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Two-Stage Fermented Feather Meal Enhances Growth Performance and Amino Acid Digestibility in Broilers

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Abstract: The study aimed to investigate the dose tolerance of enzymatically degraded feather meal (EFM) in the diet, and the effect of the two-stage fermented feather meal on the growth performance and amino acid digestibility of broilers. In trial 1, 160 one-day-old broilers were randomly assigned into 0, 10, 15, and 20% EFM groups. In trial 2, 160 one-day-old broilers were randomly assigned into control, 10% EFM, *Bacillus subtilis* var. *natto* N21 + *B. coagulans* L12 fermented EFM (BBEFM), and *B. subtilis* var. *natto* N21 + *Saccharomyces cerevisiae* Y10 fermented EFM (BSEFM) groups. Trial 3 involved 32 twenty-one-day-old male broilers randomly assigned into nitrogen-free diet, highly digestible protein, EFM, and BSEFM groups for a 7-day metabolic trial. During all of the feeding periods, increasing the EFM dosage in the diet linearly and quadratically inhibited weight gain (WG), feed intake, and feed conversion ratio (FCR) ($p < 0.05$), except the FCR at 22–35 days ($p > 0.05$). Dietary inclusion of more than 15% resulted in a negative impact on growth performance over days 1–35 ($p < 0.05$). Therefore, the EFM dose tolerance in the broiler diet is 10%. The WG, FCR, and production efficiency factor of the BSEFM group were better than those of the control group in days 1–35 ($p < 0.05$). The apparent and standardized ideal amino acid digestibility of BSEFM was higher than EFM in trial 3, except for Met, Cys, and Trp ($p < 0.05$). In conclusion, the EFM dose tolerance for the broiler diet is 10%. *Bacillius subtilis* var. *natto* N21 + *S. cerevisiae* Y10 fermentation can improve the amino acid digestibility of EFM and enhance broiler growth performance.

Keywords: amino acid digestibility; broiler; feather meal; fermentation

Citation: Yeh, R.-H.; Hsieh, C.-W.; Chen, K.-L. Two-Stage Fermented Feather Meal Enhances Growth Performance and Amino Acid Digestibility in Broilers. *Fermentation* **2023**, *9*, 128. https://doi.org/10.3390/fermentation9020128

Academic Editor: Alessandro Robertiello

Received: 8 December 2022
Revised: 16 January 2023
Accepted: 20 January 2023
Published: 29 January 2023

1. Introduction

Feather meal is a high-protein feedstuff with unbalanced amino acid composition limiting in Met, Lys, His, and Trp [1,2]. Keratin is the main feather meal protein, and is insoluble and poorly susceptible to breakdown by digestive enzymes due to its higher degree of cross-linking by disulfide bridges, hydrogen bonds, and hydrophobic interactions [2–4]. Therefore, the use of unmodified feather meal in the diet is limited.

Hydrolyzed feather meal with 70% digestible crude protein (CP) is processed with adjusted 60–70% moisture content, pressure-cooked under 207–690 kPa for 6–60 min, and then dried and crushed [5]. Some amino acid loss or denaturation occurs during processing and will have an impact on nutrient availability [6,7]. The application of enzyme or biodegradation technology can potentially improve feather meal nutritional value [8–10]. Enzymatically degraded feather meal (EFM) has great nutritional value due to reduced amino acid loss and improved digestibility [11,12]. EFM also requires lower energy inputs and results in lower environmental pollution. However, the correct tolerance dosage to incorporate into the broiler diet remains unclear.

Probiotic fermentation has the ability to decompose carbohydrates and proteins, thus improving nutritional value [13–15], potentially increasing animal growth and improving

feed taste and preservation. Therefore, inoculation of probiotics for feed fermentation has been utilized for many years [16,17]. Two-stage fermentation is a combination of aerobic and anaerobic fermentation that exerts the characteristics of different probiotics. Chen et al. [18] conducted two-stage fermentation using *B. subtilis* var. *natto* N21 (BS), which has high proteolytic capacity, for the first two days of aerobic feed fermentation, then *S. cerevisiae* Y10 (SC), which has greater acidic capacity, for the subsequent three days of anaerobic feed fermentation. This two-stage BS + SC-fermented feed improved broiler weight gain by 16% during the period from 1–39 days [18]. Under similar two-stage fermentation conditions, Yeh et al. [19] found that inoculation of *B. coagulans* L12 (BC) in the second stage could increase the content of digestible amino acids in the diet and improve broiler growth performance. Thus, two-stage fermentation has the potential to improve broiler growth performance and nutrient availability. Although BS has keratin decomposition capabilities, the ability of two-stage fermented feather meal to improve broiler growth performance has not been demonstrated.

Two-stage fermentation has the potential to be applied to feather meal. The high proteolytic ability of BS may improve the amino acid utilization of feather meal. SC and BC can provide fermentation products such as organic acids, which may improve the flavor and nutritional value of feather meal. Therefore, BS, SC and BC were selected as starters in this study. The purpose of this study is to investigate the dose tolerance of EFM in the broiler diet, and to investigate the effect of two-stage fermented EFM on growth performance, carcass traits, serum biochemical constituents, and amino acid digestibility in broilers.

2. Materials and Methods

2.1. Trial 1, the Effect of Different Doses of Enzymatically Degraded Feather Meal on Broiler Growth Performance

2.1.1. Enzymatically Degraded Feather Meal Preparation

The poultry feathers (the moisture content is 60%) were stirred for 15 min, then Allzyme FD (Alltech Inc., Kaohsiung, Taiwan) (0.5 kg/ton) and sodium sulfite (2.5 kg/ton) were added and stirred at 50 °C for 40 min. After stirring, the steam was pressurized for 10 min to keep the pressure at 2 bar for 15 min. After steaming, the feathers were dried at 65 °C using an oven and crushed. The moisture content was brought below 12%. The crude protein, lysine, methionine and cystine contents of EFM were 80%, 2.02% and 0.65%, respectively.

2.1.2. Bird Management and Experimental Design

A total of 160 one-day-old Arbor Acres broilers, with equal numbers of both sexes, were randomly assigned into 0%, 10%, 15%, and 20% EFM groups, with four replicates for each group. The starter weights were 47.1 ± 2.0 g. The feeding trial was carried out for 35 days. Feed (Tables 1 and 2) and water were provided *ad libitum*. Bird management and feed formulation refer to the Arbor Acres Broiler Management Manual [20]. All procedures used in this experiment were approved by the Institutional Animal Care and Use Committee of National Chiayi University (IACUC, approval number 105050).

Table 1. Composition of the basal diet (trials 1 and 2, 1–21 days).

Items	0%	10%	15%	20%	SEM	*p* Value
Ingredient, %						
Yellow corn, grain	45.13	59.30	66.52	73.59		
Soybean oil	3.42	2.13	1.07	0.00		
Full-fat soybean meal, 38%	22.67	9.47	5.16	0.00		
Soybean meal, 44%	25.00	15.00	8.00	2.03		

Table 1. *Cont.*

Items	0%	10%	15%	20%	SEM	*p* Value
Enzymatic degradation feather meal, 80% CP	0.00	10.00	15.00	20.00		
Dicalcium phosphate	1.59	1.51	1.47	1.41		
Limestone, pulverized	1.50	1.53	1.54	1.59		
Salts	0.33	0.33	0.33	0.33		
DL-Methionine	0.16	0.21	0.23	0.22		
L-lysine HCl	0.00	0.32	0.48	0.63		
Vitamin premix [1]	0.10	0.10	0.10	0.10		
Mineral premix [2]	0.10	0.10	0.10	0.10		
Total	100.0	100.0	100.0	100.0		
		Calculated value				
CP, %	23.0	23.0	23.0	23.1		
ME, kcal/kg	3150	3150	3150	3145		
Ca, %	0.95	0.95	0.95	0.95		
AP, %	0.47	0.47	0.47	0.47		
Met, %	0.53	0.53	0.53	0.53		
Lys, %	1.33	1.33	1.33	1.33		
		Proximate analysis [3]				
CP, %	23.6	23.5	23.6	23.5	0.1	0.791
GE, kcal/kg	4407	4394	4397	4394	9.0	0.705
Ca, %	0.99	0.97	0.98	0.98	0.02	0.840
TP, %	0.64	0.65	0.64	0.64	0.01	0.813

[1] Vitamin premix supplied per kg of diet: vitamin A, 3000 IU; vitamin D3, 400 IU; vitamin E, 10 IU; vitamin K3, 1 mg; vitamin B1, 3.6 mg; vitamin B2, 5.4 mg; vitamin B6, 7.0 mg; Ca-pantothenate, 20.0 mg; niacin, 70 mg; biotin, 0.3 mg; folic acid, 1.1 mg; vitamin B12, 0.02 mg. [2] Mineral premix supplied per kg of diet: Cu ($CuSO_4 \cdot 5H_2O$, 25.45% Cu), 8 mg; Fe ($FeSO_4 \cdot 7H_2O$, 20.09% Fe), 80 mg; Mn ($MnSO_4 \cdot H_2O$, 32.49% Mn), 60 mg; Zn (ZnO, 80.35% Zn), 40 mg; Se ($NaSeO_3$, 45.56% Se), 0.15 mg. [3] Data are means of three batches of each feed; each batch was tested in triplicate.

Table 2. Composition of the basal diet (trials 1 and 2, 21–35 days).

Items	0%	10%	15%	20%	SEM	*p* Value
Ingredient, %						
Yellow corn, grain	46.77	60.97	68.14	75.40		
Soybean oil	4.08	2.78	1.73	0.47		
Full-fat soybean meal, 38%	20.77	7.56	3.27	0.12		
Soybean meal, 44%	25.00	15.00	8.00	0.00		
Enzymatic degradation feather meal, 80% CP	0.00	10.00	15.00	20.00		
Dicalcium phosphate	1.36	1.27	1.23	1.18		
Limestone, pulverized	1.35	1.39	1.41	1.43		
Salts	0.33	0.33	0.33	0.33		
DL-Methionine	0.14	0.18	0.21	0.23		
L-lysine HCl	0.00	0.32	0.48	0.63		
Vitamin premix [1]	0.10	0.10	0.10	0.10		
Mineral premix [2]	0.10	0.10	0.10	0.10		
Total	100.00	100.00	100.00	100.00		
		Calculated value				
CP, %	22.4	22.4	22.4	22.4		
ME, kcal/kg	3200	3200	3200	3200		
Ca, %	0.85	0.85	0.85	0.85		
AP, %	0.42	0.42	0.42	0.42		
Met, %	0.50	0.50	0.50	0.50		
Lys, %	1.29	1.29	1.29	1.29		

Table 2. *Cont.*

Items	0%	10%	15%	20%	SEM	*p* Value
Proximate analysis [3]						
CP, %	22.9	22.7	22.8	22.6	0.1	0.429
GE, kcal/kg	4496	4484	4485	4484	9.0	0.739
Ca, %	0.88	0.85	0.86	0.86	0.02	0.727
TP, %	0.63	0.64	0.63	0.63	0.01	0.813

[1] Vitamin premix supplied per kg of diet: vitamin A, 3000 IU; vitamin D3, 400 IU; vitamin E, 10 IU; vitamin K3, 1 mg; vitamin B1, 3.6 mg; vitamin B2, 5.4 mg; vitamin B6, 7.0 mg; Ca-pantothenate, 20.0 mg; niacin, 70 mg; biotin, 0.3 mg; folic acid, 1.1 mg; vitamin B12, 0.02 mg. [2] Mineral premix supplied per kg of diet: Cu ($CuSO_4 \cdot 5H_2O$, 25.45% Cu), 8 mg; Fe ($FeSO_4 \cdot 7H_2O$, 20.09% Fe), 80 mg; Mn ($MnSO_4 \cdot H_2O$, 32.49% Mn), 60 mg; Zn (ZnO, 80.35% Zn), 40 mg; Se ($NaSeO_3$, 45.56% Se), 0.15 mg. [3] Data are means of three batches of each feed; each batch was tested in triplicate.

2.1.3. Measurements and Analysis

Growth Performance

Chicken body weight and feed intake were recorded each week to calculate weight gain and feed conversion ratio. When the chickens died, the body weight and feed intake were recorded, and the average weight gain and feed conversion ratio were calculated. The production efficiency factor (PEF) was then calculated using the following Formula (1) [19]:

$$\text{PEF} = (\text{A}\ (\%) \times \text{B}\ (\text{kg}))/(\text{C}\ (\text{day-old}) \times \text{D}) \times 100 \quad (1)$$

where A is the survival rate, B is the body weight, C is the age of the broiler, and D is the feed conversion ratio.

2.2. Trial 2, the Effect of Two-Stage Fermented Feather Meal on Broiler Growth Performance

2.2.1. The Feather Decomposition Ability of *B. subtilis* var. *natto* N21

The methods used followed those of Huang et al. [21]. A total of 50 mL of Tryptone Soya Broth (HIMEDIA®), which contains 3% poultry feather, was added to a 250 mL Erlenmeyer flask. After sterilization (121 °C for 20 min), 5% BS (10^8 cfu/mL) was inoculated into the Erlenmeyer flask, and suction filtration (No. 1 Qualitative Filter Paper, ADVANTEC®) was performed at 0 h, 24 h, 48 h, and 72 h. The feather decomposition rate was calculated using the following Formula (2):

$$\text{feather decomposition rate}\ (\%) = (\text{A} - \text{B})/\text{A} \times 100\% \quad (2)$$

where A is the dry weight before cultivation, and B is the dry weight after cultivation.

The filtrate was further filtered through a 0.2 μm filter funnel (NALGENE®). The 700 μL of filtrate was mixed with 6.3 mL of acetate buffer (10 mM, pH 5.5), then concentrated by a high-recovery centrifuge tube (Amicon Ultra-15 Centrifugal Filter Units, 10 kDa, Millipore) at 3500× *g* for 30 min. The filtrate was concentrated to 250 μL, then diluted to 1 mL to obtain the enzyme solution.

A total of 70 μL of the substrate solution (2.1 mg azokeratin + 14 μL 0.5 M phosphate buffer + 56 μL 2D water) and 40 μL of the enzyme solution were then mixed. The solution was allowed to stand at 50 °C for 60 min. The reaction was then stopped with the addition of 25 μL of 4 M NaOH. The solution was concentrated at 8000× *g* for 20 min. After centrifugation, 100 μL of supernatant was added to an ELISA plate. The optical density (OD) was measured at 450 nm using an automated ELISA reader (Model 680, Bio-Rad, St. Louis, MO, USA), which used 200 μL of 1 N NaOH solution as a blank, and the enzyme activity was calculated using the following Formula (3):

$$\text{keratinolytic activity}\ (\text{U/mL}) = (\text{OD} - \text{blank})/(\text{reaction time} \times 0.001) \times \text{concentration} \quad (3)$$

2.2.2. Two-Stage Fermented Enzymatically Degraded Feather Meal Preparation

In this study, we used BS for the first stage of bacterial fermentation, which has high proteolytic capacity, and then added BC or SC for the second-stage fermentation, both of which have greater acidic capacity. BS was incubated in Tryptone Soya Broth (HIMEDIA) at 37 °C at 150 rpm in a concave-bottomed Erlenmeyer flask. BC was incubated in Tryptone Soya Broth at 37 °C at 100 rpm in an Erlenmeyer flask. SC was incubated in Yeast Peptone Dextrose Broth (BD) at 28 °C at 100 rpm in an Erlenmeyer flask. After incubation the broth was centrifuged at 8000× *g* for 10 min, and the supernatant removed, with the same amount of sterile water added, then shaken. This step was repeated three times to remove the medium. Sterile water was added to the precipitate to dilute to 10^9 cfu/mL. The diluents ensured that the concentration of probiotics was higher than 10^9 cfu/mL using the standard plate count method (Tryptone Soya agar (HIMEDIA) was used for BS and BC; Yeast Peptone Dextrose agar (BD) was used for SC).

To the EFM was added 10% corn meal as substrate for fermentation. Three batches of each fermented feather meal were produced for animal experimentation. The weight of substrate for each batch of fermented feather meal was 15 kg. For the first fermentation, the substrate was sterilized (121 °C for 30 min) and then cooled to 45 °C. The substrate was supplemented with BS diluent (10^6 cfu/g of feed) and 50% water at 37 °C for a two-day aerobic fermentation. For the second fermentation, which immediately followed the first fermentation, the substrate was supplemented with BC or SC diluent (10^6 cfu/g of feed) at 28 °C for a five-day anaerobic fermentation. The fermented feather meal was dried using an oven (65 °C). The moisture content was brought below 12%. Each of the fermented feather meals were made in three batches.

2.2.3. Bird Management and Experimental Design

A total of 160 one-day-old broilers, with equal numbers of each sex, were randomly assigned into control, 10% EFM, BS + BC fermented EFM (BBEFM), and BS + SC-fermented EFM (BSEFM) groups with four replicates each. The starter weights were 35.7 ± 2.4 g. The feeding trial was carried out for 35 days. Bird management was the same as for trial 1. All procedures used in this experiment were approved by the Institutional Animal Care and Use Committee of National Chiayi University (IACUC, approval number 105050).

2.2.4. Measurements and Analysis

Fermented Feather Meal Physiological Characteristics

For measurement of the pH value, 1 g of feed was added to 9 mL of sterile water, then mixed. The pH value was measured using a pH meter PB-10 (digital pH meter, Sartorius, Taipei, Taiwan). For the bacterial count, 1 g of feed was added to 9 mL of sterile water, then mixed. The supernatants were diluted 10-fold with buffered peptone water. Next, 100 µL of supernatant was smeared onto Tryptone Soya agar, Lactobacilli MRS agar, and Yeast Peptone Dextrose agar to determine *Bacillus*-like bacteria, total lactic acid bacteria, and yeast, respectively. *Bacillus*-like bacteria were incubated at 37 °C for 24 h. Total lactic acid bacteria were incubated at 37 °C with 13% CO_2 for 48 h. The yeast was incubated at 28 °C for 48 h.

Growth Performance

The measurements were carried out as for trial 1.

Carcass Traits

Eight chicks, each from the control, EFM, BBEFM, and BSEFM groups, were euthanized at 35 days of age to measure the weights of the liver, proventriculus with gizzard, intestine (from duodenum to rectum), abdominal fat (from gizzard to celiac fat), breast (including bone and skin), and thigh (fragment from femur to tibia, including bone and skin).

Serum Biochemical Constituents

Blood samples were taken from the brachial vein of chickens withdrawn from feed and water for 12 h at 35 days of age. After centrifuging (1000× *g* for 15 min), the serum was stored at −40 °C for further analysis. Serum calcium and phosphorus concentrations and amylase, lactate dehydrogenase, glutamate oxaloacetate transaminase, creatine kinase, γ-glutamyl transpeptidase, and alkaline phosphatase activities were analyzed using an automatic blood chemical analyzer with Roche testing kits (Roche COBAS MIRA PLUS, Switzerland). Serum enzyme activity is defined as the level of international units (IU) per liter of serum [22].

2.3. Trial 3, the Effect of Two-Stage Fermented Feather Meal on Broiler Amino Acid Digestibility

2.3.1. Bird Management and Experimental Design

The methods used followed those of Adedokun et al. [23]. A total of 32 twenty-one-day-old male broilers were randomly assigned into nitrogen-free diet (NFD), highly digestible protein diet (HDP), EFM, and BSEFM groups, with four replicates each, for a nutrient digestibility trial. The feeding trial was carried out for seven days. The formulas for the semi-purified diets are shown in Table 3. The SIAAD-related literature used nitrogen-free materials such as corn starch or dextrose to prepare semi-purified diets (20% CP). Dextrose was used in this study, and EFM and BSEFM were used as the only protein sources in the diet. The corn starch in NFD and HDP diets was to reduce the impact of high glucose content on the appearance and texture of the diet. The chickens from each replicate were housed in a 30 cm × 25 cm × 40 cm cage. Feed and water were given *ad libitum*. Bird management and the approval of animal use protocol were the same as for trial 1. The feed adaptation period was three days, followed by the use of the experimental feeding diet for four days. At the end of the metabolic trial, the birds were sacrificed. Digesta samples were taken from the ileum using a distilled water wash bottle. The ileum segment was located between Meckel's diverticulum and the ileal–caecal–colonic junction. The digesta were pooled by cage, then stored at −20 °C. The digesta samples were freeze dried and ground before the component analysis. All procedures used in this experiment were approved by the Institutional Animal Care and Use Committee of National Chiayi University (IACUC, approval number 105050).

Table 3. Composition of the basal diet (trial 3).

Items	NFD [1]	HDP [2]	EFM [3]	BSEFM [4]
Ingredient, %				
Corn starch	42.1	32.1	0	0
Dextrose	40.1	40.1	59.2	59.2
Soybean oil	5.0	5.0	5.0	5.0
Casein	0	10.0	0	0
Solka-Floc [5]	5.0	5.0	0	0
$NaHCO_3$	2.0	2.0	0	0
KCl	1.2	1.2	0	0
MgO	0.2	0.2	0	0
Feather meal	0	0	31.4	0
Fermented feather meal	0	0	0	31.4
Dicalcium phosphate	1.9	1.9	1.9	1.9
Limestone	1.3	1.3	1.3	1.3
NaCl	0.2	0.2	0.2	0.2
Mineral premix [6]	0.2	0.2	0.2	0.2
Vitamin premix [7]	0.2	0.2	0.2	0.2
Choline chloride, 50%	0.3	0.3	0.3	0.3

Table 3. *Cont.*

Items	NFD [1]	HDP [2]	EFM [3]	BSEFM [4]
Cr_2O_3	0.3	0.3	0.3	0.3
Total	100.0	100.0	100.0	100.0
		Calculated values		
CP, %			20.0	20.0

[1] NFD = nitrogen-free diet. [2] HDP = highly digestible protein diet. [3] EFM = enzymatically degraded feather meal. [4] BSEFM = EFM cultures were *B. subtilis* var. *natto* N21 and *S. cerevisiae* Y10. [5] Purified cellulose, International Fiber Corp., North Tonawanda, NY. [6] Mineral premix supplied per kg of diet: Cu ($CuSO_4 \cdot 5H_2O$, 25.45% Cu), 8 mg; Fe ($FeSO_4 \cdot 7H_2O$, 20.09% Fe), 80 mg; Mn ($MnSO_4 \cdot H_2O$, 32.49% Mn), 60 mg; Zn (ZnO, 80.35% Zn), 40 mg; Se ($NaSeO_3$, 45.56% Se), 0.15 mg. [7] Vitamin premix supplied per kg of diet: vitamin A, 3000 IU; vitamin D3, 400 IU; vitamin E, 10 IU; vitamin K3, 1 mg; vitamin B1, 3.6 mg; vitamin B2, 5.4 mg; vitamin B6, 7.0 mg; Ca-pantothenate, 20.0 mg; niacin, 70 mg; biotin, 0.3 mg; folic acid, 1.1 mg; vitamin B12, 0.02 mg.

The NFD and HDP groups were used to determine ileal (basal) amino acid flow in broiler chickens. The EFM and BSEFM groups used EFM and BSEFM as the only protein sources in the diet, respectively, which were used to adjust dietary CP to 20%. The diets were mixed with 3 g/kg chromic oxide as an indicator. Digesta and feed samples were measured for CP and amino acid content.

2.3.2. Measurements and Analysis

CP Analysis

CP analyses were performed according to the AOAC [24] (method 990.03).

Amino Acid Analysis

Samples for amino acid analyses were hydrolyzed in 6 N HCl for 24 h at 110 °C conditions under a N atmosphere. Performic acid oxidation was carried out for the sulfur-containing amino acids Met and Cys before acid hydrolysis. Samples for Trp analysis were hydrolyzed using barium hydroxide [25] (method 982.30 E). The amino acids in the hydrolysate were subsequently determined using HPLC after post-column derivation.

Chromium Analysis

The samples were ashed at 600 °C for 12 h in a muffle furnace, using inductively coupled plasma mass spectrometry (ICP-AES Vista, Varian, Palo Alto, CA, USA) according to AOAC [26] (method 985.01).

The basal ileal amino acid flow (IAAF), apparent ileal amino acid digestibility (AIAAD), and standardized ileal amino acid digestibility (SIAAD) were calculated using the following Formulas (4)–(6):

$$\text{IAAF (mg/kg of DMI)} = A \times (B/C) \quad (4)$$

where A is the amino acid content (mg/kg) in the ileal digesta, B is the chromium content (mg/kg) in the diet, and C is the chromium content (mg/kg) in the ileal digesta.

$$\text{AIAAD (\%)} = [1 - (A/B) \times (C/D)] \quad (5)$$

where A is the chromium content (mg/kg) in the diet, B is the chromium content (mg/kg) in the ileal digesta, C is the amino acid content (mg/kg) in the ileal digesta, and D is the amino acid content (mg/kg) in the diet.

$$\text{SIAAD (\%)} = \text{AIAAD (\%)} + [100 \times (\text{IAAF in g/kg of DMI})/(\text{AA content diet in g/kg of DM})] \quad (6)$$

2.4. Statistical Analysis

The orthogonal polynomial contrasts were employed to test the linear and quadratic effects of the increasing levels of EFM in trial 1. Variances among the treatments were calculated using the GLM procedure [27], and the groups were compared using a one-way

ANOVA test. Duncan's new multiple-range test was used to compare the means according to Steel and Torrie [28].

3. Results

3.1. Trial 1

Growth Performance

Table 4 presents the effect of different dosages of EFM on broiler growth performance. During all of the feeding periods (1–21-, 22–35- and 1–35-day), increasing the EFM dosage in diet linearly and quadratically inhibited weight gain, feed intake, and feed conversion ratio ($p < 0.05$), except for the feed conversion ratio at 22–35 days of age ($p > 0.05$). The presence of more than 10% EFM in the diet significantly reduced weight gain ($p < 0.05$), 15% EFM inclusion had further adverse effects on the feed conversion ratio ($p < 0.05$), while 20% EFM inclusion significantly decreased feed intake ($p < 0.05$) during the 1–21-day period. During days 22–35, the 20% EFM group experienced significantly reduced weight gain and feed intake ($p < 0.05$). Over the 1–35-day period, the 15% EFM group experienced significantly reduced weight gain, feed intake, and PEF ($p < 0.05$), and the feed conversion ratio of the 20% EFM group was further impacted ($p < 0.05$). According to quadratic fit model of dietary EFM dosage and weight gain ($Y = -2.28X^2 + 21X + 1870$, $R^2 = 0.93$) from the 1–35-day period, the best dietary inclusion level of EFM is 4.61%. The inclusion of 10% EFM in the diet was used in trial 2, since there was no significant difference in the growth performance at 1–35 days between the control group and the 10% EFM group in trial 1.

Table 4. Effect of different dosages of enzymatically degraded feather meal on broiler growth performance [1] (trial 1).

Items	Enzymatically Degraded Feather Meal				SEM	*p* Value	
	0%	10%	15%	20%		Linear	Quadratic
1–21 days							
Weight gain, g/bird	786 [a]	735 [b]	634 [c]	408 [d]	9	<0.001	<0.001
Feed intake, g/bird	1070 [a]	1010 [a]	995 [a]	701 [b]	25	<0.001	<0.001
Feed conversion ratio, feed intake/weight gain	1.36 [c]	1.38 [c]	1.57 [b]	1.72 [a]	0.04	<0.001	0.010
22–35 days							
Weight gain, g/bird	1085 [a]	1114 [a]	1043 [ab]	969 [b]	28	0.014	0.028
Feed intake, g/bird	1982 [a]	1974 [a]	1838 [ab]	1763 [b]	45	0.003	0.135
Feed conversion ratio, feed intake/weight gain	1.83	1.78	1.76	1.82	0.03	0.640	0.158
1–35 days							
Weight gain, g/bird	1871 [a]	1848 [a]	1677 [b]	1376 [c]	32	<0.001	<0.001
Feed intake, g/bird	3052 [a]	2984 [a]	2833 [b]	2464 [c]	45	<0.001	<0.001
Feed conversion ratio, feed intake/weight gain	1.63 [b]	1.62 [b]	1.69 [b]	1.79 [a]	0.03	0.001	0.010
Survival rate, %	95.0	95.0	90.0	92.5	4.3	0.532	0.963
PEF [2]	319 [a]	320 [a]	262 [b]	210 [c]	16	<0.001	0.017

[1] Data are means of four pens of broilers with 10 broilers per pen. [2] PEF = production efficiency factor = (survival rate (%) × body weight (kg))/(age (day-old) × feed conversion ratio) × 100. [a–d] Means in the same row with different superscripts are significantly different ($p < 0.05$).

3.2. Trial 2

3.2.1. Feather Decomposition Ability of *B. subtilis* var. *natto* N21

Table 5 presents the effect of BS fermentation on feather degradation rate and keratinase activity. The results show that BS fermentation significantly enhanced both feather degradation rate and keratinase activity ($p < 0.05$).

Table 5. Effect of *B. subtilis* var. *natto* N21 fermentation on feather degradation rate and keratinase activity [1] (trial 2).

Items	Feather	Fermented Feather	SEM	*p* Value
Feather degradation rate, %				
24 h	2.6 [b]	33.7 [a]	0.3	<0.001
48 h	3.2 [b]	62.0 [a]	0.3	<0.001
72 h	4.5 [b]	72.6 [a]	0.7	<0.001
Keratinase activity, U/mL				
24 h	21 [b]	523 [a]	2	<0.001
48 h	17 [b]	476 [a]	1	<0.001
72 h	23 [b]	438 [a]	2	<0.001

[1] Data are means of three batches of each feather; each batch was tested in triplicate. [a,b] Means in the same row with different superscripts are significantly different ($p < 0.05$).

3.2.2. Physiological Characteristics of Fermented Feather Meal

Table 6 presents the physiological characteristics of two-stage fermented feather meal. The pH of the EFM was 5.76–5.78, but increased to 7.07–7.10, while the count of *Bacillus*-like bacteria was 8.09–8.11 log cfu/g feed after the first fermentation. After the second fermentation, the pH values were 5.45 and 5.59, the count of *Bacillus*-like bacteria was 8.06 and 8.8 log cfu/g feed, and the count of total lactic acid bacteria was 8.26 and yeast 7.95 log cfu/g feed for BBEFM and BSEFM, respectively. After drying, the pH value was 5.47 and 5.62, the count of *Bacillus*-like bacteria was 7.51 and 7.47 log cfu/g feed, and total lactic acid bacteria was 7.97 and yeast <5.00 log cfu/g feed for BBEFM and BSEFM, respectively.

Table 6. Physiological characteristics of two-stage fermented feather meal [1] (trial 2).

Items	Fermented Feather Meal [2]		SEM	*p* Value
	BBEFM	BSEFM		
pH value				
Autoclave	5.78	5.76	0.09	0.861
First fermentation	7.07	7.10	0.02	0.449
Second fermentation	5.45	5.59	0.12	0.483
Dry	5.47	5.62	0.12	0.453
Bacillus-like bacteria, log cfu/g feed				
First fermentation	8.09	8.11	0.03	0.708
Second fermentation	8.06	8.08	0.03	0.785
Dry	7.51	7.47	0.02	0.320
Total lactic acid bacteria, log cfu/g feed				
Second fermentation	8.26			
Dry	7.97			
Yeast, log cfu/g feed				
Second fermentation	7.95			
Dry	<5.00			

[1] Data are means of three batches of each fermented feather meal; each batch was tested in triplicate. [2] BBEFM = EFM cultures were *B. subtilis* var. *natto* N21 and *B. coagulans* L12; BSEFM = EFM cultures were *B. subtilis* var. *natto* N21 and *S. cerevisiae* Y10.

3.2.3. Growth Performance

Table 7 presents the effect of feeding with two-stage fermented feather meal on broiler growth performance. During the 1–21-day period, the BSEFM group experienced significantly higher weight gain and feed intake than the EFM group ($p < 0.05$), while the BBEFM

group experienced the lowest ($p < 0.05$). Over days 22–35, the BBEFM and BSEFM groups experienced a higher weight gain and feed conversion ratio than the control and EFM groups ($p < 0.05$). Over the entire period, the BSEFM group outperformed the control and EFM groups ($p < 0.05$) in weight gain, feed conversion ratio, and PEF, while the BBEFM group exhibited no difference among the control and EFM groups ($p > 0.05$).

Table 7. Effect of two-stage fermented feather meal on broiler growth performance [1] (trial 2).

Items	Control	EFM [2]	Fermented Feather Meal [3]		SEM	*p* Value
			BBEFM	BSEFM		
1–21 days						
Weight gain, g/bird	773 [a]	732 [b]	632 [c]	796 [a]	8	<0.001
Feed intake, g/bird	1053 [ab]	1007 [b]	885 [c]	1074 [a]	18	<0.001
Feed conversion ratio, feed intake/weight gain	1.36	1.38	1.40	1.35	0.02	0.250
22–35 days						
Weight gain, g/bird	1091 [b]	1106 [b]	1211 [a]	1209 [a]	25	0.006
Feed intake, g/bird	2023	2015	2031	2038	22	0.893
Feed conversion ratio, feed intake/weight gain	1.86 [a]	1.82 [a]	1.68 [b]	1.69 [b]	0.03	0.003
1–35 days						
Weight gain, g/bird	1864 [b]	1838 [b]	1842 [b]	2005 [a]	30	0.006
Feed intake, g/bird	3077 [a]	3021 [ab]	2916 [b]	3112 [a]	40	0.024
Feed conversion ratio, feed intake/weight gain	1.65 [a]	1.64 [a]	1.58 [ab]	1.55 [b]	0.02	0.026
Survival rate, %	97.5	97.5	95.0	97.5	2.6	0.873
PEF [4]	323 [b]	320 [b]	324 [b]	368 [a]	11	0.034

[1] Data are means of four pens of broilers with 10 broilers per pen. [2] EFM = enzymatically degraded feather meal. [3] BBEFM = EFM cultures were *B. subtilis* var. *natto* N21 and *B. coagulans* L12; BSEFM = EFM cultures were *B. subtilis* var. *natto* N21 and S. cerevisiae Y10. [4] PEF = production efficiency factor = (survival rate (%) × body weight (kg))/(age (day-old) × feed conversion ratio) × 100. [a–c] Means in the same row with different superscripts are significantly different ($p < 0.05$).

3.2.4. Carcass Traits

Table 8 presents the effect of two-stage fermented feather meal on broiler carcass traits. The results show that the relative weight of the dressing percentage, breast, thigh, liver, abdominal fat, intestines, and proventriculus and gizzard displayed no significant difference among treatments ($p > 0.05$).

Table 8. Effect of two-stage fermented feather meal on broiler carcass traits [1] (trial 2).

Items	Control	EFM [2]	Fermented Feather Meal [3]		SEM	*p* Value
			BBEFM	BSEFM		
Relative weight, % of body weight t						
Dressing percentage	79.5	79.9	79.3	80.0	0.9	0.941
Breast	21.3	21.4	19.9	21.4	0.8	0.518
Thigh	21.3	21.6	21.3	21.5	0.3	0.843
Liver	2.04	2.04	2.05	2.04	0.09	1.000

Table 8. *Cont.*

Items	Control	EFM [2]	Fermented Feather Meal [3]		SEM	*p* Value
			BBEFM	BSEFM		
Relative weight, % of body weight t						
Abdominal fat	1.28	1.38	1.35	1.39	0.11	0.894
Intestines	4.61	4.37	4.53	4.44	0.28	0.930
Proventriculus and gizzard	2.29	2.28	2.56	2.46	0.14	0.424

[1] Data are means of four pens of broilers; two broilers were sampled from each pen. [2] EFM = enzymatically degraded feather meal. [3] BBEFM = EFM cultures were *B. subtilis* var. *natto* N21 and *B. coagulans* L12; BSEFM = EFM cultures were *B. subtilis* var. *natto* N21 and *S. cerevisiae* Y10.

3.2.5. Serum Biochemical Constituents

Table 9 presents the effect of two-stage fermented feather meal on broiler serum biochemical constituents. There was no significant difference in serum biochemical constituents among treatments ($p > 0.05$).

Table 9. Effect of two-stage fermented feather meal on broiler serum biochemical constituents [1] (trial 2).

Items	Control	EFM [2]	Fermented Feather Meal [3]		SEM	*p* Value
			BBEFM	BSEFM		
Amylase, U/L	931	948	815	948	64	0.470
Glutamate oxaloacetate transaminase, U/L	321	274	335	286	19	0.142
Lactate dehydrogenase, U/L	2946	3177	3192	2909	217	0.708
Creatine kinase, U/L	7775	8877	9574	8501	953	0.615
γ-glutamyl transpeptidase, U/L	20.3	19.3	22.4	19.2	1.5	0.634
Alkaline phosphatase, U/L	2170	2063	2470	1915	320	0.666
Calcium, mg/dL	10.4	10.8	10.7	10.4	0.1	0.211
Phosphorus, mg/dL	8.31	9.01	8.86	8.65	0.36	0.577

[1] Data are means of four pens of broilers; two broilers were sampled from each pen. [2] EFM = enzymatically degraded feather meal. [3] BBEFM = EFM cultures were *B. subtilis* var. *natto* N21 and *B. coagulans* L12; BSEFM = EFM cultures were *B. subtilis* var. *natto* N21 and *S. cerevisiae* Y10.

3.3. Trial 3

3.3.1. Amino Acid Composition

Table 10 presents the dietary amino acid composition for the metabolic trial. CP and total amino acid content of the NFD and HDP groups were 3.6, 0.1% and 8.5, 8.5%, respectively. The limiting amino acids in the EFM were Met and His, with high Glu and Ser. The BSEFM and EFM contained similar amino acid composition ratios; however, in the former, the amount of CP and total amino acids increased by 11.7% and 21.1%, respectively.

Table 10. Dietary amino acid composition for the metabolic trial [1] (trial 3).

Items	NFD [2]	HDP [3]	EFM [4]	BSEFM [5]
CP, %	3.6	8.5	19.6	21.9
Total amino acids, %	0.1	8.5	17.1	20.7
Essential amino acids, %				
Thr	0	0.34	0.77	0.95
Val	0.01	0.48	1.15	1.30
Met	0	0.22	0.14	0.18
Ile	0	0.33	0.85	0.98
Leu	0.01	0.89	1.53	1.85
Trp	0.01	0.36	0.48	0.58
Phe	0.01	0.43	0.87	1.03
Lys	0	0.57	0.59	0.72
His	0	0.19	0.23	0.29
Arg	0	0.27	1.24	1.42

Table 10. *Cont.*

Items	NFD [2]	HDP [3]	EFM [4]	BSEFM [5]
Nonessential amino acids, %				
Asp	0.01	0.63	1.44	1.83
Ser	0	0.45	1.63	1.97
Glu	0.01	1.99	2.36	2.98
Pro	0	0.91	1.45	1.69
Gly	0.01	0.16	1.18	1.35
Ala	0.01	0.28	0.78	0.97
Cys	0.01	0.01	0.39	0.63

[1] Data are means of three batches of each feed; each batch was tested in triplicate. [2] NFD = nitrogen-free diet. [3] HDP = highly digestible protein diet. [4] EFM = enzymatically degraded feather meal. [5] BSEFM = EFM cultures were *B. subtilis* var. *natto* N21 and *S. cerevisiae* Y10.

3.3.2. Amino Acid Digestibility

Tables 11 and 12 present the AIAAD and SIAAD in chicks fed with two-stage fermented feather meal. The AIAAD of BSEFM was higher than that of EFM for all amino acids, except Met and Cys ($p < 0.05$). Similarly, the SIAAD of BSEFM was significantly higher than that of the EFM group for all amino acids, except Met and Trp ($p < 0.05$).

Table 11. Apparent ileal amino acid digestibility in chicks fed two-stage fermented feather meal [1] (trial 3).

Items	EFM [2]	BSEFM [3]	SEM	*p* Value
Total amino acids, %	64.9 [b]	77.2 [a]	0.7	<0.001
Essential amino acids, %				
Thr	57.1 [b]	70.3 [a]	1.0	<0.001
Val	66.8 [b]	73.0 [a]	0.8	0.001
Met	77.8	78.7	0.8	0.481
Ile	67.5 [b]	74.4 [a]	0.9	0.001
Leu	64.9 [b]	72.6 [a]	1.3	0.005
Trp	72.5 [b]	75.7 [a]	0.7	0.019
Phe	72.4 [b]	79.1 [a]	1.1	<0.001
Lys	64.8 [b]	76.9 [a]	0.9	<0.001
His	67.8 [b]	76.5 [a]	0.6	<0.001
Arg	68.0 [b]	74.5 [a]	0.8	0.002
Nonessential amino acids, %				
Asp	55.6 [b]	67.8 [a]	0.9	<0.001
Ser	65.1 [b]	72.2 [a]	0.7	<0.001
Glu	66.6 [b]	76.4 [a]	0.6	<0.001
Pro	61.9 [b]	71.5 [a]	0.7	<0.001
Gly	64.8 [b]	71.6 [a]	0.7	0.001
Ala	67.4 [b]	76.4 [a]	0.9	<0.001
Cys	61.9	63.6	0.8	0.186

[1] Data are means of four pens of broilers; two broilers were sampled from each pen. [2] EFM = enzymatically degraded feather meal. [3] BSEFM = EFM cultures were *B. subtilis* var. *natto* N21 and *S. cerevisiae* Y10. [a,b] Means in the same row with different superscripts are significantly different ($p < 0.05$).

Table 12. Standardized ileal amino acid digestibility in chicks fed two-stage fermented feather meal [1] (trial 3).

Items	NFD [2]		SEM	*p* Value	HDP [3]		SEM	*p* Value
	EFM [4]	BSEFM [5]			EFM	BSEFM		
Total amino acids, %	69.3 [b]	75.5 [a]	0.8	0.0016	72.0 [b]	80.1 [a]	1.2	0.003
Essential amino acids, %								
Thr	65.2 [b]	76.4 [a]	1.0	0.0002	72.5 [b]	82.5 [a]	1.1	0.001
Val	69.9 [b]	75.5 [a]	0.6	0.0005	72.7 [b]	79.0 [a]	1.1	0.008

Table 12. *Cont.*

Items	NFD [2]		SEM	*p* Value	HDP [3]		SEM	*p* Value
	EFM [4]	BSEFM [5]			EFM	BSEFM		
Met	83.4	80.5	0.9	0.0717	84.4	84.0	1.3	0.807
Ile	74.3 [b]	79.5 [a]	0.8	0.0037	74.4 [b]	80.1 [a]	1.5	0.031
Leu	69.7 [b]	78.9 [a]	0.9	0.0003	73.9 [b]	82.2 [a]	1.4	0.005
Trp	75.9	78.1	1.1	0.2010	83.4	84.0	1.3	0.747
Phe	73.5 [b]	78.3 [a]	0.9	0.0078	77.3 [b]	81.4 [a]	1.0	0.030
Lys	70.3 [b]	79.7 [a]	1.1	0.0007	76.4 [b]	80.7 [a]	1.1	0.028
His	73.9 [b]	81.7 [a]	1.0	0.0012	77.3 [b]	83.9 [a]	1.2	0.009
Arg	70.6 [b]	75.8 [a]	1.0	0.0124	73.8 [b]	78.7 [a]	1.1	0.018
Nonessential amino acids, %								
Asp	61.2 [b]	74.5 [a]	0.8	<0.0001	68.8 [b]	78.0 [a]	1.3	0.002
Ser	67.0 [b]	74.6 [a]	0.8	0.0007	70.9 [b]	77.1 [a]	0.9	0.003
Glu	69.3 [b]	79.4 [a]	1.0	0.0004	71.3 [b]	78.8 [a]	1.4	0.009
Pro	65.6 [b]	75.5 [a]	0.6	<0.0001	68.4 [b]	78.2 [a]	1.0	<0.001
Gly	67.2 [b]	74.7 [a]	0.9	0.0011	72.7 [b]	78.6 [a]	0.8	0.002
Ala	69.9 [b]	77.7 [a]	1.0	0.0012	76.2 [b]	83.0 [a]	0.7	<0.001
Cys	54.8 [b]	64.4 [a]	0.9	0.0003	59.0 [b]	64.9 [a]	1.4	0.024

[1] Data are means of four pens of broilers; two broilers were sampled from each pen. [2] NFD = nitrogen-free diet. [3] HDP = highly digestible protein diet. [4] EFM = enzymatically degraded feather meal. [5] BSEFM = EFM cultures were *B. subtilis* var. *natto* N21 and *S. cerevisiae* Y10. [a,b] Means in the same row with different superscripts are significantly different ($p < 0.05$).

4. Discussion

4.1. Trial 1

The addition of 4% feather meal in the diet had no effect on broiler growth performance; however, the addition of 5–8% feather meal resulted in inadequate levels of Lys, Met, His, and Trp, and limited growth performance [29–31]. The dietary feather meal may be added at levels up to 10% without adverse effects on broiler growth, provided that amino acids are properly supplemented [32–34].

In this trial, the content of MET and LYS in the diet was properly balanced. Although weight gain was negatively affected in the 10% group over the 1–21-day period, this result was not observed over days 22–35 and 1–35. The decline in weight gain may be attributable to the underdeveloped digestive tract of broilers during days 1–21. The inclusion of 10% EFM in the diet at 22–35 days, presumably a period with better gastrointestinal development, did not adversely affect growth performance. However, dietary inclusion of more than 15% EFM resulted in a significant impact on the growth performance over days 1–35. Therefore, the EFM dose tolerance in the broiler diet is 10%.

Feather meal has poor nutrient availability, and the amino acid composition is imbalanced [1,2,4]. This may overestimate the nutrient availability of the feed formulation. These disadvantages may result in reducing broiler growth performance linearly and quadratically with the increase in dietary EFM dosage. In addition, increasing the EFM dosage in the diet also linearly and quadratically inhibited feed intake. Lower feed intake may further exacerbate nutrient deficiencies in broilers. The above issues require further research to understand the correlation of various factors with the growth performance of broilers.

4.2. Trial 2

B. subtilis var. natto N21 was selected for its high proteolytic capacity. Inoculated BS-fermented feed can generally improve broiler growth [18]. However, feathers are mainly composed of keratin, the microbial decomposition of which presents difficulties. Feather decomposition ability for a given inoculation is usually evaluated by measuring the weight of feathers in a medium before and after fermentation [35]. In trial 2, the feather decomposition rate without BS was lower than 4.5% after 72 h of incubation. Feather decomposition rates, however, were 33.7%, 62.0%, and 72.6%, respectively, after 24 h, 48 h,

and 72 h of BS inoculation. High keratinase activity was also observed through further analysis of the filtrate derived from the medium. These results confirmed the keratinolytic ability of BS.

Bacillus subtilis natto is adapted to survive in a neutral pH environment [36] and produces alkaline metabolites during reproduction (e.g., nattokinase), causing the cultivation environment to become alkaline [37]. This is consistent with the observation from first-stage fermentation in trial 2, which exhibited a pH increase from 5.76 to 7.10. The pH of the EFM decreased from 7.07 to 5.45 upon second-stage fermentation since the inoculated microbe was BS or SC with great acidification ability. The drying process showed no significant effect on the pH of oven-dried BBEFM and BSEFM after fermentation. Chen et al. [18] indicated that lactic acid and then acetic acid, neither of which is volatile, were the main organic acids in BS + SC-fermented feed. Accordingly, the drying process did not affect the pH levels in this trial.

Bacillus coagulans and B. subtilis have the capacity to form spores [38,39] which can resist high pressure, high temperature, and low pH values [40]. Consequently, the drying process had no effect on the count of Bacillus-like bacteria in the BBEFM and BSEFM groups, nor the count of total lactic acid bacteria in the BBEFM group. SC and other microorganisms have limited heat-resistant capacity, and so their survival rate after the drying process was decreased.

Inclusion of BSEFM improved growth performance during the first three weeks to a level comparable to the control group (corn–soybean meal diets). On the other hand, the BBEFM group did not exhibit improved growth, not even reaching the EFM growth level. Our previous study confirmed that not all of the various two-stage fermented feeds could improve broiler growth performance [19]. This agrees with the result obtained in this trial. The lower growth rate of the BBEFM group over days 1–21 may be attributed to the lower feed intake; however, further study is still required. The weight gain and feed conversion ratios of the BBEFM and BSEFM groups improved significantly in comparison to the control and EFM groups during the later stage of growth (22–35 days). In general, the broiler gastrointestinal tract is better developed at 22–35 days compared with 1–21 days of age. Broilers in the BBEFM group may experience compensatory growth at the later stage, which results in a comparable level of weight gain, feed conversion ratio, and PEF to the control group over the whole period. The BSEFM group showed better growth performance during both stages; therefore, the weight gain, feed conversion ratio, and PEF were significantly higher during the entire growth period than in the control group. The nutritional value of feather meal can be augmented through fermentation technology, upgrading its feeding value to be comparable to that of soybean meal [8–10]. In this trial, the BSEFM not only partially substituted for soybean meal and full-fat soybean meal in the diet, but also improved broiler weight gain, feed conversion ratio, and PEF by 7.6%, 13.9%, and 6.1%, respectively. These results confirm that dietary inclusion of BSEFM could provide better feed value than a corn–soybean meal diet. In this trial, the weight gain of the EFM group was significantly lower than the control group during days 1–21, but not over days 22–35 or 1–35. This results are consistent with trial 1, which again confirms that the EFM dose tolerance for broiler diets is 10%.

Our previous studies showed that feeding a broiler BS + BC- or BS + SC-fermented feed could enhance the relative weight of the proventriculus and gizzard [18,19]. However, there was no significant difference in carcass traits among treatments. Although BBEFM and BSEFM are also produced from the same two-stage fermentation technology, the substrate and conditions of the fermentation differ from those of the previous fermented feed, and so the influence on carcass traits may also be different. In addition, the fermented feather meal in this study accounted for only 10% of the diet, a lower ingested amount of fermented feather meal than the fermented feed in the previous study. Therefore, BBEFM and BSEFM had no significant influence on carcass traits.

Glutamate oxaloacetate transaminase and lactate dehydrogenase are widely distributed in chicken liver, heart, kidney, and muscle, and creatine kinase is an enzyme

specific to the heart and muscle. While rising glutamate oxaloacetate transaminase and lactate dehydrogenase levels with constant creatine kinase levels signal damage to the liver and kidney, an increase in all three enzymes signals both heart and muscle damage [41]. Amylase is found in the liver, bile, saliva, and pancreas, with the pancreas being the major source. Amylase activity, therefore, can be used to diagnose pancreatic function [42]. γ-glutamyl transpeptidase is a cell membrane enzyme related to glutathione metabolism and amino acid absorption in the glomerulus and small intestine [43]. γ-glutamyl transpeptidase is an enzyme specific to poultry kidney [41]. Alkaline phosphatase is an important enzyme involved in bone mineralization [44], and along with serum calcium and phosphorus is an indicator of bone characteristics. No significant differences among carcass traits and serum biochemical constituents were observed in trial 2, indicating that diets including 10% EFM, BBEFM, or BSEFM did not show any detrimental effect on liver, heart, kidney, muscle, pancreas, or bone.

4.3. Trial 3

In order to determine the level of endogenous nitrogen in the gastrointestinal tract of the broiler, no nitrogen-containing materials were included in the NFD diet, which exhibits very low CP and amino acid content. Casein was the only source of nitrogen-containing material in the HDP diet and was assumed to be completely absorbed by birds. This thereby provides a basis for the assessment of endogenous amino acids induced by protein intake [23]. Several publications have shown that the amino acid content in feathers was limiting in Met and His, with high Glu and Ser [45,46], which is consistent with the results of our trial. After fermentation, the amino acid composition ratio of BSEFM was still similar to that of EFM; however, the CP and total amino acid content increased by 11.7% and 21.1%, respectively. Shi et al. [47] indicated that solid-state fermentation of rapeseed cake with *Aspergillus niger* significantly increased total amino acids by 24.3% and essential amino acids by 28.5%. The solid-state fermentation of cottonseed meal with *Candida tropicalis* increased total amino acids by 11.9% and essential amino acids by 12.0% [48]. Our previous study produced two-stage BS + BB-fermented feed using a corn–soybean meal diet as the substrate; this fermentation was also found to increase the total amino acid content by 6.7% [19]. Observations from the present study are also consistent with the above references. The increase in CP and amino acid content are also accompanied by loss of carbohydrate content through fermentation [47,49]. However, the increase in amino acid composition of BSEFM requires further study.

Recent publications have reported the potential for improvement in solubility and digestibility of feather meal through an enzyme or fermentation technique [10,11], techniques which were applied to EFM in this study. The use of SIAAD to evaluate the endogenous ileal amino acids is more accurate than the traditional AIAAD measurement methods [23,50]. Similar results, obtained using AIAAD and SIAAD on NFD or HDP diets in this trial, confirmed the amino acid digestibility improvement in feather meal via the BS + SC fermentation process.

The amino acid digestibility of feather meal varies greatly [51]. Bandegan et al. [45] indicated that the poorest AIAAD values among amino acids in feather were for Cys and Asp. Thr, Lys, and sulfur-containing amino acids showed the poorest values while Ile, Phe, and Val showed the highest values among the essential amino acids [45]. Our results in this trial also showed Cys and Asp to have the poorest AIAAD values. However, the Thr, Leu, Val, and Arg values were poorest while Met, Phe, and Ile showed the best AIAAD values among the essential amino acids. The abovementioned literature is partly consistent with the results of this study. Ileal amino acid digestibility values are influenced by the analytical method and animal species [52–54]. In addition, processing methods also affect amino acid composition and digestibility [29,46,51,55], which may contribute to the different results.

Summarizing the results of trials 2 and 3, the BSEFM group showed better nutritional digestibility under the same amount of feed intake, and therefore showed an improvement in weight gain and feed conversion ratio. Protein from feather meal might be decomposed

during BS + SC two-stage fermentation, thus producing microbial protein or metabolites that improve growth performance of broilers.

5. Conclusions

The EFM dose tolerance for the broiler diet is 10%. Adding 10% BS + SC two-stage fermented EFM to the diet can improve the weight gain and feed conversion ratio and can enhance the SIAAD of EFM in broilers.

Author Contributions: Conceptualization, R.-H.Y. and K.-L.C.; methodology, R.-H.Y., C.-W.H. and K.-L.C.; software, R.-H.Y.; validation, R.-H.Y. and K.-L.C.; formal analysis, R.-H.Y. and K.-L.C.; investigation, R.-H.Y. and K.-L.C.; resources, R.-H.Y., C.-W.H. and K.-L.C.; data curation, R.-H.Y. and K.-L.C.; writing—original draft preparation, R.-H.Y. and K.-L.C.; writing—review and editing, R.-H.Y. and K.-L.C.; supervision, R.-H.Y. and K.-L.C.; project administration, R.-H.Y., C.-W.H. and K.-L.C.; funding acquisition, R.-H.Y. and K.-L.C. All authors have read and agreed to the published version of the manuscript.

Funding: This study was funded by the Council of Agriculture, Executive Yuan (110AS-10.1.1-LI-L1).

Institutional Review Board Statement: The experiments were performed in accordance with regulations of the Republic of China (Taiwan) and approved by the Institutional Animal Care and Use Committee of National Chiayi University (IACUC, approval number 105050).

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are available from the first author upon request. The data are not publicly available due to institutional policy.

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

References

1. Ullrey, D.E.; Baer, C.K.; Pond, W.G. *Encyclopedia of Animal Science*, 2nd ed.; CRC Press: New York, NY, USA, 2011; pp. 169–421. ISBN 9781351238014.
2. NRC. *Nutrient Requirements of Poultry*, 9th ed.; National Academy Press: Washington, DC, USA, 1994. [CrossRef]
3. Wang, X.; Parsons, C. Order of amino acid limitation in poultry by-product meal. *Br. Poult. Sci.* **1998**, *39*, 113–116. [CrossRef]
4. Zhang, B.; Jiang, D.D.; Zhou, W.W. Isolation and characterization of a new *Bacillus* sp. 50-3 with highly alkaline keratinase activity from *Calotes versicolor* faeces. *World J. Microbiol. Biotechnol.* **2009**, *25*, 583–590. [CrossRef]
5. El Boushy, A.R.; van der Poel, A.F.B.; Walraven, O.E.D. Feather meal-A biological waste: Its processing and utilization as a feedstuff for poultry. *Biol. Wastes* **1990**, *32*, 39–74. [CrossRef]
6. Gessesse, A.; Hatti-Kaul, R.; Gashe, B.A. Novel alkaline proteases from alkaliphilic bacteria grown on chicken feather. *Enzyme Microb. Technol.* **2003**, *32*, 519–524. [CrossRef]
7. Moritz, J.S.; Latshaw, J.D. Indicators of nutritional value of hydrolyzed feather meal. *Poult. Sci.* **2001**, *80*, 79–86. [CrossRef]
8. Williams, C.M.; Lee, C.G.; Garlich, J.D.; Shih, J.C.H. Evaluation of a bacterial feather fermentation product, feather lysate, as a feed protein. *Poult. Sci.* **1991**, *70*, 85–94. [CrossRef]
9. Cedrola, S.M.L.; de Melo, A.C.N.; Mazotto, A.M.; Lins, U.; Zingali, R.B.; Rosado, A.S.; Peixoto, R.S.; Vermelho, A.B. Keratinases and sulfide from *Bacillus subtilis* SLC to recycle feather waste. *World J. Microbiol. Biotechnol.* **2012**, *28*, 1259–1269. [CrossRef]
10. Guo, G.; Chu, J.; Wang, J.G.; He, Q.X.; Liu, K.C. A two-step biotechnological process for improving nutrition value of feather meal by *Bacillus licheniformis* S6. *J. Northeast Agric. Univ.* **2013**, *20*, 71–77. [CrossRef]
11. Grazziotin, A.; Pimentel, F.A.; de Jong, E.V.; Brandelli, A. Nutritional improvement of feather protein by treatment with microbial keratinase. *Anim. Feed Sci. Technol.* **2006**, *126*, 135–144. [CrossRef]
12. Lee, G.G.; Ferket, P.R.; Shih, J.C.H. Improvement of feather digestibility by bacterial keratinase as a feed additive. *FASEB J.* **1991**, *59*, 1312.
13. Suganuma, T.; Fujita, K.; Kitahara, K. Some distinguishable properties between acid-stable and neutral types of α-amylases from acid-producing *Koji*. *J. Biosci. Bioeng.* **2007**, *104*, 353–362. [CrossRef] [PubMed]
14. Ward, O.P.; Qin, W.M.; Dhanjoon, J.; Ye, J.; Singh, A. Physiology and biotechnology of *Aspergillus*. *Adv. Appl. Microbiol.* **2006**, *58*, 1–55. [CrossRef] [PubMed]
15. Hong, K.J.; Lee, C.H.; Kim, S.W. *Aspergillus oryzae* GB-107 fermentation improves nutritional quality of food soybeans and feed soybean meals. *J. Med. Food.* **2004**, *7*, 430–435. [CrossRef] [PubMed]
16. Cumby, T.R. Design requirements of liquid feeding systems for pigs: A review. *J. Agric. Eng. Res.* **1986**, *34*, 153–172. [CrossRef]
17. Hirabayashi, M.; Marsui, T.; Yano, H.; Nakajima, T. Fermentation of soybean meal with *Aspergillus usamii* reduces phosphorus excretion in chicks. *Poult. Sci.* **1998**, *77*, 552–556. [CrossRef] [PubMed]

18. Chen, K.L.; Kho, W.L.; You, S.H.; Yeh, R.H.; Tang, S.W.; Hsieh, C.W. Effect of *Bacillus subtilis* var. natto and *Saccharomyces cerevisiae* mixed fermented feed on the enhanced growth performance of broilers. *Poult. Sci.* **2009**, *88*, 309–315. [CrossRef] [PubMed]
19. Yeh, R.H.; Hsieh, C.W.; Chen, K.L. Screening lactic acid bacteria to manufacture two-stage fermented feed and pelleting to investigate the feeding effect on broilers. *Poult. Sci.* **2017**, *97*, 236–246. [CrossRef]
20. Arbor Acres. *Broiler Management Manual*; Arbor Acres Taiwan Inc.: Taipei, Taiwan, 2012.
21. Huang, H.H.; Weng, B.C.; Hsuuw, Y.D.; Lee, Y.S.; Chen, K.L. Dietary supplementation of two-stage fermented feather-soybean meal product on growth performance and immunity in finishing pigs. *Animals* **2021**, *11*, 1527. [CrossRef]
22. Bergmeyer, H.U. *Methods of Enzymatic Analysis*, 3rd ed.; Wiley-Blackwell: Weinheim, Germany, 1983.
23. Adedokun, S.A.; Parsons, C.M.; Lilburn, M.S.; Adeola, O.; Applegate, T.J. Standardized ileal amino acid digestibility of meat and bone meal from different sources in broiler chicks and turkey poults with a nitrogen-free or casein diet. *Poult. Sci.* **2007**, *86*, 2598–2607. [CrossRef]
24. AOAC International. *Official Methods of Analysis*, 15th ed.; AOAC International: Gaithersburg, MD, USA, 1990.
25. AOAC International. *Official Methods of Analysis*, 17th ed.; AOAC International: Gaithersburg, MD, USA, 2000.
26. AOAC International. *Official Methods of Analysis*, 18th ed.; AOAC International: Arlington, VA, USA, 2005.
27. SAS Institute. *SAS/STAT User's Guide: STATISTICS*, 1st ed.; SAS Institute Inc.: Cary, NC, USA, 2004; pp. 1731–1747. ISBN 1-59047-243-8.
28. Steel, R.G.D.; Torrie, J.H. *Principles and Procedures of Statistics—A Biometrical Approach*, 2nd ed.; McGraw-Hill Book Company: New York, NY, USA, 1980.
29. Moran, E.T.; Summers, J.D.; Slinger, S.J. Keratin as a source of protein for the growing chick: 1. Amino acid imbalance as the cause for inferior performance of feather meal and the implication of disulfide bonding in raw feathers as the reason for poor digestibility. *Poult. Sci.* **1966**, *45*, 1257–1266. [CrossRef]
30. Luong, V.B.; Payne, C.G. Hydrolysed feather protein as a source of amino acids for laying hens. *Br. Poult. Sci.* **1977**, *18*, 523–526. [CrossRef]
31. MacAlpine, R.; Payne, C.G. Hydrolysed feather protein as a source of amino acids for broilers. *Br. Poult. Sci.* **1977**, *18*, 265–273. [CrossRef]
32. Baker, D.H.; Blitenthal, R.C.; Boebel, K.P.; Czarnecki, G.L.; Southern, L.L.; Willis, G.M. Protein-amino acid evaluation of steamed-processed feather meal. *Poult. Sci.* **1981**, *60*, 1865–1872. [CrossRef]
33. Cabel, M.C.; Goodwin, T.L.; Waldroup, P.W. Reduction in abdominal fat content of broiler chicks by the addition of feather meal to finisher diets. *Poul. Sci.* **1987**, *66*, 1644–1651. [CrossRef]
34. Cupo, M.A.; Cartwright, A.L. The effect of feather meal on carcass composition and fat pad cellularity in broilers: Influence of the calorie:protein ratio of the diet. *Poult. Sci.* **1991**, *70*, 153–159. [CrossRef]
35. Lucas, F.S.; Broennimann, O.; Febbraro, I.; Heeb, P. High dversity among feather-degrading bacteria from a dry meadow soil. *Microb. Ecol.* **2003**, *45*, 282–290. [CrossRef]
36. Wu, F.C.; Chou, S.Z.; Shih, I.L. Factors affecting the production and molecular weight of levan of *Bacillus subtilis* natto in batch and fed-batch culture in fermenter. *J. Taiwan Inst. Chem. Eng.* **2013**, *44*, 846–853. [CrossRef]
37. Allagheny, N.; Obanu, Z.A.; Campbell-Platt, G.; Owens, J.D. Control of ammonia during *Bacillus subtilis* fermentation of legumes. *Int. J. Food Microbiol.* **1996**, *29*, 321–333. [CrossRef]
38. Gandhi, A.B. Probiotic lactic acid bacteria. *East. Pharm.* **1994**, *37*, 43.
39. Sanders, M.E.; Morelli, L.; Bush, S. *Lactobacillus sporogenes* is not a *Lactobacillus* probiotic. *ASM News* **2001**, *67*, 385–386.
40. Palop, A.; Raso, J.; Pagán, R.; Condón, S.; Sala, F.J. Influence of pH on heat resistance of spores of *Bacillus coagulans* in buffer and homogenized foods. *Int. J. Food Microbiol.* **1999**, *46*, 243–249. [CrossRef] [PubMed]
41. Wang, C.H. Plasma and tissue enzyme activities in young chickens. *J. Chin. Soc. Vet. Sci.* **1992**, *18*, 132–138.
42. Rodeheaver, D.P.; Wyatt, R.D. Distribution of alpha-amylase activity in selected broiler tissues. *Poult. Sci.* **1986**, *65*, 325–329. [CrossRef] [PubMed]
43. Kaplan, L.A.; Pesce, A.J. *Clincal Chemistry. Theory, Analysis and Correlation*, 3rd ed.; Oxford University Press: Mosby, MO, USA, 1996; ISBN 0-8151-5243-4.
44. Bilal, T.; Atis, S.; Keser, O. The effects of microbial phytase on serum calcium and phosphorus levels and alkaline phosphatase activities in broilers fed diets containing different levels of phosphorus. *Acta. Sci. Vet.* **2015**, *43*, 1327.
45. Bandegan, A.; Kiarie, E.; Payne, R.L.; Crow, G.H.; Guenter, W.; Nyachoti, C.M. Standardized ileal amino acid digestibility in dry-extruded expelled soybean meal, extruded canola seed-pea, feather meal, and poultry by-product meal for broiler chickens. *Poult. Sci.* **2010**, *89*, 2626–2633. [CrossRef] [PubMed]
46. Papadopoulos, M.; El Boushy, A.; Roodbeen, A.; Ketelaars, E. Effects of processing time and moisture content on amino acid composition and nitrogen characteristics of feather meal. *Anim. Feed Sci. Technol.* **1986**, *14*, 279–290. [CrossRef]
47. Shi, C.; He, J.; Yu, J.; Yu, B.; Huang, Z.; Mao, X.; Zheng, P.; Chen, D. Solid state fermentation of rapeseed cake with *Aspergillus niger* for degrading glucosinolates and upgrading nutritional value. *J. Anim. Sci. Biotechnol.* **2015**, *6*, 13. [CrossRef] [PubMed]
48. Khalaf, M.A.; Meleigy, S.A. Reduction of free gossypol levels in cottonseed meal by microbial treatment. *Int. J. Agric. Biol.* **2008**, *10*, 185–190.
49. Rozan, P.; Villaume, C.; Bau, H.; Schwertz, A.; Nicolas, J.; Méjean, L. Detoxication of rapeseed meal by *Rhizopus Oligosporus* sp-T3: A first step towards rapeseed protein concentrate. *Int. J. Agric. Biol.* **1996**, *31*, 85–90. [CrossRef]
50. Angkanaporn, K.; Ravindran, V.; Bryden, W.L. Additivity of apparent and true ileal amino acid digestibilities in soybean meal, sunflower meal, and meat and bone meal for broilers. *Poult. Sci.* **1996**, *75*, 1098–1103. [CrossRef]

51. Bielorai, R.; Harduf, Z.; Iosif, B.; Alumot, E. Apparent amino acid absorption from feather meal by chicks. *Br. J. Nutr.* **1983**, *49*, 395–399. [CrossRef] [PubMed]
52. Ten Doeschate, R.A.H.M.; Scheele, C.W.; Schreurs, V.V.A.M.; Van Der Klis, J.D. Digestibility studies in broiler chickens: Influence of genotype, age, sex and method of determination. *Br. Poult. Sci.* **1993**, *34*, 131–146. [CrossRef]
53. Ravindran, V.; Hendriks, W.H. Recovery and composition of endogenous protein collected at the terminal ileum as influenced by the age of broiler chickens. *Aust. J. Agric. Res.* **2004**, *55*, 705–709. [CrossRef]
54. Garcia, A.R.; Batal, A.B.; Dale, N.M. A comparison of methods to determine amino acid digestibility of feed ingredients for chickens. *Poult. Sci.* **2007**, *86*, 94–101. [CrossRef] [PubMed]
55. Eggum, B.O. Evaluation of protein quality of feather meal under different treatments. *Acta. Sci. Vet.* **1970**, *20*, 230–234. [CrossRef]