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Effects of Dietary Inclusion of Guar Meal Supplemented by β-Mannanase on Performance of Laying Hens, Egg Quality Characteristics and Diacritical Counts of White Blood Cells

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Abstract: Problem statement: Using Guar Meal (GM) in poultry diets has being limited because of having β-mannan, one of the Nonstarch Polysaccharides (NSP). In this study we try evaluating effects of enzyme supplementation of GM-included diets on productive performance of laying hens. **Approach:** A total number of 144 Lohmann LSL-Lite hens were divided in 24 cages (n = 6). Based on a 3 × 2 factorial arrangement of treatments, six iso-caloric and iso-nitrogenous diets including 3 levels of GM (0.0, 35.0 and 70.0 g kg^{-1}) with and without enzyme (Hemicell® a β-mannanase-based enzyme, 0.0 and 0.6 g kg^{-1}) were assigned to hens in 4 cages (replicates). Data was analyzed based on completely randomized design using GLM procedure of SAS. **Results:** Dietary GM inclusion significantly affected on Egg Production (EP) on weeks 2, 4 and 6 as well as the overall trail period. Hens fed the GM-included diets did have decreased EP compared to hens fed the control diet. Almost the same trend was observed in terms of Egg Mass (EM); so that hens fed the GM-included diets showed decreased EM compared to the hens fed the control diet. Enzyme supplementation did not have significant effect on EP in the present experiment, but EM was significantly improved in the hens fed the β-mannanase-supplemented diets on weeks 3, 6 and the overall experimental period. Dietary inclusion GM increased Feed Conversion Ratio (FCR) of laying hens compared to the hens fed the control diets on weeks 2, 4, 6 and overall trial period. **Conclusion/Recommendations:** 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.

Key words: Guar meal, enzyme, β-mannanase, performance, egg characteristics, laying hens

INTRODUCTION

Guar Meal (GM), a relatively inexpensive high protein meal, produced as a by-product of guar gum manufacture. The protein content of GM ranges between 33-60% depending on fraction type. GM results from combinations of two fractions, the germ and hull. The germ and hull constitute approximately 44 and 21% of the guar bean, respectively. However, the germ and hull proportions of the guar bean are not consistent with the relative amounts of the fractions mixed in guar meal. Also, the degree of contamination of the germ and hull fractions with guar gum is not equivalent within these proportions when mixed into commercial GM. Guar gum residue contained in the meal increases the viscosity of digesta, thereby decreasing growth and feed efficiency.

Improving poultry performance by dietary manipulation has been the goal of nutritionists. Modification NRC nutrient recommendations (Maroufyan *et al*., 2010; Fanooci and Torki, 2010), using feed additives like enzymes (Zangiabadi and Torki, 2010), organic acids (Mahdavi and Torki, 2009) or medicinal plants (Ghasemi *et al*., 2010; Najafi and Torki, 2010) has been reported by other researchers. A patented enzyme product (β-mannanase, Hemicell) has been shown to improve feed conversion in corn-soybean diets fed to layers (Zangiabadi and Torki, 2009). The mechanism of β-mannanase is to degrade β-mannan, which is an antinutritional factor existing in many legumes, including soybean and canola meals. Dietary inclusion of Hemicell has improved broiler performance of broilers fed corn-soybean meal- or corn-soybean meal-palm date-included diets (Zangiabadi and Torki, 2010).

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The objectives of the present study were to investigate effects of dietary including graded levels of GM supplemented by enzyme on productive performance of laying hens, egg quality characteristics and diacritical counts of white blood cells.

MATERIALS AND METHODS

A total number of 144 Lohmann LSL-Lite hens were divided in 24 cages (n = 6) with almost equal distribution of average body weight and egg production among cages. Hens in 4 cages (replicates) were assigned to feed on one the 6 experimental diets. Based on a 3×2 factorial arrangement of treatments, 6 iso-caloric and iso-nitrogenous diets (ME =2720 Kcal kg^{-1} and CP =145 g kg^{-1}) including GM (0.0, 35.0 and 70.0 g kg^{-1}) and enzyme (0.0 and 0.6 g kg^{-1}) were formulated (Table 1). Collected data of Feed Intake (FI), Egg Production (EP), Egg Mass (EM) and calculated Feed Conversion Ratio (FCR) during 6 week trial period was analyzed based on completely randomized design using GLM procedure of SAS.

RESULTS

Effects of dietary GM inclusion and enzyme supplementation on EP, FI, FCR, Egg Weight (EW) and EM during experimental period (6 weeks) are presented in Table 1-6, respectively. There was no interaction between dietary GM inclusion and enzyme supplementation on any of productive performance and egg quality traits (p>0.05). Dietary GM inclusion significantly affected on EP on weeks 2, 4 and 6 as well as the overall trail period (weeks 1-6). Hens fed the GM-included diets did have decreased EP compared to hens fed the control diet. Almost the same trend was observed in terms of EM; so that hens fed the GM-included diets showed decreased EM compared to hens fed the control diet (Table 6).

Table 1: Composition of the experimental diets

	g /100 g diet					
Guar meal (g/100 g diet)	0.0		3.5		7.0	
Hemicel (g/100 g diet)	0.00	0.06	0.00	0.06	0.00	0.06
Feed ingredients						
Corn	61.37	61.37	60.60	60.60	59.81	59.81
Soybean meal	13.15	13.14	8.61	8.61	3.99	3.99
Date pits	10.00	10.00	10.00	10.00	10.00	10.00
Fish meal	4.42	4.42	4.42	4.42	4.42	4.42
Soybean oil	0.68	0.68	0.68	0.68	0.68	0.68
Dicalcium phosphate	1.08	1.08	1.10	1.10	1.11	1.11
Limestone	8.45	8.45	8.46	8.46	8.46	8.46
Common salt	0.23	0.23	0.23	0.23	0.24	0.24
Guar meal	0.00	0.00	3.50	3.50	7.00	7.00
Hemicell	0.00	0.06	0.00	0.06	0.00	0.06
Vit. and Min. Premix[1]	0.25	0.25	0.25	0.25	0.25	0.25
Sand	0.05	-	1.91	1.85	3.79	3.73
DL-Methionine	0.06	0.07	-	-	-	-
Calculated analyses						
ME (Kcal/kg)	2720.00	2720.00	2720.00	2720.00	2720.00	2720.00
Crude protein (%)	14.58	14.58	14.58	14.58	14.58	14.58
Ether extract (%)	3.98	3.98	4.11	4.11	4.23	4.23
Crude fiber (%)	7.13	7.13	6.96	6.96	6.77	6.77
Calcium (%)	3.75	3.75	3.75	3.75	3.75	3.75
Available P (%)	0.29	0.29	0.29	0.29	0.29	0.29
Lys (%)	0.74	0.74	0.69	0.69	0.65	0.65
Met (%)	0.33	0.33	0.30	0.30	0.33	0.33
Met and Cys (%)	0.56	0.56	0.59	0.59	0.67	0.67

[1]The vitamin and mineral premix provide the following quantities per kilogram of diet: vitamin A, 10,000 IU (all-trans-retinal); cholecalciferol, 2,000 IU; vitamin E, 20 IU (α-tocopheryl); vitamin K3, 3.0 mg; riboflavin, 18.0 mg; niacin, 50 mg; D-calcium pantothenic acid, 24 mg; choline chloride, 450 mg; vitamin B12, 0.02 mg; folic acid, 3.0 mg; manganese, 110 mg; zinc, 100 mg; iron, 60 mg; copper, 10 mg; iodine, 100 mg; selenium, 0.2 mg and antioxidant, 250 mg

Table 2: Effects of dietary guar meal inclusion (0, 35.0 and 70 g kg^{-1}) and enzyme supplementation (0.0 and 0.6 g kg^{-1}) on egg production (%)

		Egg production (%)						
Weeks of trial		Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Weeks 1-6
Treatment								
Enzyme								
0.00		77.770	63.880	67.260	67.260	68.650	67.260	68.680
0.06		79.760	68.250	73.610	70.630	75.190	74.600	73.670
Guar meal (%)								
0.00		80.950	75.590a	76.190	75.890a	76.780	75.590a	76.830a
3.50		77.670	63.390b	70.230	66.660ab	68.150	71.420ab	69.590b
7.00		77.670	59.220b	64.880	64.280b	70.830	65.770b	69.590b
Guar meal (%)	Enzyme							
0.00	0.00	79.760	75.000	74.400	76.780	75.000	74.400	75.890
0.00	0.06	82.140	76.190	77.970	75.000	78.570	76.780	77.780
3.50	0.00	74.400	60.110	66.070	61.900	63.690	64.880	65.170
3.50	0.06	80.950	66.660	74.400	71.420	72.620	77.970	74.000
7.00	0.00	79.160	56.550	61.310	63.090	67.260	62.500	64.980
7.00	0.06	76.190	61.900	68.450	65.470	74.400	69.050	69.240
SEM MSEM		6.434	7.664	10.047	9.148	9.723	7.182	6.009
Source of variation		p-values						
Guar meal		0.514	0.001	0.107	0.048	0.219	0.042	0.012
Enzyme		0.460	0.179	0.139	0.378	0.116	0.022	0.056
Enzyme × Guar meal 0.354		0.766	0.886	0.472	0.855	0.344	0.514	

a-b: Means within a column (within main effects) with no common superscript differ significantly (p<0.05), SEM = Standard Error of Means

Table 3: Effects of dietary guar meal inclusion (0, 35.0 and 70 g kg^{-1}) and enzyme supplementation (0.0 and 0.6 g kg^{-1}) on feed intake

		Feed intake (g hen^{-1} day^{-1})						
Weeks of trial		Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Weeks 1-6
Treatment								
Enzyme								
0.00		119.470a	119.470	119.730	119.890	119.200	119.780	119.590
0.06		119.040b	118.730	119.400	119.360	118.930	119.180	119.110
Guar meal								
0.00		119.410	119.770	119.830	119.370	119.240	119.280	119.480
3.50		119.220	119.130	119.770	119.920	119.270	119.490	119.470
7.00		119.120	118.400	119.090	119.580	118.700	119.670	119.090
Guar meal	Enzyme							
0.00	0.00	119.700	119.820	119.880	120.000	120.000	120.000	119.900
0.00	0.06	119.130	119.730	119.790	118.750	118.480	118.570	119.070
3.50	0.00	119.400	119.370	120.000	119.850	119.730	120.000	119.720
3.50	0.06	119.050	118.900	119.550	119.850	118.810	118.980	119.210
7.00	0.00	119.310	119.220	119.310	119.820	117.880	119.340	119.150
7.00	0.06	118.930	117.580	118.870	119.340	119.520	120.000	119.040
SEM		0.387	1.327	1.077	0.826	2.158	1.525	0.755
Source of variation		p-values						
Guar meal		0.329	0.147	0.331	0.423	0.842	0.880	0.518
Enzyme		0.013	0.192	0.467	0.136	0.765	0.352	0.136
Enzyme × Guar meal		0.847	0.492	0.929	0.263	0.322	0.372	0.646

a-b: Means within a column (within main effects) with no common superscript differ significantly (p<0.05), SEM = Standard Error of Means

Although, enzyme supplementation did not have significant effect on EP in the present experiment (Table 2), EM was significantly improved in hens fed the β-mannanase-supplemented diets on weeks 3, 6 and the overall experimental period (weeks 1-6, Table 6). Hens fed the GM-included diets had decreased FI compared to hens fed the control diet on week 1 of the trial; however, FI of hens during week 2-6 did not significantly affected by dietary GM inclusion or enzyme supplementation (Table 3). As it is presented in Table 4, including GM to diets increased FCR of laying hens compared to hens fed the control diets on weeks 2, 4, 6 and overall trial period (weeks 1-6). In the present study, dietary supplementation by β-mannanase improved FCR on weeks 3, 6 and the overall experimental period. The results of dietary treatment on the measured egg quality characteristics were shown in Table 6.

Table 4: Effects of dietary guar meal inclusion (0, 35.0 and 70 g kg^{-1}) and enzyme supplementation (0.0 and 0.6 g kg^{-1}) on Feed Conversion Ratio (FCR)

		Feed conversion ratio (g feed: g egg)						
Weeks of trial		Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Weeks 1-6
Treatment								
Enzyme								
0.00		2.430	3.08	2.960a	2.940	2.810	2.940a	2.860a
0.06		2.370	2.83	2.590b	2.760	2.560	2.560b	2.610b
Guar meal								
0.00		2.340	2.53b	2.520	2.500b	2.460	2.540b	2.480b
3.50		2.380	3.03a	2.760	2.910ab	2.840	2.730ab	2.770a
7.00		2.470	3.31a	3.040	3.130a	2.740	2.980a	2.950a
Guar meal	Enzyme							
0.00	0.00	2.420	2.58	2.650	2.470	2.540	2.650	2.550
0.00	0.06	2.270	2.48	2.390	2.530	2.390	2.440	2.420
3.50	0.00	2.490	3.19	2.930	3.100	3.010	2.990	2.950
3.50	0.06	2.280	2.87	2.590	2.720	2.680	2.470	2.600
7.00	0.00	2.390	3.48	3.290	3.240	2.880	3.170	3.080
7.00	0.06	2.560	3.14	2.790	3.020	2.600	2.780	2.820
SEM		0.192	0.393	0.404	0.445	0.356	0.314	0.249
Source of variation		p-values						
Guar meal		0.408	0.003	0.058	0.034	0.115	0.041	0.005
Enzyme		0.425	0.134	0.039	0.339	0.099	0.009	0.025
Enzyme × Guar meal		0.133	0.800	0.832	0.609	0.875	0.621	0.692

a-b: Means within a column (within main effects) with no common superscript differ significantly ($p<0.05$), SEM = Standard Error of Means

Table 5: Effects of dietary guar meal inclusion (0, 35.0 and 70 g kg^{-1}) and enzyme supplementation (0.0 and 0.6 g kg^{-1}) on average egg weight

		Egg weight (g)						
Weeks of trial		Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Weeks 1-6
Treatment								
Enzyme								
0.00		63.55	62.40	62.260	62.747	62.910	61.760	62.600
0.06		63.36	62.48	63.300	62.152	62.810	63.540	62.940
Guar meal								
0.00		63.60	62.72	63.600	63.070	63.720	62.450	63.190
3.50		64.32	62.94	62.710	62.930	62.750	62.640	63.050
7.00		62.46	61.67	62.030	61.330	62.100	62.860	62.070
Guar meal	Enzyme							
0.00	0.00	62.68ab	62.01	62.400	63.200	63.400	61.070	62.460
0.00	0.06	64.52a	63.43	64.800	62.940	64.040	63.830	63.930
3.50	0.00	64.22a	63.26	63.020	63.450	63.220	62.980	63.360
3.50	0.06	64.42a	62.62	62.400	62.420	62.280	62.300	62.740
7.00	0.00	63.76a	61.94	61.350	61.580	62.090	61.220	61.990
7.00	0.06	61.16b	61.41	62.720	61.090	62.100	64.500	62.160
SEM		1.920	2.131	2.712	2.612	2.278	3.485	2.137
Source of variation		P values						
Guar meal		0.095	0.462	0.525	0.356	0.378	0.972	0.534
Enzyme		0.780	0.926	0.357	0.583	0.914	0.225	0.703
Enzyme × Guar meal		0.040	0.564	0.537	0.954	0.787	0.481	0.622

a-b: Means within a column (within main effects) with no common superscript differ significantly ($p<0.05$), SEM = Standard Error of Means

As it is shown in Table 7, among the egg quality traits, only the thickness of egg shell was significantly affected by dietary GM inclusion. Hens fed diet included 7% GM did have lower egg shell thickness compared to hens fed the other two experimental diets. Enzyme supplementation did not have any beneficial effects on the measured egg traits in this study.

The results of dietary effects on the white blood cell count in the present trial are shown in Table 8. There was no significant effect of dietary treatment on the white blood cell count.

Table 6: Effects of dietary guar meal inclusion (0, 35.0 and 70 g kg^{-1}) and enzyme supplementation (0.0 and 0.6 g kg^{-1}) on egg mass

		Egg mass (g hen^{-1} day^{-1})						
Weeks of trial		Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Weeks 1-6
Treatment								
Enzyme								
0.00		49.360	39.750	41.640b	42.100	43.150	41.420b	42.910b
0.06		50.600	42.720	46.430a	43.780	47.060	46.970a	46.260a
Guar meal								
0.00		51.450	47.390a	48.220a	47.790a	48.760	47.160	48.460a
3.50		50.250	39.980b	43.910ab	41.740b	42.760	44.640	43.880b
7.00		48.240	36.330b	39.980b	39.280b	43.790	40.800	41.400b
Guar meal	Enzyme							
0.00	0.00	50.000	46.430	46.190	48.570	47.560	45.390	47.350
0.00	0.06	52.910	48.360	50.260	47.020	49.970	48.930	49.570
3.50	0.00	48.120	38.060	41.420	39.190	40.290	40.830	41.320
3.50	0.06	52.380	41.900	46.390	44.280	45.230	48.450	46.440
7.00	0.00	49.970	34.760	37.320	38.540	41.600	38.060	40.045
7.00	0.06	46.510	37.910	42.650	40.030	45.980	43.540	42.770
SEM		4.143	4.879	5.287	5.282	5.590	4.860	3.253
Source of variation		p-values						
Guar meal		0.316	0.000	0.020	0.013	0.099	0.053	0.001
Enzyme		0.473	0.152	0.039	0.447	0.104	0.012	0.021
Enzyme × Guar meal		0.167	0.924	0.970	0.468	0.893	0.707	0.643

a-b: Means within a column (within main effects) with no common superscript differ significantly ($p<0.05$), SEM = Standard Error of Means

Table 7: Effects of dietary guar meal inclusion (0, 35.0 and 70 g kg^{-1}) and enzyme supplementation (0.0 and 0.6 g kg^{-1}) on egg quality characteristics (egg index, yolk index, Haugh unit, egg shell weight and egg shell thickness)

	Egg quality characteristics				
	Egg index	Yolk index	Haugh unit	Egg shell weight	Egg shell thickness
Treatment					
Enzyme					
0.00	75.700	43.890	69.080	6.900	38.950
0.06	75.070	43.920	68.620	7.080	39.220
Guar meal					
0.00	75.680	43.460	69.910	7.060	39.000ab
3.50	75.400	43.920	69.670	7.030	40.180a
7.00	75.080	44.340	66.970	6.880	38.090b
SEM	2.074	0.896	2.957	0.302	1.563
Source of variation	p-values				
Guar meal	0.848	0.175	0.115	0.460	0.048
Enzyme	0.470	0.944	0.708	0.174	0.676
Enzyme × Guar meal	0.614	0.577	0.576	0.579	0.501

a-b: Means within a column (within main effects) with no common superscript differ significantly ($p<0.05$), SEM = Standard Error of Means

Table 8: Effects of dietary guar meal inclusion (0, 35.0 and 70 g kg^{-1}) and enzyme supplementation (0.0 and 0.6 g kg^{-1}) on white blood cell counts (heterophil, lymphocyte, monocyte, eosinophil and basophil)

	Heterophile	Lymphocyte	Monocyte	Eosinophile	Basophile
Treatment					
Enzyme					
0.00	26.330	71.41	0.660	0.660	1.000
0.06	26.580	70.83	1.080	0.410	1.080
Guar meal					
0.00	28.120	69.25	1.120	0.620	0.870
3.50	25.370	72.12	0.870	0.500	1.120
7.00	25.870	72.00	0.620	0.500	1.120
SEM	4.151	3.998	1.006	0.716	1.006
Source of variation	p-values				
Guar meal	0.388	0.291	0.618	0.922	0.849
Enzyme	0.884	0.725	0.324	0.404	0.841
Enzyme × Guar meal	0.971	0.989	0.849	0.786	0.849

a-b: Means within a column (within main effects) with no common superscript differ significantly ($p<0.05$), SEM = Standard Error of Means

DISCUSSION

Some studies reported that there was no negative impact on productive performance after adding GM without enzyme to diets at concentrations up to 2.5% in broiler chicks (Lee *et al*., 2003a; 2003b) or 5% in laying hen diets (Gutierrez *et al*., 2007). Different indigestible polysaccharides, such as pectin, gum Arabic, gum agar, locust bean gum and guar gum, increase intestinal viscosity, which decreases growth and increases feed conversion. Degradation of these gums could allow for by-product meals to be used in poultry diets to decrease the cost of feeding. Generally, viscosity increased with each treatment as digesta traveled through the small intestine from duodenum to jejunum to ileum. Lee *et al*. (2005) reported that GM can be used at up to 5% with β-mannanase enzyme in broilers. Jackson *et al*. (2004) reported that Hemicell improved weight gain and FCR of broilers.

Hydrolyzing the gum and decreasing digesta viscosity should increase starch digestibility in the small intestine, thereby leading to improved growth and feed efficiency. It has been reported that laying hens induced to molt by GM feeding exhibit improved resistance to *Salmonella* Enteritidis colonization when compared with hens molted by complete feed withdrawal. Furthermore, supplementation of β-mannanase (Hemicell) to diets containing high levels of GM appears to enhance resistance to *Salmonella* Enteritidis colonization in molted laying hens (Gutierrez *et al*., 2008).

In the study by Lee *et al*. (2003a), the hull fraction of gaur increased intestinal viscosity at all inclusion levels fed (0, 2.5, 5.0, 7.5 and 10.0%). In their investigation, although FCR was not affected until the inclusion rate exceeded 5.0%; whereas, the germ fraction significantly increased intestinal viscosity at 7.5 and 10% inclusion rates. Lee *et al*. (2005) reported that Hemicell significantly reduced the FCR of guar germ diets to a level comparable with the positive and negative control diets. Daskiran *et al*. (2004) added Hemicell at 0.5, 1 and 1.5% in a corn-soy-based starter diet containing 1% GM and found that Hemicell improved FCR at all inclusion levels.

The significant effect of Hemicell on immunity of broilers may be explained by the findings of Wu *et al*. (2005) who reported that substrate of Hemicell entering the intestinal tract resulted in a reduction of the β-mannan content associated with a reduction of innate immune stimulation. An important mode of action is a reduction in innate immune stimulation associated with a reduction in the β-mannan content of substrate entering the intestinal tract. β-mannans crossing the intestinal mucosa are potent stimulators of the innate immune system, resulting in increased proliferation of macrophages and monocytes and resultant cytokine production.

CONCLUSION

From the results of the present investigation it can be concluded that including GM in laying hens more than 3 % may cause decrease productive performance. In addition, supplementing corn-soybean or corn-soybean-guar meal diets by β-mannanase would have beneficial effects on performance of hens especially in terms of feed conversation ratio and egg production.

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