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Impact of varying levels of soy hulls and β-mannanase enzyme supplementation on growth performance, carcass characteristics, nutrient utilization and blood biochemical profile in broiler chickens

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ABSTRACT

Two hundred and forty day-old broilers were randomly assigned to six experimental groups in a 2 x 3 factorial design. The birds were fed diets containing soybean hulls (SH) at the levels of 4% and 6% and β-Mannanase (BM) at the concentration of 0%, 0.05% and 0.1%. Results indicated significant effects of SH and BM supplementation on body weight (BW) and weight gain (WG), with birds fed 4% SH and 0.1% BM exhibiting higher ($p < 0.05$) body weight and weight gain compared to other groups. Feed conversion ratio (FCR) was improved ($p < 0.05$) in birds receiving 4% SH with 0.1% BM supplementation. However, no significant effects were observed on carcase traits across treatment groups. Regarding nutrient digestibility, birds supplemented with 0.1% BM showed improved ($p < 0.05$) crude protein (CP) digestibility compared to those without BM supplementation. No significant variations were observed in dry matter, crude fibre and ether extract utilisation among treatment groups. Hematological and serum biochemical parameters were not influenced by SH and BM supplementation, indicating no adverse effects on blood parameters. In conclusion, these findings underscore the potential advantages of incorporating 4% SH and 0.1% BM in broiler diets for enhanced growth performance and nutrient utilisation.

HIGHLIGHTS

- Broilers with 4% SH and 0.1% BM had significantly higher body weight and weight gain.
- 0.1% BM enhanced crude protein digestibility in broiler diets.
- No adverse effects on blood parameters with SH and BM supplementation.

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Broiler chickens; soybean hulls; β-mannanase enzyme; growth performance; nutrient digestibility

Introduction

The growth performance of poultry is significantly influenced by the primary energy and protein sources in their diets, such as corn and soybean meal (SBM) (Scapini et al. 2018). However, the rising costs of these key feed ingredients have created substantial challenges for feed manufacturers, especially in developing regions. Consequently, the poultry industry has increasingly sought to reduce production costs by incorporating locally available feedstuffs (Azizi et al. 2021). These alternative ingredients, often byproducts of various industries (Azizi et al. 2021), contain considerable amounts of non-starch polysaccharides (NSPs) (Zamani et al. 2017), which can be classified as either soluble or insoluble fibres based on their water solubility (Aziz-Aliabadi et al. 2023).

Dietary fibre (DF) has traditionally been viewed as a dilutive element in chicken feed, potentially reducing voluntary energy intake and nutrient digestibility (Jiménez-Moreno et al. 2019). Additionally, poultry lack the enzymes required to hydrolyse the NSPs found in grain cell walls, leading to inefficient breakdown, which can negatively impact bird performance and feed efficiency (Kurul et al. 2020). Consequently, commercial rations, especially for young birds, are typically formulated with less than 3% crude fibre (Scapini et al. 2018).

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Soybean hulls (SH) are a byproduct of soybean oil extraction and SBM production, containing approximately 75% NSPs, with around 60% being insoluble (Mielenz et al. 2009; Scapini et al. 2018). Among these NSPs, α-galactoside and β-mannan, which exist as glucomannans and galactomannans, are significant, with β-mannan being one of the most abundant polysaccharides after xylan (Saeed et al. 2019; Yaqoob et al. 2022). The enzyme α-galactosidase enhances dietary energy by breaking down galacto-oligosaccharides in SBM (Llamas-Moya et al. 2021). However, NSPs in feed can impede nutrient utilisation by causing physical and physiological disruptions in the intestinal mucosa, making dietary carbohydrases essential for improving NSP utilisation in poultry diets (Jiang et al. 2022). Although broiler chickens exhibit high protein degradability, undigested protein remains in the intestine due to anti-nutritional factors, with most mannans in SBM being part of the NSP fraction and acting as anti-nutritional agents (Scapini et al. 2018; Jiang et al. 2022). This composition of SH highlights the challenge of hydrolysing fibre to convert it into beneficial nutrients for poultry.

Nutritional formulation of diets and the inclusion of exogenous enzymes play a crucial role in animal performance (Cozannet et al. 2021). Dietary supplementation with carbohydrases can break down NSPs, producing oligosaccharides in the gastrointestinal tract (Lin and Olukosi 2021). This approach, particularly the use of β-mannanases, is effective in enhancing the utilisation of mannan-rich poultry feed by hydrolysing hemicellulose (Chauhan et al. 2012; Yaqoob et al. 2022). β-mannanase breaks down β-mannans, glucomannans and galactomannans, reducing the viscosity of intestinal digesta and releasing bound nutrients like D-mannose, which serves as an energy source (Zou et al. 2006; Jiang et al. 2022). Additionally, β-mannanase reduces pathogenic bacteria in the intestine and has been shown to improve immunity, prevent energy depletion, and enhance energy availability in animals (Yaqoob et al. 2022). Previous studies have demonstrated the beneficial effects of β-mannanase supplementation in broiler diets containing SBM (Scapini et al. 2018; Jiang et al. 2022; Yaqoob et al. 2022). Given the importance of β-mannanase and the presence of β-mannan in SH-based diets, this trial was conducted to explore the impact of varying levels of β-mannanase supplementation in diets with higher SH content on broiler performance, nutrient digestibility and hematological parameters. The aim of the present study is to assess the supplementing broiler diets with 4% or 6% SH and varying levels of β-Mannanase will improve growth performance, carcase traits, nutrient digestibility and blood chemistry.

Materials and methods

Experimental birds, housing and management

Two hundred and forty, day-old broilers were acquired from a commercial hatchery and weighed individually prior to experimentation. Chicks were randomly divided into six experimental groups ($4SH_0$, $4SH_{0.05}$, $4SH_{0.1}$, $6SH_0$, $6SH_{0.05}$ and $6SH_{0.1}$), containing two levels of SH (4% and 6%) and three levels of β-mannanase (BM) (0, 0.05% and 0.1%) in 2×3 factorial fashion. Each group consisted of 4 replicates per 10 chicks each. β-mannanase enzyme used was a 1, 4 – β - D-mannanase (Hemicell HT® Elanco Companion Animal Health, reg No. 983410). It is obtained by fermenting *Paenibacillus lentus*. This enzyme helps in breakdown of β-mannans by randomly cleaving the main chain of galactomannan, galactogluco-mannan and mannan inside the 1, 4- β-D-mannan (Scapini et al. 2018). The enzyme contains approx. 160 million units per kg and is used in g/kg. The composition of the test diets and SH is presented in Table 1.

Prior to the commencement of the trial, the broiler house underwent meticulous disinfection and

Table 1. Composition of experimental diets (on fed basis).

Ingredients (g/100g)	$4SH_0$	$4SH_{0.05}$	$4SH_{0.1}$	$6SH_0$	$6SH_{0.05}$	$6SH_{0.1}$
Enzyme level (%)	0	0.05	0.10	0	0.05	0.10
Corn	56.0	56.0	56.0	55.0	55.0	55.0
Soybean meal	28.0	28.0	28.0	27.4	27.4	27.4
Fish meal	2.00	2.00	2.00	2.00	2.00	2.00
Canola meal	6.00	6.00	6.00	5.00	5.00	5.00
Soybean hulls[1]	4.00	4.00	4.00	6.00	6.00	6.00
Soybean oil	2.00	2.00	2.00	2.50	2.50	2.50
Dicalcium phosphate	1.00	1.00	1.00	1.00	1.00	1.00
L-Threonine	0.12	0.12	0.12	0.13	0.13	0.13
DL-Methionine	0.23	0.23	0.23	0.24	0.24	0.24
Lysine HCl	0.1	0.1	0.1	0.13	0.13	0.13
Vitamin-mineral premix[2]	0.40	0.40	0.40	0.40	0.40	0.40
Sodium Chloride	0.20	0.20	0.20	0.20	0.20	0.20
	100	100	100	100	100	100
Calculated nutrient composition						
Dry matter (%)	90	90	90	90	90	90
ME energy (kcal/kg)	3,000	3,000	3,000	3,000	3,000	3,000
Protein (%)	21.0	21.0	21.0	21.0	21.0	21.0
Total crude fibre (%)	4.67	4.67	4.67	5.55	5.55	5.55
Calcium (%)	0.82	0.82	0.82	0.84	0.84	0.84
Dig. Phosphorus (%)	0.41	0.41	0.41	0.42	0.42	0.42
Dig. Met (%)	0.56	0.56	0.56	0.56	0.56	0.56
Dig. TSAA (%)	0.91	0.91	0.91	0.9	0.9	0.9
Dig. Lys (%)	1.20	1.20	1.20	1.20	1.20	1.20
Dig. Thr (%)	0.86	0.86	0.86	0.86	0.86	0.86

[1]Composition of soybean hulls used in the ration: ME, 1203 kcal/kg; CP, 13.52%; Avg. TSSA, 0.31; Avg. lysine, 0.54%; Avg. Trp, 0.09%; Avg. Thr: 0.25; Av-Arg: 0.64, as recorded in experiment 1.

[2]Each kg of vitamin DSM premix (Parsippany, NJ) contained: Retinyl acetate (vit A), 4400 IU; Cholecalciferol (vit. D_3), 118 pg; DL-α-Tocopherol acetate (vit. E), 12 IU; Menadione sodium bisulphite (MSB, vit. K3), 2.40 mg; Thiamine (vit. B1), 2.5 mg; Riboflavin (vit. B2), 4.8 mg; Niacin (vit. B3), 30 mg; Pantothenic acid (vit. B5), 10 mg; Pyridoxine (vit. B6), 5 mg; Biotin (vit. B7), 130 µg; Folic acid, 2.5 mg; Cyanocobalamin (vit. B12), 19 µg; Iron, 80 mg; Manganese, 85 mg; Copper, 6 mg; Selenium, 130 µg; Iodine, 1 mg; Zinc, 75 mg; Ca, 0.71 g; Mg, 0.62 g.

Abbreviations: ME, metabolisable energy; SH, soybean hulls.

whitewashing. Each replicate was kept as a separate pen. The chicks were randomly allotted to these experimental pens so that to keep average means differences among the groups to minimum. The room temperature was kept at 35 °C during first week and was regularly lowered by 5 °F after each week till it reached 25 °C. Humidity was set at about 60%. Birds were housed in an open-sided house with appropriate ventilation. Each pen was equipped with a layer of sawdust as litter, approximately 2–3 inches deep. The litter was regularly stirred throughout the experiment to maintain a dry condition. Equipment like feeders and drinkers were provided to maintain uniform environmental and management conditions in the experimental house. The lighting schedule was set to 20 h of light and 4 h of darkness.

Growth traits, sampling and calculations

Each morning, a carefully calculated quantity of experimental diet was provided to chicks freely, and any remaining feed in the feeding pan was recorded weekly. Body weight was measured on weekly basis. Feed Conversion Ratio (FCR) was assessed as the amount of feed consumed per unit weight gained, calculated as the ratio of feed consumed to weight gain (Ahmad et al. 2024). Adjustments to the diet quantity were made weekly, if necessary, based on the observed consumption and growth patterns to ensure accurate assessment of feed efficiency. The experiment lasted for 35 days including a 1-week adaptation period.

Dressing Percentage was evaluated by randomly selecting two birds from each replicate at the trial's end, weighing them and then slaughtering them (Hafeez et al. 2024). The slaughter procedure adheres to humane practices outlined by the American Veterinary Medical Association, involving stunning, bleeding, monitoring for unconsciousness and post-slaughter handling to ensure animal welfare and food safety. The dressed body weight was measured after removing certain parts, including the head, feet, feathers, abdominal fat, skin and visceral organs. Carcase, breast, leg and wing was determined as the percentage of boneless breast meat in relation to the eviscerated carcase. Abdominal fat percentage was determined by weighing the remaining fat in the abdominal cavity after processing and expressing it as a proportion of the carcase weight. Internal Organs' percentage, including heart, gizzard, liver, duodenum, jejunum and caeca was determined by separately weighing each organ and expressing it as a proportion of the carcase weight.

Digestibility of nutrients

On day 35, the pooled ileal digesta samples underwent drying in a forced-air oven at 55 °C for 1 week, while both the diets and dried ileal digesta samples were ground to pass through a 0.5 mm screen using an ultracentrifugal mill (Sultan et al. 2024). The dry matter (DM) content of the diets and ileal digesta samples was determined by drying them in a drying oven at 105 °C for 24 h, following the protocol outlined by AOAC International in 2006. Nitrogen content was measured using the Kjeldahl method, as described by AOAC International in 2006, with a nitrogen analyser. The ether extract (EE) concentration was determined using a Soxhlet apparatus. Subsequently, phosphorus and chromium concentrations were determined after digestion using nitric acid and perchloric acid, following the procedure outlined by Ahsan (2024), and measured using UV spectrophotometry at wavelengths of 440 nm and 620 nm, respectively, in a plate reader.

The apparent metabolisable energy (AME) values were computed utilising a dedicated equation derived from the analysis results.

$$\text{AME(Kcal/Kg of diet)} = GE\ diet - \left[GE\ in\ digesta/excreta \times \left(\frac{Marker\ diet}{Marker\ excreta/digesta}\right)\right]$$

where GE = gross energy and Marker = chromic oxide concentration.

$$\text{Apparent digestibility} = 100 - \left[100 \times \frac{\%\ nutrient\ in\ digesta \times \%\ Cr\ in\ diet}{\%\ nutrient\ in\ diet \times \%\ Cr\ in\ digesta}\right]$$

For determination of ileal amino acids digestibility, the following formula was used.

$$\text{Apparent ileal digestibility of amino acids} = \left[1 - \left(\frac{Cr\ in\ diet}{Cr\ in\ Digesta}\right) \times \left(\frac{Amino\ acid\ in\ digesta}{Amino\ acid\ in\ diet}\right) \times 100\right]$$

Blood biochemistry

Blood samples were collected from four birds per replicate, resulting in a total of 24 samples per treatment group, at 35th day of age and transferred to an ethylene diamine tetra acetic acid (EDTA; Tiangen, Beijing, China) tube to avoid clot formation (Khan et al. 2024). The blood samples were centrifuged for 20 minutes at

3000 x g. The plasma samples were collected and stored separately at a temperature of −20 °C for subsequent analysis of blood parameters including uric acid, plasma protein, albumin and plasma total cholesterol profile.

Statistical analysis

Weekly body weight (BW) and feed intake (FI) data were recorded and compiled using Microsoft Excel Worksheet to calculate weekly body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR). Total BWG, FI and FCR were computed by summing the weekly data. Carcase, breast meat and abdominal fat yield were assessed, and the weights of internal organs and hematological parameters were documented. Analysis of variance (ANOVA) was performed using the general linear model (GLM) procedure of the Statistical Analysis System (SAS, 2006) in a 2 × 3 factorial design. Pairwise differences among the means were determined at a significance level (p) of $\leq$ 0.05 using Duncan's multiple range test.

Results

Data on growth performance of broiler chickens fed HS with exogenous enzymes supplementation is presented in Table 2. There were significant differences in the live body weight of the treatment groups with significant main effects of SH and BM on live body weight (BW) at 4% and 0.1% inclusion levels on day 14, 21 and 28. Interaction effect of SH and BM was significantly lower ($p < 0.01$) in $6SH_{0.1}$ day 14 and 35. It was evident that live body weight was significantly ($p < 0.01$) higherin $4SH_{01.}$

Effect of SH and BM on the body weight gain of broiler chicken from day 7 to day 35 is given in Tables 3 and 4. Overall body weight gain was significantly ($p < 0.01$) higher in SH4 compared to SH6. Furthermore, enzyme effect showed that 0.1% inclusion rate improved significantly ($p < 0.01$) body weight gain on day 14 and total weight gain. Further, interaction effects showed that significantly higher weight gain was observed in $4SH_{0.1}$, 6SH0 and $6SH_{0.1.}$

Table 2. Effect of soybean hulls enriched with β-mannanase on the live body weight of broiler chicken from day 7 to day 35 ($N = 240$).

		Live body weight (g)			
Treatment[1]	Level	d14	d21	d28	d35
SH	4	516.3^{a}	1041.8^{a}	1598.6^{a}	2071.5^{a}
	6	510.6^{b}	1023.6^{b}	1551.4^{b}	1898.1^{b}
Enzyme (Enz)	0	510.6^{b}	1025.8^{c}	1559.6^{c}	1932.5^{c}
	0.05	513.7^{a}	1032.8^{b}	1575^{b}	1981.1^{b}
	0.1	516.1^{a}	1039.5^{a}	1590.5^{a}	2040.8^{a}
Interaction effect					
$4SH_0$		515.0^{ab}	1035.2	1584.2	2030.5^{b}
$4SH_{0.05}$		516.0^{a}	1042.7	1599.0	2074.2^{ab}
$4SH_{0.1}$		518.0^{a}	1047.5	1612.7	2109.7^{a}
$6SH_0$		506.3^{c}	1016.3	1535.1	1834.5e
$6SH_{0.05}$		511.5^{b}	1023.0	1551.0	1888d
$6SH_{0.1}$		514.2^{ab}	1031.5	1568.2	1972^{c}
SEM		6.64	58.93	71.44	169.23
p-value	SH	0.000	0.000	0.000	0.000
	Enz	0.000	0.000	0.000	0.0001
	SH*Enz	0.040	0.260	0.730	0.0183

Mean values within each column showing the same superscript are not statistically different from each other at p-level of 0.05.
[1]SH = Inclusion of 4 and 6% soybean hulls in the diet enriched with β-mannanase @ 0, 0.05 and 0.1%, respectively.

Table 3. Effect of soybean hulls enriched with β-mannanase on the body weight gain of broiler chicken from day 7 to day 35 ($N = 240$).

		Weight gain (g)					
Treatment	Level	d14	d21	d1 - 21	d28	d35	Total
SH	4	319.60	518.80	992.60	551.20	395.00	2031.7^{a}
	6	321.00	520.00	994.40	536.60	435.50	1858.1^{b}
Enzyme (Enz)	0	321.3^{a}	516.10	988.50	539.00	418.30	1892.6^{c}
	0.05	316.3^{b}	517.30	992.40	555.30	382.00	1941.3^{b}
	0.1	323.2^{a}	524.70	999.60	537.50	445.50	2000.9^{a}
Interaction effect							
$4SH_0$		317^{b}	515.00	986.00	554.00	370.00	1990.8^{b}
$4SH_{0.05}$		315.8^{b}	518.50	993.80	563.50	350.70	2034.5^{a}
$4SH_{0.1}$		325.9^{a}	523.00	997.90	536.20	464.20	2069.9^{a}
$6SH_0$		325.6^{a}	517.20	991.00	524.00	466.70	1794.4^{e}
$6SH_{0.05}$		316.9^{b}	516.20	991.10	547.20	413.20	1848.1^{d}
$6SH_{0.1}$		320.6^{ab}	526.50	1001.20	538.70	426.70	1932.0^{c}
SEM		5.15	47.85	48.95	57.31	85.21	168.20
p-value	SH	0.352	0.782	0.667	0.197	0.368	0.000
	Enz	0.005	0.213	0.134	0.354	0.509	0.000
	SH*Enz	0.006	0.839	0.745	0.487	0.448	0.016

Mean values within each column showing the same superscript are not statistically different from each other at p-level of 0.05.
[1]SH = Inclusion of 4 and 6% soybean hulls in the diet enriched with β-mannanase @ 0, 0.05 and 0.1%, respectively.

Table 4. Effect of soybean hulls enriched with β-mannanase on feed intake of broiler chicken from day 7 to day 35 ($N = 240$).

		Feed intake (g)					
Treatment	Level	d14	d21	d7 - 21	d28	d35	Total FI
SH	4	400.9^{b}	718.2	1285.1	1133.1	1417.6^{a}	3818.3
	6	409.0^{a}	735.4	1299.4	1129.3	1362.1^{b}	3835.7
Enzyme (Enz)	0	406.4	736.4	1295.5	1132.2	1392.8	3892.2^{a}
	0.05	404.6	718.2	1293.1	1138.1	1392.4	3839.4^{ab}
	0.1	403.9	725.9	1288.1	1123.4	1384.4	3749.3^{b}
Interaction effect							
$4SH_0$		401.5	720.4	1293.1	1146.35	1418.3	3850.2^{ab}
$4SH_{0.05}$		400.7	704.7	1275.0	1133.5	1419.5	3898.2^{a}
$4SH_{0.1}$		400.5	729.5	1287.2	1119.5	1415.0	3706.5^{b}
$6SH_0$		411.4	752.3	1297.9	1118.0	1367.3	3934.3^{a}
$6SH_{0.05}$		408.5	731.7	1311.2	1142.7	1365.2	3780.5^{ab}
$6SH_{0.1}$		407.2	722.2	1289.0	1127.2	1353.7	3792.2^{ab}
SEM		4.91	23.79	18.96	50.29	41.74	103.51
p-value	SH	0.000	0.062	0.058	0.870	0.001	0.587
	Enz	0.156	0.256	0.689	0.868	0.859	0.005
	SH*Enz	0.491	0.161	0.115	0.749	0.954	0.024

Mean values within each column showing the same superscript are not statistically different from each other at p-level of 0.05.
[1]SH = Inclusion of 4 and 6% soybean hulls in the diet enriched with β-mannanase @ 0, 0.05 and 0.1%, respectively.

Table 5. Effect of soybean hulls enriched with β-mannanase on feed conversion ratio of broiler chickens from day 7 to day 35 ($N = 240$).

		Feed conversion ratio (FCR)					
Treatment	Level	d14	d21	d7 - 21	d28	d35	d7 - 35
SH	4	1.25[b]	1.38	1.29	2.06	3.82	1.88[b]
	6	1.27[a]	1.41	1.30	2.10	3.38	2.06[a]
Enzyme (Enz)	0	1.26[ab]	1.43	1.31	2.10	3.59	2.06[a]
	0.05	1.28[a]	1.38	1.30	2.05	3.93	1.98[b]
	0.1	1.25[b]	1.38	1.28	2.09	3.29	1.87[c]
Interaction effect							
$4SH_0$		1.27[a]	1.4	1.31	2.07	4.17	1.94[bc]
$4SH_{0.05}$		1.27[a]	1.36	1.29	2.02	4.24	1.92[c]
$4SH_{0.1}$		1.23[b]	1.40	1.29	2.09	3.07	1.79[d]
$6SH_0$		1.26[ab]	1.46	1.31	2.14	3.01	2.19[a]
$6SH_{0.05}$		1.29[a]	1.42	1.32	2.09	3.63	2.05[b]
$6SH_{0.1}$		1.27[a]	1.37	1.29	2.1	3.51	1.96[bc]
SEM		0.02	0.05	0.02	0.11	1.10	0.13
p-value	SH	0.01	0.136	0.226	0.337	0.341	0.000
	Enz	0.004	0.127	0.119	0.674	0.525	0.000
	SH*Enz	0.032	0.184	0.119	0.805	0.368	0.05

Mean values within each column showing the same superscript are not statistically different from each other at *p*-level of 0.05.
[1]SH = Inclusion of 4 and 6% soybean hulls in the diet enriched with β-mannanase @ 0, 0.05 and 0.1%, respectively.

However, overall weight gain was significantly ($p < 0.01$) higher in $4SH_{0.05}$ and $4SH_{0.1}$.

Effect of SH and BM on feed intake of broiler chicken from day 7 to day 35 is given in Table 4. Significantly ($p < 0.01$) higher feed intake was observed in SH6 and SH4 on day 14 and day 35 of the experiment. Enzyme effect showed that significantly ($p < 0.05$) lower feed intake was observed at the inclusion level of 0.05 and 0.1% in broilers. The interaction effect showed that total feed intake was significantly ($p < 0.01$) lower in broilers treated with $4SH_{0.1}$. $6SH_{0.05}$ and $6SH_{0.1}$.

Effect of SH and BM on FCR of 2 broiler chickens from day 7 to day 35 is given in Table 5. The results showed that FCR for the total period was significantly ($p < 0.01$) lower in 4SH and enzyme inclusion at the rate of 0.1%. The interaction of SH and enzyme inclusion showed that FCR was significantly ($p < 0.01$) lower $4SH_{0.1}$.

Effect of SH and BM on carcase traits (%) of broiler is shown in Table 6. The results showed that the levels of SH and enzyme did not affect the carcase traits in broilers.

Effect of SH and BM on nutrient digestibility of broiler chicks is given in Table 7. The results showed that enzyme inclusion at the level of 0.1% improved the digestibility of crude protein in broilers compared to no enzyme supplementation. However, SH levels alone and interaction of SH and enzyme supplementation did not affect the digestibility of nutrients in broilers.

Effect of SH and BM on blood biochemistry of broiler chickens in given in Tables 8 and 9. The results indicated that the inclusion of enzymes and SH levels had no impact on blood chemistry, demonstrating no adverse effects.

Table 6. Effect of soybean hulls enriched with β-mannanase on carcase traits (%) of broiler chicks. ($N = 36$).

Treatment	Level	Carcase	Breast	Leg	Gizzard	Heart	Liver	Abd. fat	Spleen
SH	4	70.29	22.76	22.38	2.54	0.49	2.48	0.17	0.17
	6	69.68	22.48	22.45	2.56	0.50	2.57	0.21	0.25
Enzyme (Enz)	0	70.02	22.40	22.57	2.53	0.47	2.46	0.20	0.24
	0.05	70.01	22.92	22.16	2.49	0.46	2.58	0.21	0.16
	0.1	69.92	22.54	22.51	2.64	0.55	2.52	0.17	0.23
Interaction effect									
$4SH_0$		70	22.37	22.51	2.44	0.48	2.33	0.19	0.20
$4SH_{0.05}$		70.28	23.31	22.22	2.67	0.47	2.63	0.18	0.15
$4SH_{0.1}$		70.58	22.6	22.4	2.52	0.51	2.47	0.15	0.17
$6SH_0$		70.04	22.42	22.64	2.61	0.46	2.59	0.21	0.29
$6SH_{0.05}$		69.75	22.54	22.09	2.3	0.44	2.53	0.24	0.17
$6SH_{0.1}$		69.25	22.49	22.62	2.76	0.59	2.57	0.18	0.3
SEM									
p-value	SH	0.102	0.174	0.629	0.889	0.831	0.329	0.068	0.088
	Enz	0.966	0.103	0.068	0.576	0.218	0.555	0.576	0.269
	SH*Enz	0.301	0.221	0.61	0.095	0.595	0.279	0.221	0.635

Mean values within each column showing the same superscript are not statistically different from each other at *p*-level of 0.05.
[1]SH = Inclusion of 4 and 6% soybean hulls in the diet enriched with β-mannanase @ 0, 0.05 and 0.1%, respectively.

Table 7. Effect of soybean hulls enriched with β-mannanase on nutrient digestibility of broiler chicks ($N = 36$).

Treatment	Level (%)	Dry matter	Crude protein	Ether extract	Crude fibre
SH	4	64.8	48.6	89.0	69.3
	6	65.2	48.5	89.7	72.1
Enzyme (Enz)	0	64.4	47.3[b]	89.3	70.8
	0.05	65.2	48.7[ab]	89.2	72.8
	0.1	65.4	49.6[a]	89.2	68.5
Interaction effect					
$4SH_0$		64.3	48.4	89.2	71.6
$4SH_{0.05}$		65.2	47.9	88.7	70.1
$4SH_{0.1}$		65.0	49.5	89.0	66.3
$6SH_0$		64.5	46.2	89.5	70.0
$6SH_{0.05}$		65.2	49.5	89.7	75.5
$6SH_{0.1}$		65.7	49.7	89.5	70.7
SEM		0.95	1.81	0.74	4.56
p-value	SH	0.395	0.845	0.052	0.118
	Enz	0.117	0.022	0.945	0.136
	SH*Enz	0.741	0.057	0.591	0.201

Mean values within each column showing the same superscript are not statistically different from each other at *p*-level of 0.05.
[1]SH = Inclusion of 4 and 6% soybean hulls in the diet enriched with β-mannanase @ 0, 0.05 and 0.1%, respectively.

Discussion

In this study, the growth performance of broiler chickens fed soybean hulls (SH) with exogenous enzyme supplementation demonstrated significant differences in key growth parameters. Notably, the interaction between SH and BM resulted in higher body weights, particularly in groups receiving 4% SH and 0.1% BM. Additionally, feed intake and feed conversion ratios varied significantly among treatments, with birds on the 4% SH and 0.1% BM diet exhibiting improved feed efficiency compared to those on a 6% SH diet without

Table 8. Effect of soybean hulls enriched with β-mannanase on blood cholesterol (mg/dl) and uric acid (mg/dl) of broiler chickens ($N = 36$).

Treatment	Level	Uric acid	Cholesterol	TG	VLDL	LDL	HDL	LDL/HDL
SH	4	4.54	139.3	68.42	18.25	18.74	102.3	0.48
	6	4.55	139.8	68.32	18.72	18.72	102.3	0.34
Enzyme (Enz)	0	4.60	139.3	68.62	18.42	18.55	102.3	0.49
	0.05	4.44	139.6	68.46	18.54	18.88	102.2	0.37
	0.1	4.59	139.8	68.03	18.50	18.77	102.5	0.38
Interaction effect								
$4SH_0$		4.67	139.1	68.71	18.23	18.52	102.3	0.50
$4SH_{0.05}$		4.47	139.3	68.48	18.16	18.87	102.2	0.41
$4SH_{0.1}$		4.48	139.7	68.09	18.36	18.83	102.5	0.52
$6SH_0$		4.53	139.5	68.54	18.61	18.58	102.3	0.47
$6SH_{0.05}$		4.42	139.9	68.44	18.91	18.89	102.2	0.32
$6SH_{0.1}$		4.70	139.9	67.98	18.64	18.70	102.5	0.24
SEM		0.37	3.89	2.59	0.49	0.28	2.95	0.08
p-value	SH	0.93	0.05	0.621	0.057	0.948	0.954	0.242
	Enz	0.59	0.18	0.075	0.893	0.531	0.482	0.637
	SH*Enz	0.57	0.63	0.969	0.63	0.946	0.962	0.651

Mean values within each column showing the same superscript are not statistically different from each other at *p*-level of 0.05.
[1]SH = Inclusion of 4 and 6% soybean hulls in the diet enriched with β-mannanase @ 0, 0.05 and 0.1%, respectively.
TG: triglyceride; VLD: very low density lipoprotein; LDL: low density lipoprotein; HDL: high density lipoprotein.

Table 9. Effect of soybean hulls enriched with β-mannanase on plasma proteins and enzymes of broiler chickens ($N = 36$).

Treatment	Level (%)	AST (U/L)	ALT (U/L)	Total protein (g/dl)	Albumen (g/dl)	Globulin (g/dl)
SH	4	295.23	1.52	3.92	1.48	2.43
	6	295.01	1.61	3.91	1.56	2.34
Enzyme (Enz)	0	295.28	1.51	4.12	1.64	2.47
	0.05	293.44	1.61	3.85	1.51	2.34
	0.1	296.64	1.58	3.76	1.42	2.34
Interaction effect						
$4SH_0$		296.18	1.5	4.18	1.63	2.54
$4SH_{0.05}$		292.97	1.62	3.82	1.49	2.33
$4SH_{0.1}$		296.54	1.46	3.75	1.34	2.41
$6SH_0$		294.38	1.53	4.06	1.66	2.4
$6SH_{0.05}$		293.91	1.6	3.88	1.54	2.34
$6SH_{0.1}$		296.75	1.7	3.77	1.5	2.27
SEM		6.13	0.04	0.76	0.5	0.4
p-value	SH	0.863	0.396	0.941	0.456	0.391
	Enz	0.138	0.729	0.169	0.227	0.489
	SH*Enz	0.657	0.501	0.894	0.859	0.8

Mean values within each column showing the same superscript are not statistically different from each other at *p*-level of 0.05.
[1]SH = Inclusion of 4 and 6% soybean hulls in the diet enriched with β-mannanase @ 0, 0.05 and 0.1%, respectively.

enzyme supplementation. Although research on the effects of β-mannanase in SBM diets is limited, prior studies have explored the impact of NSP-degrading enzymes on broiler performance. For example, Jiang et al. (2022) observed significantly higher body weight gain in birds supplemented with β-mannanase and protease during the first 2 weeks. Similarly, Jackson et al. (2004) reported improved weight gain in broilers fed β-mannanase in starter and grower pelleted rations. The β-mannan content in SBM-based diets varies with SBM levels; for instance, a diet with 35% dehulled SBM may contain 0.44% β-mannan, and levels would increase with the inclusion of SH. Thus, when formulating poultry diets, it's essential to account for the substantial β-mannan content in SBM and the potential benefits of exogenous enzymes like β-mannanase, which can enhance energy production by reducing fibre viscosity, improving passage rates, and enhancing nutrient digestibility (Hsiao et al. 2006).

Mateos et al. (2012) observed that enzymes interact more effectively with nutrients in the presence of a moderate amount of dietary fibre, enhancing gizzard function and gastroduodenal reflux. Conversely, Scapini et al. (2018) and Azarfar (2013) reported no improvements in weight gain, feed intake, or feed conversion in broilers fed corn and SBM-based diets with β-mannanase supplementation (400 mg/kg and 0.5 to 1.0 g/kg, respectively). This discrepancy may be due to variations in β-mannan concentrations among soybean varieties (Hsiao et al. 2006) and the higher β-mannan content in soy hulls (SH), which could be more prevalent in corn-SBM-SH diets than in corn-SBM diets (Scapini et al. 2018). Although the β-mannanase used in our study was designed for corn-SBM diets, a higher dosage (>0.1%) might be more effective for diets with elevated SH levels. Aziz Ur Rahman et al. (2021) found that exogenous enzymes did not sufficiently improve feed conversion ratios when unconventional feed ingredients, like potato peels, were

included at levels above 5%, leading to reduced weight gain and poorer nutrient digestibility. Hajati (2010) noted improved leg meat quality in broilers fed a combination of xylanase and β-glucanase enzymes. Singh et al. (2019) suggested that the impact of enzyme supplementation might vary with the birds' age due to changes in microbiota and physiological factors. Typically, commercial broiler diets contain 3% or less crude fibre (CF), which promotes organ growth and enhances the secretion of digestive acids and enzymes (Back Knudesen 2001; Sklan et al. 2003). However, our trial included a higher CF level of 8%, which might have posed challenges for the birds. The discrepancies with previous findings may stem from differences in experimental conditions such as variations in SH quality, enzyme activity, diet formulation, or bird genetics. Additionally, the lower body weight and weight gain observed in groups with 6% SH might be due to the higher fibre content potentially hindering nutrient digestion and absorption despite enzyme supplementation.

The analysis of carcase traits in broiler chicks fed soybean hulls (SH) enriched with β-mannanase (BM) revealed that the BM supplementation did not exert a significant effect on any carcase trait across the treatments. These results are consistent with recent studies by various researchers (Attia et al. 2014; Aziz Ur Rahman et al. 2021). Azarfar (2013) observed higher liver weights in broilers supplemented with 0.05 g/kg BM compared to those supplemented with 1.0 g/kg BM, while Hajati (2010) found no significant changes in organ weights of 44–day-old broilers supplemented with xylanase and β-glucanase in an enzyme blend. The lack of effect of SH and BM on carcase traits in broilers could be due to the possibility that the inclusion levels of SH and BM were not high enough to influence carcase characteristics. Carcase traits are often more stable and less sensitive to changes in diet compared to growth performance metrics. Additionally, the broilers might have been able to efficiently utilise the nutrients from the diets regardless of SH and BM levels, leading to no noticeable changes in carcase traits. It's also possible that the duration of the study was not long enough to observe any significant impact on carcase composition.

In the current study, as detailed in Table 7, the nutrient digestibility data for broiler chickens fed soybean hulls (SH) with exogenous enzyme supplementation revealed significant improvements in crude protein (CP) digestibility with 0.1% β-mannanase enzyme, which increased digestibility to 49.6% compared to lower enzyme levels. However, there were no significant effects on dry matter (DM), crude fibre (CF), or ether extract utilisation among the different treatment groups. While research on the effects of SH and enzyme supplementation on nutrient digestibility in broilers is limited, related studies indicate that various enzymes, including xylanase, amylase, glucanase, phytase, protease, pectinase and mannanase, as well as additives like probiotics and organic acids, enhance nutrient digestibility (Singh et al. 2022). The observed improvement in CP digestibility aligns with findings from Singh et al. (2022), Romero et al. (2013) and Cowieson and Adeola (2005), who noted enhanced nutrient digestibility with enzyme supplementation. Yaqoob et al. (2022) reported significant improvements in DM and CF digestibility with β-mannanase at lower dietary ME levels. Singh et al. (2022) also found that adding XAP (xylanase, amylase and protease) improved digestibility of gross energy, CP and starch in both high and low fibre diets. Similarly, Kong and Adeola (2010) observed better DM digestibility and energy utilisation in broilers fed diets with 25 g/kg SH and barley malt during the starter phase. These studies underscore that the inclusion of specific enzymes and feed ingredients can positively affect nutrient digestibility and utilisation in broiler diets. The improvement in crude protein digestibility with 0.1% enzyme inclusion suggests that β-mannanase effectively broke down NSPs, enhancing nutrient availability. However, the lack of effect from varying SH levels and the interaction between SH and enzyme supplementation could be due to the fibre content in SH not being high enough to impose a significant barrier to nutrient absorption. Additionally, the enzyme might have primarily targeted specific NSPs in the diet, which were not abundant in the SH, leading to minimal impact on overall nutrient digestibility. Furthermore, the dietary SH levels used might not have been sufficient to cause a noticeable challenge to digestion that the enzyme could mitigate.

In this study, serum biochemistry data for broilers fed soybean hull (SH)-based diets with β-mannanase (BM) supplementation showed no significant effects on parameters including triglycerides, total cholesterol, serum protein, albumin, HDL, LDL, VLDL, AST and ALT. This result is consistent with findings by Masoudi and Bojarpour (2020), who reported no changes in blood parameters with varying SH or corn hull levels. Sayehban et al. (2016) also found no interactions in blood parameters with different levels of olive pulp and enzyme supplementation. Conversely, Hashemi et al. (2017) observed changes in VLDL and HDL profiles when feeding corn or wheat/barley-based diets with NSP-degrading enzymes, suggesting that such

enzymes can affect lipid metabolism. Rodríguez et al. (2012) further demonstrated that xylanase and β-glucanase improved fat digestibility, with fat accumulation linked to higher VLDL and cholesterol levels (Hashemi et al. 2017). Despite these insights, the current study did not observe significant impacts on serum biochemistry, indicating that the specific levels of SH and BM used did not influence these parameters. The lack of impact on blood chemistry from the inclusion of enzymes and varying levels of soybean hulls (SH) may be due to the broilers' ability to effectively metabolise the dietary components without causing significant stress or disruption to their physiological balance. Additionally, the enzyme and SH levels used might not have been sufficient to alter blood biochemical parameters, which could explain the absence of noticeable effects. This outcome is consistent with the possibility that these dietary modifications are safe and do not negatively influence the birds' overall health.

The practical implications of this study suggest that incorporating 4% SH with 0.1% β-mannanase in broiler diets can enhance growth performance and protein digestibility without negatively impacting carcase traits or blood chemistry. This formulation offers a cost-effective alternative by utilising soybean hulls, a byproduct, thereby potentially reducing feed costs for poultry producers while maintaining or improving bird performance. The findings support the commercial viability of this feed strategy, contributing to more sustainable and economical poultry production.

Conclusion

The inclusion of 4% SH enriched with 0.1% BM led to significant improvements in growth performance and protein digestibility compared to diets without enzyme supplementation.

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Ethics statement

The study was approved by the ethical committee The University of Agriculture, Peshawar, Pakistan (Approval No. 20/AN/2023).

Consent form

All authors are agreed to submit the article to this journal.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Data availability statement

The data of the current experiment can be obtained from corresponding author when needed. The relevant data is provided in the paper. However, any other relevant data/information regarding the research would be provided.

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