

EFFECT OF FERMENTED SOYBEAN MEAL ON GROWTH PERFORMANCE, ECONOMIC EFFICIENCY, AND CECAL MICROBIOTA IN BROILER CHICKENS

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Abstract: This study was conducted to assess the effect of fermented soybean meal (FSBM) on growth performance, economic efficiency, and cecal microbial activity in broilers. One hundred and twenty Cobb 500 broiler chicks, one-day-old, with initial weight (44.75 g/bird) were used. In a complete randomized design, the birds were weighted and randomly assigned to six groups from T₁ to T₆ treated with different levels of FSBM as 0%, 2%, 4%, 6%, 8%, and 10 % respectively. For a treatment, five replicates with four chicks in each one were used. Results showed a significant increase ($P < 0.05$) in the body weight (BW) in T₂, T₅ and T₆ group which increased by 16.76%, 15.77% and 17.96% than the control group, respectively. Birds fed diet contain 2% FSBM had the best feed conversion ratio (FCR) as it improved with 12.43% than the control. There was a significant increase ($P < 0.05$) in the abdominal fat%, liver% and heart% in T₆ and no significant differences were detected in other groups compared to the control. Also T₃ showed a significant increase in intestine% compared to control. The economic efficiency, net profit and total return were significantly ($P < 0.05$) higher in T₂, T₃, T₅, and T₆ than control group. There was a significant ($P < 0.05$) decrease in the number of coliform bacteria in all groups compared to the control. But there was a numerically increase in the number of lactic acid bacteria in T₂ and T₆ than the control. There was a significant increase in the cholesterol level in a level dependent manner. No significant differences in total protein and albumin were detected in T₂ compared to control group. Collectively, our results revealed that 2% FSBM had benefit effects on growth performance of birds with a good economic efficiency.

Key words: broilers; fermented soybean meal; cecal microbiota; growth

Introduction

Diet is an important aspect of chicken production, and different poultry species or lines have variable nutritional needs based on genetics, environment, age, and health state. The high positive link between early live weight and body weight (BW) at the end of the production cycle has great interest in early nutrition research in recent years (1, 2). The major goal of a balanced pre-starter ration is to meet the nutritional requirements of the baby chick while facilitating the transition from the yolk sac to the first feed. This transition is critical because the bird's metabolism

transitions from lipid-rich yolk metabolism to a solid carbohydrate and protein-based diet upon hatch. Nutrient availability is crucial for growth and development just after hatching. Pre-starter meals can be designed to be highly digestible or to give certain nutrients at a higher concentration to fulfill the bird's nutritional needs. The ability of specially formulated pre-starter or starting diets to improve bird performance up to market age is dependent on the potential carryover impact (3).

Soybean meal (SBM) is the most commonly used vegetable protein source in animal feeding due to its low cost and great nutritional content (4). Soybean meal is the material left over after

the oil is extracted from soybean flakes; it contains 40 to 50% protein and is high in amino acids including lysine, tryptophan, threonine, isoleucine, and valine (5). Although the desolventization-toasting procedure decreases anti-nutritional factors (ANFs) in raw soybeans to a low level, residual ANFs may still interfere with nutrient digestion, absorption, and utilization (6, 7). microbial fermentation improves the nutritional quality of SBM by degrading anti-nutritional factors (ANFs) like tannins, galacto-oligosaccharides, trypsin inhibitors (TI), lectins, non-starch polysaccharides (NSPs), and allergen proteins (glycinin, β -conglycinin), which restrict the use of SBM in monogastric animals, especially young animals also have harmful effects on animal health (8-10).

Fermentation is a dynamic process that converts complex substrates into simpler molecules by involving microbes, substrates, and ambient factors (11). Fermentation results have been observed to be highly diverse and dependent on the nature and characteristics of the substrates utilized (12, 13). Fermentation produces different final products depending on the type of microorganisms involved, such as lactic acid, ethanol, or acetic acid, as different microbes respond differently to each substrate (11, 13) for example *Lactobacillus* produces lactic acid, mould gives citric acid, and yeasts release ethanol and carbon dioxide (14). Solid state fermentation (SSF) and submerged fermentation (SmF) are the two main types of fermentation processes (14, 13). In the absence of free-flowing liquid, the SSF technique uses solid substrates such as grains, rice, rice bran, and wheat bran (14, 15). SSF is gaining traction as a result of its higher yields and better product qualities than SmF (14, 16). Soybean meal benefits from two stages of fermentation (fungal and bacterial fermentation). *Aspergillus* is the most preferred species because of its ability to produce enzymes such as hemicellulases, hydrolases, pectinases, protease, amylase, lipases, and tannases (17). Several *Aspergillus* species, like as *A. oryzae*, have been utilized to ferment SBM (18-20). *Lactobacillus plantarum* is a bacterial strain that has been utilized to ferment SBM on a regular basis (21). Their ability to produce lactic acid distinguishes them from other bacteria. Probiotics are

helpful microorganisms that modify the microflora balance in the intestine. *Lactobacillus plantarum* suppresses the growth of pathogenic bacteria, improves digestion, and strengthens the immune system (22). Therefore the purpose of this study was to evaluate the growth performance, carcass traits, economic efficiency, cecal microbiota, and serum biochemical parameters of broiler chickens fed diet with FSBM.

Materials and methods

All experimental procedures were conducted according to the guidelines of the Committee of Animal Welfare and Research Ethics Zagazig University Egypt with approval number ZU-IACUC/2/F/98/2021.

Preparation of fermented soybean meal (FSBM)

FSBM was prepared following the method used by Sun *et al* (23) Prior to fermentation, *Lactobacillus plantarum* DSM-20174 was cultivated for 16 hours at 37°C in liquid MRS (De Man, Rogosa and Sharpe) medium and *A. oryzae* AUMC42 CYA was sub-cultured with the help of potato dextrose agar then kept at room temperature till the beginning of spore formation then this culture was kept in refrigerator at 4°C before being used as a substrate for a two-stage fermentation of feed. The fermentation process was started by soaking SBM in water until it reached a moisture level of 40-45 percent. After inoculating the soaked SBM with *Aspergillus oryzae* (10^8 spores /g) at a rate of 20 g/kg SBM, the mixture was fermented for 24 hours at 30°C in a bed-packed incubator (single stage fermentation). The initial fermented mixture was reconstituted with water to contain (58-60 %) moisture content and treated with *Lactobacillus plantarum* (10^9 CFU/g) at a rate of 4 g/kg SBM after single stage fermentation was completed. This combination was allowed to ferment for 48 hours at 37°C under anaerobic conditions (second stage fermentation). The fermented SBM was dried at 55-60°C in hot air oven to a moisture level of about 10% after fermentation.

Birds, housing, diets, and experimental design

One hundred-twenty- one- day- old chicks (Cobb 500 broiler) were purchased and weighted

at the start of the experiment with average BW of (44.75 gm), and at the end of each period as following: prestarter (1-7 day), starter (8-14 day), grower (15-28 day) and finisher (29-42 day). They were randomly assigned to 6 groups with 5 replicates each contain 4 birds in completely randomized design. Six treatments (T1- T6) were formulated with 0%, 2%, 4%, 6%, 8%, and 10% FSBM in broiler chickens diets respectively. Throughout the experiment, all birds were given free access to food and fresh drinking water. The average rate of temperature during the experimental period was recorded. Chicks were reared under the same management, sanitary and ecological conditions all over the experimental period. Usual health and vaccination protocols were done against Newcastle (at the 4th and 14th days) and Gumboro diseases (at 7th and 22th day). The ingredient composition (%) of the experimental diets was shown in Table 1 and 2 formulated according to Tillman *et al.* (24).

Growth performance

The birds were weighed individually on the first day of life to determine the initial BW, and then again at 7, 14, 28, and 42 days to determine the average BW of the birds in each groups. The body weight gain (BWG) was calculated as $W_2 - W_1$, where W_2 is the final BW at the intended

period and W_1 is the initial BW at the same period. Feed intake (FI) of each replicate was recorded as the difference between weight of the feed offered and residues left and then divided by the number of birds in each replicate to find out the average FI per bird. FCR was calculated (25).

Carcass characteristics study

On the final day of the feeding study, the birds were starved of food overnight, but drinking water was provided. Each bird was tagged and weighed before and after slaughter to determine the live and bled weights. The liver, heart, digestive tract (gizzard, intestine, abdominal fat and spleen) and bursa were selected, weighed and expressed as % of live body weight.

$$\text{Dressing \%} = \text{Dressed weight} / \text{Live weight} \times 100\%$$

Economic importance

Collective efficiency measures were calculated (26, 27, and 28), including total return, total costs, variable costs and net profit. Also, the performance index (PI) was calculated (29). Feed costs, as well as expenses for a one-day-old chick, litter, labour, veterinary services, electricity, and other incidental expenses were incorporated into the total cost (USD/bird).

Table 1: Ingredient composition (%) of the experimental diets used in the pre-starter and starter stage

Ingredient	Experimental diets											
	Pre-starter (1-7day)					Starter (8-14 day)						
	Control	2% FSBM	4% FSBM	6% FSBM	8% FSBM	10% FSBM	Control	2% FSBM	4% FSBM	6% FSBM	8% FSBM	10% FSBM
Yellow corn	48	48.15	48.5	49.15	49.35	50.52	50	50.12	50.58	51.41	51.93	52.38
Soybean meal, 46%	37.5	35.5	33.3	31	29	26.2	35.26	33.22	31	28.5	26.2	24
Fermented soybean meal	0	2	4	6	8	10	0	2	4	6	8	10
Corn gluten, 60%	5	5	5	5	5	5	5	5	5	5	5	5
Soybean oil	4.49	4.35	4.2	3.85	3.65	3.3	5.22	5.14	4.9	4.57	4.35	4.1
Calcium carbonate	0.68	0.68	0.68	0.68	0.68	0.75	0.5	0.5	0.5	0.5	0.5	0.5
Calcium dibasic phosphate	2.65	2.65	2.65	2.65	2.65	2.55	2.5	2.5	2.5	2.5	2.5	2.5
Common salt	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Sodium bicarbonate	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Premix	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
L-Lysine HCL, 78%	0.31	0.3	0.3	0.3	0.3	0.31	0.23	0.23	0.23	0.23	0.23	0.23
DL-Methionine, 98%	0.25	0.25	0.25	0.25	0.25	0.25	0.21	0.21	0.21	0.21	0.21	0.21
L-Threonine, 98.5%	0.13	0.13	0.13	0.13	0.13	0.13	0.09	0.09	0.09	0.09	0.09	0.09
Choline chloride, 60%	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07
Antimycotoxin	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Anticoagulant	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Chemical composition % **												
ME (kcal/kg diet)	3019.14	3022.34	3027.36	3022.54	3022.58	3024.49	3094.18	3101.24	3101.56	3100.07	3102.83	3101.92
Crude protein	23.91	24.02	24.07	24.09	24.21	24.06	22.92	23.02	23.07	23.02	23.03	23.08
Calcium	1	1.01	1.01	1.01	1.01	1.01	0.90	0.90	0.91	0.91	0.91	0.91
Available Phosphorus	0.46	0.46	0.47	0.47	0.47	0.46	0.44	0.44	0.44	0.45	0.45	0.45
Digested Lysine HCL	1.30	1.30	1.30	1.30	1.30	1.30	1.18	1.19	1.19	1.19	1.19	1.19
Digested Methionine	0.59	0.59	0.59	0.59	0.59	0.59	0.54	0.54	0.54	0.54	0.54	0.54
Digested Threonine	0.91	0.91	0.91	0.91	0.92	0.91	0.84	0.84	0.84	0.84	0.84	0.84

¹Muvco premix: Each 2.5 kg contain vit. A (10, 000000 IU), vit. D3 (2, 000000 IU), vit. E (10 g), vit.k3 (1000 mg), vit. B1 (1000 mg), vit. B2 (5 g), vit.B6 (1.5 g), pantothenic acid (10 g), vit. B12 (10 mg), niacin (30 g), folic acid (1000 mg), biotin (50 g), fe (30 g), Mn (60 g), Cu (4 g), I (300 mg), Co (100 mg), Se (100 mg) and Zn (50 g). FSBM: fermented soybean meal; Control: fed basal diet, 2%: fed basal diet with 2% FSBM, 4%: fed basal diet with 4% FSBM, 6%: fed basal diet with 6% FSBM, 8%: fed basal diet with 8% FSBM, 10%: fed basal diet with 10% FSBM; ** according to Tillman et al (24); The chemical composition of SBM(2200 metabolizable energy(ME) Kcal/kg, 89.5% dry matter, 45% crude protein (CP), 1.6% ether extract and 6% crude fiber(CF)) while that of FSBM (2750 metabolizable energy(ME) Kcal/kg, 89% dry matter, 50.50% crude protein (CP), 0.85% ether extract and 3.40% crude fiber(CF))

Table 2: Ingredient composition (%) of the experimental diets used in the grower and finisher stage

Ingredient	Experimental diets											
	Grower (15-28 day)					Finisher (29-42day)						
	Control	2% FSBM	4% FSBM	6% FSBM	8% FSBM	10% FSBM	Control	2% FSBM	4% FSBM	6% FSBM	8% FSBM	10% FSBM
Yellow corn	57	57.2	57.94	58.5	59.12	59.75	59.79	60.25	60.7	61.47	62.07	62.57
Soybean meal, 46%	31.45	29.45	27	24.7	22.35	20	30.5	28.2	26	23.5	21.2	18.85
Fermented soybean meal	0	2	4	6	8	10	0	2	4	6	8	10
Corn gluten, 60%	2	2	2	2	2	2	0	0	0	0	0	0
Soybean oil	5.4	5.2	4.91	4.65	4.38	4.1	5.98	5.82	5.57	5.3	5	4.85
Calcium carbonate	0.36	0.37	0.37	0.37	0.37	0.37	0.2	0.2	0.2	0.2	0.2	0.2
Calcium dibasic phosphate	2.3	2.3	2.3	2.3	2.3	2.3	2.15	2.15	2.15	2.15	2.15	2.15
Common salt	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Sodium bicarbonate	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Premix	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
L-Lysine HCL, 78%	0.17	0.16	0.16	0.16	0.16	0.16	0.1	0.1	0.1	0.1	0.1	0.1
DL-Methionine, 98%	0.23	0.23	0.23	0.23	0.23	0.23	0.22	0.22	0.22	0.22	0.22	0.22
L-Threonine, 98.5%	0.12	0.12	0.12	0.12	0.12	0.12	0.09	0.09	0.09	0.09	0.09	0.09
Choline chloride, 60%	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Antimycotoxin	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Anticoagulant	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Chemical composition %												
ME (kcal/kg diet)	3150.51	3150.09	3150.24	3150.35	3150.48	3150.05	3197.50	3203.09	3202.63	3204.43	3202.35	3209.05
Crude protein	19.97	20.08	20.05	20.06	20.06	20.06	18.50	18.51	18.50	18.50	18.52	18.51
Calcium	0.81	0.81	0.81	0.80	0.81	0.81	0.71	0.71	0.71	0.71	0.71	0.71
Available Phosphorus	0.40	0.41	0.41	0.41	0.42	0.42	0.38	0.38	0.39	0.39	0.39	0.39
Digested Lysine HCL	1.03	1.02	1.02	1.02	1.02	1.02	0.93	0.93	0.93	0.93	0.93	0.93
Digested Methionine	0.50	0.51	0.51	0.51	0.51	0.51	0.47	0.47	0.47	0.47	0.47	0.47
Digested Threonine	0.76	0.76	0.76	0.76	0.76	0.76	0.68	0.68	0.68	0.68	0.68	0.68

¹Muvco premix: Each 2.5 kg contain vit. A (10, 000000 IU), vit. D3 (2, 000000 IU), vit. E (10 g), vit.k3 (1000 mg), vit. B1 (1000 mg), vit. B2 (5 g), vit.B6 (1.5 g), pantothenic acid (10 g), vit. B12 (10 mg), niacin (30 g), folic acid (1000 mg), biotin (50 g), fe (30 g), Mn (60 g), Cu (4 g), I (300 mg), Co (100 mg), Se (100 mg) and Zn (50 g); The chemical composition of SBM(2200 metabolizable energy (ME) Kcal/kg, 89.5% dry matter, 45% crude protein (CP), 1.6% ether extract and 6% crude fiber (CF)) while that of FSBM (2750 metabolizable energy (ME) Kcal/kg, 89% dry matter, 50.50% crude protein (CP), 0.85% ether extract and 3.40% crude fiber (CF))

Cecal microbial assay

The cecal contents were collected aseptically and homogenised in sterile bags. The samples were weighed, and sterile 0.9% saline dilutions of 1:4 weight/volume were prepared. In a sterile 96 well flat-bottom plate, tenfold dilutions of each sample from each group were prepared. Dilutions were plated on duplicate selective agar media to count the number of target bacterial strains. Coliforms and Lactobacillus spp. were counted using MacConkey agar (Hi-media, Mumbai, India) and MRS agar (Hi-media, Mumbai, India), respectively. After each plate was incubated at 37°C for 24 to 72 hours, either anaerobically (lactobacillus spp) or aerobically (coliforms spp), colonies were counted as described by Chiang *et al.* (30) and Mountzouris *et al.* (31), respectively. The results were expressed as log₁₀ colony-forming units (CFU) per gram of digesta for statistical analysis.

Serum parameters measured

Blood samples were collected in labelled tubes at the end of the experiment, allowed to clot, and centrifuged at 3500 g for 15 minutes. Sera samples were separated and kept at -20°C until biochemical testing. Serum glucose was measured according to Trinder (32). Serum total cholesterol (33), triglyceride (34), high density lipoprotein

cholesterol (35) and low density lipoprotein cholesterol (36). Serum total protein (37), albumin (38), and globulin (39) were estimated. Serum aspartate-aminotransferase (AST) and alanine-aminotransferase (ALT) were determined as described by Reitman and Frankel (40).

Statistical analysis

The data were analyzed by SPSS version 25 (Armonk, NY: IBM Corp). The results were reported as Mean ± SE. Data were screened and Shapiro-Wilk test was applied to test normality assumption, Levene's test also was run to evaluate homogeneity of variance. Welch's ANOVA was run for variables violate homogeneity condition. While One-way ANOVA for assumption met data was applied to test differences among groups. Significant results followed by Tukey's honesty significant difference test. $P < 0.05$ statistically considered significant.

Results

Growth performance

The obtained result revealed that there was significant ($P < 0.05$) higher final BW and absolute BWG was found in T2, T5, and T6 which increased by 16.76%, 15.77% and 17.96% than the control group, respectively. There was a significant increase ($P < 0.05$) in the FI as supplementation of FSBM increase in the diet.

Table 3: Overall performance of broilers fed on fermented soybean meal (FSBM) (mean \pm SE)

Trait studied*	Experimental diets + groups					
	Control (T1)	2% FSBM (T2)	4% FSBM (T3)	6% FSBM (T4)	8% FSBM (T5)	10% FSBM (T6)
BW (g)	2709.45 \pm 15.34 ^c	3163.44 \pm 38.06 ^a	2926.11 \pm 19.44 ^b	2846.66 \pm 72.01 ^{bc}	3136.67 \pm 14.37 ^a	3196.11 \pm 31.12 ^a
BWG (g)	2664.69 \pm 15.24 ^c	3119.53 \pm 37.55 ^a	2881.03 \pm 19.49 ^b	2801.67 \pm 71.88 ^{bc}	3092.08 \pm 13.96 ^a	3151.28 \pm 30.91 ^a
FI (g)	4510.08 \pm 14.88 ^c	4611.58 \pm 22.78 ^{bc}	4572 \pm 49.03 ^{bc}	4697.25 \pm 6.74 ^{ab}	4654.33 \pm 46.25 ^b	4807.75 \pm 2.50 ^a
FCR	1.69 \pm 0.004 ^a	1.48 \pm 0.01 ^c	1.59 \pm 0.01 ^b	1.68 \pm 0.04 ^a	1.51 \pm 0.01 ^{bc}	1.53 \pm 0.02 ^{bc}

a, b, c Means within same row with different superscript are statistically different at $P < 0.05$ according to Tukey's Honesty significant difference test; *BW = final body weight; BWG = body weight gain; FI = feed intake; FCR = feed conversion ratio

Table 4: Carcass traits relative to the live weight of broilers fed on fermented soybean meal (FSBM) (mean \pm SE)

Trait studied	Experimental diets + groups					
	Control (T1)	2% FSBM (T2)	4% FSBM (T3)	6% FSBM (T4)	8% FSBM (T5)	10% FSBM (T6)
Live BW (g)*	2718.75 \pm 87.85 ^c	3277.50 \pm 63.33 ^a	2920 \pm 78.63 ^{bc}	2885 \pm 61.17 ^{bc}	3110 \pm 61.95 ^{ab}	3301.25 \pm 40.54 ^a
Dressing%	74.21 \pm 1.15	73.92 \pm 2.36	78.94 \pm 3.89	78.65 \pm 3.87	70.92 \pm 2.18	74.93 \pm 0.86
Liver%	1.74 \pm 0.07 ^b	1.90 \pm 0.06 ^{ab}	1.93 \pm 0.06 ^{ab}	1.79 \pm 0.08 ^b	2.04 \pm 0.06 ^{ab}	2.14 \pm 0.09 ^a
Gizzard%	2.63 \pm 0.07	2.67 \pm 0.14	3.19 \pm 0.07	2.64 \pm 0.20	2.59 \pm 0.19	2.70 \pm 0.18
Intestine%	5.45 \pm 0.30 ^{ab}	4.93 \pm 0.28 ^b	6.04 \pm 0.24 ^a	5.29 \pm 0.09 ^{ab}	4.58 \pm 0.16 ^b	4.76 \pm 0.38 ^b
Spleen%	0.17 \pm 0.01	0.15 \pm 0.05	0.10 \pm 0.003	0.16 \pm 0.02	0.09 \pm 0.001	0.11 \pm 0.01
Heart%	0.46 \pm 0.02 ^b	0.49 \pm 0.05 ^{ab}	0.61 \pm 0.06 ^b	0.42 \pm 0.02 ^b	0.43 \pm 0.01 ^b	0.43 \pm 0.01 ^a
Abdominal fat%	1.29 \pm 0.19 ^b	1.60 \pm 0.06 ^{ab}	1.22 \pm 0.14 ^b	1.49 \pm 0.06 ^{ab}	1.75 \pm 0.11 ^{ab}	2.03 \pm 0.18 ^a
Bursa%	0.12 \pm 0.01	0.14 \pm 0.03	0.18 \pm 0.10	0.16 \pm 0.05	0.14 \pm 0.01	0.11 \pm 0.06

a, b, c Means within same row with different superscript are statistically different at $P < 0.05$ according to Tukey's Honesty significant difference test; *Live BW = Live body weight

The highest value of FI recorded with T6 and the lowest value with the control group. Birds fed diet supplemented with 2% FSBM recorded lowest value of feed conversion ratio and highest weight gain and this indicate best utilization of food (Table 3).

Carcass quality traits

As demonstrated in Table (4), there was a significant increase ($P < 0.05$) in the abdominal fat%, liver% and heart% in T6 and no significant differences were detected in other groups compared to the control. Also T3 showed a significant increase

in intestine% compared to control. But, no significant differences on weight percentages of dressing, spleen, gizzard, bursa relative to live BW between all groups compared to the control one.

Economic importance

As noticed in Table (5), there was a significant increase ($P < 0.05$) in the final BW in T2, T5, and T6 compared to control. A significant decrease in the feed cost and a significant increase in the total return, net profit, economic efficiency and performance index recorded in T2, T5, and T6 rather than other groups.

Table 5: Economic efficiency of broilers fed on fermented soybean meal (FSBM) (mean \pm SE)

Trait studied	Experimental diets + groups					
	Control (T1)	2% FSBM (T2)	4% FSBM (T3)	6% FSBM (T4)	8% FSBM (T5)	10% FSBM (T6)
Final body weight (kg)	2.71 \pm 0.02 ^c	3.16 \pm 0.04 ^a	2.93 \pm 0.02 ^b	2.85 \pm 0.07 ^{bc}	3.14 \pm 0.01 ^a	3.20 \pm 0.03 ^a
Total costs (L.E)	53.54 \pm 0.11 ^d	54.69 \pm 0.17 ^c	54.70 \pm 0.37 ^c	55.91 \pm 0.05 ^b	55.90 \pm 0.36 ^b	57.36 \pm 0.02 ^a
Total return (L.E)	77.86 \pm 0.43 ^c	90.60 \pm 1.07 ^a	83.93 \pm 0.54 ^b	81.71 \pm 2.02 ^{bc}	89.83 \pm 0.40 ^a	91.49 \pm 0.87 ^a
Net profit (L.E)	24.33 \pm 0.32 ^c	35.92 \pm 0.95 ^a	29.23 \pm 0.44 ^b	25.80 \pm 1.97 ^{bc}	33.93 \pm 0.12 ^a	34.13 \pm 0.89 ^a
Economic efficiency (%)	45.44 \pm 0.51 ^c	65.67 \pm 1.62 ^a	53.43 \pm 0.92 ^b	46.13 \pm 3.49 ^c	60.70 \pm 0.40 ^{ab}	59.50 \pm 1.57 ^{ab}
Performance index (%)	160.08 \pm 1.30 ^c	214.10 \pm 4.44 ^a	184.42 \pm 2.04 ^b	169.99 \pm 8.53 ^{bc}	208.40 \pm 0.62 ^a	209.54 \pm 4.22 ^a

^{a, b, c} Means within same row with different superscript are statistically different at $P < 0.05$ according to Tukey's Honesty significant difference test; The cost of one Kg of FSBM is 14 L.E; Total costs = Total fixed costs + total variable cost; Total Returns (TR) = Litter sale + Broiler sale; Litter sale = Litter sale price / No. of broilers at end of experiment; Broiler sale = Body weight/gm at end of project (6th week) \times Gram price; Net Profit = Total returns – Total costs. Measurement of Efficiency% = Net profit / Total cost*100

Table 6: The cecal microbial profiles (Log₁₀ CFU/g) of broilers fed on fermented soybean meal (FSBM) (mean \pm SE)

Trait studied	Experimental diets + groups					
	Control (T1)	2% FSBM (T2)	4% FSBM (T3)	6% FSBM (T4)	8% FSBM (T5)	10% FSBM (T6)
Lactobacillus	3.99 \pm 0.81 ^{ab}	5.52 \pm 0.15 ^a	2.79 \pm 0.4 ^b	4.00 \pm 0.00 ^{ab}	4.93 \pm 0.17 ^{ab}	5.68 \pm 0.15 ^a
Coliforms	7.91 \pm 0.02 ^a	7.51 \pm 0.03 ^c	7.55 \pm 0.02 ^{bc}	7.67 \pm 0.04 ^b	7.63 \pm 0.04 ^{bc}	7.52 \pm 0.02 ^c

^{a, b, c, d} Means within same row with different superscript are statistically different at $P < 0.05$ according to Tukey's Honest significant difference test

Cecal microbial assay

There was a significant ($P < 0.05$) decrease in the number of coliform bacteria in all groups compared to the control. But there was a numerically increase in the number of lactic acid bacteria in T2 and T6 compared to the control (Table 6).

Serum parameters measured

The serum biochemical parameters of broiler chickens fed diets with FSBM are noticed in table 7. The results revealed that the serum cholesterol level was increased with increasing the level of FSBM inclusion in the diets. The serum cholesterol level was significantly higher ($P < 0.05$) in T4, T5 and T6 compared to the control. The serum triglyceride level increased significantly ($P < 0.05$) as the amount of FSBM in the diet increased. T2 showed no significant difference

compared to control group. The highest value was recorded with 8% and 10% FSBM. The high density lipoprotein cholesterol (HDL-C) level recorded no significant difference between T2 and T3 compared to the control. There was significant decrease ($P < 0.05$) of HDL-C in groups fed T4, T5 and T6 compared to the control. The low density lipoprotein cholesterol (LDL-C) showed significant increase ($P < 0.05$) in groups fed 6%, 8%, and 10% FSBM, but noted no significant differences between T2 and T3 compared to control group. Total protein, albumin and globulin showed no significant differences ($P < 0.05$) in T2 compared to the control group. Also, transaminases (alanine and aspartate) ALT and AST showed no significant differences ($P < 0.05$) in T2 and T3 compared to control group. But, T4, T5, and T6 recorded significant increase ($P < 0.05$) compared to the control.

Table 7: Some serum biochemical parameters of broilers fed on fermented soybean meal (FSBM) (mean \pm SE)

Trait studied	Experimental diets + groups					
	Control (T1)	2% FSBM (T2)	4% FSBM (T3)	6% FSBM (T4)	8% FSBM (T5)	10% FSBM (T6)
Total cholesterol (mg/dl)	170.93 \pm 10.01 ^b	188.58 \pm 18.19 ^b	209.85 \pm 3.18 ^b	329.85 \pm 1.34 ^a	332.45 \pm 10.95 ^a	335.58 \pm 25.43 ^a
Triglyceride (mg/dl)	70.65 \pm 4.08 ^d	85.95 \pm 2.29 ^d	112.23 \pm 3.66 ^c	227.83 \pm 2.46 ^b	265.98 \pm 10.08 ^a	269.50 \pm 9.05 ^a
HDL-C (mg/dl)*	51.81 \pm 0.63 ^a	50.00 \pm 0.58 ^a	50.44 \pm 0.88 ^a	39.80 \pm 0.66 ^b	39.94 \pm 0.68 ^b	33.75 \pm 2.31 ^c
LDL-C (mg/dl)*	84.39 \pm 4.13 ^b	91.53 \pm 2.97 ^b	97.41 \pm 19.01 ^b	147.58 \pm 6.63 ^a	163.49 \pm 10.19 ^a	177.66 \pm 14.16 ^a
Total protein (g/dl)	6.67 \pm 0.45 ^a	6.21 \pm 0.28 ^a	4.96 \pm 0.21 ^b	3.35 \pm 0.15 ^c	3.91 \pm 0.53 ^{bc}	3.40 \pm 0.13 ^c
Albumin (g/dl)	4.57 \pm 0.29 ^a	3.89 \pm 0.19 ^{ab}	2.58 \pm 0.02 ^c	3.33 \pm 0.30 ^b	2.20 \pm 0.09 ^{cd}	1.70 \pm 0.03 ^d
Globulin (g/dl)	4.44 \pm 0.42 ^a	4.62 \pm 0.25 ^a	3.92 \pm 0.19 ^a	2.43 \pm 0.07 ^{bc}	2.70 \pm 0.04 ^b	1.72 \pm 0.04 ^c
ALT (u/l)*	9.29 \pm 0.43 ^c	9.38 \pm 1.04 ^c	15.53 \pm 1.02 ^c	31.83 \pm 4.37 ^b	41.45 \pm 1.78 ^a	48.44 \pm 1.44 ^a
AST (u/l)*	22.23 \pm 0.65 ^c	24.96 \pm 0.80 ^c	33.70 \pm 6.09 ^c	222.08 \pm 24.08 ^b	302.18 \pm 4.34 ^a	296.15 \pm 8.31 ^a

^{a, b, c, d} Means within same row with different superscript are statistically different at $P < 0.05$ according to Tukey's Honesty significant difference test; *HDL-C = the high density lipoprotein cholesterol; LDL-C = the low density lipoprotein cholesterol; ALT and AST = transaminases (alanine and aspartate)

Discussion

Microbial fermentation is a recent low-cost method to enhance the nutritional value of unconventional feed ingredients for broiler chickens. Also, there is a growing interest in the introduction of fermented feed to broiler diets due to its positive impacts on the gut health and growth performance (41, 42). As a result, microbial fermentation of atypical feeds like SBM could be a potential alternative for enhancing SBM nutritional content. Our results showed improvement in BW of birds fed diet contain FSBM. Birds fed diet supplemented with 2% FSBM recorded lowest value of FCR and highest weight gain, indicating best utilization of food. The chemical composition of SBM (2200 metabolizable energy (ME) Kcal/kg, 89.5% dry matter, 45% crude protein (CP), 1.6% ether extract and 6% crude fiber (CF)) while that of FSBM (2750 metabolizable energy (ME) Kcal/kg, 89% dry matter, 50.50% crude protein (CP), 0.85% ether extract and 3.40% crude fiber(CF)). As a result, increased protein content in fermented SBM could result from (1) microbial protein and enzyme synthesis during fermentation (43), (2) an increase in microbial population that is primarily composed of protein (44), and (3) improved proteolytic microorganism activities, resulting in an increase in free amino

acids and peptides in fermented products (45). Also, increasing microbial population produces fiber degrading enzymes, resulting in fiber decrease after fermentation (46).

In the diets of chicks, replacing conventional SBM with fermented SBM increased BW gain (47, 18). According to Mathivanan *et al.* (17), adding 0.5 %t FSBM fermented by *A.s niger* to the food improved BW in the 5th and 6th weeks and decreased FCR in the 3rd and 4th weeks, whereas adding 1.0 % and 1.5 % FSBM had no effect on broiler chicken growth performance. A trial with bacteria and fungi-mixed fermented SBM found that supplementing the diets of broiler chickens with 4.5–6.0% wet or dry FSBM did not show greater effects on growth performance of broiler chickens, compared with 4% fish meal, but had better effects on growth performance than 5% SBM (48). The varied microorganisms utilized for fermentation, the different processing procedures for FSBM products, or the variable supplemented levels of FSBM in the diet could all account for the differences between our findings and other observations.

Our results showed that there were significant differences on weight percentages of heart, liver, intestine and abdominal fat. But, no significant differences were detected on weight percentages of dressing, spleen, gizzard or bursa relative to

live BW between all experimental groups compared to control one. Another study showed by Kim *et al.* (49) that adding 3 % FSBM products to diet for 7 days after hatching had no effect on the relative weights of breast muscle and abdomen fat at 35th day old. Furthermore, in broiler chickens at day 37, partial substitution of SBM by FSBM (4.5–6.0%) had no effect on the relative weight of abdominal fat (48). Guo *et al.* (50) reported that FSBM with levels of 2.5, 5, and 7.5% did not significantly affect the performance and carcass yield. Also our results revealed an improvement in the economic efficiency and this was noticed with group fed 2% FSBM.

This study revealed an increase in the number of lactic acid bacteria and decrease in the coliforms bacteria. Jeong *et al.* (51) discovered that feeding fermented SBMs to weaned piglets enhanced fecal *Lactobacillus* numbers. These findings show that fermented SBMs can be employed as a probiotic carrier and as a desirable feed element for growing animals.

Sembratowicz *et al.* (52) revealed that the SBM fermentation resulted in an increase in *Lactobacillus* from 4.2 to 6.9 logs CFU/g, as well as a 30-fold increase in lactic acid concentration.

Our results of blood parameters revealed that the serum cholesterol level increased with the level of FSBM inclusion in the diets. Sembratowicz *et al.* (52) showed that broiler diet containing 3 % and 6 % FSBM increased HDL cholesterol levels, with highest level was observed in birds receiving 3 % FSBM and the lowest level was observed in birds receiving 6 % FSBM. Chachaj *et al.* (53) revealed that turkeys fed 9 and 10% FSBM had higher activity of aminotransferases (alanine and aspartate). Sembratowicz *et al.* (52) showed that broiler diets with 3% and 6% FSBM increased AST levels. Also our results showed that T2 had no significant difference with the control group of total protein.

Conclusion

In conclusion, integrating different levels of FSBM in the diet had improved the average BW and FCR. Replacing SBM in the diet with FSBM shifted the cecal microbial community of broilers towards a healthier balance by increasing the abundance of beneficial bacteria and reducing the abundance of potentially harmful bacteria. These

findings indicate that FSBM may be a new feed resource to improve growth performance and manipulate the intestinal microbial bacteria of animals. The diet containing FSBM also increased in HDL cholesterol content and positively influenced protein metabolism. Replacement of non-FSBM with FSBM at the amount of 2, 8, and 10% in the feed improved the chicken growth performance and the dressing percentage and economic efficiency. However, the replacement of a 2% dose of FSM was found to be more favorable than replacement of an 8 or 10% FSM dose because it also improved the microbiota of the intestines without harmful effect on the birds' health. These findings encourage the animal feed industry to look for cost effective opportunities to include fermented products in the diet.

None of the authors have any conflict of interest to declare.

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