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Dietary Brewer Grain Meal with Multienzymes Supplementation Affects Growth Performance, Gut Health, and Antioxidative Status of Weaning Pigs

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Abstract: We conducted a 28-day feeding study on 80 weaning pigs [(Landrace × Large White) × Duroc] to determine the effects of dietary inclusion with brewer's grain meal (BGM) and multienzymes on their growth, intestinal health, and antioxidative status. Piglets were grouped by sex and initial BW and assigned to 20 pens with four pigs each. Treatments were a corn–soybean meal-based diet with either 0.1% multienzyme addition (PC) or without (NC), and two BGM compositions fortified with 0.1% multienzyme: 10% (BGM10) and 20% (BGM20). The overall body weight, average daily weight gain, and weight gain:feed ratio were significantly greater in pigs fed BGM20 than those fed the NC diet ($p < 0.05$). Moreover, the BGM diets significantly increased the digestibility of total ash and ether extract, glucose, total protein, immunoglobulin A, total antioxidant capacity, superoxide dismutase, heart and small intestine weights, villus height: crypt depth ratio (VH/CD), and *Lactobacillus* spp. count compared with the NC diet ($p < 0.05$). The diarrheal rate, blood urea nitrogen, malondialdehyde, duodenal crypt depth, and *Salmonella* spp. count were reduced in pigs fed the BGM-supplemented diet than those fed the NC diet ($p < 0.05$). The diarrheal rate ($p = 0.010$), ether extract digestibility ($p = 0.044$), total protein ($p = 0.044$), and duodenal villus height and VH/CD ($p = 0.003$ and $p = 0.002$, respectively) decreased quadratically with the increase in BGM supplementation. Overall, diets containing up to 20% BGM with multienzyme addition improved the nutrient utilization and intestinal health in weaning pigs by suppressing pathogenic bacterial growth without compromising the overall growth of the pigs.

Keywords: brewing waste; multienzyme; gut health; redox status; productive performance; weaning pigs

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1. Introduction

Brewer's grain meal (BGM) is an abundant by-product of the brewing industry and is relatively high in protein (19–30% *w*/*w*) and various bioactive substances such as hydroxycinnamic acids, phenolic compounds, and unidentified growth factors [1]. BGM is commonly used as a low-cost protein feed for cattle and a suitable substitute for conventional protein in aquaculture feed [2,3]. Despite the benefits of BGM, its use in swine feed is limited because of its high dietary fiber content (30–50% *w*/*w*) [4]. Its main components (approximately 50% *w*/*w*) are lignocelluloses, consisting of cellulose, hemicellulose (40% arabinoxylan), and lignin (20–28% *w*/*w*) [1,5]. Amoah et al. [6] revealed that including 20% BGM in the diet of weaning pigs had detrimental effects on growth and feeding efficiency. However, Ngodigha et al. [7] did not observe any adverse effects on growth or blood metabolites of weaning/growing pigs fed a 20% BGM-supplemented diet. These

contrasting results need to be validated through further studies to ensure that a potentially beneficial dietary supplement is not unnecessarily rejected.

Many studies have shown that multienzymes can have potent effects on the feed utilization of non-starch polysaccharides (NSPs), including improved growth and animal health, owing to synergistic interactions among enzymes [8,9]. For instance, multienzyme complex (protease, α-amylase, β-glucanase, and xylanase) dietary supplementation resulted in improved feed utilization and fecal microbial diversities in the hindgut of weaned pigs [10]. However, the effects of the use of multienzymes (containing amylase, xylanase, β-lucanase, lipase, cellulase, β-mannanase, and phytase) produced from *Aspergillus niger* and *Bacillus licheniformis* with BGM in the diet of weaning pigs have not been investigated. Consequently, this study aimed to investigate the effects of two different proportions of BGM with multienzyme fortification on the growth, nutrient digestibility, intestinal morphology, blood metabolites, immunity, antioxidative capacity, and microbial count of weaning pigs.

2. Materials and Methods

2.1. Ethical Approval

The authorization for animal handling (no. IACUC-KKU122/64) was approved by the Institutional Animal Care and Use Committee of Khon Kaen University (Khon Kaen, Thailand) on 18 November 2021. Animal handling and the experimental procedures followed the relevant animal welfare guidelines and practices.

2.2. Treatments, Diets, Housing, and Management

Eighty crossbred weaned pigs ((Landrace × Large White) × Duroc) with an initial weight (BW) of 7.86 ± 0.02 kg were grouped based on BW and sex in a randomized complete block (RCB) design with five replicates of four pigs each. Dietary treatments were as follows: a corn–soybean meal (SBM)-based diet plus 0.1% multienzymes (PC), a corn–SBM-based diet with no multienzymes (NC), and two corn–SBM-based diets fortified with 0.1% multienzymes and 10% (BGM10) and 20% BGM (BGM20), respectively. The active components of the multienzyme complexes derived from *Aspergillus niger* and *Bacillus licheniformis* were amylase at 20,000,000 U/kg, xylanase at 20,000,000 U/kg, β-glucanase at 10,000,000 U/kg, lipase at 9,000,000 U/kg, cellulose at 7,000,000 U/kg, β-mannanase at 5,000,000 U/kg, and phytase at 1,000,000 U/kg. All pigs used in this study were housed in pens (2.03 m width × 2.13 m length; stocking density 1.08 m^2/pig) with a polyvinyl feeder and nipple drinker under open-housed conditions. The temperature ranged from 28 to 33 °C and relative humidity was 56–68%. A mash diet was formulated to meet or exceed the recommendations of the NRC [11] (Table 1) for pigs weighing 7 to 25 kg in a two-phase period (phase I: days 1 to 14; phase II: days 15 to 28). Fresh water and feed were made available ad libitum to the pigs during the 28-day feeding period according to relevant welfare practices. Sanitation and vaccinations were performed to control ammonia emissions and disease.

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

	Phase I (Days 1 to 14)				Phase II (Days 15 to 28)			
Ingredient	**PC**	**NC**	**BGM10**	**BGM20**	**PC**	**NC**	**BGM10**	**BGM20**
Maize	49.73	49.87	41.74	33.87	51.84	52.02	44.54	36.73
Soybean meal (43.8%)	30.14	30.10	25.20	20.63	28.48	28.40	22.79	18.30
Broken rice	8.00	8.00	8.00	8.00	10.00	10.00	10.00	10.00
Fish meal	4.00	4.00	4.00	4.00	3.00	3.00	3.00	3.00
Rice bran oil	0.40	0.40	3.20	5.60	0.68	0.68	3.20	5.60
Skim milk	5.00	5.00	5.00	5.00	3.00	3.00	3.00	3.00
Brewer's grain meal (26.5%)	0.00	0.00	10.00	20.00	0.00	0.00	10.00	20.00

Table 1. *Cont.*

Ingredient	Phase I (Days 1 to 14)				Phase II (Days 15 to 28)			
	PC	NC	BGM10	BGM20	PC	NC	BGM10	BGM20
L-lysine (78%)	0.17	0.17	0.30	0.30	0.17	0.17	0.46	0.46
DL-methionine (99%)	0.08	0.08	0.08	0.12	0.08	0.08	0.26	0.16
Threonine (99%)	0.20	0.20	0.20	0.20	0.08	0.08	0.08	0.08
Dicalcium phosphate	0.97	0.97	0.97	0.97	1.11	1.11	1.11	1.11
Limestone	0.36	0.36	0.36	0.36	0.61	0.61	0.61	0.61
NaCl	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Multi-enzyme complex [1]	0.10	0.00	0.10	0.10	0.10	0.00	0.10	0.10
Vitamin–mineral premix [2]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Total	100	100	100	100	100	100	100	100
Calculated value (%)								
Metabolizable energy (kcal/kg)	3265	3265	3265	3265	3265	3265	3265	3265
Crude protein	22.50	22.50	22.50	22.50	20.70	20.70	20.70	20.70
Lysine	1.43	1.43	1.43	1.43	1.38	1.38	1.38	1.38
Methionine + cysteine	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65
Tryptophan	0.22	0.22	0.22	0.22	0.23	0.23	0.23	0.23
Threonine	0.87	0.87	0.87	0.87	0.89	0.89	0.89	0.89
Ca	0.80	0.80	0.80	0.80	0.83	0.83	0.83	0.83
Total phosphorus	0.66	0.66	0.66	0.66	0.68	0.68	0.68	0.68
Fiber	3.42	3.42	7.07	10.75	3.36	3.36	6.99	10.68

[1] Composed of amylase at 20,000,000 U/kg, xylanase at 20,000,000 U/kg, β-glucanase at 10,000,000 U/kg, lipase at 9,000,000 U/kg, cellulose at 7,000,000 U/kg, β-mannanase at 5,000,000 U/kg, and phytase at 1,000,000 U/kg. [2] One kilogram contains 1,600,000 IU vitamin A, 400,000 IU vitamin D3, 2200 IU vitamin E, 0.3 g vitamin K3, 0.2 g vitamin B1, 0.80 g vitamin B2, 0.2 g vitamin B6, 2.4 mg vitamin B12, 2 g pantothenic acid, 3 g nicotinic acid, 60 g choline, 50 mg biotin, 30 g Fe as $FeSO_4$, 0.2 g Co as $CoSO_4$, 8 g Mn as $MnSO_4$, 32 g Cu as $CuSO_4$, 20 g Zn as $ZnSO_4$, 0.2 g I as KI, 0.02 g Se as Na_2SeO_3, 10 g ethoxyquin, 2 g silicon dioxide.

2.3. Growth and Diarrheal Incidence

Body weight and feed intake were recorded for each pig at weeks 0, 2, and 4, and these data was used to calculate the average daily weight gain (ADWG), average daily feed intake (ADFI), and weight gain:feed ratio (G:F). Diarrheal incidence was recorded daily by counting pigs that showed watery diarrhea in each pen. The diarrheal rate was calculated by dividing the number of diarrhetic pigs by the total number of pigs per pen.

2.4. Total Tract Digestibility

A total of 20 barrows (BW 10.22 ± 0.46 kg) were assigned to the four dietary treatments in a metabolic crate using a completely randomized design for a seven-day adaptation period and five days of fecal collection. A total of 0.2% Cr_2O_3 and Fe_2O_3 were homogenously mixed and used as an inert marker at the beginning and terminal days of the collection period, respectively. Fecal samples were collected at 12 h intervals and stored in a sealed plastic bag at −20 °C. Pooled feces and diets were dried in a forced-air oven (60 °C for 72 h) and finely pulverized in a grinding mill (0.88 mm sieve). AOAC [12] protocols were used to assay dry matter (DM, #930.15), crude protein (CP, #984.13), total ash (#942.15), and ether extract (EE, #920.39). Each sample was analyzed in triplicate and the average was determined for the total tract digestibility, as previously described by Adeola [13].

2.5. Sample Collection and Chemical Analyses

Five pigs per treatment with BW similar to the pen BW were euthanized on day 28 after 12 h feed withdrawal. Blood samples were performed immediately from the cranial vena cava and transferred to a vacutainer tube (5 mL/tube) coated with silica particles before centrifugation (1.872× *g*) at 4 °C for 15 min. The obtained sera were analyzed for aspartate aminotransferase (AST), glucose (BG), triglycerides (TG), total cholesterol (TC), total protein (TP), albumin, and blood urea nitrogen (BUN) concentrations using colorimetric kits (Abcam assay kits, Cambridge, UK). Immunoglobulin A (IgA), IgG, interleukine1β (IL1β), IL6, and tumor necrosis factor-alpha (TNFα) levels were assessed using an enzyme-linked immunosorbent method (R&D System, Minneapolis, MN, USA), and total antioxidant capacity (TAC), superoxide dismutase (SOD), glutathione peroxidase

(GPx), and malondialdehyde (MDA) concentrations were analyzed using commercial kits (Sigma Aldrich, St. Louis, MO, USA). All measurements were performed in triplicate following the manufacturer protocols.

After blood collection, cecum and colon (proximal, middle, and distal regions) samples were collected for hindgut pH measurement using a portable pH meter (AP 110, Fisher Scientific, Pittsburgh, PA, USA). Segments of the heart, liver, kidney, stomach, spleen, small intestine, colon and cecum were rinsed with 0.9% saline solution, blot dried, and weighed. The duodenum (50 cm posterior from the pyloric sphincter), jejunum (5 cm between pyloric region and ileocecal opening), and ileum (15 cm above the ileocecal opening) were dissected longitudinally, flushed with phosphate buffer saline, and placed in paraformaldehyde in 0.1 M cacodylate buffer for 48 h before dehydration with ethanol and xylene. These tissues were transversely sectioned (5 µm) using an automated microtome (Leica RM2235, Wetzlar, Germany) and stained with hematoxylin-eosin (Sigma-Aldrich, St Louis, MO, USA). Villus height (VH; from the luminal tip surface to the villus–crypt axis), crypt depth (CD; between brush border membrane), and the VH/CD ratio were measured.

2.6. Fecal Microbial Count

Fresh fecal samples (about 2 g) from the rectum of the pigs (5 samples per group) were suspended in 0.9% (*w/v*) NaCl solution at 1:10 dilution. The aliquots (0.1 mL) of each mixture were spread-plated in triplicate onto each specific agar. The viable counts of *Lactobacillus* spp. were isolated from Rogosa and Sharpe agar after incubation at 37 °C for 48 h under aerobic conditions, whereas the isolates of *Escherichia coli* from MacConkey agar and *Salmonella* spp. from Salmonella Shigella agar were incubated at 37 °C for 24 and 48 h, respectively. The average growth of microbial enumerations was log-transformed and represented as $\log_{10}$ CFU/g of digesta.

2.7. Statistical Analyses

Data were analyzed using the MIXED procedure in the SAS software suite (v.9.4, SAS Institute, Cary, NC, USA) in an RCB design that considered "pen" the experimental unit for growth and diarrheal rate, and "individual pig" the experimental unit for digestibility trials, blood criteria, hindgut pH, organ weight, intestinal morphology, and microbial count. The statistical model considered the experimental diets as the fixed effect and block as the random effect as the following formula:

$$Y_{ij} = \mu + \alpha_i + \beta_{j+} \varepsilon_{ij} \quad (1)$$

where Y_{ij} = jth observation of the ith treatment (i = 1,2, … ,5; j = 1,2, … ,5), μ = the population mean, α_i = the treatment effect of the ith treatment, β_j = replication effect of the jth replicate, and ε_{ij} = the random effect. Significant differences between treatment groups were tested with Duncan's new multiple range test at a probability level of $p < 0.05$. Orthogonal polynomial contrast was defined as the linear and quadratic effects as the addition level of BGM increased at $p < 0.05$.

3. Results

3.1. Growth and Diarrheal Incidence

The BW, ADWG, and ADFI showed no significant differences between PC and NC diet groups, except for a greater G:F ratio associated with the PC diet ($p < 0.05$; Table 2).

However, pigs fed the BGM20 diet with multienzyme fortification showed greater overall BW and ADWG than those fed the PC diet ($p < 0.05$). A linear increase in growth and linear reduction of diarrheal rate were also observed as the BGM proportion increased in the diet ($p < 0.05$).

Table 2. Effect of dietary BGM with multienzyme addition on growth performance in weaning pigs [1,2].

Item	Treatment				SEM	*p*-Value		
	PC	NC	BGM10	BGM20		Treatment	Linear	Quadratic
BW (kg)								
Day 0	7.89	7.86	7.84	7.84	0.018	0.299	0.376	0.718
Day 14	11.06	10.91	11.09	11.28	0.462	0.956	0.586	0.992
Day 28	17.05 ^{b}	16.66 ^{b}	17.34 ^{ab}	18.13 ^{a}	0.266	0.014	0.007	0.878
ADWG (g)								
Days 1 to 14	226	218	232	246	32.511	0.939	0.551	0.999
Days 15 to 28	428	410	447	489	39.674	0.554	0.151	0.939
Overall	327 ^{b}	314 ^{b}	339 ^{ab}	368 ^{a}	9.532	0.011	0.006	0.896
ADFI (g)								
Days 1 to 14	340	362	387	369	13.959	0.179	0.689	0.213
Days 15 to 28	801	899	841	832	38.276	0.362	0.282	0.639
Overall	570	631	614	601	21.003	0.249	0.368	0.955
G:F ratio								
Days 1 to 14	0.668	0.653	0.606	0.675	0.109	0.968	0.888	0.669
Days 15 to 28	0.552	0.457	0.544	0.592	0.057	0.436	0.089	0.755
Overall	0.584 ^{a}	0.506 ^{b}	0.556 ^{ab}	0.614 ^{a}	0.024	0.044	0.016	0.899
Diarrhea rate (%)	6.01	8.96	5.15	4.18	1.217	0.079	0.010	0.288

BW = body weight; ADGW = average daily weight gain; ADFI = average daily feed intake; G:F = weight gain:feed ratio. [1] Corn–soybean meal-based diet with 0.1% multienzyme addition (PC); corn–soybean meal-based diet without multienzyme addition (NC); NC + 10% (BGM10) with 0.1% multienzyme addition; NC + 20% BGM (BGM20) with 0.1% multienzyme addition. [2] Values shows means of five replicates (pen) per treatment. ^{a,b} Values in rows without a common superscript are significantly different ($p < 0.05$).

3.2. Total Tract Digestibility

BGM supplementation in the diet increased the digestibility of total ash ($p = 0.039$) and EE ($p = 0.037$) compared with the NC diet (Table 3). Furthermore, a high BGM diet showed linear and quadratic effects on DM, total ash, and EE digestibility ($p < 0.05$), including a linear effect on CP digestibility ($p = 0.080$).

Table 3. Effect of dietary BGM with multienzyme addition on apparent total tract digestibility in weaning pigs [1,2].

Item	Treatment				SEM	*p*-Value		
	PC	NC	BGM10	BGM20		Treatment	Linear	Quadratic
Apparent total tract digestibility (%)								
Dry matter	90.56	86.88	92.75	91.58	1.334	0.059	0.045	0.071
Crude protein	87.85	84.33	90.41	89.09	1.595	0.109	0.080	0.108
Total ash	60.31 ^{ab}	51.54 ^{b}	74.10 ^{a}	72.28 ^{a}	5.120	0.039	0.013	0.055
Ether extract	78.33 ^{ab}	67.65 ^{b}	84.59 ^{a}	81.08 ^{a}	3.487	0.037	0.028	0.044

[1] A corn–soybean meal-based diet with 0.1% multienzyme addition (PC); corn–soybean meal-based diet without multienzyme addition (NC); NC + 10% (BGM10) with 0.1% multienzyme addition; NC + 20% BGM (BGM20) with 0.1% multienzyme addition. [2] Data are shown as group mean ± SEM (5 pigs per treatment). ^{a,b} Values in rows without a common superscript are significantly different ($p < 0.05$).

3.3. Blood Profiles, Immunity, and Antioxidant Status

Serum metabolic profiles did not differ significantly between the PC and NC groups (Table 4). However, pigs fed BGM10 showed higher TG and TP concentrations than those fed the control ($p < 0.05$). Pigs fed BGM-supplemented diets also had lower BUN than those fed the PC diet ($p < 0.05$). Moreover, there were linear and quadratic effects on BG ($p = 0.017$ and $p = 0.099$, respectively) and TP ($p = 0.016$ and $p = 0.044$, respectively), including a quadratic effect on TG in response to the BGM level ($p = 0.004$).

Table 4. Effect of dietary BGM with multienzyme addition on blood profiles, immunity, and oxidative status in weaning pigs [1,2].

Item	Treatment				SEM	*p*-Value		
	PC	NC	BGM10	BGM20		Treatment	Linear	Quadratic
AST (U/L)	73.87	67.36	73.47	64.14	7.576	0.759	0.754	0.396
Glucose (mg/dL)	137.79 ^{ab}	129.02 ^{b}	150.05 ^{a}	149.31 ^{a}	4.365	0.015	0.017	0.099
Triglyceride (mg/dL)	83.93 ^{b}	78.37 ^{b}	98.91 ^{a}	85.77 ^{ab}	4.281	0.032	0.161	0.004
Total cholesterol (mg/dL)	102.22	90.03	89.55	90.70	6.324	0.459	0.933	0.905
Total protein (mg/dL)	6.68 ^{bc}	6.15 ^{c}	7.54 ^{a}	7.33 ^{ab}	0.265	0.012	0.016	0.044
Albumin (mg/dL)	5.12	4.28	5.41	5.01	0.462	0.396	0.316	0.231
BUN (mg/dL)	20.25 ^{a}	19.39 ^{ab}	17.31 ^{b}	17.16 ^{b}	0.822	0.050	0.104	0.386
IgA (μg/mL)	24.36 ^{bc}	19.79 ^{c}	26.57 ^{ab}	30.72 ^{a}	1.554	0.003	<0.001	0.423
IgG (g/L)	0.86	0.88	0.95	1.03	0.077	0.416	0.136	0.931
IL1β (pg/mL)	296.37	322.97	286.37	307.13	27.675	0.810	0.707	0.438
IL6 (pg/mL)	120.5	128.67	123.15	115.99	4.861	0.352	0.112	0.896
TNFα (pg/mL)	49.33 ^{b}	68.78 ^{a}	55.09 ^{b}	47.98 ^{b}	3.913	0.010	0.003	0.469
TAC (U/mL)	10.62 ^{b}	13.74 ^{ab}	16.15 ^{a}	15.94 ^{a}	1.250	0.029	0.298	0.468
SOD (U/mL)	72.47 ^{b}	81.12 ^{ab}	98.23 ^{ab}	105.86 ^{a}	8.116	0.047	0.016	0.521
GPx (U/mL)	694.24	881.45	901.16	866.69	68.198	0.174	0.878	0.745
MDA (nmol/mL)	7.11 ^{a}	6.06 ^{ab}	6.11 ^{ab}	4.99 ^{b}	0.407	0.024	0.021	0.111

AST = aspartate aminotransferase; BUN = blood urea nitrogen; IgA = immunoglobulin A; IgG = immunoglobulin G; IL1β = interleukine 1β; TNFα = tumor necrosis factor-alpha; TAC = total antioxidant capacity; SOD = superoxide dismutase; GPx = glutathione peroxidase; MDA = malondialdehyde. [1] Corn–soybean meal-based diet with 0.1% multienzyme addition (PC); corn–soybean meal-based diet without multienzyme addition (NC); NC + 10% (BGM10) with 0.1% multienzyme addition; NC + 20% BGM (BGM20) with 0.1% multienzyme addition. [2] Data are shown as group mean ± SEM (5 pigs per treatment). $^{a–c}$ Values in rows without a common superscript are significantly different ($p < 0.05$).

The blood-related immunity and proinflammatory cytokine levels did not differ between PC and BGM-supplemented groups ($p > 0.05$; Table 4). However, the pigs in BGM20 produced greater IgA ($p = 0.003$) and lower TNFα concentrations ($p = 0.010$) than those in the NC group, with a linear response to BGM proportion ($p = 0.001$ and $p = 0.003$, respectively).

Serum oxidative capacity did not differ between the control and NC vs. BGM-supplemented groups (Table 4). However, the BGM diets significantly improved the TAC by 51.12% ($p = 0.029$), and the BGM20 group produced greater SOD (46.07%, $p = 0.047$) and lower MDA (29.82%, $p = 0.024$) than the PC group. Furthermore, the concentrations of SOD ($p = 0.016$) and MDA ($p = 0.021$) showed linear responses to an increase in BGM in the feed.

3.4. Hindgut pH

The pH of the cecum ($p = 0.013$) and proximal colon ($p = 0.026$) was lower in the BGM-supplemented groups than the PC group (Table 5), but was comparable to that in the NC group. No differences in hindgut pH were recorded in the middle or distal colons ($p > 0.05$).

Table 5. Effect of dietary BGM with multienzyme addition on hindgut pH in weaning pigs [1,2].

Item	Treatment				SEM	*p*-Value		
	PC	NC	BGM10	BGM20		Treatment	Linear	Quadratic
Cecum	6.34 ^{a}	5.49 ^{b}	5.08 ^{b}	5.19 ^{b}	0.244	0.013	0.423	0.419
Colon								
Proximal colon	6.09 ^{a}	5.32 ^{ab}	5.03 ^{b}	4.71 ^{b}	0.279	0.026	0.199	0.971
Middle colon	6.12	5.46	5.26	5.53	0.349	0.409	0.899	0.603
Distal colon	5.95	5.37	5.66	5.71	0.376	0.754	0.461	0.762

[1] A corn–soybean meal-based diet with 0.1% multienzyme addition (PC); corn–soybean meal-based diet without multienzyme addition (NC); NC + 10% (BGM10) with 0.1% multienzyme addition; NC + 20% BGM (BGM20) with 0.1% multienzyme addition. [2] Data are shown as group mean ± SEM (5 pigs per treatment). ^{a,b} Values in rows without a common superscript are significantly different ($p < 0.05$).

3.5. Organ Weight

Pigs fed the PC diet had a heavier small intestine weight than those fed the NC diet ($p = 0.044$), but were comparable to those fed the BGM-supplemented diet (Table 6).

Table 6. Effect of dietary BGM with multienzyme addition on organ weights in weaning pigs [1,2].

Item	Treatment				SEM	*p*-Value		
	PC	NC	BGM10	BGM20		Treatment	Linear	Quadratic
Relative organ weight (g/kg BW)								
Heart	3.64 [b]	3.54 [b]	4.28 [a]	4.18 [a]	0.168	0.019	0.015	0.045
Liver	29.24	25.96	26.98	30.82	0.462	0.519	0.076	0.516
Kidney	3.87	4.24	3.82	3.52	0.322	0.497	0.172	0.896
Stomach	8.29	8.52	8.96	9.73	0.491	0.227	0.112	0.786
Spleen	2.05	1.92	2.24	2.15	0.174	0.623	0.295	0.273
Small intestine	45.95 [a]	41.24 [b]	47.02 [a]	47.23 [a]	1.462	0.044	0.017	0.144
Colon	16.34 [b]	15.49 [b]	19.91 [a]	18.17 [ab]	0.959	0.029	0.101	0.039
Cecum	2.39	2.44	2.61	2.66	0.155	0.587	0.389	0.779

[1] A corn–soybean meal-based diet with 0.1% multienzyme addition (PC); corn–soybean meal-based diet without multienzyme addition (NC); NC + 10% (BGM10) with 0.1% multienzyme addition; NC + 20% BGM (BGM20) with 0.1% multienzyme addition. [2] Data are shown as group mean ± SEM (5 pigs per treatment). [a,b] Values in rows without a common superscript are significantly different ($p < 0.05$).

The BGM10 showed greater weights of the heart ($p = 0.019$) and colon ($p = 0.029$) than those fed the control diets. Additionally, linear and quadratic responses of heart weight ($p = 0.015$ and $p = 0.045$, respectively) and linear response for relative weights of the liver ($p = 0.076$) and small intestine ($p = 0.017$) were observed as the BGM content in the feed increased. No significant differences were detected in the weights of the kidney, stomach, or spleen among the four dietary treatments.

3.6. Intestinal Morphology

Pigs fed BGM10 had greater VH and VH/CD in the duodenum than those that consumed the NC diet ($p < 0.05$, Table 7), and there was a reduction in duodenal CD with increased BGM supplementation ($p = 0.016$). The increased BGM content also produced linear and quadratic effects on duodenal VH, CD, and VH/CD ($p < 0.05$). However, the intestinal morphology did not differ between the PC and NC groups.

Table 7. Effect of dietary BGM with multienzyme addition on intestinal morphology in weaning pigs [1,2].

Item	Treatment				SEM	*p*-Value		
	PC	NC	BGM10	BGM20		Treatment	Linear	Quadratic
Duodenum								
Villus height (μm)	593.96 [b]	550.64 [b]	707.73 [a]	623.78 [ab]	28.316	0.013	0.056	0.003
Crypt depth (μm)	328.41 [ab]	351.73 [a]	303.78 [b]	306.72 [b]	9.822	0.016	0.012	0.067
VH/CD	1.82 [bc]	1.57 [c]	2.34 [a]	2.04 [ab]	0.122	0.005	0.010	0.002
Jejunum								
Villus height (μm)	549.85	512.11	588.67	589.95	62.460	0.789	0.409	0.640
Crypt depth (μm)	248.53	246.76	243.71	240.01	18.299	0.988	0.658	0.980
VH/CD	2.34	2.08	2.46	2.47	0.313	0.799	0.389	0.645
Ileum								
Villus height (μm)	483.78	466.87	493.94	512.94	38.445	0.859	0.456	0.934
Crypt depth (μm)	197.21	209.84	174.69	188.61	14.972	0.437	0.240	0.129
VH/CD	2.66	2.23	2.91	2.73	0.320	0.517	0.217	0.214

VH/CD = villus height-to-crypt depth ratio. [1] A corn–soybean meal-based diet with 0.1% multienzyme addition (PC); corn–soybean meal-based diet without multienzyme addition (NC); NC + 10% (BGM10) with 0.1% multienzyme addition; NC + 20% BGM (BGM20) with 0.1% multienzyme addition. [2] Data are shown as group mean ± SEM (5 pigs per treatment). [a–c] Values in rows without a common superscript are significantly different ($p < 0.05$).

3.7. Fecal Microbial Count

The number of *Lactobacillus* spp. count was higher in pigs fed the BGM-supplemented diets than those fed the PC diet ($p = 0.034$). The BGM20 showed lower *Salmonella* spp. count than those fed the NC diet ($p = 0.044$, Table 8). A linear reduction in *Salmonella* spp. count was observed as BGM supplementation increased ($p = 0.018$). However, the *E. coli* was unaffected by dietary treatment ($p > 0.05$).

Table 8. Effect of dietary BGM with multienzyme addition on microbial counts (log_{10} CFU/g) in weaning pigs [1,2].

Item	Treatment				SEM	*p*-Value		
	PC	NC	BGM10	BGM20		Treatment	Linear	Quadratic
Lactobacillus spp.	6.15 [b]	7.93 [ab]	9.37 [a]	9.71 [a]	0.808	0.034	0.150	0.585
Escherichia coli	5.84	4.27	4.79	5.18	0.744	0.519	0.443	0.949
Salmonella spp.	7.08 [ab]	8.21 [a]	6.56 [ab]	5.31 [b]	0.627	0.044	0.018	0.817

[1] A corn–soybean meal-based diet with 0.1% multienzyme addition (PC); corn–soybean meal-based diet without multienzyme addition (NC); NC + 10% (BGM10) with 0.1% multienzyme addition; NC + 20% BGM (BGM20) with 0.1% multienzyme addition. [2] Data are shown as group mean ± SEM (5 pigs per treatment). [a,b] Values in rows without a common superscript are significantly different ($p < 0.05$).

4. Discussion

The observed improvements in growth performance and feed efficiency of weaned pigs fed the BGM diet are consistent with previous findings [14]. Multienzymes containing NSPase, protease, and lipase have shown potent effects on increasing nutrient utilization [15,16]. Young pigs benefit from greater nutrient availability, which supports their growth while the gastrointestinal tract is immature [15]. BGM contains approximately 50% fiber [4], which may provide a substrate for enzyme function, thereby improving the growth rate of weaned pigs. Furthermore, the hydrolysis of non-digestible mannan-oligosaccharide (MOS) and xylo-oligosaccharide (XOS) may activate the growth of beneficial lactic acid bacteria to control pathogens in the gastrointestinal tract [17,18]. This possibility was confirmed by the reduction in diarrheal rate in weaned pigs that were fed a BGM-supplemented diet in our study.

Previous reports demonstrated that multienzyme fortification improved nutrient utilization by increasing the release of several encapsulated nutrients in the feed [19,20]. According to Li et al. [21], adding multienzymes containing protease, α-amylase, cellulase, and β-glucanase to the diet significantly increased the apparent total tract digestibility of acid detergent fiber and neutral detergent fiber in weaned pigs. The lack of significant effects on the digestibility of EE and CP may be attributed to differences in feeding conditions, enzymes, and dietary composition. The improvements in DM, CP, and EE digestibility may be attributed to NSPs and other nutrient-degrading actions. Indeed, the increased digestibility of total ash may be due to increased digestibility of phosphorus because of multienzyme-containing phytase, which has potential health benefits of a lower insoluble complex with phytate in the gastrointestinal tract [22]. These results suggest that the inclusion of a 10–20% BGM diet fortified with 0.1% multienzymes might efficiently increase nutrient utilization in weaning pigs.

Changes in blood metabolites can reflect the nutritional status and metabolic activity of an animal. An increase in BG and TG levels could provide energy to weaning pigs, and this is in line with the observation of Ao et al. [23]. Furthermore, the pigs fed a BGM-supplemented diet had higher TP levels, which indicated efficient protein utilization. This result agrees with the increase in CP digestibility and lower BUN concentration observed in the current study. It is well established that a lower BUN concentration is an efficient indicator of protein and amino acid adequacy and amino acid utilization in the body [24]. This positive outcome may be explained by the fact that multienzymes can strongly interact with NSPs and produce small peptides and amino acids that can be directly absorbed in

the small intestine [21,25]. Such interactions may result in high CP digestibility and VH in pigs fed a BGM-supplemented diet.

Gut mucosal immunity has a greater impact on the production of immune cells, which contain considerable amounts of lymphocytes and antibodies, than on other tissues in the animal body [26]. It is beneficial to the host in preventing an inflammatory response against pathogenic invasion by secreting IgA in the mucosal wall [27]. It is typically transported by immature epithelial cells in the crypts and binds to the mucus layer of the epithelial surface in cooperation with innate non-specific immune cells [27]. Increased secretion of IgA antibodies into the intestinal lumen is a major defense mechanism of the mucosal immune system, which prevents the adherence of antigens and neutralizes their toxins or enzymes [28]. The decline in the serum IgA concentration of weaned pigs fed the NC diet may well be linked to their high susceptibility to pathogenic infection and the altered TNFα production found in this study. However, the reason for the similarity in antioxidant status of pigs fed the BGM and NC diets is unclear; thus, further investigation is needed to explore the factors involved in this effect. According to Stefanello et al. [29], the structure of brewer's grain comprises a lignified cell wall with high polyphenol and flavonoid content (7.36 mg GAE/g and 0.21 mg CE/g, respectively). These active compounds are powerful free radical scavengers that transfer H-atoms from their hydroxyl groups to the free radicals, thus inhibiting lipid peroxidation. This finding is consistent with that of a previous observation on broiler chickens, which showed that increasing the dietary BGM could activate GPx function and inhibit MDA secretion [30]. This positive effect may modulate both enzymatic and non-enzymatic reactions in free radical scavenging [31,32]. This is consistent with the increased levels of TAC and SOD and decreased level of MDA observed in the current study.

Reduced hindgut pH can inhibit the growth of opportunistic pathogens in weaning pigs [33]. A previous study demonstrated that supplementing the diet with 15% fiber significantly decreased hindgut pH [34]. In the present study, the BGM-supplemented diet contained both digestible and resistant starches, which may escape and reach the hindgut and be used for microbial fermentation. The end-product of fermentation (short-chain fatty acids) is possibly used by the lactic acid-producing bacteria as an energy source [35]. It suggests that a maximum of 20% BGM-supplementation plus enzyme combinations can be considered in the diet of weaning pigs to achieve a physiological reduction in the pH of the large intestine [36]. However, the reason underlying the observed increase in relative heart weight in pigs fed the BGM-supplemented diet in the present study is not fully understood. It may be associated with an increased metabolic rate or cardiovascular failure [37,38]. Buddington et al. [39] reported that the heart grows with increasing body weight, which is consistent with our findings of greater BW in pigs fed the BGM-supplemented diet. Furthermore, the increase in weight of the small intestine and colon could increase the activity of amylase, lipase, and NSPase in the digestion of several nutrients in response to the multienzymes in the BGM-supplemented diet. According to Agyekum et al. [40], ingestion of a high-fiber diet induces the secretion of digestive juices and enzymes owing to an increased workload of the secretory organs, which ultimately increases hypertrophy. Agyekum et al. [41] observed that pigs fed multienzymes in a high-fiber diet (30%) showed greater colon weight. This positive effect would ultimately result in the use of ingestible fiber as a substrate for short-chain fatty acid production. This might explain the greater abundance of *Lactobacillus* spp. observed in weaning pigs fed the BGM-supplemented diet. Previous reports showed that *Lactobacillus* are prevalent in the feces of piglets and tend to decline during the weaning transition period [42]. However, a high-BGM diet with multienzyme supplementation can successfully hydrolyze several NSP components into NSP-releasing oligosaccharides, such as XOS and MOS. The *Lactobacillus* spp. may effectively utilize both compounds, further altering the synergistic effects of beneficial gut microbes, including their antibacterial functions [21,34,43]. Similarly, Borowsky et al. [44] observed that MOS was highly agglutinated, with type-1 fimbriae of *Salmonella* spp. and

S. enterica in particular. This theory is consistent with the lower abundance of *Salmonella* spp. and lower hindgut pH.

Intestinal morphology indicates the absorptive capacity and health status of weaning pigs. Higher values of VH and VH/CD reflect greater digestion and absorption of nutrients, whereas diminished CD reflects epithelial cell destruction and inflammation [45]. Weaning stress is typically caused by impairment of intestinal VH and villus structure, which can induce cell death and decrease the rate of cell renewal [46]. In this study, we observed a positive increase in VH and VH/CD in pigs fed the BGM diet with multienzyme fortification. This result agrees with the findings of Kim et al. [47], who reported that 0.1% multienzymes containing amylase, proteinase, phytase, mannanase, and xylanase increased the VH and VH/CD in weaning pigs. One possible reason is that the mature apical enterocytes in pigs fed a BGM-supplemented diet may increase nutrient supply to the intestinal tract. Additionally, the indigestible MOS and XOS may contribute to the control of pathogen invasion, thus lowering intestinal sloughing. However, as the BGM content in the diet increases, it is necessary to provide additional multienzymes to maintain the gut health of weaning pigs.

5. Conclusions

Supplementing the diets of weaned pigs with 20% BGM plus 0.1% multienzymes is an effective approach to improve their growth, nutrient digestion and absorption, immunity, antioxidant capacity, and *Lactobacillus* spp. It also reduces pro-inflammatory cytokines, lipid peroxidation, hindgut pH, and *Salmonella* spp. count without hepatic alterations.

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