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Effects of dietary guar meal with or without beta-mannanase on the egg yolk fatty acids, cholesterol, and some blood parameters of laying hens

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Abstract: In this study, the effects of adding enzyme to the diets containing guar meal (GM) on the egg yolk fatty acids, cholesterol, and some blood plasma parameters of hens were examined. This study was carried out in the chicken coop of the Experimental Animals Ethics Committee, Faculty of Agriculture. This study constitutes the second phase of the study titled "Effects of dietary guar meal with or without beta-mannanase on performance and egg quality traits in laying hens", previously published. The experimental period lasted 126 days. Ninety-six Lohman Brown hens at the age of 56 weeks were and kept in individual cages. They were divided into 8 treatment groups as of: 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 lighting period of 16.5 h light and 7.5 h dark was applied throughout the experiment. As a result of the study, it was found that the effects of the treatments on the egg yolk total cholesterol, egg yolk total saturated fatty acids, total unsaturated fatty acids, blood triglyceride, blood glucose, blood total cholesterol, and blood calcium amounts were significant ($p < 0.05$) and their effects on the blood total protein and blood phosphorus amount were not significant ($p > 0.05$). According to the results of this study, it was concluded that using up to 8% GM in the diets did not cause a negative effect on the blood parameters of the hens.

Key words: Guar meal, laying hen, egg yolk, fatty acids, cholesterol, blood parameters

1. Introductıon

Guar (*Cyamopsis tetragonoloba*) is a drought-tolerant annual legume plant. It is grown especially for its galactomannan gum. Guar meal (GM) is produced as a result of the process of extracting gum from guar. Having high protein content (33%–60%), GM [1,2] is also very rich in arginine (4.76%–6.01%), lysine (1.66%–1.99%), and methionine (0.47%–0.51%) [3, 4]. GM contains 10.9–11.3 mega joules of metabolic energy in dry matter [5,6].

GM also contains various antinutritional factors such as saponin [7], trypsin inhibitor [8], hemagglutinins, hydrocyanic acid [9], and phytic acid [10]. Gum is a very strong antibacterial factor for the monogastric animals and 13%–18% of GM is gum [11]. Therefore, it may cause some problems in feeding the laying hens. The enzymes such as cellulase, hemicellulase, and beta-mannanase are added to the diets [12,13] or some heat treatments [14] are applied in order to eliminate the antinutritional effect of GM and improve its nutritional value.

Saponins in the structure of feeds have hypocholesterolemic effects on animals [15]. Furthermore, the polyphenolic compounds are effective in reducing the cholesterol due to their adsorbent properties [16]. Since GM contains gum, it reduces the blood cholesterol level in animals [17,18]. Guar was found to cause a decrease in plasma glucose [19] and plasma cholesterol levels in rats [20,21] due to the high viscosity of the gum it contained.

Adding GM to the diets can change the blood values in the laying hens. In a previous study, it was found that feeding the chickens with the diets containing GM did not cause a change in their fasting blood sugar, triglyceride, HDL, LDL, and phosphorus levels [22]. The plasma cholesterol and glucose levels of the broiler quails which were fed with the heat-treated GM with and without enzyme supplementation were found to be lower [23]. The blood albumin, globulin, and protein content of the broiler chickens and quails fed with the diets containing guar meal were not affected [24].

In this study, the effects of diets containing different levels of GM and beta-mannanase on laying hens' egg yolk fatty acids, cholesterol and some blood parameters were investigated.

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2. Materials and methods

2.1. Housing of the experimental animals

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 Kahramanmaraş Sütçü İmam University, 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 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. Feedstuffs, experimental diets and nutrient contents

The chemical composition of guar meal, corn, soybean meal, and soybean oil are given in the Tables 1 and 2. The feed ingredients and nutrient composition of the experimental diets are given in the Table 3.

It was found that the GM used in the study contained 27.92% total saturated fat (ΣSFA), 23.50% mono unsaturated fatty acids (MUFA), 48.58% poly unsaturated fatty acids (PUFA), and 72.08% total unsaturated fatty acids (ΣUFA) (Table 2).

2.4. Chemical analysis of feedstuffs

The chemical composition (dry matter, crude ash, crude protein, crude oil, starch, and sugar) of the corn, guar

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

Parameter	Guar meal	Corn	Soybean meal	Soy oil
Analyzed nutrients				
Crude 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 tanen, %	0.97	0.15	0.24	-
*Metabolizable energy, MJ/kg	10.95	14.03	9.61	36.76
Calculated analysis				
Calcium,%	0.24	0.01	0.25	-
Available phosphorus,%	0.15	0.10	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 [27].

Table 2. Analyzed fatty acid contents of guar meal and soy oil used in the experiment.

Fatty acids	Guar meal, %	Soy oil, %
Lauric (C12: 0)	0.68	0.02
Miristic (C14: 0)	0.56	0.08
Pentadecanoic (C15: 0)	0.19	-
Palmitic (C16: 0)	16.43	10.85
Heptadecanoic (C17: 0)	0.22	-
Stearic (C18: 0)	7.96	4.38
Arachidic (C20: 0)	0.53	0.48
Heneicosanoic (C21: 0)	0.10	-
Behenic (C22: 0)	0.87	0.57
Tricosanoic (C23: 0)	0.30	0.14
Lignoceric (C24: 0)	0.08	0.06
Total saturated fatty acids	27.92	16.58
Palmitoleic (C16: 1)	0.27	0.22
Heptadecanoic (C17: 1)	0.12	-
Oleic (C18: 1n9c)	22.88	25.37
Eicosenoic (C20: 1)	-	0.29
Erucic (C22: 1n9)	0.23	-
Total monounsaturated fatty acids	23.50	25.88
Linolelaidic (C18: 2n6t)	0.13	-
Linoleic (C18: 2n6c)	39.30	51.67
Gama-linolenic (C18: 3n6)	1.27	
Alfa-linolenic (C18: n3)	3.62	5.77
Eicosatrienoic (C20: 3n3)	0.45	-
Arachidonic (C20: 4n6)	0.08	-
Eucosapentaenoic (C20: 5n3)	1.92	-
Decosahexaenoic (C22: 6n3)	1.81	-
Total polyunsaturated fatty acids	48.58	57.44
Total unsaturated fatty acids	72.08	83.32

meal, and soybean meal used in the experimental diets were analyzed according to the methods of 934.01, 942.05, 990.03, 920.39, 920.40, and 923.09, respectively [25]. The crude cellulose contents of these components were analyzed using the Gerhardt Fibre bag method. The condensed tannin content was determined according to the method reported by Makkar et al. [26]. The metabolic energy contents of the components used in the experimental diets were computed using the formula [ME (Kcal kg^{-1}) = 38 × [(1 × crude protein, g kg^{-1}) + (2.25 × crude fat, g kg^{-1}) + (1.1 × starch, g kg^{-1}) + (1.05 × sugar, g kg^{-1}) + 53] reported by Carpenter and Clegg [27].

The nutrient contents of feed raw materials used in this study were analyzed prior to the trial. The diets were prepared as isonitrogenic (180 g HP/kg) and isocaloric (11.71 MJ/kg) by means of loading these nutritional values into the diet prepadiet package program. In addition, it was ensured that all the feed diets were similar in terms of the methionine and lysine content. The methionine, lysine, calcium, and phosphorus contents of the feeds were determined based on the values specified in NRC [28].

2.5. Fatty acids analysis of egg yolk

The fatty acids analysis was carried out using the method specified by Folch et al. [29] by means of injecting the fatty acid samples, extracted from the egg yolks and esterified, into the gas chromatography (GC-2025; Shimadzu, Kyoto, Japan) equipped with a flame ionizing detector. Supelco 37 component mix certified standard was used in the total fatty acid analysis of the egg yolk. TR-CN 100 column (Teknokroma, Barcelona, Spain) with a length of 60 m, a film thickness of 0.25 microns, and an internal diameter of 0.20 mm was used in analyzing the samples. The total analysis time, injector temperature, detector temperature, and the flow rate of carrier gas (helium) were 61 min, 240 °C, 250 °C, and 30 mL/min, respectively.

2.6. Cholesterol analysis of egg yolk

In the 9th and 18th weeks of the study, 3 eggs randomly selected from each group were boiled (10 min) and their egg yolks were separated. The homogenized egg yolk samples were first dissolved in alcohol (n-propanol) and then the filtered samples were centrifuged after being kept in the water bath. The cholesterol kit was added onto the supernatant obtained from the centrifugation and kept in the water bath at 37 °C for 10 min and then the reading was carried out on the spectrophotometer at 520 nm. The cholesterol amount was determined by means of putting the values obtained in the reading into the formula [30].

$$\text{Amount of cholesterol in the extract (mg/dL)} = \frac{\text{Sample optical density}}{\text{Standard optical density}} \text{ x Standard concentration}$$

$$\text{Egg yolk cholesterol (mg / dL)} = \frac{(\text{Amount of cholesterol in the extract / 100}) \times 4}{\text{Amount of the sample (g)}}$$

2.7. Blood analysis

In the study, the blood samples taken from the *vena cutanea ulnaris* of the 3 hens randomly selected from each group were placed into the special tubes. These tubes were centrifuged at 3000 rpm for 10 min. The plasmas obtained were kept frozen at –80 °C (Elcold Lab 11) until the analysis.

The triglyceride, glucose, total cholesterol, total protein, Ca, and P contents of the blood plasma samples were determined using the photometric method. These analyses were carried out on an auto analyzer (Olympus AU400

Table 3. Feed ingredients of experimental diets and their nutrient analysis.

Ingredients	Experimental diets (g/kg)							
	GM0	GM0E	GM8	GM8E	GM16	GM16E	GM24	GM24E
Corn	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
Hemicell (beta mannanase)	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
Methionine	1.07	1.07	1.12	1.12	1.16	1.16	1.21	1.21
Lysin	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
	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
ME, MJ/kg	11.71	11.71	11.71	11.71	11.71	11.71	11.71	11.71
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
	analysis							
Calcium, %	3.50	3.48	3.50	3.48	3.50	3.48	3.50	3.48
Available phosphorus, %	0.42	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 K3: 5 IU, Vitamin B1: 3 mg, Vitamin B2: 6 mg, Vitamin B6: 5 mg, Vitamin B12: 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 [28].

chemistry analyzer - OLY-AU400; Mishima Olympus Co. Ltd., Shizuoka, Japan) using the commercial kits (OSR; Beckman Coulter, Brea, CA, USA).

2.8. Statistical analysis

The experimental data were analyzed using the one-way ANOVA in IBM SPSS 25.0 for Windows (IBM Corp., Armonk, NY, USA). The means were expressed in mean ± standard error of mean (SEM). The means were compared using Duncan's multiple range test in the same software [31]. The significant was set at $p < 0.05$.

3. Results and discussion

3.1. Saturated fatty acids in the egg yolk

In this study, it was found that the effect of the guar level and guar-enzyme interaction on the amount of palmitic acid (C16: 0) and stearic acid (C18: 0) in the egg yolk was

significant ($p < 0.05$) and the effect of the enzyme level on it was not significant ($p > 0.05$). The effect of guar level on the total saturated fatty acids levels of the groups was found to be significant ($p < 0.01$), whereas the effect of enzyme level and guar-enzyme interaction on them was not significant ($p > 0.05$) (Table 4).

There was a significant difference between the control group and the groups of GM8E and GM24E in terms of PA content in the egg yolks. Compared to the control group, there was a decrease in the egg yolk PA amounts of the groups depending on the the increase in the GM amount. On the other hand, compared to the control group, there was an increase in the egg yolk SA amounts of the groups depending on the increase in the GM amount in the diet. Compared to the control group, an increase was observed only in the egg yolk ΣSFA contents of the GM8 and GM8E groups, while the ΣSFA contents of other groups were found to be close to each other.

The diets containing a high content of saturated fatty acids increase the amount of stearic and palmitic fatty acids in the egg yolk in the laying hens [32]. On the other hand, the raw materials containing a high content of polyunsaturated fatty acids such as soybean oil cause a decrease in the saturated fatty acids in the egg yolk [33].

In this study, it is thought that the raw material compositions of diets were effective in the increase or decrease in the saturated fatty acid contents of egg yolks. Furthermore, there was an increase or decrease in the egg cholesterol levels depending on the egg yolk fatty acid profiles (saturated or unsaturated) (Figure 1). It is thought that the diet contents were effective in the change in the egg yolk PA, SA, and ΣSFA values of the groups (Figure 1).

3.2. Unsaturated fatty acids in the egg yolk

In this study, it was found that the effect of the guar level and guar-enzyme interaction on the oleic acid (OLA), nervonic acid (NA), and linoleic acid (LA) contents in the egg yolk was significant ($p < 0.05$) and the effect of the enzyme level on them was not significant ($p > 0.05$). The effect of guar level on the amounts of palmitoleic and erucic fatty acids was found to be significant ($p < 0.01$); whereas the effect of enzyme level and guar-enzyme interaction on them was not significant ($p > 0.05$), (Table 5). About 2/3 of the yolk consists of unsaturated fatty acids.

In the study, there was a decrease in the OLA amounts and an increase in the LA and POA amounts in the egg yolks in proportion to the increase in the amount of GM in the diet. It is thought that this increase in the amounts of fatty acids in the egg yolks might be related to the raw material profile of the diets.

Compared to the control group, there was a 4% decrease in the amount of oleic acid in the egg yolks in the group having the highest amount of GM (24%). It is thought that this decrease was caused by the raw material content of the diets and the feed consumption level of the groups. The amount of linoleic acid egg yolk increased in proportion to the increase in the GM amount in the diets. The linoleic acid content of GM in the diets (39.30%) might have caused this increase. In the studies previously carried out on chickens [34,35], it was found that the diet PUFA increased the ß-oxidation and inhibited the novo fatty acid synthesis. The fact that PUFA is oxidized before SFA [36] explains the lower fat accumulation in the chickens fed

Table 4. Effect of diets containing GM and enzymes on egg yolk saturated fatty acid amounts (n = 6).

Groups	Parameters		
	Palmitic acid	Stearic acid	ΣSFA
GM0	25.46b	7.74c	33.20b
GM0E	25.60b	8.08bc	33.68ab
GM8	25.60b	8.53ab	34.13a
GM8E	27.54a	7.75c	35.29a
GM16	25.50b	8.16bc	33.66ab
GM16E	25.38b	8.16bc	33.54ab
GM24	24.90bc	8.62ab	33.52ab
GM24E	24.18c	8.72a	32.90b
SEM	0.27	0.16	0.33
Main effects			
Guar levels, %			
0	25.53b	7.91b	33.44b
8	26.57a	8.14b	34.71a
16	25.44b	8.16b	33.60b
24	24.54c	8.67a	33.21b
SEM	0.19	0.11	0.23
Enzyme levels, %			
0	25.36	8.26	33.62
0.5	25.67	8.18	33.85
SEM	0.13	0.08	0.16
Probability (P)			
Guar × enzyme	0.01	0.02	0.09
Guar level	0.01	0.01	0.01
Enzyme level	0.13	0.47	0.36

GM: Guar meal. E: Enzyme (HC: Hemicell). Treatment groups: GM0: 0% guar meal. GM0E: 0% GM + 0.05% beta – mannanase. GM8: 8% GM. GM8E: %8 GM + 0.05% beta – mannanase. GM16: 16% GM. GM16E: 16% GM + 0.05% beta – mannanase. GM24: 24% GM. GM24E: 24% GM + 0.05% beta - mannanase.
[abc] Means in a column without a common superscript letter differ significantly ($p < 0.05$).

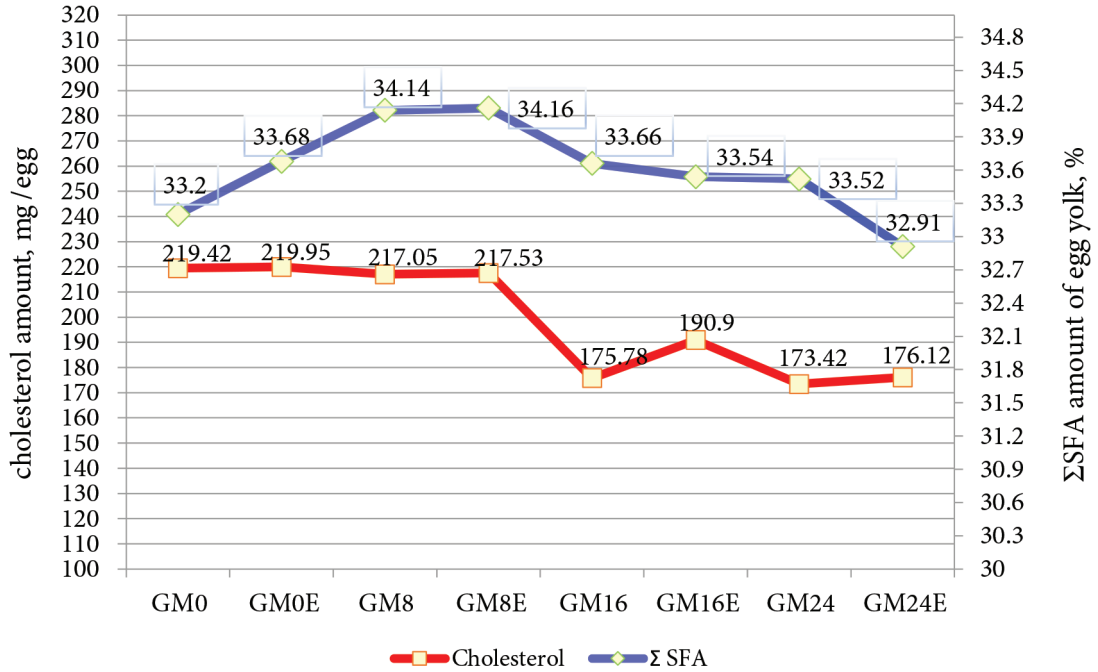

Figure 1. The relationship between egg yolk ΣSFA amounts and cholesterol amounts. Treatment groups: GM0 (0% GM), GM0E (0% GM + 0.05% HC), GM8 (8% GM), GM8E (8% GM + 0.05% HC), GM16 (16% GM), GM16E (16% GM + 0.05% HC), GM24 (24% GM) and GM24E (24% GM + 0.05% HC. GM: Guar meal. E: Enzyme (HC: Hemicell)

with the diets rich in PUFA. In chickens, depending on the decrease in ß-oxidation and the increase in the novo synthesis of fatty acids, the amount of saturated fat in the body tissues increases compared to the unsaturated fats. It was reported that feeding the chickens with the diets rich in C18: 2, especially MUFA, reduced the body fat accumulation [37].

The nutritional manipulations are effective in the production of the eggs rich in unsaturated fatty acids. Furthermore, the polyunsaturated fatty acids have been proven to have some cholesterol-lowering effects [38]. Therefore, the eggs rich in polyunsaturated fats have some positive effects in preventing the cardiovascular diseases, arthritis, and diabetes [39].

3.3. Egg yolk cholesterol

The effect of guar level on the cholesterol ratios of egg yolk was found to be significant ($p < 0.05$), whereas the effect of enzyme level and guar-enzyme interaction on them was not significant ($p > 0.05$). A 21.1% decrease was observed in the amount of cholesterol in the GM24 group compared to the control group (Table 6). This result was not similar to that of Hasani et al. [40].

While 75% of the blood cholesterol is synthesized in the body, 25% of it is obtained from the foods of animal origin [41]. Majority of the cholesterol in the egg yolk is synthesized in the liver of hens, transferred to the follicles a few days before the ovulation, and stored there [42]. Various nutrients contribute to the cholesterol production in different amounts. Proteins, fats, and carbohydrates are broken down into acetate during the energy production cycle, and this acetate leads to excess amounts of fatty acids and cholesterol in the body [43].

In this study, a decrease was observed in the feed consumption of the groups depending on the GM content of the diets. It is thought that this decrease contributed to the decrease in the cholesterol amounts in the egg yolks (Figure 2).

In the study, the cholesterol amount in the egg yolk of the laying hens was found to decrease in direct proportion to the increase in the ratio of GM in the diets. It is believed that the polyphenolic content of GM caused this decrease. Because the polyphenolic compounds are also effective in reducing the cholesterol due to their adsorbent properties [18,16]. Being present in GM in the ratio of 5%–13%, [44,45] saponin also causes a decrease in the cholesterol level in animals [15]. Tannins also reduce the cholesterol absorption in the gut and lower the amount of plasma cholesterol [46] by means of inhibiting 3-hydroxy-methyl-glutaryl-CoA reductase, which is necessary for the cholesterol biosynthesis [47,48] or increasing the bile acid excretion [49].

3.4. Results of the blood analysis

According to the results of the study, the effect of different treatments on the total triglyceride (TG), glucose (Glu),

Table 5. Egg yolk unsaturated fatty acid amounts (n = 6).

Groups	Parameters					
	MUFA				PUFA	ΣUFA
	POA	OLA	ERA	NA	LA	
GM0	3.20a	47.19ab	1.02b	0.50cd	12.41c	64.32a
GM0E	3.06a	48.23a	1.26b	0.46d	11.09d	64.10a
GM8	3.00ab	46.57bc	1.09b	0.50d	12.67c	63.83a
GM8E	2.77ab	45.60c	1.16b	0.62ab	12.10cd	62.25b
GM16	3.04a	46.81b	1.23b	0.53bcd	12.62c	64.23a
GM16E	3.03ab	48.08a	1.24b	0.44d	11.86cd	64.65a
GM24	2.36bc	44.08d	1.62a	0.58abc	15.42b	64.06a
GM24E	2.01c	43.25d	1.82a	0.66a	16.69a	64.43a
SEM	0.20	0.34	0.10	0.03	0.38	0.29
Main effects						
Guar levels, %						
0	3.13a	47.71a	1.14b	0.47b	11.75b	64.20a
8	2.89a	46.08b	1.12b	0.56a	12.39b	63.04b
16	3.03a	47.45a	1.23b	0.49b	12.24b	64.44a
24	2.19b	43.66c	1.72a	0.62a	16.05a	64.24a
SEM	0.14	0.24	0.07	0.02	0.27	0.20
Enzyme levels, %						
0	2.90	46.16	1.24	0.53	13.28	64.11
0.5	2.72	46.29	1.37	0.54	12.93	63.85
SEM	0.10	0.17	0.05	0.01	0.19	0.14
Probability (P)						
Guar × enzyme	0.87	0.01	0.64	0.01	0.02	0.01
Guar level	0.01	0.01	0.01	0.01	0.01	0.01
Enzyme level	0.22	0.60	0.09	0.44	0.21	0.23

MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, POA: palmitioleic acid, OLA: oleic acid, LA: linoleic acid, ERA: erucic acid, NA: nervonic acid, UFA: unsaturated fatty acids. GM: Guar meal. E: Enzyme (HC: Hemicell). Treatment groups: GM0: 0% guar meal. GM0E: 0% GM + 0.05% beta – mannanase. GM8: 8% GM. GM8E: %8 GM + 0.05% beta – mannanase. GM16: 16% GM. GM16E: 16% GM + 0.05% beta – mannanase. GM24: 24% GM. GM24E: 24% GM + 0.05% beta - mannanase. ^{abc} Means in a column without a common superscript letter differ significantly ($p < 0.05$). SEM: Standard error of the mean.

and total cholesterol (TC) values in the blood plasma was found to be significant ($p < 0.05$). The effect of guar level on the decrease in the blood TG, Gl, and TC levels was significant ($p < 0.05$), while the effect of enzyme level and guar-enzyme interaction on it was negligible (Tables 7). It is thought that the decrease in the total TG, Glu, and TC amounts in the blood plasma of the groups might be related to the ratio of GM in the diet and the feed consumption levels. The diets rich in polyunsaturated fatty acids are also known to lower the blood cholesterol level [50].

In this study, blood cholesterol and blood triglyceride contents were affected depending on the GM level of the diets ($P < 0.05$). Among these, blood cholesterol results were compatible with the results of Hasani et al. [40].

In this study, a decrease was observed in the blood triglyceride levels of the groups due to the increase in the amount of GM in the diets. This result was not compatible with the result of the study [40] in which blood triglyceride amount was not affected ($p > 0.05$) depending on the level of GM. In addition, the result was not in line with the results

Table 6. Effect of different levels of GM and enzyme contain diets on egg cholesterol content.

Groups	Egg yolk cholesterol values by weeks, mg/egg		
	9. week	18. week	0–18 weeks
GM0	221.13ab	217.7a	219.42a
GM0E	224.66a	215.23ab	219.95a
GM8	226.16a	207.93abc	217.05ab
GM8E	234.23a	200.83abc	217.53ab
GM16	168.43c	183.13cd	175.78c
GM16E	191.56bc	190.23abcd	190.90bc
GM24	179.10c	167.73d	173.42c
GM24E	172.73c	179.5d	176.12c
SEM	10.06	9.85	8.47
Main effects			
Guar levels, %			
0	222.9a	216.5a	219.7a
8	230.2a	204.4ab	217.3a
16	180.0b	186.7bc	183.3b
24	175.9b	173.6c	174.8b
SEM	7.11	6.96	5.93
Enzyme levels, %			
0	198.7	194.1	196.4
0.5	205.8	196.5	201.1
SEM	5.03	4.92	4.23
Probability (P)			
Guar × enzyme	0.54	0.76	0.79
Guar level	0.01	0.01	0.01
Enzim level	0.33	0.74	0.44

GM: Guar meal. E: Enzyme (HC: Hemicell). Treatment groups: GM0: 0% guar meal. GM0E: 0% GM + 0.05% beta – mannanase. GM8: 8% GM. GM8E: %8 GM + 0.05% beta – mannanase. GM16: 16% GM. GM16E: 16% GM + 0.05% beta – mannanase. GM24: 24% GM. GM24E: 24% GM + 0.05% beta - mannanase. ^{abcd} Means in a column without a common superscript letter differ significantly ($p < 0.05$). SEM: Standard error of the mean.

of the studies in which GM did not cause any change in the blood triglyceride levels in the laying hens [22] and it caused an increase in the blood triglyceride levels in the broilers [51]. In this study, the blood glucose and cholesterol values of the groups decreased in parallel with the increase in the amount of GM in the diets ($p < 0.05$). These results showed similarities with the results reached by Dinani et al. [23].

The antinutrient factors of GM reduce the digestibility of feeds and decrease protein quality [52]. According to the findings of the research; the effects of treatments on blood total protein value were insignificant ($p > 0.05$). This result was similar to the results [24,23] of the study that feeding diets containing GM did not cause any change in the total blood protein value of the chickens. However, it was not similar to the result [51] of the research that feeding with diets containing GM reduced the total protein amount of blood in laying hens (Table 8).

The effect of guar level on blood calcium amount was significant, the effect of enzyme level and guar x enzyme interaction was found insignificant.

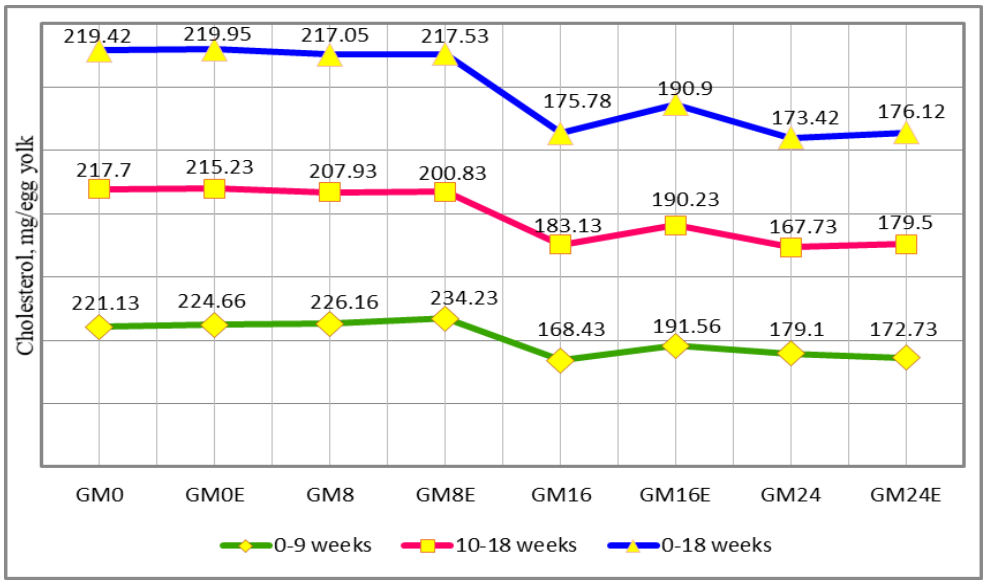

Figure 2. Egg yolk cholesterol content. Treatment groups: GM0 (0% GM), GM0E (0% GM + 0.05% HC), GM8 (8% GM), GM8E (8% GM + 0.05% HC), GM16 (16% GM), GM16E (16% GM + 0.05% HC), GM24 (24% GM) and GM24E (24% GM + 0.05% HC. GM: Guar meal. E: Enzyme (HC: Hemicell)

Table 7. Blood plasma values of treatment groups (n = 3).

Groups	Parameters		
	∑Triglyceride (mg/dL)	∑Glucose (mg/dL)	∑Cholesterol (mg/dL)
GM0	95.66a	279.00a	191.00
GM0E	96.00a	247.33b	177.00
GM8	93.63a	250.00b	131.66
GM8E	80.50ab	248.33b	100.33
GM16	74.03ab	239.00b	146.00
GM16E	74.93ab	234.00b	90.00
GM24	48.56b	227.33b	90.00
GM24E	55.90b	228.33b	101.66
SEM	10.77	7.21	30.64
Main effects			
Guar levels, %			
0	95.53a	263.16a	184.00a
8	87.06a	249.16ab	116.00ab
16	74.48ab	236.50bc	118.00ab
24	52.23b	227.83c	95.83b
SEM	7.62	5.10	21.64
Enzyme levels, %			
0	77.82	248.83	139.66
0.5	76.83	239.5	117.25
SEM	5.38	3.60	15.30
Probability (P)			
Guar × enzyme	0.81	0.12	0.73
Guar level	0.01	0.01	0.05
Enzyme level	0.89	0.08	0.31

GM: Guar meal. E: Enzyme (HC: Hemicell). Treatment groups: GM0: 0% guar meal. GM0E: 0% GM + 0.05% beta – mannanase. GM8: 8% GM. GM8E: %8 GM + 0.05% beta – mannanase. GM16: 16% GM. GM16E: 16% GM + 0.05% beta – mannanase. GM24: 24% GM. GM24E: 24% GM + 0.05% beta - mannanase. [abc] Means in a column without a common superscript letter differ significantly ($P < 0.05$). SEM: Standard error of the mean.

Table 8. Blood plasma values of treatment groups (n = 3).

Groups	Parameters		
	ΣProtein (g/dL)	Calcium (mg/dL)	Phosphorus (mg/dL)
GM0	4.66	22.22a	6.56
GM0E	5.30	21.26a	5.50
GM8	5.66	19.47a	6.30
GM8E	4.86	17.50ab	6.50
GM16	5.23	17.39ab	6.10
GM16E	5.70	15.62ab	5.23
GM24	5.06	10.99b	4.56
GM24E	5.36	12.00b	4.46
SEM	0.59	2.19	0.67
Main effects			
Guar levels, %			
0	4.98	21.74a	6.03a
8	5.26	18.48ab	6.40a
16	5.46	16.50b	5.66ab
24	5.21	11.49c	4.51b
SEM	0.42	1.55	0.47
Enzyme levels, %			
0	5.15	17.51	5.88
0.5	5.30	16.59	5.42
SEM	0.29	1.09	0.33
Probability (P)			
Guar × enzyme	0.62	0.90	0.75
Guar level	0.87	0.01	0.06
Enzyme level	0.72	0.56	0.35

GM: Guar meal. E: Enzyme (HC: Hemicell). Treatment groups: GM0: 0% guar meal. GM0E: 0% GM + 0.05% beta – mannanase. GM8: 8% GM. GM8E: %8 GM + 0.05% beta – mannanase. GM16: 16% GM. GM16E: 16% GM + 0.05% beta – mannanase. GM24: 24% GM. GM24E: 24% GM + 0.05% beta - mannanase. [abc] Means in a column without a common superscript letter differ significantly ($P < 0.05$). SEM: Standard error of the mean.

The effect of all treatments on blood phosphorus content was insignificant ($p > 0.05$).

In this study, there was a decrease in the blood calcium content of the groups depending on the amount of GM. It is believed that this decrease was caused by the antinutritional factors of GM preventing the absorption of minerals [52].

4. Conclusion

Using GM instead of soybean meal in the diets positively contributes to the feed cost. On the other hand, due to its antinutritional content, the high rates of GM caused a significant change in some blood parameters by means decreasing the feed consumption of the laying hens.

The GM-containing diets lowered the egg yolk fatty acids profile (saturated and unsaturated), cholesterol level, and the values of blood triglyceride, glucose, total cholesterol, and calcium, but had no effect on the total protein and phosphorus amounts.

Especially in the groups containing 16% and above GM, a decrease was observed in the egg yolk cholesterol and the blood parameters of triglyceride, glucose, cholesterol, Ca, and P.

In the groups containing 24% GM, the egg yolk cholesterol content significantly decreased in proportion to the increase in the amount of egg yolk polyunsaturated fatty acids.

The increases and decreases in the egg yolk saturated fatty acids and the egg yolk cholesterol levels were found to be in parallel with each other.

A decrease occurred in the amounts of egg yolk and blood cholesterol in the laying hens fed with the GM-containing diets. Therefore, it is possible to benefit from this positive effect of GM in producing the low cholesterol eggs. The low cholesterol eggs are thought to contribute positively to the people with cardiovascular problems.

Adding or not adding mannanase to the GM-containing diets was similar in terms of their effect on the blood parameters.

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