Effects of dietary protein, energy and β-mannanase on laying performance, egg quality, and ileal amino acid digestibility in laying hens

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ABSTRACT β-mannan is a nonstarch polysaccharide found in hulled and dehulled soybeans that can survive drying-toasting phase of processing soybeans and have antinutritive effects in poultry. β-mannanase is an active enzyme (endohydrolase) that can hydrolyze β-mannan to reduce its antinutritional effects. Two experiments were conducted to evaluate the effects of β-mannanase supplementation in low energy/protein diets on egg production, egg quality, and apparent ileal digestibility of the dry matter (**DM**), crude protein (**CP**), and amino acids in 21-week-old Single Comb White Leghorn hens (Hy-Line W-36). A total of 192 hens (8 replicates of 6 hens per treatment) for a production study (Exp. 1) and a total of 64 hens (8 replicates of 2 hens per treatment) for a digestibility study (Exp. 2) were randomly allocated to 4 experimental treatments in a 2 × 2 factorial arrangement. Four dietary treatments were control (**CS**) based on corn and 44% CP soybean meal (ME: 2,850 kcal/kg CP: 18.5%) and CS-low energy/protein (**CSL**) (ME: 2,750 kcal/kg CP: 17.5%), with or without 0.05% β-mannanase enzyme. Hens were fed the experimental diets for 14 d for the digestibility study and 8 wk for the production study. Hen-day egg production (**HDEP**), weekly feed intake, FCR, and biweekly egg quality parameters were measured. Significant interaction on feed intake ($P < 0.01$) was observed between energy/protein and enzyme. At 3, 6 and 8 wk, the feed intake and FCR of CSL with enzyme were significantly lower than those of CSL without enzymes. The main effects indicated that birds fed diets without inclusion of β-mannanase had higher feed intake than those fed diets with enzymes at 4, 7, and 8 wk. The inclusion of β-mannanase significantly increased ($P < 0.05$) HDEP at 2, 3, 5, and 7 wk. However, there was no significant effect of nutrient density or enzyme supplementation on egg quality parameters. The digestibility study showed that the inclusion of β-mannanase significantly improved ($P < 0.01$) apparent ileal digestibility of lysine, histidine and tryptophan in the diet. The results of these experiments indicate that supplementation of β-mannanase could reduce the feed intake and FCR and improve HDEP and apparent ileal digestibility of key amino acids in corn/soy diets fed to laying hens.

Key words: laying hen, β-mannanase, feed intake, egg production, amino acid digestibility

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INTRODUCTION

Nonstarch polysaccharides (**NSP**) present in the plant-derived feed ingredients are complex high molecular weight carbohydrates which act as antinutrition factors and reduce feed nutrient digestion and performance of poultry (Choct, 2002; Hsiao et al., 2004; Meng et al., 2005). β-mannan is one of the NSPs found in dehulled and hulled soybeans that can survive drying-toasting phase of processing soybeans (Dale, 1997; Hsiao et al., 2006). Soybean meal (**SBM**) is a primary source of vegetable protein that contains 3% of soluble NSP and 16% of insoluble NSP (Irish and Balnave, 1993), which consist of mainly mannans and galactomannans (Slominski, 2011). B-mannans present in nondehulled SBM are between 1.33 and 2.14% and in SBM dehulled between 1.02 and 1.51% (Hsiao et al., 2006).

β-galactomannan or β-mannan is a polysaccharide with reiterating units of mannose. Both galactose and glucose are commonly found attached to the β-mannan backbone (Carpita and McCann, 2000; Hsiao et al., 2006). The solubility of β-mannan in water increases as the number galactose molecules on the mannan backbone increases. β-mannan is commonly found in a wide

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variety of feedstuffs, including SBM, palm kernel meal, copra meal, guar gum, and sesame meal (Rogel and Vohra, 1983; Dierick, 1989; Hsiao et al., 2006). It has been reported that β-mannan significantly reduced growth and increased feed:gain ratio in broilers (Ray et al., 1982; Daskiran et al., 2004; Arsenault et al., 2017). Moreover, β-mannan in hen diets decreased feed intake, egg weight, and egg production (Patel and McGinnis, 1985).

Addition of feed enzymes has been widely documented as a cost-effective method to reduce antinutritional factors such as NSP in order to achieve more production benefit in broilers (Choct, 2001; Hsiao et al., 2004; Arsenault et al., 2017). β-mannanase is an enzyme that can hydrolyze β-mannan to reduce its antinutritional factor. Inclusion of β-mannanase in broiler diets has resulted in an increase in apparent metabolizable energy and body weight gain along with an improved feed conversion ratio (Daskiran et al., 2004; Jackson et al., 2004; Lee et al., 2005; Zangiabadi and Torki, 2010; Liu et al., 2017). Data from a laying hen study with second cycle laying hens indicate that use of β-mannanase significantly increased average egg production and egg mass of hens fed low −energy corn-soy diet (Wu et al., 2005). In addition, it has been reported that use of β-mannanase can help reduce negative effects of β-mannan in poultry diets, optimizing production in broilers and laying hens. However, few studies have been conducted to investigate the effect of β-mannanase on performance of laying hen and digestibility of energy and amino acids when laying hens are fed corn and SBM diets containing varying level of energy and protein during a peak production period. Thus, the objectives of this study were: 1) to evaluate the effects of β-mannanase on feed intake, egg production and egg quality in laying hens fed the diets with different levels of energy/protein during a peak production period, and 2) to evaluate the effects of β-mannanase on apparent ileal digestibility of dry matter, crude protein, and amino acids in laying hens fed the diets with different levels of energy/protein during a peak production period.

MATERIALS AND METHODS

All protocols relating to the care and use of live animals were approved by Institutional Animal Care and Use Committee of the University of Georgia.

Housing, Birds, and Treatments

The study was conducted at the Poultry Research Center of the Department of Poultry Science at the University of Georgia. Two experiments were conducted to evaluate the effects of β-mannanase supplementation in low energy/protein diets on egg production, egg quality, and apparent ileal digestibility of the dry matter (**DM**), crude protein (**CP**), and amino acids in 21-week-old Single Comb White Leghorn hens (Hy-Line W-36). For experiment 1 (Exp. 1), a total of 192 hens (8 replicates of 6 hens per treatment) were used for an 8-wk production study, and experiment 2 (Exp. 2) had a total of 64 hens (8 replicates of 2 hens per treatment) for a 14-d digestibility study. Four dietary treatments were control (**CS**) based on corn and 44% CP soybean meal and CS-low energy/protein (**CSL**) with (**CS+Enz** or **CSL+Enz**) or without 0.05% β-mannanase enzyme (CTCZYME: CTCBIO Inc., Seoul, Republic of Korea)

Experimental Diets, Sampling, and Analysis

Diets were formulated to meet or above the recommendations established for Hy-Line hens (Hy-Line, 2016). Diets were formulated to provide 18.5% crude protein; 2,850 kcal/kg metabolizable energy (**ME**) for CS and CS+Enz and 17.5% crude protein; 2,750 kcal/kg ME for CSL and CSL+Enz (**Table 1**). For Exp. 1, hens were individually housed in metabolic cages located in an environment-controlled layer facility. The lighting schedule was followed by the Hy-line North America lighting program (Hy-Line, 2016). Feed and water were provided to permit ad libitum consumption. Egg production was recorded daily, feed intake, FCR/dozen of eggs were measured weekly, and egg quality parameters (egg weight, Haugh Unit, specific gravity, shell weight, and eggshell thickness) were measured biweekly.

In Exp. 2, hens were kept in a control diet for 14 d to adapt to the cages. Chromium oxide (Cr_2O_3) was used as an indigestible marker at a level of 3g/kg. Hens were given the treatment diets for 14 d. On d 14, the hens were euthanized by cervical dislocation, and the distal part of ileum beginning at Meckel's diverticulum up to 2 cm anterior to the ileocecocolonic junction was immediately removed. The intestinal content was emptied in a container. Contents were pooled within the 2 hens of one cage, immediately frozen at -20C, freeze-dried, and ground using coffee grinders (Kitchen Aid 3-oz, 200-Watt Blender; Benton Harbor, MI). The analyses performed on diets and ileal digesta samples were in duplicates.

Apparent Ileal Nutrient Digestibility

The apparent ileal nutrient digestibility was calculated according to the following equation (Al-Masri, 1995).

$$C = [1 - (Cri/Cro) \times (No/Ni)] \times 100$$

Where Cri = the concentration of chromium in the diet, Cro = the concentration of chromium in the ileal digesta or excreta, Ni = the concentration of the nutrient in the diet, and No = the concentration of the nutrient in the ileal digesta or excreta. The values were expressed as a percentage of feed basis.

Hen Performance and Egg Quality Measurement

Egg production and mortality were recorded daily. Feed intake and FCR/dozen of eggs were measured weekly, and egg quality parameters (egg weight, Haugh

Table 1. Feed formulation and nutrient composition.

Ingredients	CS	CS ± Enz	CSL	CSL Enz
Corn	52.17%	52.17%	58.00%	58.00%
Soybean meal w/ hulls (44%)	30.37%	30.37%	27.00%	27.00%
Soybean oil	4.50%	4.50%	2.08%	2.08%
Limestone	7.56%	7.56%	7.56%	7.56%
Oyster shells	1.50%	1.50%	1.50%	1.50%
Defluor. Phos.	2.24%	2.24%	2.25%	2.25%
Lysine (79%)	0.020%	0.020%	0.020%	0.020%
DL-Met	0.60%	0.60%	0.55%	0.55%
[1]**Mineral premix**	0.16%	0.16%	0.16%	0.16%
Filler (sand)	0.18%	0.13%	0.18%	0.13%
Salt	0.20%	0.20%	0.20%	0.20%
[2]**Vitamin premix**	0.50%	0.50%	0.50%	0.50%
[3]**β-mannanase**	0.00%	0.05%	0.00%	0.05%
Total	100.00%	100.00%	100.00%	100.00%
ME (kcal/kg)	2,850	2,850	2,750	2,750
Dry matter%	79.23	79.23	78.94	78.94
CP%	18.49	18.49	17.49	17.49
Ca%	4.258	4.258	4.2521	4.2521
Avail-P%	0.50	0.50	0.5	0.5
Linoleic acid %	2.025	2.025	1.682	1.682
Na%	0.20	0.20	0.20	0.20
Dig. Lys%	1.10	1.10	1.03	1.03
Dig. Thr%	0.67	0.67	0.63	0.63
TSAA%	1.16	1.16	1.09	1.09
Dig. Met%	0.87	0.87	0.81	0.81
β-Mannan (g/kg)	4.86	4.86	4.32	4.32
β-Mannanse activity (U/kg)	0	400	0	400

[1]Provided per kg of Mineral premix: Ca 0.72 g, Mn 3.04 g, Zn 2.43 g, Mg 0.61 g, Fe 0.59 g, Cu 22.68 g, I 22.68 g, Se 9.07 g.
[2]Provided per kg of DSM Vitamin premix: Vit. A 2,204,586 IU, Vit. D_3 200,000 ICU, Vit. E 2,000 IU, Vit. B12 2 mg, Biotin 20 mg, Menadione 200 mg, Thiamine 400 mg, Riboflavin 800 mg, d-Pantothenic Acid 2,000 mg, Vit. B6 400 mg, Niacin 8,000 mg, Folic Acid 100 mg, Choline 34,720 mg.
[3]B-mannanase (CTCZYME: CTCBio, Inc., Seoul, Republic of Korea).

Unit, specific gravity, shell weight, eggshell thickness) were measured biweekly at 2, 4, 6, and 8 wk. The specific gravity of eggs was determined using salt solutions of different concentrations, as previously described (Bennett, 1993). Albumen height was measured with a tripod meter (AMES Co., Waltman, MA) above albumin while avoiding chalaza and yolk. For eggshell thickness, shells were washed to remove albumin and dried at room temperature overnight. Three pieces from different parts of the eggshell was measured (mm), and the average of 3 measurements was taken to compute the eggshell thickness value.

Statistical Analysis

Data on apparent ileal nutrient digestibility, hen performance, and egg quality parameters measurement were subjected to analysis using 2-way ANOVA following the general linear model procedure of SAS (SAS Institute Inc., Cary, NC). Significant differences between treatments were determined using Tukey's honestly significant difference test. Data were considered significantly different if $P \leq 0.05$.

RESULTS AND DISCUSSION

Laying Hen Performance and Egg Quality

For Exp. 1, there were significant interactions in feed intake at wk 3, 6, and 8 and cumulative feed intake for 5 to 8wk in the current study (**Table 2**). The CSL treatment with the enzyme β-mannanase (CSL+Enz) had significantly lower feed intake when compared to CSL without the enzyme β-mannanase. CSL treatment had significantly higher feed intake compared to CS in order to meet body energy need; however, with the enzyme the energy utilization was possibly met with the lower feed intake observed. The main effects also indicated that the β-mannanase supplementation significantly reduced feed intake at wk 4 and 8 and cumulatively for 0 to 4 and 5 to 8 wk of the study with accumulatively (1-mo and 2-mo of study period**;** **Table 2**), indicating that β-mannanase supplementation potentially improves energy utilization by breaking down β-mannans in the diets. It has been reported that β-mannanase supplementation improved metabolizable energy in broilers by intestinal viscosity caused by β-mannans in the diets (Latham et al., 2018). Furthermore, in the current study, there were significant interactions in feed conversion ratio at wk 3, 6, and 8 (**Table 5**). The CSL treatment with the enzyme β-mannanase (CSL+Enz) had significantly lower feed conversion ratio when compared to both CS (wk 3) and CSL (wk 3, 6, and 8) without the enzyme β-mannanase. Similar results were found by Wu and Hendriks (2004) and Ryu et al. (2017) where dietary supplementation with β-mannanase improved feed conversion of laying hens. Factors identifying the inhibition of nutrient utilization are a key for selecting the appropriate inclusion of enzymes to improve the nutritive value of feedstuffs and ultimately poultry

Table 2. Effect of B-mannanase on feed intake of laying hens measured weekly for 8 wk.

Energy	Enzyme	[2]WK 1	WK 2	WK 3	WK 4	WK 5	WK 6	WK 7	WK 8	0−4 WK	5−8 WK
CS[1]		92.84	76.52	91.48	99.19	95.10	101.68	105.48	86.06	90.01	97.08
CSL		93.79	76.12	93.21	97.97	97.70	102.60	105.44	89.90	90.27	98.91
SEM		1.407	0.951	0.990	1.221	1.232	0.925	1.188	1.037	0.892	0.951
***P* value**		0.638	0.772	0.226	0.486	0.147	0.488	0.981	0.014	0.834	0.185
	-	94.98	77.10	93.20	100.6^{a}	97.27	102.89	107.81^{a}	90.92^{a}	91.48^{a}	99.72^{a}
	+	91.66	75.54	91.49	96.52^{b}	95.54	101.38	103.11^{b}	85.04^{b}	88.80^{b}	96.27^{b}
SEM		1.407	0.951	0.990	1.221	1.232	0.925	1.188	1.037	0.892	0.951
***P* value**		0.106	0.255	0.232	0.024	0.331	0.259	0.009	0.003	0.043	0.016
CS	-	94.52	77.01	90.63^{b}	101.14	94.24	100.46^{ab}	106.48	85.74^{b}	90.82	96.73^{b}
	+	91.17	76.03	92.32^{ab}	97.24	95.96	102.90^{ab}	104.48	86.39^{b}	89.19	97.43^{b}
CSL	-	95.44	77.19	95.77^{a}	100.13	100.29	105.32^{a}	109.13	96.10^{a}	92.13	102.71^{a}
	+	92.14	75.05	90.66^{b}	95.81	95.12	99.87^{b}	101.75	83.70^{b}	88.41	95.11^{b}
SEM		1.989	1.345	1.400	1.726	1.742	1.308	1.680	1.467	1.261	1.345
***P* value**		0.991	0.668	0.022	0.904	0.058	0.005	0.121	0.0001	0.416	0.005

[a,b,c]Means with different superscripts within each row are significantly different ($P < 0.05$).
[1]CS, Standard Corn/SBM diet with 2,850 kcal/kg ME and 18.5% CP; CSL, CS with 2,750 kcal/kg ME and 17.5% CP.
[2]Unit in g/bird/d.

production. Beta-mannan or β-galactomannan is found in a variety of feed ingredients, including soybean meal, which is one of the primary ingredients used in poultry diets. Beta-mannan inhibits animal performance, compromising weight gain and FCR (Anderson and Warnick, 1964; Latham et al., 2018; Lee et al., 2018). This also holds true for glucose and water absorption (Rainbird et Al, 1984). The advantageous effect of enzymatic degradation of β-mannan with the addition of a β-mannanase has been well-documented in broilers fed diets containing soybean meal and corn (Jackson et al., 2004; Latham et al., 2018; Lee et al., 2018) but lacks in laying hens. This could be due to the fact that typically broiler diets containing higher levels of CP with addition of increased SBM for improving growth support compared to laying hens with less CP because of alternative needs to focus on egg production (Ryu et al. 2017 and Lee et al., 2018).

Included in (**Table 1**), were the levels of the β-mannan levels recovered from the CS (4.86 g/kg) and CSL (4.32 g/kg) control diets. The difference in the β-mannan levels from the control diets due to CS having higher SBM in diet, did have increased feed intake for wk8 for CSL. However, no differences for the control diets were observed for the other parameters such as feed conversion ratio, egg production and quality and ileal digestibility. Other common ingredients that can possibly be used for CP inclusion in poultry diets are palm kernel, copra meal and guar meal that could possibly have a greater effect on performance parameters in control diets because these ingredients have higher β-mannan levels. Rogiewicz et al. (2016) also found that the structure of β-mannans in SBM was different to that of β-mannans found in palm kernel, copra meal and guar meal, where the enzyme supplementation would be more effective. Although SBM has traceable levels of β-mannans, copra meal and guar meal have higher β-mannan levels (60−80 and 200−250 g/kg, respectively); the beneficial effects of β-mannanase supplementation would be higher in the diets containing copra meal or guar meal compared to those containing SBM (Saeed et al., 2019).

The main effects showed the inclusion of enzyme significantly increased egg production at wk 2, 3, 5, 7 and for 5 to 8 wk. (**Table 3**). There was no interaction in HDEP. The overall egg production at mo 1 and 2 was significantly higher in birds fed the diets supplemented with β-mannanase enzyme. This result is in agreement with a study where dietary supplementation with higher inclusion of β-mannanase at (0.8 g/kg diet) improved egg production in laying hens (Ryu et al., 2017). However, in the present study, there were no significant results observed for egg quality including egg weight, yolk weight, albumin weight, eggshell weight, and eggshell thickness, Haugh Unit, and egg specific gravity among the treatments (**Table 4**).

Laying Hen Apparent Ileal Digestibility of Dry Matter, Crude Protein, and Amino Acids

For Exp 2, no interaction effect on ileal digestibility of nitrogen, crude protein and amino acids was observed between energy and enzyme (**Tables 6 and 7**). However, the main effects of enzymes showed that the inclusion of enzyme significantly increased apparent ileal digestibility of essential amino acids, lysine, histidine, and tryptophan, in laying hens (**Table 6**). The main effects of inclusion of enzyme also had a significant increase in apparent ileal digestibility of a nonessential amino acid, serine, in laying hens (**Table 7**). In this current study with layers and in Caldas et al. (2018) with broilers, partial improvement of apparent ileal digestibility of essential amino acids were observed depending on different levels β-mannanase enzyme being added. Higher ileal amino acid digestibility is observed for broilers with inclusion of β-mannanase, possibly due to broiler diets containing higher levels of CP with more SBM in diet compared to layer diets (Sundu et al., 2006).

However, there was no effect of enzyme in other amino acids, nitrogen and CP. There was no significant effect

Table 3. Effect of B-mannanase on hen day production of laying hens measured weekly for eight weeks.

Energy	Enzyme	[2]WK 1	WK 2	WK 3	WK 4	WK 5	WK 6	WK 7	WK 8	0−4 WK	5−8 WK
CS[1]		71.28	86.01	88.84	91.82	91.37	91.67	93.16	93.96	84.49	92.54
CSL		71.58	86.46	89.58	89.59	88.99	90.33	91.37	93.13	84.30	90.95
SEM		2.703	2.634	2.955	2.612	2.329	2.297	2.245	1.794	2.231	1.918
***P* value**		0.938	0.906	0.860	0.551	0.476	0.683	0.578	0.745	0.953	0.564
	-	69.20	81.85[b]	84.08[b]	87.95	86.31[b]	87.80	88.84[b]	91.25	80.77[b]	88.55[b]
	+	73.66	90.63[a]	94.35[a]	93.45	94.05[a]	94.20	95.69[a]	95.83	88.02[a]	94.94[a]
SEM		2.703	2.634	2.955	2.612	2.329	2.297	2.245	1.794	2.231	1.918
***P* value**		0.253	0.026	0.021	0.147	0.026	0.059	0.040	0.082	0.029	0.026
CS	-	68.16	81.55	83.04	89.88	88.39	88.69	90.18	92.08	80.66	89.84
	+	74.41	90.48	94.64	93.75	94.35	94.65	96.13	95.83	88.32	95.24
CSL	-	70.24	82.14	85.12	86.01	84.23	86.91	87.50	90.42	80.88	87.26
	+	72.92	90.77	94.05	93.16	93.75	93.75	95.24	95.83	87.72	94.64
SEM		3.822	3.726	4.179	3.694	3.293	3.248	3.175	2.537	3.156	2.713
***P* value**		0.644	0.968	0.751	0.661	0.592	0.892	0.781	0.745	0.898	0.718

[a,b,c]Means with different superscripts within each row are significantly different ($P < 0.05$).
[1]CS, Standard Corn/SBM diet with 2,850 kcal/kg ME and 18.5% CP; CSL, CS with 2,750 kcal/kg ME and 17.5% CP.

Table 4. Effect of β-mannanase on egg quality parameters for wk 8.

Energy	Enzyme	[2]Egg wt.	[2]Yolk wt.	[2]Albumin wt.	[2]Egg shell wt.	[3]Egg shell thick.	Haugh unit	Specific gravity
CS[1]		56.79	14.05	37.36	5.29	36.14	102.6	1.091
CSL		57.44	14.32	37.70	5.32	36.34	103.4	1.092
SEM		0.37	0.144	0.341	0.039	0.184	0.738	0.0006
***P* value**		0.225	0.198	0.492	0.566	0.433	0.461	0.536
	-	57.52	14.30	37.84	5.29	36.50	102.5	1.092
	+	56.71	14.06	37.22	5.32	35.98	103.4	1.091
SEM		0.37	0.144	0.341	0.046	4.913	0.738	0.0006
***P* value**		0.138	0.239	0.211	0.633	0.055	0.404	0.440
CS	-	57.31	14.30	37.72	74.51	36.0838	102.3	1.091
	+	56.26	13.80	37.00	76.54	36.1888	102.9	1.091
CSL	-	57.73	14.31	37.96	72.91	36.9163	102.8	1.092
	+	57.16	14.32	37.44	77.63	35.77	104.0	1.091
SEM		0.53	0.204	0.482	1.92	0.2598	1.044	0.001
***P* value**		0.655	0.226	0.836	0.492	0.023	0.791	0.170

[a,b,c]Means with different superscripts within each row are significantly different ($P < 0.05$).
[1]CS, Standard Corn/SBM diet with 2,850 kcal/kg ME and 18.5% CP; CSL, CS with 2,750 kcal/kg ME and 17.5% CP.
[2]Unit in grams, Weight= wt.
[3]Unit in mm, Thickness= thick.

Table 5. Effect of B-mannanase on FCR[2] measured weekly for 8 wk.

Energy	Enzyme	WK 1	WK 2	WK 3	WK 4	WK 5	WK 6	WK 7	WK 8
CS[1]		1.986	1.442	1.689	1.631	1.631	1.728	1.726	1.413
CSL		1.891	1.283	1.436	1.596	1.485	1.568	1.631	1.341
SEM		0.0836	0.0431	0.0614	0.0541	0.0447	0.0468	0.0460	0.0244
***P* value**		0.995	0.2245	0.0985	0.3475	0.3014	0.0784	0.4987	0.0865
	-	1.781	1.237	1.484	1.426	1.426	1.523	1.521	1.208
	+	1.703	1.094	1.247	1.408	1.296	1.380	1.443	1.153
SEM		0.0851	0.0446	0.0629	0.0556	0.0462	0.0483	0.0475	0.0259
***P* value**		0.7125	0.0987	0.0742	0.1085	0.0934	0.0847	0.1478	0.1047
CS	-	1.866	1.322	1.569[a]	1.511	1.511	1.608[a]	1.606	1.293[a]
	+	1.781	1.173	1.326[b]	1.486	1.375	1.458[ab]	1.521	1.231[ab]
CSL	-	1.817	1.274	1.502[a]	1.576	1.575	1.616[a]	1.630	1.285[a]
	+	1.782	1.131	1.141[c]	1.361	1.475	1.290[b]	1.515	1.142[b]
SEM		0.0941	0.0536	0.0719	0.0646	0.0552	0.0573	0.0565	0.0349
***P* value**		0.9139	0.1219	0.0024	0.2446	0.1277	0.004	0.4739	0.0265

[a,b,c]Means with different superscripts within each row are significantly different ($P < 0.05$).
[1]CS, Standard Corn/SBM diet with 2,850 kcal/kg ME and 18.5% CP; CSL, CS with 2,750 kcal/kg ME and 17.5% CP.
[2]FCR, Feed conversion ratio/dozen of eggs.

on energy density in digestibility of amino acids, Nitrogen, and CP.

Exogenous NSP enzymes may not always show positive effects on growth performance and nutrient utilization because their beneficial effects depend on the type and amount of NSP contained in diets (Adeola and Cowieson, 2011). Thus, the effect of β-mannanase supplementation can be amplified when poultry fed feed ingredients containing high levels of mannans, such as soybean, guar, palm kernel, and olive pulps. In the present study, we conducted 2 experiments to evaluate the effects of β-mannanase supplementation in low energy/

Table 6. Effect of β-mannanase on apparent ileal digestibility % of essential amino acids measured at the end of study in laying hens.

Energy	Enzyme	Val[2]	Met	Ile	Leu	Thr	Phe	Lys	His	Arg	Trp
CS[1]		69.12	90.10	72.90	75.53	61.67	75.67	76.50	77.18	81.63	75.39
CSL		67.36	90.69	71.13	75.27	60.14	74.19	75.85	76.31	80.23	76.37
SEM		1.76	0.68	1.58	1.36	1.95	1.39	1.38	1.08	1.14	1.15
***P* value**		0.485	0.543	0.435	0.895	0.585	0.457	0.741	0.572	0.392	0.552
	-	66.43	90.50	70.20	73.71	58.29	73.44	73.67[b]	75.09[b]	79.84	73.66[b]
	+	70.05	90.30	73.84	77.09	63.52	76.42	78.69[a]	78.40[a]	82.02	78.11[a]
SEM		1.76	0.68	1.58	1.36	1.95	1.39	1.38	1.08	1.14	1.15
***P* value**		0.156	0.834	0.114	0.090	0.069	0.140	0.016	0.040	0.186	0.011
CS	-	68.31	90.99	72.24	74.51	60.64	75.06	74.81	76.41	81.07	73.87
	+	69.93	89.22	73.56	76.54	62.71	76.28	78.20	77.96	82.19	76.92
CSL	-	64.55	90.01	68.16	72.91	55.95	71.82	72.53	73.77	78.62	73.45
	+	70.18	91.37	74.11	77.63	64.34	76.56	79.18	78.84	81.85	79.30
SEM		2.48	0.96	2.23	1.92	2.76	1.96	1.95	1.53	1.61	1.63
***P* value**		0.425	0.115	0.308	0.492	0.263	0.377	0.41	0.261	0.514	0.398

[a,b,c]Means with different superscripts within each row are significantly different ($P < 0.05$).
[1]CS, Standard Corn/SBM diet with 2,850 kcal/kg ME and 18.5% CP; CSL, CS with 2,750 kcal/kg ME and 17.5% CP.
[2]Val, valine; Met, methionine; Ile, isoleucine; Leu, leucine; Thr, threonine; Phe, phenylalanine; Lys, lysine; His, histidine; Arg, arginine; Trp, tryptophan.

Table 7. Effect of β-mannanase on apparent ileal digestibility % of nonessential amino acids measured at the end of study in laying hens.

Energy	Enzyme	DM[2]	CP	Asp	Ser	Glu	Pro	Gly	Ala	Tyr	Cys
CS[1]		60.56	66.77	71.35	66.87	79.64	74.88	64.44	72.53	74.57	59.53
CSL		62.45	67.39	70.52	67.37	79.71	74.11	62.91	72.68	73.08	56.71
SEM		1.52	1.66	1.42	1.75	1.12	1.35	1.81	1.50	1.44	2.99
***P* value**		0.124	0.794	0.682	0.841	0.964	0.689	0.552	0.946	0.470	0.511
	-	65.68	65.32	69.17	64.08[b]	78.11	73.13	61.83	70.59	72.99	57.98
	+	65.41	68.83	72.70	70.17[a]	81.24	75.85	65.52	74.62	74.65	58.26
SEM		1.96	1.66	1.42	1.75	1.12	1.35	1.81	1.50	1.44	2.99
***P* value**		0.486	0.147	0.090	0.021	0.059	0.164	0.159	0.068	0.423	0.947
CS	-	59.77	65.34	70.71	65.88	78.95	74.11	64.00	71.27	73.91	58.96
	+	63.76	68.20	71.98	67.87	80.33	75.65	64.89	73.79	75.23	60.09
CSL	-	64.28	65.31	67.63	62.28	77.27	72.16	59.66	69.90	72.07	56.99
	+	66.61	69.46	73.41	72.47	82.15	76.06	66.15	75.46	74.08	56.43
SEM		1.712	2.35	2.00	2.48	1.59	1.90	2.55	2.12	2.04	4.23
***P* value**		0.066	0.786	0.270	0.109	0.282	0.540	0.283	0.479	0.867	0.844

[a,b,c]Means with different superscripts within each row are significantly different ($P < 0.05$).
[1]CS, Standard Corn/SBM diet with 2,850 kcal/kg ME and 18.5% CP; CSL, CS with 2,750 kcal/kg ME and 17.5% CP.
[2]DM, dry matter; CP, crude protein; Asp, aspartate; Ser, Serine; Glu, glutamate; Pro, proline; Gly, glycine; Ala, alanine; Tyr, tyrosine; Cys, cysteine.

protein diets on key production and nutrient utilization parameters, such as egg production, egg quality, and apparent ileal digestibility of the DM, CP, and amino acids in laying hens fed a corn and soybean meal diet. The improved performance in egg production of laying hens fed diets supplemented with β-mannanase was observed in the present study, which corresponds with a study where improved egg production was observed in laying hens fed corn-soybean or palm kernel meal-based diets with β-mannanase (Lee et al., 2013); in particular, palm kernel meal contains approximately 40% mannan which significantly reduces nutrient digestibility in poultry (Sundu et al., 2006). Other studies also reported that dietary supplementation with β-mannanase improved egg production and growth performance in layers and broilers fed soybean meal or olive pulps (Wu et al., 2005; Zangiabadi and Torki, 2010, 2011).

Excessive NSPs in poultry diets often lead to the growth performance reduction because they increase the growth of potential harmful intestinal microbiota and intestinal viscosity and reduce gut health, immunity, and nutrient digestion and absorption (Spring et al., 2000; Davis et al., 2004; Mourão et al., 2006; Baurhoo et al., 2007; Choct et al., 2010). It is noteworthy that the reduction of the intestinal viscosity is one of the main purposes of the use of beta-mannanase and it showed to be linked with the enhanced intestinal ecosystem as well as intestinal nutrient absorption (Adeola and Cowieson, 2011; Latham et al., 2018; Lee et al., 2018). A possible reason why the increased levels of NSP in poultry impair nutrient digestibility is that an increase in digesta viscosity by NSP prevents interaction between substrates and digestive enzymes and then reduces nutrient digestibility (Campbell and Bedford, 1992). It has been previously reported that supplementation of β-mannanase in high NSP diets can reduce the gut viscosity and improve nutrient utilization in broilers (Lee et al., 2005). Moreover, the present study and others confirmed that β-mannanase supplementation improves nutrient digestibility in laying hens as well (Wu and Hendriks, 2004; Ryu et al., 2017). Although viscosity was not measured in this current study, β-mannanase supplementation increased the amino acid digestibility by breaking down β-mannans and potentially reducing viscosity. The current study was in agreement with that of Kong et al. (2011) showing reduced intestinal viscosity and improved ileal digestibility of amino acids in broiler chickens fed soybean meal diets with β-mannanase. Lee et al. (2018) also observed that β-mannanase improved true metabolizable energy in broilers fed a soybean meal based diet. Similarly, Latham et al. (2018) also reported that β-mannanase supplementation improved growth performance and feed energy utilization and reduced intestinal viscosity in broilers fed a corn and soybean meal based diet.

In conclusion, this study indicates that the supplementation of β-mannanase enzyme in low energy/low protein diets improved egg production, feed conversion ratio, and apparent ileal digestibility of specific amino acids at peak production of laying hens. It is also warranted to evaluate long-term effects of β-mannanase supplementation in laying hens in the future.

DISCLOSURES

The authors declare no conflicts of interest.

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