Pakistan J. Zool., vol. 56(3), pp 1079-1087, 2024 DOI: https://dx.doi.org/10.17582/journal.pjz/20220424140433

Effect of Dietary Inclusion of Soybean Hulls in Basal Diet on Digesta Viscosity, Fecal Consistency, Hematology, Serum Biochemistry, and Intestinal Morphometric Parameters in the Laying Hens During Peak Egg Production Stages

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Article Information
Received 24 April 2022
Revised 18 June 2022
Accepted 01 July 2022
Available online 13 October 2022 (early access)
Published 27 March 2024

Authors' Contribution
MS animal trial, laboratory experiment, statistical analysis, study design, and writing. AH study design, feed formulation, data evaluation, manuscript review. WKK data evaluation, manuscript review. AK data analysis, manuscript review. AS data analysis, manuscript review.

Key words
Soybean hull, Proximate analysis, Viscosity, Hematology, Histomorphology

ABSTRACT

This study aimed to investigate the effect of soybean hull (HS) in the diet of laying hens on digesta viscosity, fecal consistency, hematology, serum biochemistry, and intestinal morphometrics during peak egg production periods. A total of 160, 28-weeks old golden misri (brown) laying hens were distributed in the following groups. A basal diet of the corn-soybean meal was formulated as a control group and a soybean meal in the basal diet was replaced with 3%SH, 6%SH, and 9% SH respectively. The results of the feces proximate analysis showed significantly higher crude protein during phases 1 and 2, while crude fiber during phase1 and 3 for the SH treatment groups. The crude fat had a significantly higher value for the control group than all SH treatment groups during all (three) phases. The ash amount was significantly higher in the 3 and 6% SH groups during phase 1 while 6% and 9% SH groups during phase 3 than in all other groups. The control group had (P<0.05) lower gut contents viscosity during all phases than the SH treatment groups. During all phases, the feces consistency was normal (dry and cone farming) for the control and 3% SH groups while loose droppings but no free water for the 6% and 9%SH treatment groups. The hematological and serum biochemistry parameters were not affected during all phases. The control group had significantly higher duodenum villus height and crypt depth during all phases while ileum villus height was significantly lower in the 9%SH treatment group than in all other groups. In conclusion, the dietary supplementations of soybean hulls increased the digesta viscosity, and have no adverse effect on fecal consistency, hematological and serum biochemistry, and intestinal morphometric parameters in the laying hens during the peak egg production period.

INTRODUCTION

Soybean hull is the byproduct of Soybean seed after the extraction of oil and a very valuable feed ingredient available on farm feeding for cattle and other species including poultry birds. Soybean hulls contain different quantities of celluloses (29-51 %), hemicelluloses (10-25 %), proteins (11-15 %), lignin (1-4 %), and pectins (4-8 %) (Mielenz *et al*., 2009) which is associated with the de-hulling process (Rojas *et al*., 2014). These outer coverings are very valuable for ruminant animals due to their fibrous nature and hence, fail to find any room in human food (Ipharraguerre and Clark, 2003). Especially in regards to the welfare of laying and breeding hens and feed restriction programmers, the use of soybean hulls is quite common these days (Esonu *et al*., 2005). Soybean hulls are abundantly available in the market at very low costs and the successful feed manufacturing business of

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0030-9923/2024/0003-1079 $ 9.00/0

the country has moved to bring in whole grain in place of soybean meal from Brazil and the USA (Khurshid *et al*., 2017). Soybean hull contains both soluble and insoluble fiber components and the soluble fiber (i.e., pectins, gums, and mucilages) can hold water and increase the viscosity of the digesta and change the nutrient absorption (Langhout *et al*., 2000; Owusu-Asiedu *et al*., 2006; Tellez *et al*., 2014; Perera *et al*., 2019). The non-starch polysaccharides (NSPs) are not only poorly digested by birds but also negatively affect bird physiology, and those adverse effects include altered intestinal transit time, change in intestinal mucosal structure, and hormonal deregulation (Vahouny, 1982). Hens are also known more tolerant to the high-fiber diet compared to fast-growing broilers (Walugembe *et al*., 2014) The interest in the effect of high-fiber content in diets on the digestive physiology of animals has increased, especially among monogastric animals, in which the knowledge of microorganisms involved in fiber breakage is still limited when compared with polygastric species (Castro Júnior *et al*., 2005). Due to high fiber concentration, soybean hulls are not commonly a part of poultry regimes; however, positive inclusion of soybean hulls has been reported in poultry rations (Muir *et al*., 1985; Newkirk, 2010). Therefore, the present study aimed to determine the effect of the inclusion of soybean hulls in different concentrations in the basal diet on digesta viscosity, fecal consistency, hematology, serum biochemistry, and intestinal morphometric parameters in laying hens during peak egg production stages.

MATERIALS AND METHODS

Availability of experimental diets

The experiment was approved by the ethical committee of the Faculty of Animal Husbandry and Veterinary Science, The University of Agriculture Peshawar, Pakistan. Four types of experimental feed were prepared on Sadiq brother Company (Rawalpindi). A control group containing a basal diet (corn-soybean meal) and 3%, 6%, and 9% SH treatment diets were formulated by replacing soybean meal in the basal diet with 3, 6, and 9% soybean hulls (SH). The chemical composition of the experimental diets is presented in Table I.

Housing and experimental birds

A total of 160, 28 weeks old golden misri (brown) layer birds were distributed in a complete randomized design with 4 dietary treatments and 4 replicates of 10 birds each. All the birds were reared together in cages from 29 weeks to 40 weeks of age. The experimental diets were fed to birds in three phases: (phase-1=week 29 to 32, phase-2=week 33 to 36, and phase-3=week 37 to 40). Each cage (120cm long×72cm wide×46cm height) was equipped with one drinker and one feeder, providing *ad libitum* access to water. The room temperature was kept at 75°F and was equipped with sufficient light (17h /day). Routine vaccination schedules and uniform environmental and management conditions were provided to all the birds in the experimental house.

Table I. Experimental diet composition.

Nutrient %	Control	3% SH[1]	6% SH	9% SH
Corn	53.12	53.18	50.90	48.46
Canola meal	4.00	4.00	4.00	5.00
Soybean meal	24.34	23.28	22.56	21.84
Guar meal	1.00	0.00	0.00	0.00
Soybean hull	0.00	3.00	6.00	9.00
PBM Hi fat	2.00	1.02	1.02	00
Poultry oil/fat	2.79	2.79	2.79	2.97
Salt sodium	0.32	0.32	0.32	0.32
Bicarbonate/soda	0.10	0.10	0.10	0.10
Limestone/chips	11.19	11.19	11.19	11.19
DCP	0.77	0.77	0.77	0.77
DLM	0.08	0.08	0.08	0.08
Choline chloride (70%)	0.10	0.10	0.10	0.10
Vitamin premix[2]	0.07	0.07	0.07	0.07
Mineral premix	0.06	0.06	0.06	0.06
Phytase	0.01	0.01	0.01	0.01
Enramycin	0.02	0.02	0.02	0.02
Ethoxyquin/antioxidant	0.01	0.01	0.01	0.01
NSPs	0.02	0.00	0.00	0.00

[1]SH= abbreviated for soybean hull. [2]To provide one kg of diet: Retinyl acetate, 4400 IU; DL-α-tocopheryl acetate, 12 IU; Cholecalciferol, 118µg; Thiamine, 2.5mg; Menadione sodium bisulphite, 2.40 mg; Niacin, 30mg; vit.B_2, 4.8 mg; D-Pantothenic acid, 10 mg; vit. B_6, 5 mg; vit. B_7, 130 µg; Cyanocobalamine, 19 µg; vit.B_9, 2.5 mg; Mn, 85 mg; Zinc, 75 mg; Fe, 80 mg; Iodine, 1 mg; Selenium, 130 µg; Copper, 6 mg.

Proximate analysis of feces

Proximate analysis was performed for excreta samples. The samples were properly thawed and mixed to create homogenous representative samples and then air-dried in an oven at 60°C for 3 days. The excreta samples were ground in Thomas-Willey mill up to 1 mm particle size and stored in labeled bottles at room temperature. Excreta samples were subjected to the proximate analysis for crude protein (CP), crude fiber (CF), dry matter (DM), ether extract (EE), and ash according to the procedure explained by (AOAC, 2000).

Gut viscosity and fecal consistency

The viscosity of intestinal contents was measured with a Brookfield digital viscometer (Model: LVDV-E, P40 adaptor). Total intestinal contents were taken from the gizzard to Meckel's diverticulum (proximal samples) and from Meckel's diverticulum to the ileo-ceco-colic junction (distal samples). For viscosity analysis, 1.5 g of fresh digesta was instantly put in a microcentrifuge tube and centrifuged for 5 min at 12,700 x g. The supernatant was removed and viscosity was determined using a Brookfield digital viscometer at a shear rate of 42.5 sec-1 at 40°C (Bedford and Classen, 1993). On the last day of each phase, excreta were visually observed and scored: (1) normal dry droppings and coning; (2) loose droppings, some coning, but no free water; (3) loose droppings with slight coning and some free water; (4) extremely loose droppings with no coning and large amounts of free water as described by Roland *et al*. (1985).

Hematology and serum biochemistry

Blood samples were collected from four birds of each replicate on the last day of each phase and examined for white blood cells (WBCs) count, red blood cells (RBCs) count, the concentration of hemoglobin, and packed cell volume (PCV). Wright and Giemsa stain fixed monolayer blood films were used for the estimation of differential white blood cells. Haemocytometer was used for the manual calculation of total white blood cells and total red blood cells (Campbell, 1995). PCV was measured by a standard manual technique using micro hematocrit capillary tubes and centrifuged at 2,500 rpm for 5 min. Cyanmethemoglobin methodology was implemented for the estimation of hemoglobin (Hb) concentration in blood. Erythrocyte indices mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentrations (MCHC) were calculated from total red blood cells (TRBC), PCV, and Hb. Other blood parameters, for instance, complete picture blood, heterophile to lymphocyte ratio, blood protein, and plasma total cholesterol were examined in the collected blood samples. Zak-Henly and the chemical methods were adopted for the estimation of cholesterol and protein in the blood, respectively (Lowry *et al*., 1951; Zak, 1957; Henly, 1957). Commercially available diagnostic kits (LAB KIT, (Barcelona) Spain) were used for the determination of serum attributes such as total cholesterol (TC), high-density lipoprotein (HDL), and low-density lipoprotein (LDL). The equation used for the calculation of LDL and Very-Low-Density Lipoprotein (VLDP) in the plasma of collected blood samples is LDL + VLDL = TC - HDL.

Intestinal morphometric parameters

The parameters of intestine morphology were studied according to the procedure explained by Feng *et al*. (2007). In brief, small pieces from the central portions of the intestinal duodenum, jejunum, and ileum were obtained and placed in 10% formalin after careful flushing and detachment of the part. At least four cross-sections were prepared for every sample of the intestinal part taken, using the embedding technique in the microtome. Width, height, crept depth, and surface area of the intestine villus was evaluated at Veterinary Research Institute (VRI) Peshawar using an imaging microscope (Nikon Eclipse 50, Nikon Corporation Japan), specially designed for the intestinal morphometric measurements.

Table II. Proximate analysis of feces during different phases.

Item %	Con-trol	3% SH[1]	6% SH	9% SH	SEM	P value
Phase 1						
Dry matter	72.77	72.50	73.51	73.61	0.52	0.689
Crude protein	23.03^{c}	26.21^{b}	28.14^{ab}	29.92^{a}	0.60	0.023
Crude fiber	11.91^{b}	12.47^{ab}	13.68^{a}	13.93^{a}	0.21	0.014
Crude fat	1.80^{a}	1.69^{b}	1.66^{bc}	1.64^{c}	0.05	0.001
Ash	31.55^{b}	34^{a}	34.19^{a}	32.84^{b}	0.96	0.037
Phase 2						
Dry matter	71.34	71	70.40	70.17	0.43	0.306
Crude protein	25.27^{b}	27.32^{a}	27.63^{a}	28.96^{a}	0.52	0.023
Crude fiber	12.85	13.66	14.02	14.66	0.45	0.201
Crude fat	1.72^{a}	1.64^{b}	1.61^{c}	1.60^{c}	0.04	0.017
Ash	31.51	33.43	32.74	31.66	0.67	0.056
Phase 3						
Dry matter	69.79	72.21	72.58	71.20	0.67	0.541
Crude protein	24.89	25.44	26.11	26.49	0.91	0.117
Crude fiber	11.91^{b}	12.79^{c}	13.88^{ac}	15^{a}	0.62	0.021
Crude fat	1.76^{a}	1.67^{b}	1.64^{bc}	1.62^{c}	0.28	0.030
Ash	29.71^{c}	30.14^{c}	33.91^{a}	34.4^{a}	1.17	0.013

Means in the same row with different superscripts are significantly different ($P<0.05$). [1]SH, Abbreviated for Soybean hull.

RESULTS

The result for feces proximate analysis during different phases is presented in Table II. Dry matter (DM) during all phases remained non-significant among the treatment groups. During phase-1 and 2, crude protein (CP) had ($P<0.05$) higher values in the 9% SH group than in the control group, whereas during phase-3 there was no difference in CP ($P>0.05$). Crude fiber (CF) was recorded ($P<0.05$) higher in the 9% SH group during phase-1 and 3 than in the control group, however, there was no difference

(P>0.05) during phase-2. Similarly, Fat % was (P<0.05) higher in the control group compared to all other treatment groups during all phases. During phase-1, the 6% SH group had (P<0.05) higher ash amount as compared to the control and 9% SH groups (P<0.05), whereas there was no significant difference in phase-2 but the 9% SH group had higher (P<0.05) ash in phase-3 than that of the control and 3% treatment groups. The effect of soybean hulls on gut viscosity and feces consistency is presented in Table III. During all phases, the gut viscosity of the 9% SH group was (P<0.05) higher as compared to all other groups. Feces consistency during all phases for the control and 3% SH treatment groups were observed normal (dry droppings and coning), while 6% and 9%SH treatments had loose droppings, some coning, but no free water. Results for the effect of soybean hulls on hematological and serum biochemistry parameters are presented in Table IV. During all phases, there were no significant differences (P>0.05) in RBCs, WBCs, PCV, HB, MCHC, MCV, TC, HDL, LDL, VLDL, heterophile, lymphocytes, and heterophile to

Table III. Effect of dietary inclusion of soybean hull in the diet on digesta viscosity and fecal consistency during different phases.

Item	Phases	Con-trol	3% SH[1]	6% SH	9% SH	SEM	P. value
Gut viscosity (cp)	1	5.33^d	5.50^c	5.63^b	5.83^a	0.02	0.019
	2	5.35^d	5.55^c	5.67^b	5.88^a	0.04	0.004
	3	5.36^d	5.48^c	5.59^b	5.78^a	0.01	0.003
Feces consist-ency[2]	1	1	1	2	2	-	-
	2	1	1	2	2	-	-
	3	1	1	2	2	-	-

Means in the same row with different superscripts are significantly different (P<0.05). [1]SH= Abbreviated for soybean hull. [2]For feaces consistency: 1, shows normal dry droppings and coning, while 2, show loose droppings, some coning, but no free water.

Table IV. Effect of dietary inclusion of soybean hull in the diet on hematology and serum biochemistry during different phases.

Parameters	Con-trol	3% SH	6% SH	9% SH	SEM	P value
Phase 1						
RBCs (10^6µl)	2.88	2.94	2.99	3.04	0.09	0.841
WBCs (10^3µl)	3.13	3.04	3.01	2.99	0.19	0.967
PCV	26.75	26	25	24	0.65	0.060
HB (g/dl)	8.63	8.59	7.90	7.74	0.27	0.113
MCHC (g/dl)	30.02	31.88	30.43	29.93	0.98	0.060
MCV(fL)	99.81	91.82	87.05	96.09	3.22	0.190
Total chol (mg/dl)	120.81	120.05	118.96	117.9	1.78	0.727
HDL (mg/dl)	80.54	79.65	79.47	80.49	1.27	0.063
LDL (mg/dl)	24.49	27.80	25.34	23.65	1.39	0.265
VLDL (mg/dl)	13.77	14.89	14.13	12.92	0.81	0.091
Heterophile (%)	25.25	28.50	27.50	26.75	1.24	0.364
Lymphocyte (%)	60.50	62.75	63.50	63	1.51	0.150
Hetrop:Lympho	0.43	0.45	0.43	0.42	0.02	0.568
Protein (mg/dl)	4.86	4.84	4.82	4.80	0.05	0.065
Phase 2						
RBCs (10^6µl)	2.69	2.66	2.54	2.49	0.06	0.274
WBCs (10^3µl)	3.08	3.00	2.97	2.97	0.19	0.984
PCV	28.25	27.75	26.50	26	0.7	0.145
HB (g/dl)	10.02	9.80	9.53	9.36	0.30	0.545
MCHC (g/dl)	36.26	37.18	37.62	36.4	1.96	0.998
MCV (fL)	104.95	104.85	104.28	104.23	3.92	0.998
Total chol (mg/dl)	117.7	116.4	116	115.2	2.08	0.065
HDL (mg/dl)	78.47	77.35	75.16	75.2	1.48	0.063
LDL (mg/dl)	25.22	25.34	23.47	22.85	1.16	0.444
VLDL (mg/dl)	14.84	14.13	13.75	13.37	0.79	0.682
Heterophile (%)	27.50	26.75	25.25	26	1.01	0.533
Lymphocyte (%)	59.75	60.75	59.25	58	1.25	0.589
Heterop:Lympho	0.44	0.44	0.42	0.44	0.01	0.860
Protein (mg/dl)	4.70	4.69	4.68	4.66	0.30	0.904
Phase 3						
RBCs (10^6µl)	2.78	2.72	2.66	2.60	0.13	0.860
WBCs (10^3µl)	3.11	3.06	3.03	3	0.14	0.968
PCV	27.5	28.5	28	27	0.90	0.066
HB (g/dl)	11	10	10.05	9.89	0.30	0.065
MCHC (g/dl)	36.74	36.29	35.21	35.75	1.19	0.067
MCV(fL)	108.4	102.41	106.9	111.3	5.93	0.284
Total chol (mg/dl)	123.6	120.3	117.8	117.7	1.52	0.068
HDL (mg/dl)	81.52	80.20	79.58	78.32	0.95	0.072
LDL (mg/dl)	22.99	23.72	25.50	26.40	0.80	0.064
VLDL (mg/dl)	16.07	16.38	14.60	14.12	0.79	0.203
Heterophile (%)	29.5	28	26.5	25.75	1.04	0.107
Lymphocyte (%)	56	55.75	56	58	2.35	0.550
Heterop:Lympho	0.52	0.50	0.47	0.42	1.60	0.128
Protein (mg/dl)	4.79	4.77	4.76	4.73	0.12	0.500

Means in the same row with different superscripts are significantly different (P<0.05). [1]SH= Soybean hull. [2]Abbreviation for: RBCs, red blood cells; WBC, white blood cells; PCV, packed cell volume; MCV, mean corpuscular volumes; MCHC, mean corpuscular hemoglobin concentration; HB, hemoglobin; HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein.

Table V. Effect of dietary inclusion of soybean hull in the diet on histomorphology during different phases.

Phase	Parameters	Control	3%SH[1]	6%SH	9%SH	SEM	P value
1	**Duodenum villus**						
	Width (µm)	72	85	81	78	4.51	0.292
	Height (µm)	621.7[a]	610.7[b]	597.7[bc]	583.2[c]	3.05	0.004
	Crypt depth (µm)	110.2[a]	103.2[b]	94[bc]	85.7[c]	3.45	0.041
	Surface area (µm^2)	0.14	0.16	0.15	0.14	8.90	0.342
	Jejunum villus						
	Width (µm)	57.5	59.2	61.5	63.7	4.31	0.761
	Height (µm)	441.7	446.7	453.5	459.2	7.07	0.134
	Crypt depth (µm)	60.7	62.5	65.2	67.7	3.26	0.304
	Surface area (µm^2)	0.082	0.085	0.087	0.090	6.25	0.493
	Ileum villus						
	Width (µm)	54.7	53.5	51.7	51	3.67	0.887
	Height (µm)	389.5[a]	378.5[ac]	368.2[c]	345[b]	7.15	0.006
	Crypt depth (µm)	50.75	48.75	48.25	47	2.97	0.844
	Surface area (µm^2)	0.066	0.063	0.059	0.055	4.33	0.311
2	**Duodenum villus**						
	Width (µm)	93	106	102	99	4.51	0.292
	Height (µm)	642.7[a]	631.7[b]	618.7[bc]	604.25[c]	6.03	0.004
	Crypt depth (µm)	131.2[b]	124.2[a]	115[ac]	99[c]	5.33	0.006
	Surface area (µm^2)	0.187	0.210	0.198	0.187	9.33	0.343
	Jejunum villus						
	Width (µm)	75.5	77.2	79.5	81.7	4.31	0.761
	Height (µm)	464.7	468.7	471.5	475.2	7.07	0.134
	Crypt depth (µm)	76.7	79.5	83.2	85.7	3.26	0.304
	Surface area (µm^2)	0.112	0.115	0.118	0.121	6.60	0.437
	Ileum villus						
	Width (µm)	69.7	68.5	66.7	66	3.67	0.887
	Height (µm)	404.5[a]	393.5[ac]	383.2[c]	360[b]	7.16	0.006
	Crypt depth (µm)	65.7	63.7	63.2	62	2.97	0.844
	Surface area (µm^2)	0.088	0.084	0.080	0.074	4.58	0.222
3	**Duodenum villus**						
	Width (µm)	110	123	119	116	4.51	0.292
	Height (µm)	677.7[a]	666.7[b]	653.7[bc]	639.2[c]	6.02	0.004
	Crypt depth (µm)	145.2[a]	138.2[c]	129[bc]	120.7[b]	5.56	0.041
	Surface area (µm^2)	0.234	0.257	0.244	0.232	9.93	0.342
	Jejunum villus						
	Width (µm)	89.5	91.25	93.5	95.75	4.31	0.761
	Height (µm)	494.7	497.7	501.5	506.2	7.07	0.134
	Crypt depth(µm)	89.75	91.5	95.25	97.75	3.26	0.304
	Surface area (µm^2)	0.140	0.143	0.147	0.151	7.08	0.409
	Ileum villus						
	Width (µm)	80.75	79.5	77.75	77	3.67	0.887
	Height (µm)	431.5[c]	422.5[ac]	415.2[c]	407[b]	7.15	0.006
	Crypt depth (µm)	81.75	79.75	79.25	78	2.97	0.844
	Surface area (µm^2)	0.109	0.104	0.100	0.097	4.94	0.190

Means in the same row with different superscripts are significantly different (P<0.05). For abbreviations see Table IV.

lymphocytes ratio (H:L) and total protein concentration among all the groups. All the blood and serum biochemistry parameters were recorded in the normal range. The result regarding intestinal morphometric parameters is presented in Table V. During all phases, the 3% SH group had higher duodenal villus width and surface area than all other groups while duodenum villus height and crypt depth were (P<0.05) higher in the control group than in all SH containing diet groups. The jejunum morphological parameters were not affected during the entire period (P>0.05). During all phases, the ileum villus width, crypt depth, and surface area were recorded as non-significant among all the groups. The villus height of the ileum in all phases was calculated (P<0.05) higher in the control group than in the 9% SH treatment group.

DISCUSSION

The effect of soybean hulls on digesta viscosity, fecal consistency, hematology, serum biochemistry, and intestinal morphometric parameters in laying hens during peak egg production period with three different periods (1, 2, and 3) were determined. The proximate analysis of feces showed a non-significant effect on the dry matter during all phases which is in line with the findings of Jarret *et al.* (2012) where a non-significant effect on feces dry matter was observed in swine-fed high fiber feed. The crude protein levels during phas1 and 2 were recorded (P<0.05) higher in SH-containing groups which is in agreement with the findings of Jarret *et al.* (2012) who recorded significantly higher nitrogen in feces of swine-fed high fiber feed. Soybean hulls containing treatment groups had (P<0.05) higher crude fiber (CF) and ash contents during phase-1 and 3 which is similar to the findings of Jarret *et al.* (2012) who described higher feces CF in swine fed high fiber feed than the control group. The low fiber digestibility which was recorded during the present study has possibly resulted in higher feces fiber excretion. Higher (P<0.05) crude fat contents in the control group than all SH treatment groups were recorded during all phases which are possibly due to the lower fat contents of the soybean hulls containing diets while the higher ash contents in feces of SH treatment groups is due to the comparatively higher DM contents of the feces of SH treatment groups than the control group. The viscosity of the intestinal contents during all phases was recorded (P<0.05) higher for the soybean hull containing diet groups which are similar to the findings of Tejeda and Kim (2021) who observed an increase in the intestinal viscosity for SH-containing diets during the entire rearing period for broiler when fed soybean hulls in the diet at the level of 8%. The findings of the present study are also in agreement with the statement of Langhout *et al*. (2000); Owusu-Asiedu *et al*. (2006); Tellez *et al*. (2014); Perera *et al*. (2019) that soluble fiber sources contain hygroscopic compounds (i.e., pectins, gums, and mucilages) with the ability to trap water and increase the viscosity of the digesta and cause changes in passage rate and nutrient absorption. Soybean hulls contain both soluble and insoluble water portions of fiber. The water-soluble portion of NSPs is notorious for forming a gel-like viscosity in the intestinal tract (Burnett, 1966; Gohl and Gohl, 1977). The water-soluble NSP fraction reduces or prolongs digesta passage rate through the intestinal tract due to its ability to form gel-like solid and thickened consistency and the feed consumption of young broilers declined (Salih *et al*., 1991; Almirall and Esteve, 1995) which is similar to the results observed in the present study. During phase-1 and 2, PCV had a higher value for the control group than SH containing treatment groups which are according to the result of Esonu *et al.* (2010) who in layer hen reported higher PCV in the control group than the group containing 30% soybean hull in feed. Total cholesterol during all phases was calculated (P<0.05) higher for the control group than all SH containing treatment groups which is similar to the report of He *et al*. (2015) who recorded lower total cholesterol in geese when fed different fibers source in feed as compared to control group with normal feed. Similarly, Mcnaughton (1978) stated that crude fiber inclusion in the diet reduces serum cholesterol levels and Regar *et al*. (2019) also reported lower cholesterol in broiler when fed different fiber sources (coffee pulp, Rice bran, and coconut oilcake) than that of the control group. The control group showed HDL during all phases which are in agreement with the findings of Regar *et al*. (2019) who reported higher HDL levels in the broiler for the control group compared to groups fed different fiber sources (coffee pulp, Rice bran, and coconut oilcake) in feed. The values of the hematological indices appeared similar to those earlier reported as normal for poultry. According to Esonu *et al.* (2010), the physiological responsiveness of the animal to its external and internal environment serves as a veritable tool for monitoring animal health and described that 30% inclusion of soybean hull (with/without supplementation enzyme) had no serious effect on the internal physiology of the layers. Similarly in the present study, it followed that up to 9% inclusion of soybean hull in the feed had no adverse effect on the internal physiology of the laying hens. All hematological and serum biochemistry values were recorded within the Thai indigenous chickens reference value range (Jain, 1993; Simaraks *et al*., 2004).

During all phases, the duodenum villus height and crypt depth were (P<0.05) higher in the control group than in all other SH treatments groups which is in agreement with the results of Tejeda and Kim (2020) who reported higher duodenum villus height in the broiler for the control group compared to 4, 6, and 8% Solka-floc (SF) and 4 and 6% SH in the feed while higher villus crept depth for the control group than that of SF (6 and 8%) and 4% SH in feed. During all phases, jejunum villus height, width, crypt depth, and surface area were not affected in all treatment groups which is in line with the report of, Tejeda and Kim (2020) where jejunum villus height was recorded as non-significant in broiler fed 4%SH in the feed while villus crypt depth was not affected in Solka-floc (4 and 8%) and soybean hull (8%SH) in feed. Similarly, Lai *et al*. (2015) in broiler reported non-significant jejunum villus height on supplementation of 0.5% FSBH (fermented soybean hull), 0.5% FSHP (Fermented soybean hull Pleurotus Eryngii), and 0.1% FSHP in feed. During all phases, the ileum villus height was (P<0.05) higher in the control group as compared to soybean hull diet groups which is in line with the result of Tejeda and Kim (2020) who documented higher ileum villus height in the broiler for the control group than both 8% SF and 8% SH in feed. Sadeghi *et al*. (2015) also reported shorter ileal villus height in broilers fed 3% sugar beet pulp in the diet than in the control and rice hull fed groups. Similar to the present study result, Tejeda and Kim (2020) in broiler recorded the highest ileal villus height for the control group compared to the 4 and 8% cellulose and SH containing feed groups. Contrary to the present study result, Lai *et al*. (2015) recorded significantly higher ileum villus height for 0.5% FSBH (fermented soybean hull), 0.5% FSHP (fermented soybean hull Pleurotus Eryngii), and 0.1% FSHP group than the control group. The impairment in the development of ileal villus could, therefore, be associated with the increase in bacterial activity that interferes with normal intestinal development (Pan and Yu, 2014). According to (Stein *et al*., 2008), the soybean hull contains 50% hemicellulose, 30% pectin, and 20% cellulose and the mix of different types of fibers appears to have a marked effect on intestinal morphology. Therefore, higher inclusions of such water-soluble carbohydrates reduce villus height in the ileum which might be associated with the lack of abrasive stimulus that is generally seen in such fibers compared with insoluble fibers (Rezaei *et al*., 2018). Anti-nutritional factors in soybean had been reported to have adverse effects on the morphology and function of digestive tracts in animals (Li *et al*., 1991). Furthermore, stressors that are present in the digesta can lead relatively quickly to changes in the intestinal mucosa due to the proximity of the mucosal surface and the intestinal content which is similar to the current study result for soybean hull groups having decreased intestinal villus and crypt depth height. Therefore, there is a possibility that the birds might have adapted to increasing levels of SH by increasing the passage rate through the digestive tract rather than increasing the size. However, this speculation needs further investigation. Any additional tissue turnover will increase nutrient requirements for maintenance, and will therefore lower the efficiency in terms of poor growth performance of the animal (Giannenas *et al*., 2010).

CONCLUSION

It is concluded that the effect of different levels of soybean hulls replacing soybean meal in the basal diet on various parameters was not adverse. Among soybean hulls treatment groups, the 3% and 6% SH diet group showed better effects for various parameters than that of the 9% SH group and the inclusion of this level will help to provide the least-cost feed ingredients to the poultry industry to generate revenue and provide quality protein in the form of eggs to common people with better/low prices.

ACKNOWLEDGMENTS

We acknowledge the staff of the Department of Poultry Science and Faculty of Animal Husbandry and Veterinary Science (FAHVS), The University of Agriculture, Peshawar Pakistan, and Sadiq Brother (SB) Company (Rawalpindi, Punjab) who provided technical and laboratory facilities.

Animal welfare statement

The experiment was approved by the ethical committee of the Faculty of Animal Husbandry and Veterinary Science, The University of Agriculture Peshawar, Pakistan

Funding

This study was financially supported by the Higher Education Commission (HEC) of Pakistan through (HEC Indigenous Scholarship) grant.

Statement of conflict of interest

The authors have declared no conflict of interest.

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