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Effects of dietary guar meal with or without beta-mannanase on performance and egg quality traits in laying hens

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Abstract: In this study, the effects of beta-mannanase supplementation to laying hens' diets with different guar meal (GM) ratios on performance and egg quality traits were investigated. The experimental period lasted 126 days. Ninety-six Lohman Brown hens at the age of 56 weeks were kept in individual cages. They were divided into 8 treatment groups as follows: 0% guar meal (GM0) (control); 0% GM + 0.05% beta-mannanase (GM0E); 8% GM (GM8); 8% GM + 0.05% beta-mannanase (GM8E); 16% GM (GM16); 16% GM + 0.05% beta-mannanase (GM16E); 24% GM (GM24); and 24% GM + 0.05% beta-mannanase (GM24E). A photoperiod of 16.5 h light and 7.5 h dark was applied throughout the experiment. The present treatments had significant effects on changes in final live body weight, feed consumption, feed efficiency, egg production, egg weight, shell thickness, eggshell weight, shape index, yolk index, Haugh unit, and yolk pigmentation values ($P < 0.05$). However, albumen index, shell strength, and mortality were not affected significantly by any of the treatments ($P > 0.05$). According to our results, guar meal might be used maximally at 16% in laying hen diets, because of its negative effects on laying performance and egg quality traits.

Key words: Guar meal, laying hen, performance, egg quality, beta-mannanase

1. Introduction

Guar (*Cyamopsis tetragonoloba*) is a drought-tolerant annual leguminous crop. It has been cultivated for its galactomannan content in particular. It has been consumed by humans and used in the food industry [1,2]. Guar consists of 30%–33% shell, 27%–30% endosperm, and 43%–47% seed [3]. Guar meal (GM) is obtained as a byproduct of the gum production process. GM is rich in protein (33%–60%) [4,5], with high arginine (4.76%–6.01%), lysine (1.66%–1.99%), and methionine (0.47%–0.51%) contents [6,7]. GM contains metabolizable energy (ME) of between 10.9 and 11.3 MJ per kg dry matter [8,9]. Despite such high nutritional value, it is quite a cheap material. Therefore, GM has mostly been preferred for poultry diets. However, its antinutritional factors have limited the use of GM in poultry nutrition. GM contains antinutritional factors such as 5%–13% saponin [10], 0.058%–0.18% trypsin inhibitor, hemagglutinins, and hydrocyanic acid [11–13], and 0.151%–0.298% phytic acid [14]. However, the inhibitory effect of trypsin is lower than that of soybean meal (SM) [15]. It has been reported that GM contained 5.5–19.2 mg hydrocyanic acid (HCN) in 100 g dry matter [16]. The gum content of GM (13%–18%) is a very strong antinutritional factor for monogastric animals [15,17]. Guar gum (a galactomannan), inhibits trypsin and chymotrypsin activity in poultry [18–20], increasing viscosity in the small intestine, affecting digestion and consequently causing negative performance [21]. Saponins in GM reduce the intestinal motility of ruminants [22], inhibit gastric emptying [23], negatively influence mucosal enzyme activity in the lowest part of the intestine, reduce digestibility of the diet [24,25], and consequently reduce animal growth rates [26]. Saponins also reduce taste, inhibit the use and absorption of minerals, and reduce protein digestibility [27,28]. Saponins, together with tannin, cause a decrease in the feed intake of domestic fowl [29] as well. The tannin content of guar endosperm is 4.5% while raw guar seeds contain about 1.75% tannin [30]; the tannin content of different sections of guar seeds varies between 0.59% and 0.78% in dry matter [11]. Domestic fowl are adversely affected by high tannin levels [31]. Tannins in feeds inactivate digestive enzymes [32] by combining with carbohydrates [33], proteins, glycoproteins [34], and metal ions [35]. It has been reported that 0.5% of tannic acid suppressed growth in chicks, while 2% tannin depressed appetite, slowed growth, and worsened feed utilization [36]. About 0.94% dietary tannin did not affect egg production and feed efficiency in laying hens [37]. Antinutritional

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factors such as saponins and polyphenols in GM cause damage to the liver, kidneys, and small intestines of mice and rats [38,39]. In order to eliminate the antinutritional effects of guar meal and to increase its nutritional values, appropriate enzymes such as cellulase, hemicellulose, and beta-mannanase have been supplemented into the diets [15,21,40–43], or heat treatments have been applied [12,44].

It has been reported that dietary mannanase supplementation into GM-containing diets reduced intestinal viscosity in laying hens [45,46]; additionally, the hydrolyzed galactomannan improved growth [47] and feed efficiency [48]. Because of its antinutritional contents, GM can be used at 5%–10% in poultry diets [6,8] with attention to possible side effects [21]. According to the findings of Gutierrez et al. [13], a 5% dietary GM without enzyme supplementation did not affect egg yield, feed consumption, or eggshell quality of chickens but worsened FCR, egg weight, and total egg mass. A 10% dietary GM decreased egg production, FCR, and egg yolk color.

Higher dietary inclusion of GM into laying hen diets worsened feed efficiency and egg production [5]. Because of its antinutritional contents, it is necessary to know the optimum dietary inclusion rate of GM to prevent its adverse effects on the growth of poultry [13]. Therefore, in this study, the effects of beta-mannanase supplementation into poultry diets with different ratios of guar meal (GM) on performance and egg quality traits of laying hens were investigated, and optimum dietary inclusion of GM to replace soybean meal was determined.

2. Materials and methods

2.1. Bird housing

The experiment was conducted in the environmentally controlled Poultry Research Unit of Kahramanmaraş Sütçü İmam University, Kahramanmaraş, Turkey. This study was conducted according to the third article of the decision of the Local Ethics Committee on 24 April 2015. All procedures performed on hens in the current study were consistent with the ethical standards indicated in directive 2010/63/EU, and the experimental protocols were approved by the Animal Experimentation Ethics Committee of the University of Kahramanmaraş, Faculty of Agriculture (Protocol No: 2015/03-1).

Laying hens were housed in a cage system made of plastic, which consisted of compartments having dimensions of 29.5 × 44 × 36 cm. Experiments lasted for 126 days. The guar meal (Indian origin) used in this experiment was obtained from a commercial firm. The analysis results of GM are given in Table 1.

The byproduct of gum extraction from guar germ material is referred to as guar meal. The granular form of beta-mannanase enzyme (HC: Hemicell) was used in this study. Endo 1,4 beta-mannanase from *B. lentus* not

Table 1. Chemical analysis of experimental feed ingredients (as feed basis).

Parameter / Ingredients	Guar meal	Corn meal	Soybean meal	Soy oil
Analyzed nutrients				
Dry matter, %	91.73	88.60	91.20	99.94
Crude protein, %	45.07	7.50	45.10	-
Crude fat, %	4.70	3.10	0.50	99.90
Crude ash, %	5.40	1.20	5.80	-
Starch, %	3.00	64.1	4.00	-
Sugar, %	8.14	1.80	8.05	-
Crude cellulose, %	10.00	2.70	5.80	-
Condensed tannin, %	0.97	0.15	0.24	-
Calculated analysis				
* Metabolizable energy, MJ/kg	10.95	14.03	9.61	36.76
Calcium, % Available phosphorus, %	0.24 0.15	0.01 0.10	0.25 0.32	- -
Methionine, %	0.54	0.20	0.61	-
Lysine	1.97	0.20	2.67	-

* Metabolizable energy values of feed ingredients were calculated according to the formula given by Carpenter and Clegg (1956).

less than 160 million units kg^{-1}, where 1 million units is defined as the quantity of enzyme capable of producing 0.72 μg of mannose per min from a mannose-containing substrate at pH 7.0 and a temperature of 40 °C.

2.2. Experimental design

Experiments were conducted in a factorial experimental (4 × 2) design with 8 treatment groups. These were 0% GM (control = GM0), 0% GM + 0.05% beta-mannanase (GM0E), 8% GM (GM8), 8% GM + 0.05% beta-mannanase (GM8E), 16% GM (GM16), 16% GM + 0.05% beta-mannanase (GM16E), 24% GM (GM24), and 24% GM + 0.05% beta-mannanase (GM24E).

A total of 96 Lohmann brown laying hens of 56 weeks of age were equally divided into 8 treatment groups for 12 replicates of each treatment. A photoperiod of 16.5 h light and 7.5 h dark was applied throughout the experiments. Feed and water were offered to birds ad libitum. Each hen was considered a replicate.

2.3. Data collection and analysis

The body weights were determined at the beginning and end of the experiment, while feed intake, egg weight, and feed conversion ratios were recorded on a weekly basis. The hen–day egg production was recorded as % daily. The feed conversion ratio (FCR) was calculated by dividing g feed consumption by the produced g egg. Egg internal–external quality measurements were made once. Internal and external egg quality traits were determined by using 12 egg samples for each group. After removing the shell membranes by hand, the remainder was weighed on a precision scale and the shell weight was determined. Egg shell thicknesses were determined using a micrometer (±0.01 mm). Averages of 3 measurements made on pointed, blunt, and middle parts of the shell were taken. The yolk colors of egg samples were determined with a DSM color fan. Yolk diameters and albumen lengths were measured with a tripod micrometer (1/100). Albumen lengths and widths were measured with a digital caliper. These values were used in the proposed formulas [49,50] to calculate yolk index, albumen index, and Haugh unit.

2.4. Chemical analysis

Chemical composition (dry matter, crude ash, crude protein, crude fat, starch, and sugar) of corn, guar meal, and soybean meal used in the experimental diets was analyzed according to the methods of 934.01, 942.05, 990.03, 920.39, 920.40, and 923.09, respectively [51]. Crude fiber contents of these ingredients were analyzed according to the Gerhardt Fibrebag System method. The condensed tannin contents were determined according to the method of Makkar et al. [52]. The metabolizable energy (ME) contents of ingredients used in experimental diets were estimated using the formula [53 + 38 (% crude protein + 2.25 × % ether extract + 1.1 × % starch + % sugar)] suggested by Carpenter and Clegg [53].

Before the experiment, the feedstuffs used in this study were analyzed with respect to their nutritional content. All experimental diets were prepared as isonitrogenous (180 g HP/kg) and isocaloric (11.71 ME, MJ/kg) using the analyzed nutritional values and diet calculator of an Excel worksheet. Furthermore, it was ensured that the methionine and lysine contents of the diets were similar to each other. The theoretical values of methionine, lysine, calcium, and phosphorus, which could not be analyzed, are used according to the values given in NRC [54]. In this study, 0, 27.2%, 54.4%, and 81.7% of the protein obtained from soybean meal was obtained from GM (0, 8%, 16%, and 24%, respectively).

The chemical composition of guar meal, corn, soybean meal, and soy oil are provided in Table 1. Feed ingredients and nutrient composition of the experimental diets are given in Table 2.

2.5. Statistical analysis

Experimental data were subjected to the one-way ANOVA procedure of SPSS (Windows version 25) software. Means were expressed as mean ± standard error of mean (SEM). Means were compared by Duncan's multiple range test within the same software with $P < 0.05$ [55].

3. Results

3.1. Growth performance

There were significant differences in the final live weights (FLW) of the experimental groups ($P < 0.05$). The greatest FLW was observed in the GM0E group, and the lowest live weight was observed in the GM24E group. Decreasing live weights were observed with increasing GM levels in the diets (Table 3). Such a decrease in live weights may be attributed to the dietary GM levels of the diets.

The effects of enzyme levels and guar × enzyme interactions on live weight changes were not found to be significant. The GM rates of more than 8% caused a decrease in live weights of the chickens. Compared to the control group, there was a 30% decrease in live weight of the treatment group supplied with 24% dietary GM. Therefore, 24% GM level was considered the critical limit for poultry diets. The present findings were similar with the results of some previous studies [20,56–58], but contradictory with the findings of Jackson et al. [59].

3.2. Feed intake and feed conversion ratio

Increasing dietary GM levels decreased feed intake by about 34.30%. The effects of guar levels, enzyme levels, and guar × enzyme interactions on feed intake were found to be significant ($P < 0.05$). The decrease in feed intake was 4–6 g at the 16% GM level and 32–38 g at the 24% GM level (Table 3). This might be attributed to the taste of saponins in GM [27].

Present findings on feed intakes agree with those of Al-Hsawi [58]. Dietary GM (0, 2.5%, and 5%) and beta-

Table 2. Feed ingredients of experimental diets and their nutrient analysis.

Ingredients	Experimental diets (g/kg)							
	GM0	GM0E	GM8	GM8E	GM16	GM16E	GM24	GM24E
Corn meal	578.31	578.31	580.86	580.86	583.57	583.57	586.28	586.28
Soybean meal	290.71	290.71	211.58	211.58	132.24	132.24	52.91	52.91
Guar meal	0.00	0.00	80.00	80.00	160.00	160.00	240.00	240.00
Enzyme (Hemicell)	0.00	0.50	0.00	0.50	0.00	0.50	0.00	0.50
Crude soy oil	18.99	18.99	15.02	15.02	10.98	10.98	6.94	6.94
Limestone	87.01	86.51	87.44	86.94	87.88	87.38	88.31	87.81
Dicalcium phosphate	18.41	18.41	17.84	17.84	17.28	17.28	16.71	16.71
DL-Methionine	1.07	1.07	1.12	1.12	1.16	1.16	1.21	1.21
L-Lysin HCl	0.50	0.50	1.14	1.14	1.89	1.89	2.64	2.64
NaCl	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Vitamin + mineral premix	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Total (kg)	1000	1000	1000	1000	1000	1000	1000	1000
	Analyzed nutrients							
Dry matter, %	90.33	90.33	90.22	90.22	90.55	90.55	90.00	90.00
Crude protein, %	18.00	18.00	18.00	18.00	18.00	18.00	18.00	18.00
Crude fat, %	3.86	3.86	3.81	3.81	3.73	3.73	3.67	3.67
Crude ash, %	13.23	13.23	13.17	13.17	13.12	13.12	13.06	13.06
Crude cellulose, %	2.72	2.72	3.21	3.21	3.70	3.70	4.19	4.19
Condensed tannin, %	0.16	0.16	0.20	0.20	0.27	0.27	0.33	0.33
	Calculated analysis							
ME, MJ/kg	11.71	11.71	11.71	11.71	11.71	11.71	11.71	11.71
Calcium, %	3.50	3.48	3.50	3.48	3.50	3.48	3.50	3.48
Available phosphorus, %	042	0.42	0.41	0.41	0.40	0.40	0.39	0.39
Lysine, %	1.02	1.02	1.01	1.01	1.01	1.01	1.01	1.01
Methionine, %	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Sodium, %	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22

ME: Metabolizable energy. MJ: Mega joule. GM: Guar meal. HC: Hemicell. Treatment diets: GM0 (0% GM), GM0E (0% GM + 0.05% HC), GM8 (8% GM), GM8E (8% GM + 0.05% HC), GM16 (16% GM), GM16E (16% GM + 0.05% HC), GM24 (24% GM), and GM24E (24% GM + 0.05% HC.

Each kg diet contains: Vitamin A: 12.000 IU, Vitamin D3: 2000 IU, Vitamin E: 35 mg, Vitamin K_3: 5 IU, Vitamin $B_{1:}$ 3 mg, Vitamin $B_{2:}$ 6 mg, Vitamin B_6: 5 mg, Vitamin $B_{12:}$ 0.015 mg, Vitamin C: 50 mg, D - Biotin: 0.045 mg, Niacin: 20 mg, Calcium D pantothenate: 6 mg, Folic acid: 0.75 mg, Choline chloride: 12.5 mg, Manganese: 80 mg, Iron: 60 mg, Zinc: 60 mg, Copper: 5 mg, Iodine: 1 mg, Cobalt: 0.2 mg, Selenium: 0.15 mg. Canthaxanthin: 15 mg, ß-apo-8'-carotenoic acid ethyl ester: 5 mg (synthetic pigment). Calcium, available phosphorus, lysine, and methionine content of diets were calculated according to National Research Council (NRC) [54].

mannanase (0 and 0.4%) did not affect the feed intake as they did in Shahbazi's work [60]. The decrease in feed intake may be attributed to antinutritional factors in guar meal.

The effects of guar meal levels and guar × enzyme interactions on feed conversion ratio (FCR) were found to be significant ($P < 0.05$). The enzyme levels alone did not have significant effects on FCR ($P > 0.05$). FCR is directly related to feed consumption, egg yield, and egg weight. Dietary GM levels influenced feed intake, egg weight, and egg yield.

The best FCR value was observed in the GM0 group and the worst FCR values were observed in the GM24 and GM24E groups ($P < 0.05$). Beta-mannanase

Table 3. The effects of dietary GM and enzyme supplementation into the diets on productive parameters of laying hens.

Groups	Parameters					
	TSW, g	FLW, g	FI, g	FCR, g/g	EW, g	EP, %
GM0	1784.83	1896.50a	111.99a	2.29c	62.90a	77.55a
GM0E	1769.83	1901.92a	108.73b	2.87b	59.27bc	63.82c
GM8	1912.25	1769.92ab	109.19b	2.53bc	59.93b	71.96abc
GM8E	1926.08	1701.75b	108.34b	2.46c	60.28b	72.95ab
GM16	1918.33	1624.17b	105.93c	2.78bc	57.81bc	65.81bc
GM16E	1910.58	1694.92b	105.97c	2.74bc	58.54bc	66.01bc
GM24	1910.08	1451.33c	80.33d	3.75a	56.61c	37.76d
GM24E	1915.25	1365.83c	73.57e	3.64a	57.48bc	35.10d
SEM	61.75	49.69	0.79	0.18	0.928	2.67
Main effects						
Guar meal level, %						
0	1777.33b	1899.20a	110.36a	2.58b	61.09a	70.69ab
8	1919.16a	1735.83b	108.77b	2.49b	60.10a	72.45a
16	1914.45a	1659.54b	105.95c	2.76b	58.17b	65.91b
24	1912.66a	1408.58c	76.95d	3.69a	57.05b	36.43c
SEM	43.67	35.13	0.55	0.13	0.65	1.89
Enzyme level, %						
0	1881.37a	1685.47a	101.86a	2.95	59.31a	63.27a
0.5	1880.43a	1666.10a	99.15b	3.02	58.89a	59.47b
SEM	30.88	24.84	0.39	0.09	0.46	1.33
Source of variation P values						
Guar × enzyme	0.99	0.37	0.00	0.02	0.05	0.02
Guar level	0.07	0.00	0.00	0.00	0.00	0.04
Enzyme level	0.983	0.583	0.03	0.58	0.52	0.04

TSW: Trial start weight. FLW: Final live weight. FI: Feed intake. FCR: Feed conversion ratio. EW: Egg weight. EP: Egg production.

[abcd] Means in a column without a common superscript letter differ significantly ($P < 0.05$). SEM: Standard error of the mean. P value (probability).

supplementation did not improve feed conversion during overall experimental period.

Galactomannan and the other antinutritional factors in GM may affect the feed efficiency of chickens negatively. In this study, the dietary enzyme supplementation of poultry diets did not have any beneficial effects on feed efficiency. Present FCR values were similar with values reported by Al-Hsawi [58] and Shahbazi [60], but were not similar to those reported by Ehsani and Torki [5] or Ashraf et al. [61].

The heaviest egg weight (62.90 g) was obtained from the GM0 group, while the lightest egg weight (56.61 g) was obtained from the GM24 group. Egg weights of the GM-containing groups (except for GM24) were significantly similar to each other. The only difference was observed in control groups compared to GM groups. The current egg weights were classified as middle class according to acceptable standards.

The effects of guar meal levels and guar × enzyme interactions on egg weights were found to be significant ($P < 0.05$), but the enzyme levels did not have any significant effects on egg weights ($P > 0.05$) (Table 3).

The current findings on egg weights were consistent with those of Zang [62] and Al-Hsawi [58], but disagree with those of Ehsani and Torki [5], Shahbazi [60], Gutierrez et al. [13], and Rama Rao et al. [7].

3.3. Egg yield performance

According to the results of the study (18 weeks); the effects of guar levels, enzyme levels, and guar × enzyme interactions on egg yields were found to be significant ($P < 0.05$). It has been observed that there was a decrease in egg yield with increasing dietary GM levels. GM24 and GM24E treatments caused a 50% reduction in egg production compared to the other treatments. GM0E treatments decreased egg production compared to the control. With respect to egg yield, GM0, GM8, and GM8E were similar to each other. GM0, GM0E, GM16, GM16E, GM24, and GM24E were significantly different ($P < 0.05$). It was also observed that up to 16% dietary guar meal supplementation did not have any adverse effects on egg production, but 24% GM resulted in a significant decrease in egg production. These results were similar to the results of Hasani et al. [63], but were not similar to the ones reported by Rama Rao et al. [64] Dietary enzyme supplementation in GM groups did make any significant difference in egg yield. The present findings were not similar to those of Ehsani and Torki [5]. Findings regarding egg production were similar with the findings of a study conducted with the dietary use of guar meal (0, 2.5%, and 5.0%) and hemicell (0%, 0.04%) by Shahbazi [60], using 20% dietary guar meal [57].

In this study, the mortality rate was zero; in other words, there were no mortalities throughout the experiments. All birds were healthy because antinutritional substance levels of guar meal (saponin, tannin, etc.) were not sufficient to affect their health. Hassan et al. [2] also reported no mortality. However, present findings on mortality do not comply with the findings of Hassan [57], Verma and McNab [12], and Patel and McGinnis [48].

3.4. Egg quality traits

The effects of guar meal levels, enzyme levels, and guar × enzyme interactions on eggshell weight were found to be significant ($P < 0.05$). The greatest shell weight was obtained from GM0E, followed by GM16E, GM24E, and GM8 treatments. The enzyme-supplemented control group had a higher shell weight (6.52 g) than the control group without enzyme (5.84 g), indicating that enzyme addition had a positive effect on shell weight (Table 4).

Eggshell weights vary with age, season, and feeding conditions [65]. The tannin content of GM might bond with minerals (chelates), especially calcium, causing lower Ca efficiency. The current results for eggshell weight were not similar to those of Ehsani and Torki [5], who used GM and mannanase-containing diets and found insignificant effects on eggshell weights.

The effects of guar meal levels and enzyme levels on eggshell thickness were found to be significant ($P < 0.05$), while the effects of guar × enzyme interactions were not significant ($P > 0.05$). It was observed that enzyme addition increased eggshell thickness when compared to the control (no enzyme) (Table 4). The phytic acid causes a decrease in bioavailability of minerals [66]. Tannins bind minerals and form complexes [67,31] that reduce mineral efficiency in the metabolism. Therefore, enzymes were also used in this study to see whether there would be a positive effect on minimizing the negative effects of antinutritional factors of GM on eggshell quality. For table eggs, it is desirable that the eggshell thickness should be 0.33–0.35 mm at least. In this study, eggshell thickness varied between 0.34–0.38 mm.

Shell breaking strength is an important external quality criterion of the egg. This quality trait is affected by genotype, maintenance, nutrition, and environmental factors. Sufficient calcium must be accumulated in the eggshell for shell resistance. Therefore, along with the feed, minerals such as calcium, phosphorus, sodium, chlorine, and vitamin D_3 should be supplied to birds sufficiently. Tannins and other antinutritional factors in GM may have the potential to affect mineral utilization and eggshell strength value negatively. The effects of treatments on the shell strength were not found to be significant (Table 4). Present findings on shell breaking strength comply with the findings of Rama Rao et al. [7], Ehsani and Torki [5], and Shahbazi [60].

The effects of guar meal levels and enzyme levels on egg shape index values were also found to be significant ($P < 0.05$), while the effects of guar × enzyme interactions were not found to be significant. The shape index values were between 75.67% and 78.00% (Table 4). Present findings on egg shape index were in line with those of Ehsani and Torki [5], but the effect of guar × enzyme interaction on egg shape index was not significant in that study.

The effects of guar × enzyme interactions, guar meal levels, and enzyme levels on egg albumen index were not found to be significant ($P > 0.05$; Table 4). Egg freshness is generally judged by the viscosity of the albumen. Therefore, albumen height is commonly measured to see if eggs are fresh [68]. The albumen index value is obtained through dividing the solid albumen height by the albumen width. If the albumen of a broken egg on a flat surface is spread over a small area, the egg is considered fresh; if the albumen is spread over a larger area, then the egg is considered old or out of date [69].

The effects of guar meal levels on egg yolk index were found to be significant ($P < 0.05$), while enzyme levels and guar × enzyme interactions did not have any significant effects on yolk index (Table 4). This result is in agreement with the findings of Youssef and Hoda [70].

Ehsani and Torki [5] reported that different guar (0, 3.5%, 7.0%) and enzyme levels (0 and 0.6%) did not have any significant effects on yolk index. The yellow or yolk index reflects the quality of the egg yolk and the upright

Table 4. Effects of dietary GM and enzyme supplementation in the diets on internal and external egg quality traits.

Groups	Parameters							
	ESW, g	ST, mm	ESS, g/cm^2	SI, %	AI, %	YI, %	HU	CFV
GM0	5.84c	0.35bc	2.56	75.67c	8.69	41.86ab	90.88ab	10.08a
GM0E	6.52a	0.36b	2.53	76.42abc	8.45	42.21ab	89.64ab	10.17a
GM8	6.03bc	0.34bc	2.50	75.75bc	8.52	41.47b	85.22b	10.17a
GM8E	5.85c	0.36b	2.47	76.75abc	8.57	41.62b	89.34ab	9.92a
GM16	6.01bc	0.38a	2.42	76.42abc	8.79	42.58ab	87.95b	9.00b
GM16E	6.32ab	0.37a	2.39	77.50ab	9.02	42.57ab	94.50a	8.58b
GM24	5.78c	0.34c	2.43	77.33abc	8.87	43.57a	94.09a	5.75d
GM24E	6.03bc	0.35b	2.41	78.00a	8.44	43.11ab	90.20ab	7.33c
SEM	0.12	0.00	0.26	0.56	0.16	0.54	1.87	0.28
Main effects								
Guar meal level, %								
0	6.18a	0.35a	2.55	76.04b	8.57	42.04b	90.26	10.12a
8	5.93ab	0.34b	2.49	76.25b	8.55	41.54b	87.26	10.04a
16	6.16a	0.37b	2.41	76.96ab	8.90	42.57ab	91.07	8.79b
24	5.90b	0.34b	2.42	77.67a	8.66	43.34a	92.01	6.54c
SEM	0.08	0.00	0.05	0.39	0.11	0.38	1.32	0.20
Enzyme level, %								
0	5.91a	0.35a	2.48	76.29a	8.72	42.37	89.47a	8.75
0.5	6.18b	0.36b	2.45	77.17b	8.62	42.38	90.83b	9.00
SEM	0.06	0.00	0.03	0.28	0.08	0.27	0.93	0.14
Source of variation P values								
Guar × enzyme	0.01	0.29	0.32	0.97	0.19	0.89	0.03	0.00
Guar level	0.04	0.00	0.22	0.02	0.13	0.01	0.07	0.00
Enzyme level	0.00	0.04	0.63	0.03	0.40	0.98	0.30	0.22

ESW: Egg shell weight. ST: Shell thickness (mm). ESS: Egg shell strength. SI: Shape index. AI: Albumen index. YI: Yolk index.
HU: Haugh unit. CFV: Color fan value.
[abcd] Means in a column without a common superscript differ significantly ($P < 0.05$).

position of the yolk. Yolk index values of fresh eggs should be between 40 and 46 [65]. In this study, the yellow index values of the treatment groups ranged from 40.58 to 44.43, which are within the limits specified in the food codex (Table 4).

The effects of guar × enzyme interactions on Haugh Unit (HU) were found to be significant, while the effects of guar meal levels and enzyme levels were insignificant ($P > 0.05$, Table 4). The freshness of an egg is determined by breaking the eggs over a flat surface. In fresh eggs, the yolk is in the center and albumen is homogeneous and elastic. In old (out of date) eggs, the yellow part is close to the shell and the white part becomes layered. Haugh unit is a very important criterion to be used to determine the freshness of the egg. The effects of guar levels on HU were found to be significant. Present findings on HU were similar to those of Shahbazi [60], whose diets contained 0, 2.5%, and 5.0% guar meal. The current experimental eggs were in the AA class with respect to HU values according to the available standards.

The effects of guar meal levels and guar × enzyme interactions on egg yolk color values were found to be significant ($P < 0.05$), while the effects of enzyme levels were not significant. Egg yolk color values decreased significantly with increasing GM levels (Table 4). The effects of GM on yolk color were more remarkable at supplementation ratios greater than 16%. This would be due to the lowered feed intake, since the hens would have

consumed fewer feed ingredients containing pigments. In this study, egg yolk color values varied between 5.75 and 10.17. When compared to the control group, there was a 56.5% decrease in the egg yolk color value in hens fed a diet containing 24% GM. This was likely due to decrease in feed intake. It was noticed that egg yolk color value was restored by the addition of enzyme at this GM level, likely due to the beneficial effects of the enzyme on pigmentation. Dietary GM affecting yolk color negatively was found by Hassan [57], Al-Hsawi [58], and Zang [62]. However, Rama Rao et al. [7] found that dietary GM did not affect egg yolk color.

4. Discussion

Because of antinutritional substances, GM supplementation in poultry diets yielded a considerable decrease in feed consumption. Such a decrease then significantly influenced laying performance of the hens adversely.

In this study, the addition of beta-mannanase enzymes into diets containing nonheat–treated GM did not positively affect the performance and egg quality traits of laying hens. Therefore, it was thought that there might be no need to add enzymes into laying-hen diets containing raw GM.

The reduction in egg yolk color value was largely attributed to the increase in dietary inclusion of GM and lower feed intake.

It has been concluded based on present findings that 24% dietary GM affected egg production performance negatively. If the antinutritional substances of GM were eliminated, it would be possible that a higher proportion of the protein would be replaced by GM. Due to the decrease in performance by a higher inclusion rate of GM, its level should be lower than 16%.

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Conflict of interest

There are no conflicts of interest.

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