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Effect of β-Mannanase and Soybean Hulls on the Nutrient Digestibility, Digesta Viscosity, Feces Consistency and Intestinal Histomorphology During Early Peak Production Period in Laying Hens

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ABSTRACT

This study was designed to determine the combined effect of fiber degrading enzymes (β-Mannanase) and soybean hulls on the egg quality parameters in the laying hens during the early (29 to 32 weeks) peak production period. Two hundred golden brown (RIR×Fayoumi) layer birds of age 28 weeks were used for the experimental purpose and were assigned into five groups CON, T1, T2, T3, and T4. Each group contained 4 replicates with 10 birds per replicate. The CON group had a corn-soybean basal diet while the T1 group had 3%SH+20mg/kg enzyme, T2 3%SH+30mg/kg enzyme, T3 9%SH+20mg/kg enzyme, and T4 group 9%SH+30mg/kg enzyme in the feed. The result showed significantly higher digestibility of crude protein (CP) in the T1 group and crude fat in the T1 and T2 groups while the digestibility of dry matter (DM), crude fiber (CF), and ash were not effected (P>0.05). Digesta viscosity and feces consistency were not effaced (P>0.05) but duodenum, jejunum, and ileum villus width, height, crypt depth, and surface area were recorded higher (P<0.05) in the T2 diet group. It is concluded that the replacement of soybean meal in the diet of laying hens by 3%SH in combination with enzyme (β-Mannanase) at the level of 20 and 30mg/kg feed has a positive effect on the nutrient digestibility, digesta viscosity, feces consistency and intestinal histomorphology during early peak egg production period in the laying hens.

INTRODUCTION

Over the last many years, the poultry feed industry has become a profitable enterprise due to its countless opportunities and integral possibilities for creating revenue and services. Feed shares more than 70% of the total cost of production and any effort to reduce the cost of feed may result in a significant decrease in the total cost of production (Ayanrinde *et al*., 2020; Hinrichs *et al*., 2007). Besides all the advantages, merits, and massive investment capacity, the feed industry faces various problems. These include seasonal unavailability of certain ingredients too, causing forced use of certain expensive ingredients in feed. As a result, it leads to an augmented price of production. Animal nutritionists are thus always in search of alternate ingredients which give them a cushion for the least-cost formulation without affecting the performance of birds and animals. It is, therefore, necessary to expand the scientific information for evaluating low-price locally prevailing agro-industrial by-products in poultry feed to decrease the cost of feed (Thirumalaisamy *et al*., 2016). Poultry feed protein sources are mainly composed of animal and plant origin. Among plant sources of protein, soybean meal is a common plant protein source. Soybean hulls are the by-product of soybean seed when used for the extraction of oil and their chemical composition may vary due to the

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efficiency of the de-hulling process (Rojas *et al*., 2014), hence, the soybean hulls might contain varying quantities of celluloses (29-51%), hemicelluloses (10-25%), proteins (11-15%), lignin (1-4%) and pectin (4-8%) (Mielenz *et al*., 2009; Shuaib *et al*., 2022). Soybean hulls, therefore are, mainly lignocellulose physical entities and poultry birds cannot produce enzymes for the breakdown of non-starch polysaccharides (NSPs) existing in the cell wall of the grains and are kept un-hydrolyzed, resulting in low feed efficacy. For the last 5 decades, enzymes have played a vital role in the poultry feed industry by augmenting the nutritive worth of the feed ingredients and they are incorporated into poultry diets to reduce feed costs without compromising weight gain and feed efficiency (Walters, 2019). According to Lima *et al*. (2007), the addition of exogenous enzymes to animal feed has goals like the elimination or hydrolysis of anti-nutritional components, NSP breakdown, enhanced nutrient digestibility, and supplementing of endogenous enzymes. Thus, exogenous enzymes in addition to enabling feed efficiency utilization can increase the use of low-cost ingredients for animal feed because the viscosity of the digest a reduces with use, potentiating the activity of endogenous enzymes on specific substrates (Ribeiro *et al*., 2011). 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. It was therefore assumed that the addition of enzyme β-mannanase (Hemicell^{TD}) in a soybean hulls-based diet may compensate for the negative effect of the soybean hulls-based diet. Hulls have been used as a feed ingredient in many experimental trials conducted on animals including broilers. However, according to our knowledge, the inclusion of soybean hulls in layer feed has not been studied in detail, especially at peak production periods in laying hens. The current research was therefore designed to determine the effect of fiber degrading enzymes added in soybean hulls on the nutrient digestibility, digesta viscosity, feces consistency and intestinal histomorphology at the early peak production period in laying hens

MATERIALS AND METHODS

Housing and experimental environment

The study was performed at the University of Agriculture Peshawar Poultry Farm. Two hundred golden brown (RIR×Fayoumi) layer birds of age 28 weeks were used for the experimental purpose and were randomly assigned into five groups of 40 birds each. Every group was further subdivided into four experimental replicates with 10 birds each and randomly assigned to one of the 5 treatments. The experimental diets were formulated in the sadiq brother (SB) feed mill (Rawalpindi). The CON group had a basal diet (corn-soybean meal) while the T1 group contained 3%SH+20mg/kg enzyme, T2 3%SH+30mg/kg enzyme, T3 9%SH+20mg/kg enzyme, and the T4 group 9%SH+30mg/kg enzyme (β-Mannanase (Hemicell™), USA) in the feed. All the birds were provided with similar environmental and managementmental conditions in the experimental house as explained by Shuaib *et al*. (2022). The composition of experimental diets is shown in Table I.

Table I. Experimental diet.

Nutrient %	Diet				
	CON	T1	T2	T3	T4
Corn	53.1	52.1	52.1	50.5	50.5
Canola meal (34%)	4.15	3.85	3.67	2.16	2.14
Soybean meal (44%)	24.3	23.6	23.6	22.2	22.2
Guar meal	1.00	0.00	0.00	0.00	0.00
Soybean hull	0.00	3.00	3.00	9.00	9.00
β-Mannanase (Hemicell)	0.00	2.00	3.00	2.00	3.00
PBM Hi fat	2.00	1.02	0.34	0.00	0.00
Poultry oil	2.79	2.79	2.71	2.67	2.67
Salt	0.32	0.32	0.28	0.26	0.26
Sodium bicarbonate	0.10	0.10	0.10	0.10	0.10
Limestone/Chips	11.1	10.1	10.1	10.0	9.16
DCP	0.77	0.77	0.75	0.77	0.62
DLM	0.08	0.08	0.08	0.07	0.08
Choline chloride (70 %)	0.10	0.10	0.10	0.10	0.10
Vitamin premix broiler	0.07	0.07	0.07	0.07	0.07
Mineral premix	0.06	0.06	0.06	0.06	0.06
Phytase	0.01	0.01	0.01	0.01	0.01
Enramycin	0.02	0.02	0.02	0.02	0.02
Ethoxyquin/Antioxidant	0.01	0.01	0.01	0.01	0.01
NSPs	0.02	0.00	0.00	0.00	0.00
Total	100	100	100	100	100

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 5mg; 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. CON, Control; T1, 3%SH+20mg/kg enzyme β-Mannanase; T2, 3%SH+30mg/kg enzyme β-Mannanase; T3, 9%SH+20mg/kg enzyme β-Mannanase; T4, 9%SH+30mg/kg enzyme β-Mannanase (Hemicell).

Nutrient digestibility

For nutrient digestibility determination, on the last 3 days of the trial, three birds of the same average weight in each group were transferred to metabolic cages and a weighted quantity of feed was provided as explained by

Shuaib *et al* (20202). Celite (Sigma Aldrich) was added at 1 % to all the diets as an inert digestibility marker. From each cage feather and feed particle-free excreta were weighed and 20% of these samples were stored at 4°C for analysis. According to the procedure laid out by (AOAC, 2000), feed and extra samples were subjected to the proximate analysis of crude protein (CP), crude fiber (CF), dry matter (DM), and ether extract (EE). It was properly thawed and mixed to create a homogenous representative sample and then air-dried in an oven at 60°C for 3 days. The diet and excreta samples were ground in a Thomas Willey mill up to 1 mm particle size. The apparent Digestibility of nutrients was determined by the formula;

Apparent Digestibility (%) = 100 − ((Concentration of marker in feed/ concentration of marker in digesta) × (Concentration of nutrient in digesta/ Concentration of nutrient in feed) × 100).

Gut viscosity and fecal consistency

The viscosity of intestinal contents was measured with the help of a viscometer. 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, fresh digesta of About 1.5 g was instantly put in a micro centrifuge tube and centrifuged for 5 min at 12,700 x g. The supernatant was removed and viscosity was determined using a brookfield digital viscometer (Model: LVDV-E, P40 adaptor) at a shear rate of 42.5 sec-1 at 40 °C (Bedford and Classen, 1993). Feces consistency (how feces hold together) was observed and noted. On the last day of each phase, excreta were visually observed and scored, where: (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).

Intestinal morphometric parameters

Intestine histomorphology parameters were studied according to the procedure explained by (Feng *et al*., 2007). A short description of the method is that pieces from the central portion of the duodenum in the small intestine were obtained and placed in 10% formalin after careful flushing and detachment of the part. A similar procedure was followed for the collection of samples from the jejunum as well as the ileum. At least four cross-sections were prepared for every sample of the intestinal part taken, using the embedding technique in the microtome. Width, height, crypt depth, and surface area of the villus of duodenum, jejunum, and ilium of the intestine were evaluated at the Veterinary Research Institute (VRI) Peshawar using an imaging microscope (Nikon Eclipse 50, Nikon Corporation Japan), specially designed for the intestinal morphometric measurements

Statistical analysis

The data on performance, egg quality, hematological parameters, gut morphology, and nutrient digestibility were subjected to the analysis of variance (ANOVA) technique using a completely randomized design (CRD). The general linear model (GLM) procedure (Steel *et al*., 1997) of SPSS 21.0 was used to analyze the data statistically. Tukey's test was applied to compare the significance of mean differences at a 5% level of significance.

Table II. Effect of dietary inclusion of soybean hull and enzyme on apparent nutrients digestibility, digesta viscosity and feces consistency.

Parameter	Diets						P value
	CON	T1	T2	T3	T4	SEM	
Dry matter (%)	78.0	80.2	80.4	78.0	80.0	1.02	0.206
Ash (%)	56.4	58.2	58.4	57.0	57.4	0.87	0.305
Crude protein (%)	66.7^{c}	70.2^{b}	73.2^{a}	67.0^{c}	69.7^{b}	1.20	0.012
Crude fiber (%)	68.0	69.3	70.0	68.0	69.0	0.79	0.553
Crude fat (%)	76.0^{c}	79.3^{ab}	81.1^{a}	77.1^{bc}	78.3^{b}	1.03	0.027
Viscosity (cP)	5.00	4.90	4.66	5.08	5.06	0.23	0.724
Feces consistency[1]	1	1	1	1	1		--

Means in the same row with different superscripts are significantly different (P<0.05). [1]For feces consistency, '1' shows normal dry droppings and cone forming. cP, centipoise; CON, Control; T1, 3%SH+20mg/kg enzyme (β-Mannanase); T2, 3%SH+30mg/kg enzyme (β-Mannanase); T3, 9%SH+20mg/kg enzyme (β-Mannanase); T4, 9%SH+30mg/kg enzyme (β-Mannanase).

RESULTS

The results regarding the nutrients digestibility, digesta viscosity and fecal consistency are shown in Table II. Digestibility of DM, CF, and ash were recorded as non-significant (P>0.05) while the digestibility of CP in the T1 group and crude fat in T1 and T2 groups were calculated significantly higher (P<0.05) than in the remaining groups. Digesta viscosity was not effected (P>0.05) and feces consistency was observed normal (dry with cone forming) among all the groups. The results regarding intestinal histomorphology are indicated in Table III. Duodenum villus width (VW), villus height (VH), surface area (SA), and crypt depth (CD) were recorded higher (P<0.05) in the T2 group as compared to all other groups. The jejunum VW, VH, and

Table III. Effect of dietary inclusion of soybean hull and enzyme on the intestinal histomorphology.

Parameters		Diets						P-value
		CON	T1	T2	T3	T4	SEM	
Duodenum villus	Width (µm)	68.0^{d}	76.0^{b}	79.0^{a}	72.0^{c}	74.0^{bc}	2.65	0.048
	Height(µm)	609^{d}	625^{b}	632^{a}	615^{cd}	620^{c}	13.5	0.029
	Crypt depth (µm)	105^{c}	111^{b}	114^{a}	107^{c}	102^{d}	4.15	0.029
	Surface area (µm^2)	0.130^{e}	0.148^{b}	0.157^{a}	0.138^{d}	0.143^{c}	4.63	0.026
Jejunum villus	Width (µm)	60.0^{c}	62.0^{b}	64.0^{a}	60.0^{c}	62.0^{b}	2.26	0.031
	Height (µm)	430^{e}	437^{c}	451^{a}	433^{d}	444^{b}	6.84	0.038
	Crypt depth (µm)	55.0^{c}	59.0^{b}	63.0^{a}	58.0^{b}	63.0^{a}	2.45	0.045
	Surface area (µm^2)	0.080^{c}	0.084^{b}	0.091^{a}	0.081^{c}	0.086^{b}	1.83	0.032
Ileum villus	Width (µm)	56.0^{ab}	56.0^{ab}	57.0^{a}	54.0^{c}	55.0^{bc}	2.15	0.042
	Height (µm)	384^{c}	387^{b}	391^{a}	379^{d}	382^{cd}	5.94	0.023
	Crypt depth (µm)	52.0^{ab}	53.0^{a}	53.0^{a}	51.0^{b}	52.0^{ab}	1.91	0.031
	Surface area (µm^2)	0.067^{ab}	0.067^{ab}	0.069^{a}	0.064^{c}	0.066^{bc}	2.44	0.019

Means in the same row with different superscripts are significantly different (P<0.05). CON, Control; T1, 3%SH+20mg/kg enzyme (β-Mannanase); T2, 3%SH+30mg/kg enzyme (β-Mannanase); T3, 9%SH+20mg/kg enzyme (β-Mannanase); T4, 9%SH+30mg/kg enzyme (β-Mannanase).

SA were calculated higher (P<0.05) in the T2 group while CD (P<0.05) was lower in the CON group as compared to all other groups. The ileum VW had a higher (P<0.05) value in the T2 group than in the T3 and T4 groups while VH in the T2 group than in the remaining all groups but SA and CD were calculated significantly lower in the T3 group than in the all other groups.

DISCUSSION

The effect of fiber degrading enzymes β-Mannanase and soybean hulls in different concentrations on the egg quality parameters in the golden brown (RIR × Fayoumi) laying hens was determined at the early peak egg production period. The digestibility of DM, CF, CP and crude fat were calculated higher in the soybean hull and enzyme treatment groups. To support the results of the present study, Esonu *et al*. (2005) described higher DM digestibility for 30% soybean hull meal and 2% cellulitic enzyme (Safzyme) group than the control group and higher digestibility of ash in 10, 20, and 30% soybean hull meal with 2% cellulitic enzyme (Safzyme) groups respectively, than the control group. In pigs and poultry, the effect of enzyme supplementation on dry matter digestibility depends on the type of diet and the kind of animal used. In general, enzymes increase dry matter digestibility in poultry from 0.9 to 17% (Schutte *et al*., 1995). When enzymes are introduced to poultry diets containing grains, they improve performance and nutritional digestibility (Marquardt *et al*., 1994). Rice hulls (RH), oat hulls (OH), sunflower hulls, and soybean hulls (SH) are insoluble fiber sources that have been found to improve nutrient utilization and live performance in broilers (Gonzalez-Alvarado *et al*., 2010). The deleterious effects of NSPs can be mitigated through dietary changes such as supplementing of diets with appropriate exogenous enzyme preparations (Creswell,1994). The increased digestibility of crude fiber observed with enzyme supplementation is due to the breakdown of NSP in the soybean hull by the enzyme. Exogenous enzymes in feed additives can complement endogenous enzymes in the digestive tract during harsh climatic conditions and at a young age, to play a greater role in digestion which is similar to the results described in the present study and the better nutrient digestibility in the soybean hull and enzyme treatment groups is due to the beneficial effect of the enzyme (β-Mannanase) on the gastrointestinal tract and its ability to break down the cell wall of the soybean hull into easily digestible components. Digesta viscosity was not affected among all the groups, although comparatively low viscosity was recorded in the T2 group (3%SH+30mg/kg enzyme) while the consistency of feces was normal (dry and cone farming) in all the groups. In line with the results of the present study, one-year-old cockerels fed with 60 percent barley diets experienced increased (P<0.05) intestinal viscosity which was subsequently decreased (P<0.05) with enzymes (Almirall *et al*., 1995). Soybean hulls contain both soluble and insoluble NSPs. The water-soluble portion of NSPs is notorious for forming a gel-like viscosity in the intestinal tract (Gobl and Gohl, 1977). Supplementation of enzymes breaks down the cell walls

of the particle feed, so it will be easier to digest by enzyme digestion and capable of lowering the viscosity in the feed (Abu, 2019). Supplementation of the diets with appropriate enzymes minimizes intestinal viscosity and enzymes work by partially degrading the soluble NSP arabinoxylans and mixing link beta-glucans into smaller fragments (Sliva *et al*., 1983). This, in turn, lessens the overlapping and the formation of mesh-like hydrogels. Water holding capacity is decreased with fragmentation, which leads to a reduction in digesta moisture (Almirall *et al*., 1995) and wet feces (Gohl *et al*., 1978) from hens fed viscosity inducing ingredients (Graham, 1994). Several trials find that the greatest benefit of multi-enzyme supplementation occurs during the latter half of the bird's life. Multi-enzyme supplementation is the most economical and effective means to reduce intestinal viscosity. Duodenum, jejunum, and ileum villus width, height, crypt depth, and surface area were recorded significantly (P<0.05) higher in the T2 group (3%SH+30mg/kg enzyme). Similarly, Tejeda and Kim (2020) recorded an increase in the jejunum crypt depth in the 4% soybean hull group than the control group in the broiler. In the current study, there is a dropped in the ileal and jejunal villus height with the increasing level of soybean hull in the feed in all phases which is similar to the findings of Tejeda and Kim (2020) and a clear dropped in the ileal and jejunal villus height was occurred in 6 and 8% SH groups as compared to the 4% SH (crude fiber) which shows that 4% SH is sufficient for stimulating the broiler intestinal villus growth. Dietary inclusion of a moderate amount of different insoluble fiber sources has been shown to improve growth performance in broiler chickens by increasing the development of gastrointestinal organs and gut health (Mateos *et al*., 2012). Increasing villus height implies not just a larger surface area capable of absorbing more accessible nutrients, but also enhanced gut health (Baurhoo *et al*., 2007). The crypt, on the other hand, can be thought of as the villus factory, with a big crypt indicating rapid tissue turnover and a high energy need for new tissue (Awad *et al*., 2009). Rezaei *et al*. (2018) stated that having more dietary fiber in the gastrointestinal system increases organ size (i.e., gizzard, intestines) to compensate for the higher volume of feed going through the intestines (i.e., bulky diets) which is similar to the more feed intake in the T2 group. The increase in the size of the duodenum, jejunum, and ileum villus width, height, crypt depth, and surface area in the T2 group is due to the beneficial effect of the moderate level of soybean hull and enzyme on these organs and to provide more and more surface area for the better absorption of all available digestible nutrients.

CONCLUSIONS AND RECOMMENDATIONS

The findings of the present study revealed the overall better effect of the soybean hulls (3% SH) along with β-Mannanase (20 and 30mg/kg feed) on the nutrient digestibility, digesta viscosity, feces consistency, and intestinal histomorphology and therefore recommended for laying hens at the early peak production period without a negative effect on the above mentioned parameters.

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IRB approval

This study was approved by the animal welfare and care committee of the Faculty of Animal Husbandry and Veterinary Sciences, The University of Agriculture, Peshawar, Pakistan, and all the measures and tools was considered to minimize the pain and discomfort of birds during the conduction of this experiment.

Ethical statement

The study was approved by the ethical committee of the Faculty of Animal Husbandry and Veterinary Sciences (FAHVS), The University of Agriculture Peshawar, before the conduction of this experiment.

Statement of conflict of interest

The authors have declared no conflict of interest.

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