received financial support from the National Science, Research and Innovation Fund (NSRF).

Institutional Review Board Statement: All animal protocols were reviewed and approved by the Institutional Animal Care and Use Committee of Khon Kaen University (Khon Kaen, Thailand; authorization no. IACUC-KKU 123/64, approved on 18 November 2021).

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: We would like to sincerely thank Kantaphon Farm (Nakhonphanom, Thailand) for providing the animals and research facilities.

Conflicts of Interest: The authors declare no conflict of interest.

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