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Effect of Fiber Degrading Enzymes Added in Soybean Hulls on the Egg Quality Parameters During Early Peak Production Period in Laying Hens

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MS designed animal trial, laboratory experiment, perfomed statistical analysis, and wroting the manuscript. AH designed study, formulated feed and evaluated data. MSU, AS, HU and AH reviewed the manuscript. MSU, AS and HU analysed the data.

Key words
Soybean hull, β-mannanase, Laying hens, Egg, Haugh unit

ABSTRACT

This study was designed to determine the combined effect of fiber degrading enzymes (β-Mannanase) and soybean hulls (SH) 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, 28 weeks old, 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. Results indicated overall significantly lower egg shell thickness in the control group while all other overall internal and external egg quality parameters were not affected (P>0.05). It is concluded that the replacement of soybean meal in the diet of laying hens by 3 and 9%SH in combination with enzyme (β-Mannanase) at the level of 20 and 30mg/kg feed has a positive effect on the egg quality parameters during early peak egg production period in the laying hens.

INTRODUCTION

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). Exogenous enzyme studies have become increasingly popular, owing to their unique properties. 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, non-starch polysaccharides (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 digesta reduces with use, potentiating the activity of endogenous enzymes on specific substrates (Ribeiro *et al*., 2011). In general, fibrous feedstuff can be introduced to the diet at a rate of 3 to 5% without affecting nutrient digestibility or growth performance in many poultry species (Jimenez-Moreno *et al*., 2009). Soybean hulls (SHs) are the by product of soybean seed when used for the extraction of oil and their chemical composition may vary due to the efficiency of the de-hulling process (Rojas *et al*., 2014), hence, the SHs 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). SHs, therefore are, mainly lignocellulose physical entities and poultry birds cannot produce enzymes for the breakdown of NSPs existing in the cell wall of the grains and are kept un-hydrolyzed, resulting in low feed efficacy. Studies in recent years have recommended that

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the unwanted influences of NSPs can be overcome by nutritive modifications which include supplementation of appropriate synthetic enzymes (cellulase and hemicellulase) provisions in the diet of birds. These enzymes break down the NSPs and reduce intestinal adhesiveness and eventually improve the digestibility of nutrients by improving gut health and performance (Creswell, 1994). Due to high fiber concentration SHs are not commonly a part of poultry regimes, however, positive inclusion of soybean hulls has been reported in poultry rations. Feeding SHs up to 20%, laying hens produced eggs at a lower cost, which was further raised to 30% with the supplementation of cellulolytic enzymes without negatively affecting the performance of the birds (Esonu *et al*., 2010). Improved ($P \leq 0.022$) metabolizable energy (MEn) and N digestibility of prime hulls at 7.5% and California-type hulls at 7.5 and 15% levels in Hy-Line W36 hens were determined by (Wang *et al*., 2021). 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 SHs 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 SHs in layer feed has not been studied in detail, especially at peak production periods in laying hens. The current research was therefore planned with the objectives to investigate the effect of fiber degrading enzymes added in SHs on the egg quality parameters during the early peak production period in laying hens.

MATERIALS AND METHODS

Housing and experimental environment

The study was conducted 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 distributed into five groups of 40 birds each. Every group was further subdivided into four experimental replicates of 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. Uniform environmental and managementmental conditions were provided to all the birds in the experimental house. The room temperature was kept at 75°F and was equipped with sufficient light (17 h/day). The flock was provided with a routine vaccination schedule. The composition and proximate analysis of experimental diets is shown in Table I.

Table I. Feed composition and proximate analysis of experimental diets.

Nutrient %	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
Proximate analysis of feed					
Dry matter	89.4	90.5	90.6	91.0	91.1
Crude protein	17.7	17.5	17.5	17.0	17.0
Crude fiber	2.85	2.87	2.87	2.90	2.90
Crude fat	4.82	4.76	4.77	4.72	4.72
Ash	13.5	13.7	13.7	13.3	13.4
Moisture	10.5	9.48	9.38	9.00	8.84
Nitrogen free extract	51.6	49.0	49.0	45.8	45.8
ME (kcal/kg)	2750	2744	2746	2736	2739

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).

Egg quality traits

From each replicate, four eggs weekly were randomly

collected to determine the egg quality parameters as described by Shuaib *et al*. (2022). Digital balance was used to record the weight of the eggs. A screw gauge without zero error was used for the calculation of shell thickness (without shell membranes) at different places and averaged. Digital balance was used to determine the egg shall weight. First, the albumin and yolk were removed, and then in the incubator, the shell of the egg was dried off for a night. For egg yolk weight determination, the egg was broken carefully and the yolk was poured into the petri dish with the help of squeezing an empty plastic bottle which create negative suction force, and then the egg yolk was weighed. The height of albumin was recorded by using a transparent plastic rod and compared with the ruler. By digital balance, egg albumin was weighted. The weight was also recorded by the following formula. Egg albumin weight = total weight of egg – (weight of egg shall + weight of egg yolk). Haugh unit shows the height of egg white and it was recorded using (Silversides *et al*., 1993) method. H.U= 100 × Log (height of albumin -7.57 -1.7 x Egg weight $^{0.37}$).

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 percent level of significance.

RESULTS

The results of the use of soybean hulls and enzyme (β-Mannanase) on the egg external quality parameters are shown in Table II**.** The overall and weekly egg weight and shell thickness remained non-significant (P>0.05) among all the groups. The shell weight remained non-significant (P>0.05) in all weeks except for week 31 where shell weight was significantly higher (P<0.05) in the T2 group as compared to the CON group. The overall had a significantly higher (P<0.05) shell weight in the T2 group as compared to the CON group. The results regarding the internal quality parameters e.g., yolk weight, albumen weight, albumen height, and Haugh unit (HU) are described in Table III. Egg yolk weight was not affected (P>0.05) in weeks 29 and 31 but at week 30 was calculated significantly higher (P<0.05) in the T3 group as compared to the T4 group, and similarly at week 32 in the T2 group than in the T4 group. Egg albumen weight remained unaffected (P>0.05) in weeks 29 and 31 but at week 30 was significantly higher (P<0.05) in the T4 group as compared to the CON group, but at week 32 in the T4 group than in the T3 group. Overall yolk weight and albumen weight was not affected but overall as well as weekly albumen height and haugh unit remained non-significant (P>0.05) among all the groups.

Table II. Egg weight, Shell weight, Shell thickness (Mean±SD).

Parameters	Week	CON	T1	T2	T3	T4	P value
Egg weight (g)	29	52.0±0.91	53.5±0.64	54.0±0.91	52.5±0.64	53.2±0.85	0.444
	30	53.2±0.75	54.2±0.85	55.0±0.57	53.5±0.64	54.0±0.81	0.507
	31	53.0±0.91	55.0±0.70	55.0±0.40	53.5±0.64	54.7±0.75	0.188
	32	55.0±0.70	55.7±0.47	56.5±0.95	55.2±1.84	56.0±0.40	0.845
	Overall	53.3±0.70	54.6±0.16	55.1±0.29	53.6±0.64	54.5±0.25	0.088
Shell weight (g)	29	5.78±0.09	5.80±0.07	5.88±0.09	5.79±0.07	5.85±0.09	0.063
	30	5.82±0.30	5.96±0.09	6.05±0.06	5.88±0.07	5.94±0.08	0.584
	31	5.70±0.09^{b}	5.88±0.15^{a}	5.90±0.04^{a}	5.77±0.07^{a}	5.85±0.08^{a}	0.021
	32	5.50±0.07	6.07±0.05	6.13±0.28	5.68±0.20	5.66±0.25	0.151
	Overall	5.70±0.12^{b}	5.92±0.03^{a}	5.98±0.04^{a}	5.78±0.07^{ab}	5.82±0.09^{ab}	0.031
Shell thickness (mm)	29	0.33±0.03	0.32±0.04	0.30±0.02	0.32±0.01	0.33±0.03	0.355
	30	0.33±0.02	0.33±0.05	0.30±0.03	0.33±0.02	0.33±0.04	0.475
	31	0.34±0.03	0.33±0.03	0.31±0.02	0.33±0.01	0.34±0.02	0.392
	32	0.33±0.04	0.32±0.03	0.30±0.01	0.33±0.04	0.33±0.04	0.471
	Overall	0.34±0.03	0.33±0.05	0.30±0.02	0.33±0.01	0.33±0.03	0.469

Means in the same row with different superscripts are significantly different (P<0.05). For composition of diet, see Table II.

Table III. Egg yolk, albumin weight, albumen height and haugh unit (Mean±SD).

Parameters	Week	CON	T1	T2	T3	T4	P value
Yolk weight (g)	29	15.9±0.96	16.4±0.20	16.8±0.28	16.2±0.20	15.9±0.23	0.067
	30	16.2±0.21 ^{ab}	17.0±0.26^{a}	16.8±0.63 ^{a}	17.1±0.20 ^{a}	15.1±0.23 ^{b}	0.006
	31	15.9±0.27	16.6±0.41	16.7±0.30	16.1±0.20	16.4±0.39	0.073
	32	16.5±0.21 ^{ab}	17.5±0.15^{ab}	18.0±0.35^{a}	17.6±0.58 ^{ab}	16.3±0.37 ^{b}	0.011
	Overall	16.1±0.32	16.9±0.15	17.1±0.22	16.7±0.20	16.0±0.11	0.072
Albumen weight (g)	29	30.8±0.51	31.1±0.37	31.4±0.52	30.5±0.36	31.4±0.52	0.062
	30	30.7±0.42 ^{b}	31.1±0.49^{ab}	32.4±0.42^{ab}	30.4±0.36 ^{b}	32.9±0.49 ^{a}	0.006
	31	31.8±0.54	32.3±0.72	32.1±0.36	31.5±0.36	32.4±0.43	0.082
	32	33.0±0.42 ^{ab}	32.0±0.27^{ab}	32.7±0.55 ^{ab}	31.5±1.05 ^{b}	33.1±0.24 ^{a}	0.054
	Overall	31.6±0.38	31.6±0.08	32.2±0.19	31.0±0.36	32.7±0.09	0.061
Albumen height (mm)	29	6.32±0.13	6.60±0.29	6.75±0.18	6.42±0.16	6.47±0.22	0.664
	30	6.02±0.17	6.30±0.24	6.45±0.19	6.12±0.15	6.17±0.28	0.660
	31	6.40±0.19	6.67±0.22	6.75±0.24	6.55±0.21	6.62±0.23	0.882
	32	6.50±0.19	6.77±0.29	6.85±0.26	6.65±0.20	6.72±0.29	0.868
	Overall	6.31±0.15	6.58±0.19	6.70±0.17	6.43±0.18	6.50±0.25	0.689
Haugh unit	29	76.6±1.44	78.5±2.07	79.1±1.73	77.7±1.54	78.2±2.04	0.883
	30	77.4±1.41	79.2±2.03	79.8±1.70	78.4±1.51	78.9±2.00	0.887
	31	76.1±1.04	78±2.11	79.1±1.36	76.8±1.23	77.1±1.67	0.677
	32	73.8±1.09	75.8±2.22	77.0±1.43	74.5±1.29	74.9±1.76	0.677
	Overall	76.0±1.14	77.9±1.38	78.8±1.23	76.9±1.39	77.3±1.82	0.699

Means in the same row with different superscripts are significantly different (P<0.05). CON, Control. See Table I for composition of diet.

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. Egg weight, shell thickness, yolk and albumen weight, albumen height, and Haugh unit were not affected (P>0.05) although numerically higher egg weight was calculated in the T2 group (3%SH and 30mg/kg enzyme). Similar to the current study results, higher egg weight was recorded by Jia *et al*. (2008), Danang and Tintin (2016) when treatments were provided with enzyme supplementation between 0.1-0.5% in the diet. Simons and Versteegh (1991) also recorded positive effects on egg weight when an enzyme (phytase) was added to the layer diets. Similarly, Mathlouthi *et al*. (2003) described no effect on egg weight when the enzyme was provided in the feed. Similar to our results, Esonu *et al*. (2005) recorded a non-significant effect on the egg shell thickness in laying hens (brown) when using 10 and 20 and 30% soybean hull meal and 2% enzyme (Safzyme) in the feed. Sousa *et al*. (2019) also recorded no effect on the egg shell weight and thickness when used fiber sources (wheat bran and soybean hull) and enzyme (xylanase) in the laying hens. Similar to our result, Sousa *et al*. (2019) described no effect on egg yolk and albumen weight in laying hens when used different fiber sources (wheat bran, soybean hulls, and coffee husks) and enzyme (xylanase) 0.075 g/kg in the feed. Costa *et al*. (2015) also concluded that yolk weight was not affected with the inclusion of 1.93g/100g linoleic acid than with 1.48 g/100g 59.5 g vs. 59.0 g and 19.5% vs. 15.5%, respectively. Similar to our study results, Costa *et al*. (2015) observed no differences between treatments for percentages of yolk, albumen, and shell. In support of our results, Costa *et al*. (2015), Jalal and Scheideler (2001) concluded that the use of the enzyme complex in the diet of medium-heavy laying hens' gives a reduction in nutritional density without compromising production performance or egg quality. Similarly, Cufadar *et al*. (2009) reported that supplementation of NSP enzymes and phytase to the basal diet (maize and soybean meal-based diets) did not affect the Haugh unit score. Similar to our results, Sousa *et al*. (2019) recorded no effect on haugh unit in the laying hens when used different fiber sources (wheat bran, soybean hulls) and enzyme xylanase

(0.075 g/kg) in the feed. Esonu *et al*. (2005) documented a non-significant effect on the haugh unit of laying hens (brown) when used 10 and 20 and 30% soybean hull meal and 2% enzyme (Safzyme) in the feed. According to Leeson and Summers (2005), protein, amino acid, and fat contents are the most important nutritional factors that affect egg weight and, consequently, the proportion of its components, especially in younger birds. As in this study, the laying hens received diets formulated to contain almost similar nutrient contents and are matured (older) birds, probably the nutrients ingested by the birds were sufficient for the egg components to remain stable. The relatively higher egg weight in the T2 group is due to the better feed intake in this group which provides the essential nutrients in adequate amounts for the egg with higher weight while the no effect on the egg shell weight and thickness is due to the no significant effect of the soybean hull and enzyme (β-Mannanase) on the egg weight and also these portions of the egg require a small amount of protein.

CONCLUSIONS AND RECOMMENDATIONS

The findings of the present study showed the overall better effect of the soybean hulls along with β-Mannanase on the egg's external and internal quality parameters. Therefore, 3 and 9% SH along with β-Mannanase at 20 and 30 mg/kg in feed is recommended for laying hens at the early peak production period without negatively effect on the egg quality.

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Animal welfare statement

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.

IRB approval

The study was approved by Advanced Studies and Research Board (ASRB), (No.1145/ASRB/UAP, dated 22/07/2020).

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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