Effect of feeding processed Soybean Meal on broiler's performance, intestinal morphology, cecal microbial population and immune response

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ABSTRACT

This study aimed to assess the effects of different levels of processed soybean meal (PSBM) on the growth performance, intestinal morphology, cecal bacterial count, and immune responses of broilers. A total of 560 Ross 308 broiler chickens were randomly assigned to seven groups. The control group received a basal diet based on standard soybean meal (SBM). Groups S1 (starter 1), G1 (grower 1), and F1 (full period 1) received diets containing 2.5% PSBM, while Groups S2, G2, and F2 received diets containing 5% PSBM at each life phase, respectively. The results demonstrated that average daily weight gain and feed conversion ratio were significantly improved in the groups fed the 5% PSBM diet. Additionally, PSBM replacement did not affect villus length in any intestinal region. Nevertheless, increasing dietary PSBM content reduced intestinal goblet cell number (P<0.01) and cecal *Clostridia* population (P<0.01). Furthermore, PSBM enhanced bronchitis antibody titers and immune responses against PHA at 24 and 48 hours after injection, respectively (P<0.01). In conclusion, adding 5% PSBM to regular SBM is recommended, particularly in the grower phase, due to its positive effects on broilers.

Introduction

Today, the growth rate of broilers has remarkably increased due to advancements in nutrition and genetics. To meet the needs associated with this high production potential, diets containing high levels of energy and protein are typically consumed. Enhancing the economic efficiency of broiler breeding is of great importance for both breeders and nutritionists, and endeavors to improve this efficiency are essential for reducing production costs (15).

One of the most essential protein sources in broiler diets is soybean meal (SBM). Economically, SBM supplies about 50% of the protein and 75% of the amino acids needed for broilers. In addition to being a rich protein source, SBM also contains high amounts of energy, with a metabolic energy concentration 11-25% higher than that of other oilseed meals due to its lower fiber content (22).

SBM contains anti-nutritional compounds, such as protease, saponins, phytoestrogens, allergenic proteins, phytic acid, oligosaccharides, guatraza, and lectins. These inhibitors can impact the use of SBM in broiler diets, particularly in the early growth period. These agents disrupt digestive enzymes and inhibit the desirable digestion and absorption of food (18, 25).

To improve the nutritional value of SBM in poultry, attention should primarily be paid to the inactivation of these anti-nutritional compounds. Most anti-nutritional substances in SBM are heat-sensitive, and if heat processing is applied to SBM at the appropriate temperature, time, pressure, and humidity, it can eliminate these anti-nutritional factors (ANFs). In addition to destroying heat-sensitive ANFs, proper heat processing also changes the tertiary structure of proteins, further enhancing the digestibility of dietary protein (30). Given the commercial significance of this procedure, researchers have investigated the effects of different thermal processes on ANFs in soybean and SBM. Autoclaving, roasting, cracking, micronizing, short wavelength radiation, extrusion, and puffing under steam are among the most crucial and widely used thermal processes (23).

Numerous studies have demonstrated that partial or total replacement of SBM with heated SBM improves growth performance, digestive enzyme activity, and intestinal morphology in broilers (26, 27, 29).

One of the most essential and effective thermal processes is the extrusion process. During extrusion, the pressure stemming from the machine's spiral and the heat it generates help to destroy anti-nutritional inhibitors in protein materials.

Jahanian and Rasouli (12) reported that complete SBM replacement with extruded SBM improved growth performance. In another experiment, they reported that extruded SBM reduced protein content in the diet by 9% without causing any negative effect on the functional components. Extrusion can be performed using wet, dry, and roasting methods. To increase the efficiency of this thermal process and use the benefits of steam in reducing anti-nutritional compounds, the wet extrusion method combined with steam is now commonly used to process materials. Previous studies have reported that wet extrusion can increase the metabolizable energy of SBM by 2.8% compared to the other methods (13).

Given the significance of SBM in broiler diets and the effect of extrusion on the quality features and increased digestibility of materials, the current experiment aimed to investigate the effects of different levels of processed SBM (PSBM) on growth performance, intestinal morphology, cecal bacterial abundance, and immune response in broilers.

Materials and Methods

***Chickens and Experimental Treatments*:** This experiment was conducted in the autumn of 2018 at a poultry farm in Kordkoy City, Golestan Province, Iran (36.57 N, 54.047 E). A total of 560 one-day-old Ross 308 broiler chickens (mixed gender) with an average initial body weight of 45 ± 1 g were assigned to seven groups (20 chickens each) using a completely randomized design with four replications. The chickens were housed in pens with wheat straw floors within a closed system that allowed for controlled temperature, moisture, and ventilation.

A conical drinking trough was used in the first 3 days of breeding, and a nipple drinking trough was used for the remainder of the period. The chickens were kept at a temperature of 32˚C for the first 3 days of the experiment, which was then reduced by one degree per day until it remained constant at 21˚C from the 12th day onwards. The exposure schedule of 23 hours of light and 1 hour of darkness was implemented during the experiment. The vaccination schedule was followed according to the conditions recommended in the breeding instructions. All diets provided to the chickens were isocaloric and isonitrogenous, and both feed and water were available *ad libitum*.

PSBM was prepared by extrusion at a temperature of 150 ± 2 °C for 20 seconds using a single-shaft extruder with a speed of 450 rpm and a diameter of 10 cm (Amandus Kahl, Expander, OEE 32, GmbH & Co., KG, Germany). The extruded meal was subsequently dried and ground (9, 18).

During the starter (S) period (1-21 days) and the grower (G) period (22-42 days), the chickens were fed diets in which SBM was replaced with varying levels of PSBM. The control group (C) received a basal diet containing only SBM. Groups S1, G1, and F1 received diets containing 2.5% PSBM during the initial period, the growth period, and the entire experimental period, respectively. However, Groups S2, G2, and F2 received diets containing 5% PSBM during the initial and growth periods. The diets used in the experiment were prepared to meet the nutritional requirements of Ross-308 strain broilers. The ingredients and chemical composition of the diets for the starter and grower stages are given in Table 1.

***Growth Performance and Sampling*:** The broilers' weight gain and feed consumed according to daily mortality were recorded to examine performance at the end of Periods S (1-21 days old) and G (22-42 days old), as well as for the entire period (1-42 days old). The weight gain of each unit per time period was determined by calculating the difference between the weights at the end and beginning of the breeding period. The amount of feed consumed by each experimental unit was calculated by subtracting the amount of feed remaining at the end of each breeding stage from the total feed given during the period. The feed conversion ratio was estimated by calculating the ratio of daily feed consumption to daily weight gain for each period.

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Table 1. Composition of the diets containing PSBM at different levels in the starter and grower periods.

Ingredients (%)/Treatments	Days (1-21)							Days (22-42)						
	C	S1	G1	F1	S2	G2	F2	C	S1	G1	F1	S2	G2	F2
Corn	55.30	55.30	55.30	55.30	55.30	55.30	55.30	65.5	65.5	65.5	65.5	65.5	65.5	65.5
Soybean	39	36.5	39	36.5	34	39	34	29	29	26.5	26.5	29	24	24
Digesta	0	2.5	0	2.5	5	0	5	0	0	2.5	2.5	0	5	5
Plant Oil	1.7	1.7	1.7	1.7	1.7	1.7	1.7	1.9	1.9	1.9	1.9	1.9	1.9	1.9
DL-methionine	0.26	0.26	0.26	0.26	0.26	0.26	0.26	0.22	0.22	0.22	0.22	0.22	0.22	0.22
L-lysine HCL	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.14	0.14	0.14	0.14	0.14	0.14	0.14
L-Threonine	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Dicalcium phosphate	1.9	1.9	1.9	1.9	1.9	1.9	1.9	1.6	1.6	1.6	1.6	1.6	1.6	1.6
Calcium carbonate	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.6	0.6	0.6	0.6	0.6	0.6	0.6
Salt	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Bicarbonate	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.25	0.25	0.25	0.25	0.25	0.25	0.25
premix[a]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Choline chloride	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Calculated nutrients (% unless otherwise stated)														
ME (kcal/kg)	2900	2900	2900	2900	2900	2900	2900	3000	3000	3000	3000	3000	3000	3000
CP	22	22	22	22	22	22	22	18	18	18	18	18	18	18
Lysine	1.267	1.267	1.267	1.267	1.267	1.267	1.267	1.03	1.03	1.03	1.03	1.03	1.03	1.03
Methionine	0.585	0.585	0.585	0.585	0.585	0.585	0.585	0.492	0.492	0.492	0.492	0.492	0.492	0.492
Met + Cys	0.923	0.923	0.923	0.923	0.923	0.923	0.923	0.78	0.78	0.78	0.78	0.78	0.78	0.78
Threonine	0.856	0.856	0.856	0.856	0.856	0.856	0.856	0.717	0.717	0.717	0.717	0.717	0.717	0.717
Calcium	0.894	0.894	0.894	0.894	0.894	0.894	0.894	0.757	0.757	0.757	0.757	0.757	0.757	0.757
Available phosphorus	0.477	0.477	0.477	0.477	0.477	0.477	0.477	0.378	0.378	0.378	0.378	0.378	0.378	0.378

C, control; S1 and S2, SBM was respectively replaced with 2.5%, and 5.0% of PSBM in starter period. G1 and G2, SBM was respectively replaced with 2.5%, and 5.0% of PSBM in grower period F1, and F2, SBM was respectively replaced with 2.5%, and 5.0% of PSBM in whole period.

Abbreviations: CP, crude protein; ME, metabolizable energy; Met + cys, Methionine + Cystine.

[a]Supplied per kilogram of diet: vitamin A, 11000 IU; vitamin D3, 4500 IU; vitamin E, 65 IU; vitamin B1, 2.5 mg; vitamin B6, 3.2 mg; Biotin, 0.2 mg; vitamin B12, 0.017 mg; vitamin K, 3 mg; riboflavin, 6.5 mg; pantothenic acid, 18 mg; niacin, 60 mg; folic acid, 1.9 mg; zinc oxide, 110 mg; manganese oxide, 120 mg; cooper sulfate, 16 mg; iron sulfate, 20 mg; calcium iodine, 1.25 mg; sodium selenate, 0.3 mg.

***Intestinal Morphology*:** At the end of the breeding period, two birds out of every five were slaughtered. After the carcass was opened, the small intestine was removed, and 2 cm long pieces were taken from the middle of the duodenum, jejunum, and ileum. After washing with phosphate-buffered saline (PBS) solution, the samples were transferred into plastic containers containing 10% formalin. Following de-watering and clarifying, the paraffin wax method was used to prepare tissue slides with low thickness. A microtome device (Leica Microtome Model: Jung RM 2045) was used to cut the paraffin mold into approximately 6-micrometer-thick sections, which were then stained with hematoxylin-eosin. Then, the villus height (from the top of the villus to its base), width (at the lowest section where it connects to the crypt), and depth (from the villus base to the end of the glands) of each of the slides were measured using a light microscope (Olympus BX41 model, Tokyo, Japan). The ratio of villus length to crypt depth was calculated, and the number of goblet cells was counted in five tissue images of intestinal villi at 400x magnification (28).

***Bacterial Population of Ileocecal Contents*:** To evaluate the bacterial content of the ileocecal region, the contents were immediately collected in sterile plates, placed on ice, and transferred to the laboratory. The bacterial suspension was cultured using the pour-plate technique on specific culture media, including Luria-Bertani agar, Xylose Lysine Deoxycholate agar, and MacConkey agar. Subsequently, all plates were incubated under microaerophilic conditions at 38-42 ˚C for 48 hours. The cecal bacterial count was then determined (16).

***Immune Responses*:** To assess the concentration of total antibody titer against sheep red blood cells (SRBC) at 24 and 35 days of age, two chickens from each replicate were immunized with 0.5 mL of a 5% SRBC suspension administered into the thigh muscle. Chicken blood

samples were collected from the wing vein on the 30th and 42nd days and measured using the hemagglutination method to evaluate the anti-SRBC antibody response (24).

To determine the antibody response against Newcastle disease virus (NDV) and infectious bronchitis virus (IBV) at 42 days of age, two birds from each replicate were randomly selected, and about 1 mL of blood was collected via the wing vein. After separating the serum from the blood clot, the samples were transferred to the laboratory. The anti-NDV antibody response was determined using the hemagglutination inhibition (HI) method, while the anti-IBV antibody response was measured using the enzyme-linked immunosorbent assay (ELISA) method (24).

The cell-mediated immune response was assessed using phytohemagglutinin-M (PHA-M). On the 42nd day, two broilers were randomly selected from each experimental group. Subsequently, 0.1 mL of PHA-M solution (0.1 mg/mL) was subcutaneously injected into the third and fourth toes of each chicken's right and left feet. After 24 and 48 hours, the thickness of the injected regions was measured. The difference in toe thickness between the right and left feet was used as a measurement criterion to evaluate the level of T-cell proliferation in the cell-mediated immune system (7).

***Statistical Analysis*:** Data analysis was performed using the GLM procedure in SAS software version 9.4 (SAS Institute Inc., Cary, NC, USA). Prior to analysis, the data were examined for normal distribution using the univariate procedure and the Shapiro-Wilk test.

Mean treatments were compared using Duncan's test at a 5% significance level. The statistical model of the design was $Yij = \mu + Ti + \varepsilon ij$, where *Yij* denotes the numerical value of each experimental observation, μ shows the population mean, *Ti* is the treatment effect, and *Eij* indicates the error effect of the experiment.

Results

***Growth Performance*:** The effects of PSBM inclusion on FI, WG, and FCR in broiler chickens are presented in Table 2. The results indicate that increasing the PSBM level did not significantly affect FI throughout the experimental periods ($P > 0.05$). However, WG was influenced by the increasing amount of PSBM in the diet across all phases ($P < 0.05$), with the highest WG observed in broilers fed 5% PSBM ($P < 0.001$). Additionally, the control treatment exhibited the lowest average body weight ($P < 0.001$). Moreover, including PSBM in the diets decreased FCR during the G and F periods ($P < 0.05$). However, no significant difference in FCR was observed among the various treatments during the S period ($P > 0.05$).

***Intestinal Morphology*:** Table 3 presents the morphological measurements of the small intestinal mucosa in chickens fed the control and test diets. The treatments did not have a significant effect on VL in any region of the intestine ($P > 0.05$), but they did significantly impact CD in the duodenum ($P < 0.05$). The highest and lowest VL/CD ratios were observed in the F2 and control groups, respectively, in the initial parts of the intestine ($P < 0.05$). However, this ratio was similar between the treatment groups and the control chickens in the ileum ($P > 0.05$). Additionally, the goblet cell count in each region was affected by the level of PSBM in the diets ($P < 0.01$). Increasing the PSBM content resulted in a decreasing trend in goblet cell numbers from the control to the F2 treatments.

Table 2. Effect of PSBM replacement level on FI, WG, and FCR in the broiler chickens.

Treatment	FI (g/chicken/d)			WG (g/chicken/d)			FCR		
	Starter Period	Grower Period	Whole Period	Starter Period	Grower Period	Whole Period	Starter Period	Grower Period	Whole Period
C	1197.22	3586.92	4784.14	907.77^{bc}	1766.60^{d}	2674.38^{c}	1.32	2.03^{a}	1.79^{a}
S1	1182.21	3609.83	4792.04	914.90^{bc}	1804.58^{dc}	2719.48^{bc}	1.29	2^{a}	1.76^{a}
G1	1127.06	3714.62	4841.69	899.14^{c}	1829.39^{bcd}	2728.53^{bc}	1.25	2.03^{a}	1.77^{a}
F1	1228.53	3657.68	4886.22	925.87^{ab}	1839.54^{bc}	2765.41^{b}	1.32	1.99^{ab}	1.76^{a}
S2	1170.75	3646.27	4817.03	908.79^{bc}	1867.57^{bc}	2776.36^{b}	1.28	1.95^{abc}	1.73^{ab}
G2	1188.06	3635.98	4824.04	914.22^{bc}	1938.04^{a}	2852.26^{a}	1.30	1.87^{c}	1.69^{b}
F2	1196.14	3584.98	4781.13	939.81^{a}	1899.49^{ab}	2839.30^{a}	1.27	1.89^{bc}	1.68^{b}
SEM	9.42	18.81	19.91	3.48	13.82	14.75	0.009	0.016	0.011
P value	0.1331	0.5990	0.8553	0.0156	0.0014	0.0002	0.3475	0.0165	0.0304

$^{a-c}$ Means in each column with uncommon superscripts are significantly different $P < 0.05$.

C, control; FCR, Feed conversion ratio; FI, Feed intake; WG, weight gain; S1 and S2, SBM was respectively replaced with 2.5%, and 5.0% of PSBM in starter period (days 1-21). G1 and G2, SBM was respectively replaced with 2.5%, and 5.0% of PSBM in grower period (days 22-42); F1, and F2, SBM was respectively replaced with 2.5%, and 5.0% of PSBM in whole period (days 1-42).

Table 3. Effect of the PSBM content of diet on intestinal morphology of the broilers.

Treatments	Duodenum				Jejunum				Ileum			
	VL (µm)	CD (µm)	VL/CD	GCC	VL (µm)	CD (µm)	VL/CD	GCC	VL (µm)	CD (µm)	VL/CD	GCC
C	1699.70	280.05^{a}	6.07^{b}	6.93^{a}	1037.99	199.89	5.19^{c}	6.31^{a}	620.41	120.30	5.16	5.87^{a}
S1	1776.13	265.25^{ab}	6.69^{a}	6.56^{ab}	1077.77	194.12	5.55^{bc}	6.13^{ab}	630.42	122.14	5.17	6.11^{a}
G1	1769.20	259.01^{b}	6.84^{a}	5.66^{dc}	1057.96	199.44	5.33^{bc}	6.18^{ab}	635.19	113.03	5.63	5.80^{a}
F1	1771.48	263.95^{ab}	6.72^{a}	5.12^{d}	1121.88	185.61	6.04^{ab}	6.04^{ab}	620.59	114.95	5.40	5.23^{b}
S2	1737.55	250.14^{b}	6.94^{a}	5.96^{bc}	1071.42	192.81	5.57^{bc}	5.95^{bc}	609.24	109.02	5.65	5.20^{b}
G2	1795.98	263.55^{ab}	6.81^{a}	5.22^{dc}	1105.30	190.56	5.82^{abc}	6.02^{b}	623.77	110.15	5.69	4.93^{b}
F2	1813.53	254.90^{b}	7.12^{a}	5.24^{dc}	1112.10	173.54	6.41^{a}	5.73^{c}	613.12	106.93	5.74	4.89^{b}
SEM	14.12	2.64	0.09	0.16	11.37	2.61	0.11	0.04	3.73	1.82	0.09	0.10
P value	0.4504	0.0481	0.033	0.0003	0.4294	0.0723	0.0269	0.0054	0.5911	0.1904	0.4351	<.0001

$^{a-c}$ Means in each column with uncommon superscripts are significantly different P < 0.05.

C, control; CD, Crypt depth; GCC, Goblet cell count; S1 and S2, SBM was respectively replaced with 2.5%, and 5.0% of PSBM in starter period (days 1-21). G1 and G2, SBM was respectively replaced with 2.5%, and 5.0% of PSBM in grower period (days 22-42); F1, and F2, SBM was respectively replaced with 2.5%, and 5.0% of PSBM in whole period (days 1-42).

Table 4. Effect of PSBM content of diet on cecal bacterial counts in the broilers ([log2 (cfu/g)]).

Treatment	*Lactobacilli*	*Clostridia*	*Campylobacters*	*Bifidobacteri*
C	7.02	7.93^{a}	8.12	7.30
S1	7.24	7.00^{b}	8.23	6.95
G1	7.69	6.79^{bc}	7.47	7.25
F1	7.57	6.50^{bc}	7.63	7.66
S2	7.63	7.06^{b}	7.69	8.02
G2	7.56	6.30^{c}	7.33	8.15
F2	8.08	6.20^{c}	7.50	8.25
SEM	0.12	0.13	0.15	0.14
P value	0.3823	0.0006	0.7382	0.1077

$^{a-c}$ Means in each column with uncommon superscripts are significantly different P < 0.05.

C, control; S1 and S2, SBM was respectively replaced with 2.5%, and 5.0% of PSBM in starter period (days 1-21). G1 and G2, SBM was respectively replaced with 2.5%, and 5.0% of PSBM in grower period (days 22-42); F1, and F2, SBM was respectively replaced with 2.5%, and 5.0% of PSBM in whole period (days 1-42).

Table 5. Effect of the dietary PSBM concentration on the antibody response to various antigens and cell-mediated immunity (µm) following PHA-P injection in the broilers.

Treatment	SRBC (1st Injection) [log2]	SRBC2 (2nd injection) [log2]	After PHA-P injection			NDV (Log2)	IBV (Log2)
			4h	24h	48h		
C	1.66	2.66	0.66	0.66^{c}	0.55^{c}	4.65	741.42^{c}
S1	1.66	3	0.66	0.66^{c}	0.57^{c}	4.73	922.83^{bc}
G1	2	3	0.70	0.70^{b}	0.66^{b}	4.72	1051.29^{ab}
F1	2	3	0.73	0.73^{ab}	0.72^{a}	4.68	1100.81^{ab}
S2	1.66	3.66	0.75	0.75^{a}	0.74^{a}	4.85	1087.56^{ab}
G2	1.66	2.66	0.70	0.70^{b}	0.70^{a}	4.58	1110.27^{ab}
F2	2	3.33	0.76	0.76^{a}	0.73^{a}	4.79	14271.04^{a}
SEM	0.14	0.18	0.01	0.008	0.01	0.032	41.89
P value	0.9850	0.8530	0.3735	<.0001	<.0001	0.3952	0.0063

$^{a-c}$ Means in each column with uncommon superscripts are significantly different P < 0.05.

C, control; IBV, Infectious bronchitis virus; SRBC, Sheep red blood cell; NDV, Newcastle disease virus; S1 and S2, SBM was respectively replaced with 2.5%, and 5.0% of PSBM in starter period (days 1-21). G1 and G2, SBM was respectively replaced with 2.5%, and 5.0% of PSBM in grower period (days 22-42); F1, and F2, SBM was respectively replaced with 2.5%, and 5.0% of PSBM in whole period (days 1-42).

***Cecal Bacterial Count*:** Table 4 presents the impact of feeding 2.5% and 5% PSBM on cecal bacterial count (frequency) in broilers. The groups fed 5% PSBM during the grower and entire periods showed the most significant decrease in *Clostridium* counts ($P < 0.001$). However, no significant differences were observed between treatments in the populations of *Lactobacillus*, *Campylobacter*, and *Bifidobacteria* ($P > 0.05$).

***Immune Response Evaluation*:** Dietary supplementation with PSBM significantly affected cell-mediated immunity 24 and 48 hours after inoculation compared to the control ($P < 0.01$), but not 4 hours after inoculation ($P > 0.05$; Table 5). Additionally, a significant increase was observed in the IBV antibody titer following the inclusion of 5% PSBM compared to 2.5% PSBM and control ($P < 0.01$). However, the treatments had no effect on SRBC antibody levels for primary and secondary responses, as well as on the anti-NDV titer ($P > 0.05$).

Discussion and Conclusion

***Growth Performance*:** In this study, an increase in broiler WG was observed with the use of PSBM at a 5% replacement level, while broiler FI remained unaffected by the source of SBM. These findings are consistent with previous studies showing increased growth performance with PSBM replacement for SBM (12, 23). The enhanced growth performance observed after PSBM consumption can be attributed to improved absorption of certain nutrients, particularly lipids, and the activity of digestive enzymes.

It has been previously found that SBM contains trypsin and lectin inhibitory compounds, as well as oligosaccharides such as raffinose and stachyose. Trypsin inhibitors, the most essential anti-nutritional substances, inhibit the conversion of zymogens into active proteases of trypsin and chymotrypsin. This inhibition reduces proteolytic activity in birds, leading to decreased protein digestibility and reduced access to amino acids, particularly lysine, which can result in diminished growth in birds (6).

In addition, lectins, another anti-nutritional substance in SBM, inhibit the amylase activity of the pancreas and decrease the digestion of starch, subsequently leading to decreased body growth (25). In the current experiment, extrusion may have increased the broilers' body weight by mitigating the negative effects of anti-nutritional substances in SBM.

According to the results of previous experiments, extrusion increases the solubility of soybeans by 85%. This increase in fiber solubility improves both fiber and energy digestibility in SBM. In addition to minimizing the anti-nutritional substances in SBM during extrusion, various cells are disintegrated due to the mechanical pressure, making nutrients more available to chickens. Also, extrusion enhances the digestibility of carbohydrates due to the changes in the structure of water-soluble non-starch polysaccharides (NSP), which leads to improved fiber digestibility and increased metabolizable energy for poultry, ultimately resulting in a higher growth rate in birds (23).

However, Guo et al. (10) reported no significant effect on the growth performance of chickens with partial PSBM replacement. They suggested that this discrepancy could be due to variations in the microbial fermentation process, different processing methods for PSBM products, and varying levels of PSBM supplementation in the diet (10).

The FCR results in the present study are consistent with findings from several researchers (5, 12). It is widely acknowledged that improving FCR in chickens fed high concentrations of PSBM is strongly correlated with the positive effect of PSBM on amino acid digestibility (5, 12).

In the group fed SBM, the presence of ANFs due to the incomplete processing of SBM resulted in an increased FCR. These ANFs inhabited protein synthesis and lipogenesis, reducing energy and protein use and decreasing nutrient digestibility. The lack of adequate energy and protein for maintenance and growth caused the birds to increase their feed intake. Consequently, this higher feed intake and decreased growth caused an increased FCR (19, 20).

***Intestinal Morphology*:** In line with the results of the present experiment, it has been reported that PSBM consumption increases intestinal VL and the VL/CD ratio (17, 25). Kim et al. (14) observed a 29% increase in jejunal villus height following the consumption of a diet containing PSBM. Similarly, Foltyn et al. (9) found that consuming extruded soybeans increased the VL/CD ratio by 11%, which is consistent with the results observed in the duodenum and jejunum in the present study.

These researchers found that PSBM affects fermentation patterns and increases saccharolytic activity compared to pectinolytic activity in the intestine. This shift reduces cecal ammonia levels and elevates butyric acid levels. As a result, the energy required for intestinal epithelial cells is provided, leading to improved growth performance and intestinal health (9, 17, 25).

According to the results of the present study, during the growth period, chickens fed PSBM had a significantly reduced number of goblet cells in all three parts of the small intestine compared to the control. Moreover, duodenal CD decreased in chickens receiving PSBM. This reduction could be attributed to the diminished destruction and atrophy of villus tip epithelial cells, which results from mitigating the adverse effects of antigenic proteins,

lectins, and protein inhibitors. Consequently, there is a decrease in the recirculation of mucosa-producing cells in the depth of the crypts (11).

Some studies have reported a decline in the VL/CD ratio after consuming conventional SBM, which may be attributed to the high levels of salicylic acid in unprocessed SBM. It has been suggested that a connection exists between the antigenic compounds in soybean protein and intestinal villus atrophy, leading to an increase in crypt cell mitosis, crypt hyperplasia, and changes in intestinal cell morphology (25). Additionally, the high levels of lectins and trypsin inhibitors lead to an increase in the number of goblet cells, which bind to specific molecules on the brush border membrane surface of the intestinal mucosa (8). This excess secretion of endogenous proteins can ultimately damage the intestinal epithelium and microvilli (25).

***Cecal Bacterial Count*:** Adding PSBM to diets resulted in a significant decrease in *Clostridium* bacterial count and an increase in the number of *Lactobacillus* and *Bifidobacteria*. PSBM replacement for SBM resulted in a balanced and healthy cecal microbial population in broilers, characterized by an increased abundance of beneficial bacteria and potentially decreased harmful bacteria.

Li et al. (16) further supported these findings, revealing a higher relative frequency of Lactobacillus in groups fed 25% and 50% PSBM compared to the control. Previous reports have indicated that the presence of trypsin inhibitors in the gastrointestinal tract leads to changes in the microbial population (21). *Clostridium* bacteria grow more rapidly in environments containing proteins, such as low-digestible feed. Trypsin inhibitors induce the highest level of disturbance in protein digestion. Incomplete digestion and the passage of feed protein can promote the activity of *Clostridium perfringens*, especially toward the end of the digestive tract, culminating in intestinal necrotic inflammation (21). Previous reports indicate that SBM contains more NSP than PSBM. The ability of NSP to create and increase viscosity is the mechanism by which these compounds impose their anti-nutritional effects (2, 3, 16). This increased viscosity traps nutrients and makes them less accessible for digestion, resulting in less nutrient absorption. Consequently, more nutrients are available for pathogenic bacteria, raising the risk of microbial overgrowth in the intestines. In addition, the heightened activity of pathogenic microbial flora increases the production of compounds such as amine, ammonia, and some toxins, thereby eliminating the environment necessary to grow beneficial microbial flora (16). Therefore, including PSBM in diets represents a nutritional strategy that can promote the growth of beneficial intestinal bacteria and enhance overall intestinal health in chickens.

***Immune Response Evaluation*:** Consistent with the results of the present experiment, previous studies have shown that the use of PSBM enhances immune responses in poultry (4). Research has proven that lysine, an essential amino acid abundant in SBM, is an influential and efficient regulator of immunological processes. Extruding SBM with steam not only improves protein and amino acid digestibility but also reduces damage to lysine. Since birds are susceptible to essential amino acids at early ages, providing them with more accessible amino acids during this period can reinforce their immune responses (4).

This improvement in immune function can be attributed to the higher digestibility and bioavailability of nutrients, which enhances immune responses to various antigens (1).

As a result, this could be a crucial factor contributing to the observed increase in the PHA response, indicating a stronger cell-mediated immune response with higher levels of dietary PSBM.

Existing research highlights soybean ANFs, particularly glycinin and conglycinin, as potential immunosuppressive agents in poultry. These ANFs are associated with reduced immune responses through various mechanisms, including decreased immunoglobulin production, thymus atrophy, diminished lymphoblastic cell production, delayed leukocyte migration, and impaired chemotaxis (19, 26). Additionally, exposure to ANFs, particularly glutenin, agglutinin, and lectin, triggers the expression of many different cytokine genes, resulting in elevated levels of pro-inflammatory cytokines (26). Furthermore, the allergenic compounds in inadequately processed soybeans impair lymphocyte proliferation, likely due to oxidative stress, which decreases DNA synthesis and alters the chicken's immune system. Therefore, thermal processing of SBM by inactivating ANFs can lead to stronger immune responses in birds.

Another critical issue with SBM is their contamination with mycotoxins. Previous studies have demonstrated that fungal toxins or mycotoxins negatively impact the function of lymphoid organs. The impacts of aflatoxin on immunosuppression have been proven. Destructive effects on complement systems and interferons cause a weakened humoral immune system. They also adversely affect macrophages and the phagocytosis process. Furthermore, aflatoxins can destroy the thymus, spleen, and bursa and suppress immunoglobulins A and G. One physical method to reduce mycotoxins is heating (18, 19). In the current experiment, the higher immune response in birds fed

PSBM suggests that extrusion may have caused the reduction of mycotoxins and sterilization of SBM.

In summary, adding 5% PSBM to the broiler diet improved WG and FCR during the grower and entire periods without significantly affecting the average FI. Also, increasing the PSBM level improved the intestinal morphology parameters, including CD, VL/CD, and goblet cells. Importantly, the 5% PSBM regimen also decreased the population of *Clostridium* in the cecal contents without negatively affecting the count of beneficial cecal bacteria. Furthermore, this PSBM concentration contributed to elevated IBV antibody titers and a strengthened immune response to PHA at 24 and 48 hours after administration. Substituting 5% PSBM for SBM is a promising strategy for broiler health as it positively affects growth and various health parameters.

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Ethical Statement

This experiment was approved by the Animal Care Committee of Islamic Azad University, Science and Research Branch of Tehran, Iran. The Animal Ethics Committee approval number was IR-SRBIAU-AEC 2022N18.

Conflict of Interest

The authors declared that there is no conflict of interest.

Author Contributions

AN and MC conceived and planned the experiments. FF and AS carried out the experiments. MA took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Animal Welfare

The authors confirm that they have adhered to ARRIVE Guidelines to protect animals used for scientific purposes.

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