

## Dietary Inclusion of Guar Meal Supplemented by B-Mannanase II) Evaluation Egg Quality Characteristics and Blood Parameters of Laying Hens

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**Abstract:** To assess effects of dietary inclusion of guar meal (GM) supplemented with a commercial enzyme product with main activity of  $\beta$ -mannanase on egg quality characteristics and blood parameters of laying hens, one hundred and forty four 74-week-old Lohmann LSL-Lite hens, with an average laying rate of  $86.3 \pm 3.8\%$  (late production phase) and  $1410 \pm 18$  g live body weight, were divided in 24 cages ( $n=6$ ). Hens in 4 cages (replicates) were randomly assigned to feed on one of the six experimental diets. Based on a  $3 \times 2$  factorial arrangement, six iso-caloric and iso-nitrogenous diets (ME=2720 kcal/kg and CP=145.8 g/kg) including three levels of guar meal (0.0, 25 and 50.0 g  $\text{kg}^{-1}$ ) with and without enzyme (Hemicell®, 0.0 and 0.4 g  $\text{kg}^{-1}$ ) were formulated. To determine blood biochemical parameters and differentiable count of white blood cells, one hen per replicate was bled via wing vein on day 35 of trial. To determine egg quality parameters, all eggs during three frequent days were collected on week 4 of trial. Collected data of blood biochemical parameters and egg quality traits was analyzed based on completely randomized design using GLM procedure of SAS. Adding GM to diet of laying hens did not significant effect on egg traits including egg index, yolk index, Haugh unit, egg shell weight and thickness. Diet enzyme supplementation decreased yolk index and egg shell thickness. Interactions between diet GM inclusion and enzyme supplementation on white blood cell count and plasma level of IgG were not statistically significant. There was no significant effect of diet GM inclusion on white blood cell count and plasma level of IgG. Enzyme supplementation decreased the blood counts of heterophill and basophil and increased lymphocyte. None of the blood biochemical parameters except for cholesterol was affected by diet GM inclusion and enzyme supplementation. Adding GM to diet of laying hens increased the serum level of cholesterol.

**Key words:** Guar Meal • Enzyme • Egg Production • Feed Intake • Feed Conversion Ratio • Laying Hens

### INTRODUCTION

Guar (*Cyamopsis tetragonoloba*) is a drought tolerant legume most prominently produced in India and Pakistan. Isolation of the galactomannan gum yields a high protein by-product containing guar hull, germ and residual gum [1].

Galactomannan gum in turn is used as pharmaceuticals as well as in oil well drilling mud, ore flotation, paper making and even explosives. The imported guar gum are usually in the form of guar splits rather than whole seeds as the by-product guar meal (GM) usually remains as a source of protein for use in animal feeds. Efforts are underway to increase production of guar as it is an excellent drought tolerant rotational crop with cotton and sorghum that does not require irrigation. Guar meal usually sells for almost half that of soybean meal and is most commonly used in cattle feedlot operations.

Increased production of guar beans may offer expanded opportunities for use in least cost poultry feeds. The crude protein content of GM varies from 35 to 47.5% on a dry matter basis [2]. Verma and McNab [3] reported that about 88% crude protein in GM was found to be present as true protein and rich in arginine. However, methionine and lysine concentrations were comparatively lower than concentrations typically found in soybean meal [4] and inadequate for optimum rat growth [5]. Ambegaokar *et al.* [2] suggested that tryptophan, methionine and threonine were the first three deficient amino acids of GM when compared to whole egg protein. The gross energy of raw and autoclaved GM were reported as 4.837 and 4.861 kcal/g while the N-corrected ME values of raw and autoclaved GM were 2.005 and 2.069 kcal/g respectively [6]. Guar meal also contains chemical compounds called saponins that are ranged from 5 to 13% by weight of dry matter [7, 8]. Saponins are currently being investigated for

antibacterial [8, 9], antiprotozoal [10] and antifungal [11] activities. Since both saponins and protein are concentrated in the hull and germ fractions of the guar bean, guar meal [2, 12] may be useful as a practical ingredient for poultry feed [13-15].

Excessive concentrations of GM in poultry diets cause diarrhea, depresses growth rate and increases mortality of broilers [16-20] and decreases egg production and feed efficiency of laying hens [20-23]. Severe depression in egg production to cessation of lay were observed by Zimmermann *et al.* [24] who fed laying hens 10 and 15% GM to induce a molt and later obtained a satisfactory post-molt laying performance. Bakshi *et al.* [25] proposed that GM contains two deleterious factors: trypsin inhibitor and guar gum residue. The trypsin inhibitor was listed as a deleterious factor because the chicks fed GM had been reported to present pancreatic hypertrophy [26] which can also be found in chickens fed un-heated soybean meal. However, the trypsin inhibitor was not universally accepted as a primary factor for the deleterious effects of feeding guar product to poultry [27,28]. Verma and McNab [19] reported that neither heating the GM directly nor steam pelleting diets containing GM had much effect on the performance of the broiler chicks, which was in agreement with the findings of Nagpal *et al.* [29] who reported autoclaving GM did not improve its gross protein value for chicks.

Guar gum is a galactomannan polysaccharide consisting of a 1-4-linked  $\beta$ -D-mannopyranose backbone with branched 1-6- $\alpha$ -D-galactopyranose. The residual gum content of typical GM is approximately 18 to 20% [29, 30]. A series of feeding experiments conducted by Vohra and Kratzer [28] demonstrated that as little as 1% guar gum in broiler chicken diets causes a depression of growth. When the diet contained 2% guar gum, the relative growth of broiler chickens was 61 to 67.4% of controls. Improving poultry performance by dietary manipulation has been the goal of nutritionists. Using feed additives like enzymes [31, 32], organic acids [33] or medicinal plants [34,35] has been reported by other researchers. Addition of feed enzymes to improve dietary nutrient utilization has become popular during the last 10 yr. There are growing interests in the potential of other enzyme products to improve performance of poultry provided with corn-soybean meal based diets. Hemicell is a fermentation product of *Bacillus lentus*. Its active ingredient is  $\beta$ -mannanase, which can hydrolyze  $\beta$ -mannan in feed.  $\beta$ -Mannan in ingredients such as guar, soybean meal and sesame meal, is a powerful antinutritional factor. Guar gum can dramatically alter the viscosity of the contents of the gastro-intestinal tract

resulting in significant physiological effects. High viscosity is generally connected with delayed gastric emptying and increased small intestinal transit time, hence inhibiting the absorption of nutrients [36]. This investigation was conducted to evaluate effects of dietary inclusion of GM supplemented with  $\beta$ -mannanase enzyme on egg quality characteristics and blood parameters of laying hens.

## MATERIALS AND METHODS

A total number of one hundred forty four 74-week-old Lohmann LSL-Lite hens, with an average laying rate of  $86.3 \pm 3.8\%$  (late production phase) and  $1410 \pm 18$  g live body weight, were divided in 24 cages (n=6). Hens in 4 cages (replicates) were randomly assigned to feed on one of the six experimental diets. Based on a 3 $\times$ 2 factorial arrangement, six iso-caloric and iso-nitrogenous diets (ME=2720 kcal/kg and CP=145.8 g/kg) including three levels of guar meal (0.0, 25 and 50.0 g kg<sup>-1</sup>) with and without enzyme (Hemicell®, 0.0 and 0.4 g kg<sup>-1</sup>) were formulated. The hens were housed in laying cages made from galvanized metal wire which provided approximately 430 cm<sup>2</sup>/hen. The cages were located in a windowless and environmentally controlled room with the room temperature kept at 21-23°C and the photoperiod set at 16 h of light (incandescent lighting, 10 lux) and 8 h dark. Each cage had a nipple waterer. Water was available *ad libitum* throughout the experiment. Feed consumption was measured on a weekly basis. To determine blood biochemical parameters and differentiable count of white blood cells, one hen per replicate was bled via wing vein on day 35 of trial. Collected data of blood biochemical parameters and egg quality traits was analyzed based on completely randomized design using GLM procedure of SAS. All statements of significance are based a probability of less than 0.05. The mean values were compared by Duncan's multiple range test.

## RESULTS AND DISCUSSION

Effects of adding GM to laying hens' diet with and without enzyme on egg quality characteristics are presented in table 1. Interactions between diet GM inclusion and enzyme supplementation on egg quality traits were not statistically significant, except for Haugh unit. Adding GM to diet of laying hens did not significant effect on egg traits including egg index, yolk index, Haugh unit, egg shell weight and thickness. Diet enzyme supplementation decreased yolk index and egg shell thickness. The interior and exterior quality of eggs were

Table 1: Effect of dietary inclusion of guar meal (0, 25 and 50 g/kg) and enzyme supplementation (0 and 0.4g/kg) on egg quality traits (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	74.48	43.19 <sup>a</sup>	70.43	7.16	39.18 <sup>a</sup>
	0.06	75.22	42.58 <sup>b</sup>	70.96	7.01	37.77 <sup>b</sup>
Guar meal						
	0.00	74.67	42.95	71.78	7.15	37.96
	2.50	75.38	42.92	69.62	7.05	38.90
	7.00	74.51	42.76	70.68	7.08	38.56
Guar meal	Enzyme					
	0.00			71.47 <sup>ab</sup>		
	0.00			72.09 <sup>a</sup>		
	2.50			71.59 <sup>ab</sup>		
	2.50			67.6 <sup>5c</sup>		
	5.00			68.23 <sup>bc</sup>		
	5.00			73.13 <sup>a</sup>		
	SEM	0.92	0.27	1.09	0.13	0.71
	CV	2.46	1.28	3.09	3.56	3.71
Source of variation		Probability				
Guar meal	0.609	0.764	0.171	0.724	0.431	
Enzyme	0.337	0.014	0.560	0.161	0.025	
Enzyme × Guar meal		0.963	0.111	0.003	0.101	0.459

a-b Means within a column (within main effects) with no common superscript differ significantly (P <0.05), SEM= Standard error of means

Table 2: Effect of dietary inclusion of guar meal (0, 25 and 50 g/kg) and enzyme supplementation (0 and 0.4g/kg) on white blood cell counts (heterophil, lymphocyte, monocyte, eosinophil, basophile and heterophil to lymphocyte ratio) and Immunoglobulin Igg(g/l).

		Hetrophil	lymphocyte	Monocyte	Eosinophil	Basophile	IgG (g/l)
Treatment							
Enzyme							
	0.00	30.50 <sup>a</sup>	66.50 <sup>b</sup>	0.83	0.50	1.66 <sup>a</sup>	0.751
	0.04	26.58 <sup>b</sup>	71.83 <sup>a</sup>	0.25	0.33	0.75 <sup>b</sup>	0.765
Guar meal							0.00
		28.25	69.12	0.62	0.50	1.50	0.72
	2.50	28.50	69.87	0.37	0.12	0.87	0.77
	5.00	28.87	68.50	0.62	0.62	1.25	0.77
	SEM	2.21	2.38	0.44	0.31	0.45	0.02
	CV	15.49	6.89	164.26	149.66	74.92	7.87
Source of variation		Probability					
Guar meal		0.960	0.847	0.812	0.273	0.399	0.402
Enzyme		0.043	0.013	0.125	0.521	0.023	0.572
Enzyme × Guar meal		0.093	0.071	0.447	0.159	0.705	0.529

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: Effect of dietary inclusion of guar meal (0, 25 and 50 g/kg) and enzyme supplementation (0 and 0.4g/kg) on blood parameters

Treatment		<sup>1</sup> FBS	<sup>2</sup> TG	Cholesterol	<sup>3</sup> HDL	<sup>4</sup> LDL	Phosphor	Uric acid
Enzyme								
0.00		235.67	1742.6	181.42	32.33	61.41	5.90	4.36
0.04		216.50	1666.5	170.75	32.75	60.75	5.62	4.79
Guar meal								
0.00		231.50	1170.7	146.25 <sup>b</sup>	29.25	53.00	5.51	4.47
2.50		220.00	1879.1	164.75 <sup>ab</sup>	31.50	61.50	5.46	4.82
5.00		226.75	2063.8	217.25 <sup>a</sup>	36.87	68.75	6.31	4.43
Guar meal	Enzyme							
0.00	0.00	238.75	1194.75	144.75	26.00	51.00	5.60	4.27
0.00	0.04	224.25	1146.62	147.75	32.50	55.00	5.42	4.67
2.50	0.00	213.75	2102.25	183.00	33.75	65.75	6.15	4.77
2.50	0.04	226.25	1656.00	146.50	29.25	57.25	4.77	4.87
5.00	0.00	254.50	1930.75	216.50	37.25	67.50	5.95	4.05
5.00	0.04	199.00	2196.75	218.00	36.50	70.00	6.67	4.82
SEM		16.04	244.99	25.10	5.29	6.94	0.78	0.58
CV		14.19	49.86	28.51	32.52	22.74	27.14	25.25
Source of variation					Probability			
Guar meal		0.774	0.114	0.029	0.355	0.104	0.489	0.766
Enzyme		0.161	0.828	0.609	0.924	0.907	0.672	0.382
Enzyme × Guar meal		0.131	0.708	0.677	0.582	0.625	0.421	0.845

<sup>1</sup>Fasting blood sugar, <sup>2</sup>triaclyglycerol, <sup>3</sup>low density lipoprotein, <sup>4</sup>high density lipoprotein

-b Means within a column (within main effects) with no common superscript differ significantly (P <0.05), SEM= Standard error of means.

reported not to be deleteriously affected by GM feeding [17, 20, 24, 37] except that the yolk color index decreased with the inclusion of guar meal in laying diets [3]. Feeding of GG or GM did not affect egg weight or shell quality, but decreased the egg yolk color and Haugh units. Guar increased absolute and relative liver weight, but did not affect the weights of the pancreas, spleen, or the incidence of fatty liver or liver hemorrhage. Feeding 10% GM depressed feed consumption and increased body weight loss. Feeding 15% GM severely depressed egg production followed by a recovery of production after returning to 0% GM feeding [38].

Effects of adding GM to laying hens' diet with and without enzyme on white blood cell count as well as plasma level of IgG are presented in table 2. Interactions between diet GM inclusion and enzyme supplementation on white blood cell count and plasma level of IgG were not statistically significant. There was no significant effect of diet GM inclusion on white blood cell count and plasma level of IgG. Enzyme supplementation decreased the blood counts of heterophils and basophils and increased lymphocytes. As it is presented in table 3, none of the blood biochemical parameters was affected by diet GM inclusion and enzyme supplementation, except for

cholesterol. Adding GM to diet of laying hens increased the serum level of cholesterol. However, in other studies have been reported that the high viscosity of guar gum may contribute to some beneficial physiological functions including decreasing postprandial serum glucose [39, 40] and attenuating postprandial hypotension in type 2 diabetes patients [41-42], decreasing plasma cholesterol [43-47].

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