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Effects of Different Levels of Guar Meal and β-Mannanase on Performance, Yolk Cholesterol Concentration and Blood Lipid Parameters of Laying Hens in Second-Cycle of Production

Research Article

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ABSTRACT

A 3 × 2 factorial arrangements with completely randomized design, with a total of 72 Leghorn Hy-line (W-36) laying hens in the second cycle (98 wk old) of production were randomly assigned to 24 wire cages (45.7×30×30 cm^3) and fed with diets containing three levels of guar meal (GM; 0, 4 and 8%) and two levels of β-mannanase (Hemicell®) enzyme (0.00 and 0.05%) for 12 wk period. There were four replicates per treatment with three hens in each replicate. Egg yolk cholesterol and serum lipids (triglyceride, cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C)) concentrations were measured at 110 wk of age. No significant differences were observed when feeding GM on egg production (EP), feed intake (FI) and feed conversion ratio (FCR). Supplementation of enzyme significantly reduced FI and improved FCR ($P<0.05$). Significant interaction was observed between enzyme and GM on egg weight (EW) and egg mass (EM) ($P<0.05$). Adding enzyme, decreased EW and EM in 4% GM diet but had diverse effect in control diet. The results of this study indicated that GM can be used in laying hens diets in the second-cycle of production up to 8% without any adverse effects on performance. The GM diets reduced serum triglyceride ($P<0.05$) and slightly egg yolk cholesterol concentrations. Supplementation of beta-mannanase enzyme significantly improved FCR by decreasing FI and also has significantly effect on decreasing egg yolk cholesterol and serum triglyceride ($P<0.05$).

KEY WORDS cholesterol, enzyme, guar meal, laying hen, performance.

INTRODUCTION

Guar meal (*Cyamopsis tetragonoloba*), a by-product of guar gum extraction, contains 36 to 45% protein, which is valued by livestock producers (Conner, 2002; Zhang, 2004). In poultry nutrition, the use of GM was limited because of its adverse effects, such as diarrhea, depressed growth rate, and increased mortality, when fed at relatively high levels (Verma and McNab, 1982; Patel and McGinnis, 1985). Residual guar gum, a highly viscous galactomannan, is probably the primary factor responsible for the reported adverse effects (Verma and McNab, 1982; Conner, 2002; Lee *et al*. 2003). Patel and McGinnis (1985) found that *β*-mannan significantly decreased EP, EW, and FI in laying hens. Gutierrez *et al*. (2007) reported that GM can be fed to high production laying hen at levels up to 5% of the diet without adverse effects on performance. Cholesterol content of egg has recently received far more attention than before due to increased cardiovascular disease in man mainly arthrosclerosis, hypertension and coronary heart

disease. Various studies are currently being carried out in order to lower cholesterol content of egg and meat through the use of additives, dietary fiber and polyunsaturated fatty acid supplementation. El-Khier *et al*. (2009) have indicated supplementing laying hens with Gum Arabic significantly reduced serum cholesterol gradually and consequently eggs with lowered yolk cholesterol were obtained. In the other hand, Shahbazi (2012a) concluded that adding GM to diet of laying hens increased the serum level of cholesterol. Favier *et al*. (1998) reported that guar gum significantly decreased blood cholesterol in rats. Both guar gum and partially hydrolysed guar gum have been reported to depress plasma and serum triglycerides, triglyceride-rich lipoprotein, total cholesterol, and apolipoprotein E levels (Moundras *et al*. 1994; Yamada *et al*. 2003). Increased bile acid excretion seems to be essential in the cholesterol-lowering effect of soluble fibers and related compounds.

There is a little publication data with contradictory results available on effect of combination of GM and β-mannanase enzyme on blood parameters and egg yolk cholesterol concentrations of laying hens (Ehsani and Torki, 2010; Shahbazi, 2012b). This study was done to investigate the effects of different levels of GM and β-mannanase enzyme on performance, egg yolk cholesterol and serum lipid concentrations of laying hens at the second-cycle of production.

MATERIALS AND METHODS

The experiment was carried out at the Poultry Unit of Teaching and Research Farm Directorate, University of Sari, Iran. A total of 72 Hy-Line W-36 hens in the second cycle (98 wk old) were randomly assigned in a 3 × 2 factorial arrangements with completely randomized design to 24 cages (45.7×30×30 cm^3) and fed with diets containing three levels of guar meal (GM; 0, 4% and 8%) and enzyme was included at the manufacturer's recommended level of 0.05% for the 12-wk period. The dietary treatments consisted of 6 isocaloric, isonitrogenous laying hen diets with 0 (control), 4% or 8% of GM with and without addition of Hemicell®. Hemicell® is a fermentation product of *Bacillus lentus* (ATTCC 55045); its active ingredient is *β*-mannanase (EC 3.2.1.78), which hydrolyzes *β*-mannan. There were four replicates per treatment with three hens in each replicate. The experimental diets were prepared to provide 15.5% CP and 2900 kcal/kg ME (Table 1). Diets were formulated based on NRC feed ingredients table (NRC, 1994).

GM utilized in this experiment was obtained from the Aryannoosh Company, Shiraz, Iran. The composition of GM used in this study was previously determined by Conner (Conner, 2002) with amino acid analysis by Degussa-Huls Corporation (Allendale, NJ). The residual gum in GM was determined by HPLC (Hansen *et al*. 1992). Experimental feed and water were offered as *ad libitum*. After the 2-wk adaptation period, all birds received a 16L: 8D lighting schedule.

During the experiment traits such as, hen-day egg production (EP), feed intake (FI), and feed conversion ratio (FCR), egg weight (EW), and egg mass (EM) were recorded weekly. Egg yolk cholesterol level was determined weekly from one egg of each experimental unit by the method of Pasin (Pasin *et al*. 1998), thus at the end of experimental period data were averaged on the whole experimental period.

In order to determine some blood parameters, one bird from each pen was randomly chosen every week and blood samples were taken via the wing vein and centrifuged at 2000 × g for 30 min to separate serum from blood cell. Serum was separated and stored at -20 ˚C and then used to analysis serum lipids (triglyceride, cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C)) by using commercial kits (Ziestchem diagnostics, Tehran, Iran) and spectrophotometer apparatus. At the end of experimental period results were averaged as before.

Statistical analysis

In this experiment, data were analyzed as 3 × 2 factorial design with three concentrations of GM (0, 4 and 8%) and two levels of enzyme Hemicell® (0 and 0.05%). Treatment 1 was considered as a control group. The statistical model used was:

$$X_{ijk} = \mu + A_i + B_j + (AB_{ij}) + \epsilon_{ijk}$$

Where:
X_{ijk}: individual observation.
μ: experimental mean.
A_i: levels of GM effect.
B_j: enzyme effect.
AB_{ij}: levels of GM and enzyme interaction.
ϵ_{ijk}: error term.

Data were subjected to analysis of variance by using the GLM procedure of SAS (SAS, 2002). Duncan's multiple-range test was applied to separate means. Statements of statistical significance are based on a probability of ($P<0.05$).

RESULTS AND DISCUSSION

No significant differences were observed when feeding 4% or 8% GM on EP, FI and FCR ($P>0.05$) (Table 2). Supplementation of enzyme significantly reduced FCR by decreasing FI without changing EP ($P<0.05$).

Table 1 Ingredient and nutrient contents of the experimental diets

Ingredients (%)	Control	GM 4 (%)	GM 8 (%)
Corn	64.47	64.72	65.81
Guar meal (GM)[1]	0.00	4.00	8.00
Soybean meal	24.25	20.57	17.26
Fat (animal-vegetable blend)	2.37	1.61	1.00
DL-methionine	0.14	0.14	0.14
Limestone	5.50	5.50	5.50
Mono-calcium phosphate	24.7	24.8	24.9
Salt	0.30	0.30	0.30
Vitamins[2]	0.25	0.25	0.25
Trace minerals[3]	0.25	0.25	0.25
Hemicell®[4]	-	-/+	-/+
Calculated composition (%)			
Crude protein (N×6.25)	15.5	15.5	15.5
Lysine	0.82	0.82	0.82
Methionine	0.79	0.79	0.79
Crude fiber	2.62	2.88	3.10
Available phosphorus	0.46	0.46	0.46
Calcium	4.40	4.40	4.40
Metabolizable energy (kcal/kg DM)	2900	2900	2900

[1] The nutrient matrix used was: Crude protein: 43.7%; Metabolizable energy: 2200 kcal/kg; Methionine: 0.53%; Lysine: 2.00%; Calcium: 0.16% and Available phosphorus: 0.16%.
[2] Vitamin mix supplied the following per kg of diet: Retinol: 2.5 mg; Cholecalciferol: 25 mg; Tocopherol acetate: 7.34 mg; Menadione: 1.1 mg; Cyanocobalamin: 11.5 mg; Riboflavin: 5.5 mg; Ca pantothenate: 11 mg; Niacin: 53.3 mg; Choline chloride: 1.020 mg; Folic acid: 0.75 mg; Biotin: 0.25 mg; Delaquin: 125 mg and DL-methionine: 500 mg.
[3] Mineral mix supplied the following per kg of diet: Mn: 150 mg; Fe: 16.8 mg; Zn: 125.5 mg; Cu: 1.7 mg; I: 1.05 mg and Se: 0.25 mg.
[4] Hemicell® (ChemGen Crop., Gaithersburg, MD, USA) was used as the source of microbial β-mannanase to provide 70 unit β-mannanase/kg diet.

Table 2 Effects of different levels of guar meal and enzyme and their interaction on overall egg production (OEP), feed intake (FI), feed conversion ratio (FCR), egg weight (EW) and egg mass (EM) of laying hens

Main effects	Level (%)	EP (%)	FI (g)	FCR (g/g)	EW (g)	EM (g/hen)
Guar meal	0	79.6	98.7	2.10	62.3^{a}	49.6
	4	77.6	99.0	2.13	62.3^{a}	48.3
	8	78.7	98.7	2.16	61.4^{b}	48.3
SEM		0.82	0.12	0.023	0.27	0.50
P-value		0.248	0.111	0.143	0.040	0.119
Enzyme	0.00	78.7	99.1	2.17	61.8	48.5
	0.05	78.6	98.5	2.09	62.3	48.9
SEM		0.67	0.10	0.019	0.22	0.41
P-value		0.944	0.001	0.001	0.125	0.480
Interaction						
Guar meal (GM %)	Enzyme					
0	0.00	79.1	99.1	2.12	61.2^{b}	48.3^{b}
0	0.05	80.1	98.3	2.08	63.5^{a}	50.9^{a}
4	0.00	77.9	99.4	2.18	62.8^{a}	48.9^{ab}
4	0.05	77.5	98.7	2.09	61.7^{b}	47.7^{b}
8	0.00	79.2	99.0	2.22	61.3^{b}	48.4^{b}
8	0.05	78.3	98.4	2.10	61.6^{b}	48.3^{b}
SEM	-	1.6	0.17	0.033	0.39	0.71
P-value	-	0.672	0.796	0.432	0.001	0.027

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Significant difference was observed between hens fed with diets containing 8% GM and control group (0% GM) on EW.

Significant interaction was observed between enzyme and GM on EW and EM (P<0.05). Adding enzyme, decreased EW and EM in 4% GM diet but had diverse effect in control diet (P<0.05). No difference was induced by feeding 8% GM. Ehsani and Torki (2010) and Shahbazi (2012a) reported that including GM in laying hens diets more than 3% may decrease productive performance. Supplementing corn-soybean or corn-soybean-GM diets by β-mannanase would have beneficial effects on performance of hens especially in terms of FCR and EP. Guar gum residue contained in the meal increases the viscosity of digesta, thereby decreasing growth and feed efficiency. The mechanism of β-mannanase is to degrade βmannan, which is an antinutritional factor existing in many legumes (Ehsani and Torki, 2010).

GM had no significant effect on egg yolk cholesterol and blood parameters except reduced blood triglyceride levels (Table 3).

The serum triglyceride concentration of the hens receiving GM diets (with and without enzyme) was also lower (P<0.05) than the control hens. This finding is in agreement with the one reported by Shahbazi (2012b) who reported that none of the blood biochemical parameters except for cholesterol were affected by diet GM inclusion and enzyme supplementation.

The high viscosity of GM may contribute to some beneficial physiological functions including decreasing plasma cholesterol (Ehsani and Torki, 2010) but there is some report that adding GM to diet of laying hens increased the serum level of cholesterol (Shahbazi, 2012b).

Adding enzyme increased egg yolk cholesterol and blood triglyceride (P<0.05).

Interaction of GM and enzyme supplementation were not significant (P>0.05) on egg yolk cholesterol and some blood parameters measured. Hens fed with diet containing 8% GM without enzyme produced numerically eggs with lower yolk cholesterol concentration in comparison with hens fed with the control diet Turk and Barnett (1972) demonstrated that addition of certain fiber to a corn-soy laying hen diet decreased egg cholesterol concentration. Guar gum, which obtains from the endosperm of the legume, is also hypocholesterolemic (Rogel and Vohra, 1983). The active hypocholesterolemic agent of GM is a soluble fiber (guar gum residues) which can bind bile salt and other organic materials (Anderson and Chen, 1979). The last author attributed this lowered concentration of yolk cholesterol to lowered serum cholesterol that results from more cholesterol excretion. In animal experiments, Guar gum was noticed to reduce serum cholesterol, suggesting gum interference with dietary cholesterol absorption. Dietary fiber supplements are accompanied by increased fecal excretion of neutral sterols such as cholesterol and plant sterols as well as bile acids.

Table 3 Effects of different levels of guar meal and enzyme and their interaction on egg yolk cholesterol and some blood parameters of laying hens

Main effects	Level (%)	Egg yolk cholesterol (mg/g yolk)	Blood cholesterol (mg/dL)	Triglyceride (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
Guar meal	0	12.50	156.12	63.80 [a]	58.60	84.75
	4	10.99	149.10	50.54 [b]	60.66	78.33
	8	9.92	145.04	49.59 [b]	62.17	72.95
SEM		0.90	4.55	3.18	2.81	4.37
P-value		0.156	0.243	0.009	0.670	0.190
Enzyme	0	10.08	149.93	50.90	62.67	77.38
	0.05	12.19	150.24	58.39	58.28	79.97
SEM		0.74	3.07	2.61	2.29	3.57
P-value		0.049	0.953	0.047	0.191	0.614
Interaction						
Guar meal (GM %)	Enzyme					
0	0.00	11.87	157.68	63.15	60.28	84.76
0	0.05	13.13	154.56	64.45	56.92	85.75
4	0.00	9.69	151.96	46.48	61.61	81.05
4	0.05	12.29	146.23	54.60	59.7	75.6
8	0.00	8.69	141.09	43.07	66.13	66.34
8	0.05	11.15	149.00	56.12	58.22	79.56
SEM	-	1.28	6.41	4.52	3.97	6.19
P-value	-	0.847	0.539	0.442	0.736	0.323

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Fecal loss of bile salts plays a major role in the hypocholesterolemic effects of plant fiber (Anderson and Chen, 1979). It is suggested that guar gum may be effective in lowering plasma cholesterol by impairing cholesterol absorption and by accelerating the small intestine/liver cycling of bile acids, which is interestingly, accompanied by reduction of bile acid concentration in the large intestine (Favier *et al*. 1998). GM and enzyme have no significant effects on HDL and LDL (P>0.05).

CONCLUSION

The results of the present study showed that use of GM up to 8% in laying hens significantly decreased serum triglycerides concentrations (P<0.05) without any adverse effect on performance and supplementation of enzyme reduced FI, FCR, egg yolk cholesterol and serum triglyceride (P<0.05).

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