RESEARCH ARTICLE

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Partial or complete replacement of fishmeal with fermented soybean meal on growth performance, fecal composition, and meat quality in broilers

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Abstract

The current study was aimed to examine the effect of partial or complete replacement of fishmeal (FM) with fermented soybean meal (FSBM) on growth performance, fecal composition, and meat quality in broiler chickens. A total number of 240 one-day-old broiler chicks were randomly allotted into four dietary treatments with six replications and ten birds per one pen. Dietary treatments were followed as; 1) Diet incorporated with 4% FM without FSBM (Control), 2) Diet incorporated with 3% FM and 2% FSBM (FSBM2), 3) Diet incorporated with 2% FM and 3% FSBM (FSBM3) and 4) Diet incorporated with 4% FSBM without FM (FSBM4). Body weight and feed intake were recorded weekly for 35 days of the experimental period. Moreover, fecal samples were collected to evaluate moisture, ash, nitrogen, calcium and phosphorus content on day 21 post-hatch. On day 35, two birds were sacrificed from each pen to measure meat quality parameters and visceral organ weights. Results revealed that, no dietary treatment effect ($p > 0.05$) was observed either in both body weight or average daily gain of broilers within the entire experimental period while broilers fed FSBM2 increased ($p < 0.05$) average daily feed intake by 10.07% whereas FSBM4 improved ($p < 0.05$) feed efficiency ratio by 8.45% compared to birds fed other dietary treatments on day 7 post-hatch. Besides, birds fed FSBM3 obtained the improved ($p < 0.05$) feed conversion ratio over the birds fed control diet by 7.51% from hatch to day 35 post-hatch (1.60 vs. 1.73). Nevertheless, no difference ($p > 0.05$) was detected on visceral organ weight, proximate composition and physicochemical characteristics of meat while broilers offered FSBM4 obtained the lowest ($p < 0.05$) calcium and phosphorous in faces (2.27% and 1.21% respectively) over those offered

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control feed and other FSBM treatments. In conclusion, FSBM would be a better replacement for ousting FM partially or completely in broiler diet as it did not impair the growth performance and meat quality while reducing the calcium and phosphorous excretion in broilers for 35 days post-hatch.

Keywords: Broiler, Fermented soybean meal, Fish meal, Growth performance, Meat quality

INTRODUCTION

Animal feed is considered as a major sector in the global food and agriculture industry. It is essential to provide high-quality, nutritious and clean animal feed to ensure efficient and optimal livestock production [1]. In poultry, high-quality feed provides optimum energy and nutrients for growth improvement, bone development and feather growth.

Microbial fermentation of SBM using either bacteria or fungi not only improves the nutritional value and the native composition of SBM [2] but also improves nutrient utilization by fungal enzymes. Bacterial and fungal fermentation results in degradation and efficient elimination of anti-nutritional factors such as phytates, oligosaccharides, trypsin inhibitors and mycotoxins in SBM [3,4]. It also increases the content of small-sized peptides and eliminates both essential and non-essential amino acids [5]. Furthermore, as the absorption of dipeptides is more efficient than that of single amino acids, the activity of PepT1 transporters in the small intestine increases [6]. These dipeptides may be absorbed more rapidly than protein-bound amino acids because they do not have to undergo any further digestion. Several studies have reported that incorporating fermented SBM (FSBM) into diets leads to improved growth performance, gut morphology and protein digestibility while reducing immunological reactions and phosphorus excretion in non-ruminants [2,7–10].

Fish meal (FM) is mainly produced by small fish species that are not appropriate for human consumption and contain a high proportion of oil and bones [11]. FM is a highly nutritious and highly digestible feed ingredient that contains a high amount of energy, protein, energy, lipids, vitamins, and minerals, including a small amount of carbohydrates [11,12]. Furthermore, FM provides essential amino acids, especially lysine and sulfur containing amino acids [13]. Even though FM is a good source of protein, its usage is limited in broiler feed formulation because of its high cost and low availability in the market compared to plant-based protein ingredients [14,15] as well as it provides off-flavor to the broiler meat [16]. Not only that, but there is also a risk that animal originated feed ingredients normally harbor pathogenic microorganisms. Storage problems and adulteration problems have emerged as problems in FM [13,17,18].

These factors have led to the exploration of alternative, safe protein sources for incorporation into broiler diets [18]. FSBM could be a good and cost-effective alternative protein source to replace FM partially or completely in the diets of various animal species as a less expensive protein source than FM [19–21]. The majority of prior research has evaluated the supplementation of FSBM in the diet on broiler performance and the partial replacement of protein feed ingredients like FM in broiler diets with 0.5%–3% FSBM [10,21,22]. Thus, the objective of this experiment was to determine the effect of partial or complete replacement of fishmeal in broiler diets with FSBM on growth performance, meat quality, and mineral excretion of broilers.

MATERIALS AND METHODS

Birds were reared under the guidelines of the Indian River® broiler management handbook [23].

The complete experimental procedure was according to the Guidelines of Research Ethics Committee of Uva Wellassa University of Sri Lanka.

Preparation of fermented soybean meal

FSBM was prepared corresponding to the study Mathivanan et al. [22] with some modifications. Commercially available SBM was obtained and soaked in clean water for minutes. Thereafter soaked SBM was autoclaved (LS-10OHD, Jiangyin Binjiang Medical Equipment, Jiangsu, China) for 30 min at 121℃. Meanwhile, *Saccharomyces cerevisiae* culture was made by mixing commercial dry yeast (Pangoo Biotech Hebei, Hebei, China) in lukewarm (37℃–40℃) sugar solution and propagate for two hours. Then after, autoclaved SBM (after cooled down to room temperature) was inoculated with the pre-made culture solution and fermented for 3 days (72 hours) under aerobic conditions. Obtained FSBM was dried and grounded for experimental use.

Birds and housing

The current experiment was conducted in the research farm of New Hope Lanka, Sri Lanka. Two hundred and forty one-day-old "Indian River" broiler chicks were randomly allotted into 24 experimental units with approximately similar initial body weights for a 35-d feeding trial. Birds were separated into four dietary treatments and six replicates per treatment (10 birds/replicate). Floor pens were provided as housing with 930 cm^2 floor spaces for each bird. Wood shavings were used as bedding material and 10 cm thick litter layer was maintained in the cages. Initially, the outside temperature was kept at 33℃ then gradually decreased within three days to meet room temperature (25±2℃) for the rest of the experiment with a continuous lighting regime (24 hours) simultaneously. Experimental diets and fresh clean drinking water were provided *ad-libitum* basis.

Experimental design, diets and treatment

The experiment was conducted by using completely randomize design (CRD) with using four dietary treatments as; 1) Diet incorporated with 4% FM without FSBM (Control), 2) Diet incorporated with 3% FM and 2% FSBM (FSBM2), 3) Diet incorporated with 2% FM and 3% FSBM (FSBM3) and 4) Diet incorporated with 4% FSBM without FM (FSBM4). All the diets were produced based on rice and SBM to fulfil the nutrition requirement specified in Indian River® nutrition specification [23]. Three phases feeding program was practiced as broiler booster (Day 1–Day 14), broiler starter (Day 15–Day 28) and broiler finisher (Day 29 to end). Feed formulation data is presented in Tables 1, 2, and 3.

Growth performance evaluation

Weekly body weights (day 7, 14, 21, 28, and 35) were measured, during the entire experimental period and average daily weight gain per bird of each replicate was calculated. Subsequently, feed intake of each replicate was measured daily as feed disappearance in the feeder and average daily feed intake (ADFI) per bird was determined. With the usage of this data, the total average feed intake per bird and feed conversion ratio (FCR) was calculated in each replicate from hatch to day 35 post-hatch. Moreover, the daily mortality of each replicate was recorded when the death occurred.

Fecal sample collection

Clean excreta (free from feathers, feed and bedding materials) was collected separately in two times (morning and afternoon) on the day 21 post-hatch according to the treatments. Plastic sheets were placed upon the bedding in each pen to collect clean excreta. The pooled samples were then frozen.

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Table 1. Composition (%, as-fed basis) of the experimental broiler starter diet

Ingredients	Broiler starter (d 0–14)			
	Control	FSBM2	FSBM3	FSBM4
Broken rice	57.00	56.90	56.65	57.00
Dried distillers grains	5.50	5.50	5.50	5.50
Vegetable oil	1.20	1.30	1.35	1.35
Soybean meal 44%	25.61	25.10	25.00	25.00
Fermented soybean meal	0.00	2.00	3.00	4.00
Corn gluten meal 60%	3.00	2.50	2.80	3.20
Fish meal	4.00	3.00	2.00	0.00
Di-calcium phosphate	0.85	0.85	0.85	0.85
Lime stone powder	1.20	1.20	1.20	1.40
Salt	0.74	0.74	0.74	0.74
Mineral pre-mix[1)]	0.20	0.20	0.20	0.20
Vitamin pre-mix[2)]	0.04	0.04	0.04	0.04
L-Lysine 98.5%	0.27	0.27	0.27	0.31
DL-Methionine 98.5%	0.26	0.26	0.26	0.26
L-Threonine 99%	0.14	0.14	0.14	0.14
Calculated values[3)]				
ME (kcal/kg)	2,900	2,900	2,900	2,900
CP (%)	22	22	22	22
Ca (%)	1.00	0.95	0.90	0.87
Available P (%)	0.34	0.32	0.29	0.25
Total Lysine (%)	1.33	1.33	1.31	1.28
Total Met + Cys (%)	0.94	0.93	0.92	0.91
Total Thr + Trp (%)	1.23	1.24	1.24	1.22
Total Val + Arg (%)	2.68	2.69	2.70	2.66

[1)]Supplied per kilogram of total diets: Fe, 80 mg; Zn, 80 mg; Mn 80 mg; Co 0.5 mg; Cu, 10 mg; Se, 0.2 mg; I, 0.9 mg; Mg, 60 mg; K, 1 mg; Na, 0.5 mg.

[2)]Supplied per kilogram of total diets: Vitamin A, 24,000 IU; Vitamin D3, 6,000 IU; Vitamin E, 30 IU; Vitamin K, 4 mg; Thiamin, 4 mg; Riboflavin, 12 mg; Pyridoxine, 4 mg; Folacine, 2 mg; Biotin, 0.03 mg; Vitamin B8, 0.06 mg; Niacin, 90 mg; Pantothenic acid, 30 mg.

[3)]The values were calculated according to the values of feedstuffs in NRC (1994).

FSBM, fermented soybean meal; ME, metabolisable energy; CP, crude protein; Met, methionine; Cys, cysteine; Thr, threonine; Trp, tryptophan; Val, valine; Arg, arginine.

Before analysis, the samples were dried (except the samples for moisture analysis) in an air oven at 55℃ for 72 hours, followed by fine grinding and strained through a <1 mm sieve [24].

Slaughtering of birds and sample collection

Two birds that close to the mean body weight of each replicate were selected and slaughtered by bleeding and properly eviscerated at the day 35 post-hatch. Internal organs (i.e., liver, heart, gizzard, pancreas, cecum and intestine) were properly separated under the inspection of a veterinary surgeon. Carcass weight and internal organs weight (liver, heart, gizzard, pancreas, cecum and intestine) of slaughtered birds were obtained and expressed the organ weight in proportion to the live body weight.

Proximate analysis of broiler breast meat, feces and broiler feed

Moisture content and ash content of samples were analyzed by using the standard method of

Table 2. Composition (%, as-fed basis) of the experimental broiler grower diet

Ingredients	Broiler grower (d 15–28)			
	Control	FSBM2	FSBM3	FSBM4
Broken rice	55.59	55.29	55.29	54.70
Wheat shorts	2.00	2.00	2.00	2.00
Dried distillers grains	7.00	7.00	7.00	7.00
Vegetable fat	2.40	2.50	2.50	2.60
Soybean meal 44%	23.5	22.5	22.5	23.56
Fermented soybean meal	0.00	2.00	3.00	4.00
Corn gluten meal 60%	2.00	2.20	2.20	2.60
Fish meal	4.00	3.00	2.00	0.00
Di-calcium phosphate	0.45	0.45	0.45	0.45
Lime stone powder	1.45	1.45	1.45	1.45
Salt	0.47	0.47	0.47	0.47
Sodium bicarbonate	0.22	0.22	0.22	0.22
Choline chloride 60%	0.08	0.08	0.08	0.08
Mineral pre-mix[1)]	0.20	0.20	0.20	0.20
Vitamin pre-mix[2)]	0.04	0.04	0.04	0.04
L-Lysine 98.5%	0.20	0.20	0.20	0.23
DL-Methionine 98.5%	0.25	0.25	0.25	0.25
L-Threonine 99%	0.15	0.15	0.15	0.15
Calculated values[3)]				
ME (kcal/kg)	3,000	3,000	3,000	3,000
CP (%)	21	21	21	21
C (%)	1.01	0.96	0.91	0.90
Available P (%)	0.27	0.25	0.23	0.24
Total Lysine (%)	1.23	1.21	1.20	1.19
Total Met + Cy (%)	0.91	0.90	0.89	0.89
Total Thr + Trp (%)	1.19	1.19	1.19	1.20
Total Val + Arg (%)	2.56	2.58	2.58	2.60

[1)]Supplied per kilogram of total diets: Fe, 80 mg; Zn, 80 mg; Mn 80 mg; Co 0.5 mg; Cu, 10 mg; Se, 0.2 mg; I, 0.9 mg; Mg, 60 mg; K, 1 mg; Na, 0.5 mg.

[2)]Supplied per kilogram of total diets: Vitamin A, 24,000 IU; Vitamin D3, 6,000 IU; Vitamin E, 30 IU; Vitamin K, 4 mg; Thiamin, 4 mg; Riboflavin, 12 mg; Pyridoxine, 4 mg; Folacine, 2 mg; Biotin, 0.03 mg; Vitamin B8, 0.06 mg; Niacin, 90 mg; Pantothenic acid, 30 mg.

[3)]The values were calculated according to the values of feedstuffs in NRC (1994).

FSBM, fermented soybean meal; ME, metabolisable energy; CP, crude protein; Met, methionine; Cys, cysteine; Thr, threonine; Trp, tryptophan; Val, valine; Arg, arginine.

AOAC [25]. Air oven-dry method was practiced to analyse the moisture content and two grams of breast meat samples were dried at 103℃ for 16–18 hours. Loss of weight was recorded as moisture content by using the following equation, where W_1 is the weight of the empty dish, W_2 is the weight of the sample with the dish before drying and W_3 is the weight of the sample with dish after drying.

$$\text{Moisture content} = [(W_3 - W_1) / (W_2 - W_1)] \times 100\%$$

Ash content of the breast meat samples was determined, three grams of pre-dried test portions were placed in a muffle furnace (HD 230 PAD; Horno de Mufla, Tecny lab, Burgos, Spain) with

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Table 3. Composition (%, as-fed basis) of the experimental broiler finisher diet

Ingredients	Broiler finisher (d 29–35)			
	Control	FSBM2	FSBM3	FSBM4
Broken rice	50.50	49.80	49.80	50.01
Wheat shorts	2.50	2.50	2.50	2.50
Rice polish	5.00	5.00	5.00	5.00
Dried distillers grains	10.00	10.00	10.00	10.00
Vegetable fat	4.60	4.80	4.80	4.80
Soybean meal 44%	15.50	15.20	15.00	15.20
Fermented soybean meal	0.00	2.00	3.00	4.00
Corn gluten meal 60%	4.00	3.80	4.00	4.50
Fish meal	4.00	3.00	2.00	0.00
Di-calcium phosphate	0.80	0.80	0.80	0.80
Lime stone powder	1.44	1.44	1.44	1.44
Salt	0.48	0.48	0.48	0.48
Sodium bicarbonate	0.25	0.25	0.25	0.25
Choline chloride 60%	0.05	0.05	0.05	0.05
Mineral pre-mix[1)]	0.20	0.20	0.20	0.20
Vitamin pre-mix[2)]	0.04	0.04	0.04	0.04
L-Lysine 98.5%	0.29	0.29	0.29	0.35
DL-Methionine 98.5%	0.20	0.20	0.20	0.22
L-Threonine 99%	0.15	0.15	0.15	0.15
Calculated values[3)]				
ME (kcal/kg)	3,070	3,070	3,070	3,070
CP (%)	20	20	20	20
Ca (%)	1.07	1.02	0.96	0.86
Available P (%)	0.33	0.31	0.30	0.34
Total Lysine (%)	1.10	1.10	1.08	1.08
Total Met + Cys (%)	0.82	0.81	0.80	0.82
Total Thr + Trp (%)	1.06	1.07	1.06	1.05
Total Val + Arg (%)	2.21	2.25	2.25	2.23

[1)]Supplied per kilogram of total diets: Fe, 80 mg; Zn, 80 mg; Mn 80 mg; Co 0.5 mg; Cu, 10 mg; Se, 0.2 mg; I, 0.9 mg; Mg, 60 mg; K, 1 mg; Na, 0.5 mg.

[2)]Supplied per kilogram of total diets: Vitamin A, 24,000 IU; Vitamin D3, 6,000 IU; Vitamin E, 30 IU; Vitamin K, 4 mg; Thiamin, 4 mg; Riboflavin, 12 mg; Pyridoxine, 4 mg; Folacine, 2 mg; Biotin, 0.03 mg; Vitamin B8, 0.06 mg; Niacin, 90 mg; Pantothenic acid, 30 mg.

[3)]The values were calculated according to the values of feedstuffs in NRC (1994).

FSBM, fermented soybean meal; ME, metabolisable energy; CP, crude protein; Met, methionine; Cys, cysteine; Thr, threonine; Trp, tryptophan; Val, valine; Arg, arginine.

pre-weighed crucibles and ignited it at 550℃ for 4 hours until light grey ash results. Then final weights of the samples were measured after cooling down according to below equation where W_1 is the weight of the empty crucible, W_2 is the weight of the de-moist sample with crucible before igniting and W_3 is the weight of the ash with crucible after igniting.

$$\text{Ash content} = [(W_3 - W_1) / (W_2 - W_1)] \times 100\%$$

Crude fat content was determined by using the soxhlet extraction method of AOAC [25]. Five grams of de-moist breast meat samples were allowed to eight hours extraction period by using

the soxhlet apparatus (DKZW-4, China). Fat content was determined according to the following equation where W_1 is the weight of the dried sample, W_2 is the weight of the empty fat extracting flask and W_3 is the weight of the extracted fat with the flask.

$$\text{Fat content} = [(W_3 - W_2) / W_1] \times 100\%$$

"Kjedhal" method [25] was followed to calculate the crude protein content. The crude protein content of the sample was calculated by multiplying the amount of nitrogen obtained from the test by 6.25. After the determination of crude fat content by the Soxhlet method, samples were directed to evaluate crude fibre content [25].

Determination of calcium and phosphorous content in feces was done according to the AOAC [25] methodology with some modifications. Primary test solutions for Ca and P teste were prepared from residuals from ash test by adding 10 mL of HCl and three drops of Conc.HNO_3 and solutions were boiled. Then the boiled solutions were volume up to 100 mL for further use. For P test, Absorbance of 1 mL of each pre-prepared solution with 10 mL of a color developing agent (Vanadium ammonium molybdate) that volume up for 50 mL using distilled water was measured at 400 nm with a spectrophotometer (CM-3500d, Minolta, Osaka, Japan) and the amount of P was calculated using a standard curve prepared from potassium dihydrogen phosphate with nitric acid as the 50 μg/mL phosphorus standard solution.

To determine Ca content, titration was done by using ethylenediaminetetraacetic acid standard solution with the presence of calcine-methyl thyme herb phenol blue indicator to the 10 mL of primary solution which was volume up for 50 mL with 10 mL of starch solution, 2 mL of triethanolamine, 1 mL of ethylenediamine, 1 drop of malachite green, 20 mL of potassium hydroxide solution and 0.1 g hydroxylamine hydrochloride.

Determination of physicochemical properties of broiler breast meat

The pH values were determined based on the Jung et al. [26] by using calibrated electrical pH meter (pH700, Eutech instrument, Singapore) from filtered supernatant of one gram of homogenized (for one minute) sample at room temperature.

Water holding capacity (WHC) was determined according to the method stated in the study of Hamm [27] and Wilhelm et al. [28]. Two grams of each breast meat samples were placed into folded filter paper (No. 4, Whatman International, Maidstone, UK) and standard 10 kg weight was placed on the filter paper. After five minutes, samples were taken out and measured the final weight. WHC was computed as the reduction of the weight basis of the initial weight of the sample in percentage using the equation, where W_1 and W_2 are the initial and final weights of samples respectively.

$$\text{Water holding capacity} = 100 - [(W_1 - W_2) / W_1] \times 100\%$$

To determine cooking loss, vacuum-packed 25g of breast meat samples were cooked in the water bath (JONILAB® JN-WB001, JOAN Lab Instrument, Zhejiang Province, China) at 80℃ for 20 minutes and kept aside to reduce the temperature to room temperature. Finally, samples were unpacked, surface dried and weighted each cooked sample [29,30]. The cooking loss is defined as the weight loss during cooking and it interprets as a percentage of initial weight as following equation where W_1 and W_2 are the initial and final weights of samples respectively.

$$\text{Cooking loss} = [(W_1 - W_2) / W_1] \times 100\%$$

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The meat surface color value was measured from the left half of the breast by using a calibrated colorimeter (Spectrophotometer, CM-3500d, Minolta, Osaka, Japan). For each bird, three readings were taken from three different locations of the left half of the breast and then the lightness (CIE L*), redness (CIE a*), and yellowness (CIE b*) values were calculated using the average values.

Statistical analysis

Data were analyzed using one-way ANOVA technique, CRD by using the SPSS software package (Version 21; IBM SPSS 2012). Tukey's multiple range test was used to determine the significant differences between experimental groups at $p < 0.05$. The pen was considered as the experimental unit for growth performance and fecal composition measurements. Sacrificed birds were used as the experiment units for analyses carcass quality, organ weight, and meat physicochemical parameter data.

RESULTS

Growth performance

All birds remained healthy and showed adequate growth performance; the mortality rate of the birds was not affected by dietary treatments, and was below 2%. The growth performance of broilers under different FM and FSBM treatments from hatch to day 35 is summarized in Table 4. No dietary treatment effect ($p > 0.05$) was observed in either body weight or average daily gain of broilers. The FSBM2 treatment significantly improved ($p < 0.05$) the ADFI (25.03 g/d) by 10.07% compared to the other treatments on day 7. The FSBM4 diet increased the feed efficiency by reducing ($p < 0.05$) FCR by 8.45% on day 7. Cumulative FCR between the control diet and FSBM3 was different ($p < 0.05$), but there was no treatment effect ($p > 0.05$) observed on other growth performance parameters. Birds fed FSBM3 showed improved ($p < 0.05$) FCR over the birds fed the control diet by 7.51% from hatch to day 35 (1.60 vs. 1.73).

Fecal analysis

The proximate analysis of feces is presented in Table 5. Ca and P levels in feces were lowered ($p < 0.05$) by 38.77% and 32.23%, respectively, in broilers fed FSBM4 than in broilers fed other diets. Interestingly, the FSBM4 diet helped reduce ($p < 0.05$) fecal Ca and P levels in broilers by 42.73% and 41.32%, respectively, compared with that in broilers fed the control diet (2.27% vs. 3.24% and 1.21% vs. 1.71% respectively). The pairwise comparison revealed that fecal samples from birds fed the control diet had a higher ($p < 0.05$) P content than from those fed FSBM4 (1.71% vs. 1.21%), implying that the control diet increased P excretion through broiler feces by 41.32% compared with the FSBM4.

Physicochemical analysis of breast meat

No significant effect ($p > 0.05$) of treatments was observed on breast meat physicochemical parameters (Table 6). Breast meat samples from birds fed FSBM3 showed a 10.99% improvement ($p < 0.05$) in WHC compared to birds fed the control diet (77.74% vs. 70.04%). Breast meat samples from birds fed FSBM4 obtained higher ($p < 0.05$) CIE L* values than birds fed the control diet (60.69 vs 57.93), which means FSBM4 improved the CIE L* value in breast meat in broilers by 4.55%.

Breast meat proximate analysis

No diet-directed effect ($p > 0.05$) was observed in the proximate composition of breast meat in

Table 4. Effect of FSBM supplementation in to the diet to replace FM partially or completely on growth performance of broiler chickens from hatch to day 35 post-hatch[1)]

Period	Treatments				SEM[2)]	*p*-value
	Control	FSBM2	FSBM3	FSBM4		
Body weight (g)						
Day 7	187.43	187.27	185.00	182.38	1.513	0.636
Day 14	351.02	375.33	361.50	350.50	4.260	0.125
Day 21	809.33	811.98	816.67	804.67	5.148	0.886
Day 28	1,435.00	1,406.67	1,461.67	1,413.52	8.612	0.092
Day 35	2,119.33	2,118.87	2,198.00	2,108.70	16.434	0.190
Average daily gain (g/d)						
Day 7	16.36	19.14	17.65	16.81	0.438	0.111
Day 14	45.83	43.66	45.52	45.42	0.592	0.591
Day 21	62.57	59.47	64.50	60.89	0.877	0.207
Day 28	68.43	71.22	73.63	69.52	1.373	0.592
Day 35	48.30	51.97	52.50	46.67	1.369	0.380
Day 1–35	48.30	49.09	50.76	47.86	0.491	0.164
Average daily feed intake (g/d)						
Day 7	24.72[b]	25.03[b]	22.37[a]	21.14[a]	0.403	< 0.001
Day 14	71.98	74.25	72.93	71.84	0.401	0.116
Day 21	94.60	95.03	96.70	93.47	0.888	0.663
Day 28	121.87	120.25	120.39	117.88	1.486	0.839
Day 35	103.95	108.26	104.07	104.35	1.537	0.740
Day 1–35	83.42	84.57	83.29	81.73	0.681	0.562
Feed conversion ratio (g/g)						
Day 7	1.52[b]	1.33[a,b]	1.28[a]	1.26[a]	0.034	0.012
Day 14	1.58	1.71	1.61	1.59	0.023	0.127
Day 21	1.52	1.62	1.50	1.54	0.033	0.620
Day 28	1.81	1.69	1.64	1.70	0.028	0.169
Day 35	2.22	2.12	1.99	2.26	0.073	0.581
Day 1–35	1.73	1.69	1.60	1.67	0.019	0.083

	Control vs. FSBM2	Control vs. FSBM3	Control vs. FSBM4
Contrast *p*-values for complete experimental period			
BW (g)	0.993	0.174	0.840
ADG (g/d)	0.620	0.158	0.758
ADFI (g/d)	0.627	0.956	0.464
FCR (g/g)	0.562	0.033	0.288

[a,b]Values in a row with different superscripts differ significantly ($p < 0.05$).

[1)]Values are the mean of six replicates per treatment.

[2)]Pooled standard error of mean.

FSBM, fermented soybean meal; BW, body weight; ADG, average daily weight gain; ADFI, average daily feed intake; FCR, feed conversion ratio.

broiler chickens (Table 7). According to the pairwise comparisons, broilers fed the control diet showed an improvement of 33.34% in Ca content ($p < 0.05$) compared to broilers fed FSBM2. In addition, numerically higher moisture and ash contents in breast meat were reported from broilers offered FSBM2 (by 3.4% and 10.6%, respectively), compared to the other dietary treatments.

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Table 5. Effect of FSBM supplementation in to the diet to replace FM partially or completely on fecal proximate composition of chicken on day 21[1)]

Parameter (%)	Treatments				SEM[2)]	*p*-value
	Control	FSBM2	FSBM3	FSBM4		
Moisture	72.83	73.85	73.70	74.11	0.356	0.168
Ash	19.08	17.95	15.44	15.19	0.149	0.216
N	4.69	3.18	5.07	3.14	0.342	0.058
Ca	3.24[ab]	3.55[b]	2.66[ab]	2.27[a]	0.190	0.043
P	1.71[bc]	1.81[c]	1.28[ab]	1.21[a]	0.098	0.031
	Control vs. FSBM2		Control vs. FSBM3		Control vs. FSBM4	
Contrast *p*-values						
Moisture	0.140		0.593		0.109	
Ash	0.701		0.203		0.172	
N	0.132		0.732		0.126	
Ca	0.437		0.145		0.110	
P	0.571		0.179		0.044	

[a–c]Values in a row with different superscripts differ significantly ($p < 0.05$).

[1)]Values are the mean of six replicates per treatment.

[2)]Pooled standard error of mean.

FSBM, fermented soybean meal.

Table 6. Effect of FSBM supplementation in to the diet to replace FM partially or completely on breast meat physicochemical composition of chicken on day 35[1)]

Parameter	Treatments				SEM[2)]	*p*-value
	Control	FSBM2	FSBM3	FSBM4		
pH	5.91	5.95	5.87	5.89	0.031	0.851
WHC (%)	70.04	73.10	77.74	74.25	1.239	0.176
Cooking loss (%)	26.82	26.27	24.09	24.29	1.074	0.798
CIE L*	57.93	58.31	59.43	60.69	0.417	0.071
CIE a*	13.49	11.83	12.70	12.03	0.411	0.499
CIE b*	11.76	10.90	12.78	12.51	0.371	0.285
	Control vs. FSBM2		Control vs. FSBM3		Control vs. FSBM4	
Contrast *p*-values						
pH	0.648		0.758		0.840	
WHC (%)	0.362		0.035		0.275	
Cooking loss (%)	0.908		0.571		0.583	
CIE L*	0.670		0.285		0.002	
CIE a*	0.244		0.613		0.303	
CIE b*	0.355		0.392		0.534	

[1)]Values are the mean of six replicates per treatment.

[2)]Pooled standard error of mean.

FSBM, fermented soybean meal; WHC, water holding capacity.

Intestinal organ weights

The weights of internal organs, including the heart, liver, gizzard, pancreas, caeca, and intestines (relative to body weight), of slaughtered birds are presented in Table 8. The pancreas of birds receiving the control diet was 14.3% heavier ($p < 0.05$) than that of birds fed FSBM4. The control diet improved ($p < 0.05$) the intestinal weight of broilers by 19.1% compared with the FSBM3 diet.

Table 7. Effect of FSBM supplementation in to the diet to replace FM partially or completely on breast meat proximate composition of chicken on day 35[1)]

Parameter (%)	Treatments				SEM[2)]	*p*-value
	Control	FSBM2	FSBM3	FSBM4		
Moisture	79.99	84.50	81.30	83.84	0.826	0.678
Ash	5.19	5.63	4.90	5.19	0.149	0.428
CP	86.28	87.96	87.59	87.42	0.664	0.868
Fat	9.25	10.58	11.53	9.21	0.794	0.744
Ca	0.04	0.03	0.03	0.03	0.003	0.670
P	0.93	0.86	0.81	0.85	0.025	0.416
	Control vs. FSBM2		Control vs. FSBM3		Control vs. FSBM4	
Contrast *p*-values						
Moisture	0.119		0.309		0.107	
Ash	0.295		0.564		1.000	
CP	0.471		0.597		0.682	
Fat	0.636		0.311		0.985	
Ca	0.020		0.565		0.533	
P	0.145		0.053		0.405	

[1)]Values are the mean of six replicates per treatment.

[2)]Pooled standard error of mean.

FSBM, fermented soybean meal; CP, crude protein.

Table 8. Effect of FSBM supplementation in to the diet to replace FM partially or completely on internal organ weight of chicken on day 35[1)]

Parameter (%)	Treatments				SEM[2)]	*p*-value
	Control	FSBM2	FSBM3	FSBM4		
Heart	0.65	0.67	0.67	0.67	0.021	0.960
Liver	3.97	3.84	3.66	4.27	0.163	0.631
Gizzard	1.53	1.46	1.43	1.52	0.034	0.733
Pancreas	0.32	0.28	0.26	0.28	0.009	0.193
Caeca	0.58	0.50	0.54	0.64	0.028	0.346
Intestine	5.24	4.94	4.40	5.33	0.141	0.070
	Control vs. FSBM2		Control vs. FSBM3		Control vs. FSBM4	
Contrast *p*-values						
Heart	0.568		0.612		0.668	
Liver	0.731		0.581		0.543	
Gizzard	0.588		0.195		0.881	
Pancreas	0.149		0.069		0.023	
Caeca	0.368		0.497		0.393	
Intestine	0.473		0.026		0.742	

[1)]Values are the mean of six replicates per treatment.

[2)]Pooled standard error of mean.

FM, fish meal; FSBM, fermented soybean meal.

In contrast, the intestinal weight in broilers fed FSBM3 was 14.9% markedly lower ($p > 0.05$) than that in broilers fed other dietary treatments. Similarly, broilers fed FSBM4 had 11.69% substantially higher ($p > 0.05$) liver weight than broilers fed other diets.

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DISCUSSION

Chah et al. [31] and Mathivanan et al. [22] reported the improved performance of broilers receiving diets containing FSBM. Similarly, results of the present study showed that the incorporation of 2%–4% FSBM into rice-SBM basal diets improved the ADFI and FCR of broilers on day 7 and the cumulative FCR. Interestingly, total replacement of FM with FSBM optimized the feed conversion efficiency of broilers on day 7. This indicates that the combination of FSBM and FM or total replacement of FM with FSBM is profitable for large-scale farmers. Higher digestibility of threonine, lysine, leucine, and methionine in FSBM attributable to microbial fermentation may improve the growth performance of broilers [7]. Furthermore, fermentation of SBM improves the physiological characteristics of the gastrointestinal tract by optimizing gastric pH, increasing the level of short-chain fatty acids, reducing pathogenic microbial activity and improving mucosal structure in broilers [22,32]. Moreover, it has been found that FSBM contains more dipeptides and tripeptides produced by microorganisms during fermentation [9]. Tsuruki et al. [33] found that peptides derived from soybean protein have higher digestive and absorption rates. Subsequently, Truong et al. [34] found that rapid protein digestion helps improve feed efficiency in broilers.

Fermentation of SBM increases phosphorus availability and reduces phosphorus excretion in broilers [8]. In the current study, broilers fed a diet with 3%–4% FSBM reduced P excretion. The total replacement of FM with FSBM reduced P excretion by 41.3%. It has been found that the fermentation of FSBM leads to the degradation of the phytase P in SBM, which increases the availability for absorption in broilers [35,36]. Shafey et al. [37] reported that higher Ca levels in the diet could reduce P absorption in broilers and lead to the formation of insoluble calcium phosphate in their digestive tract. High Ca and P levels in the excreta of broilers fed the control diet than that in broilers fed FSBM4 may also be a result of the higher Ca level in the control diet.

Wang et al. [38] reported that fermented feed effectively improved the meat quality of fattening pigs, including the pH value, redness, muscle tenderness, and intramuscular fat content. In contrast, the results of the current study did not show any treatment effect on the above-mentioned parameters. Moreover, Lee et al. [39] reported that significantly higher WHC was in both breast and leg meat of chickens fed fermented soybean hulls which were confirmed by the result that FSBM3 improved the WHC by 10.99% compared to the control diet. It has been reported that ducks on a FSBM diet had lower pH values in the thigh and breast meat ($p < 0.05$), and the WHC of the breast meat 24 hours and 48 hours post mortem decreased by 11.26% and 8.21%, respectively [40], which contradicts the current study.

The proximate composition of the meat is also an important parameter in determining broiler meat quality, health benefits, and nutritional changes in the meat. Incorporating FSBM did not alter the proximate nutrient composition of breast meat in the present study. However, the results contradict those of Marcinčák et al. [41], who reported that feeding fermented feed appeared to improve the fat content and fatty acid composition of broiler meat. That study revealed that broilers fed with 10% fermented cornmeal (fermented with *Umbelopsis isabellina*) had improved the oleic, α-linolenic, γ-linolenic acid content, and the ratios of n-6 to n-3 polyunsaturated fatty acids in raw broiler meat. Furthermore, Weibing [40] reported that incorporation of FSBM increased the crude protein content of breast and thigh muscles by 1.52% and 1.90%, respectively. The same study reported an increase in fat content in duck breast and thigh meat by 1.17% and 2.67%, respectively.

The results of the current analysis indicate that, the incorporation of FSBM into the broiler diet with or without FM does not significantly affect the internal organ weight. These results are in agreement with those of Wang et al. [21], who indicated that the relative weights of the heart, liver, pancreas, spleen, thymus and bursa of Fabricious of broilers were not affected by dietary treatments

with FSBM inclusion, even though, pancreas samples received from birds fed the control diet were 14.29% higher than birds fed FSBM4. This counteracts the findings of Wang et al. [21], who reported a non-significant difference in pancreas weight in broilers receiving an FSBM treated diet and a control diet containing FM without FSBM. Molette et al. [42] reveal that the liver is responsible to the production of a significant amount (85%) of fat in the growing birds, and high-fat content in the feed tempt to increase the weight of the liver in birds. In the current experiment, there was no significant difference ($p < 0.05$) in liver weight between all the groups, which is consistent with Lee et al. [39], proposing that the addition of fermented feed ingredients into the broiler feed will preserve the normal immunity and health levels of the birds while preventing undesirable effects on the meat.

Decrement of wild catching of fish, high demand and low availability has been led to increase the cost of FM in recent past [43,44]. Therefore, it has been described that FSBM can be used as a highly digestible and cost-effective plant originated protein ingredient to reduce the feed cost and able to partially replace the animal-based ingredients like FM not only in broilers and swine but also in fish [4,43,45].

In conclusion, FSBM can be used as a replacement for FM (partially or completely) in broiler diets, it optimized the feed conversion efficiency while reducing Ca and P excretion without impairing meat quality parameters of broilers at 35 days of age.

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