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Research Article
Effect of Dietary Inclusion of Guar Meal with or without β-mannanase Supplementation on Broiler Performance and Immunity

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Abstract

Objective: The current study was conducted to investigate the effect of dietary inclusion of 5% Guar Meal (GM) as a partial replacement for soybean meal (SBM) with or without β-mannanase enzyme (0.03%) on performance, carcass traits, blood biochemical and histological picture of intestine as well as immunity parameters of broilers. **Materials and Methods:** In this study, about 496 one-day-old male broiler chicks (Ross 308) were divided to 4 groups with 4 replicates of 31 chicks each. The trial continued for 35 days. The data were analyzed as 2×2 factorial arrangement in a completely randomized design. **Results:** The results showed that body weight gain (BWG), dressed weight (DW), thigh weight and breast weight were significantly lowered in birds fed diet containing 5% GM than those fed SBM diet ($p<0.05$). Villus height were significantly ($p<0.05$) reduced in birds fed diet containing 5% GM compared with those fed SBM. β-mannanase supplementation of GM diet significantly improved BWG, DW, breast weight, thigh weight, feed conversion ratio (FCR), villus height and increased thymus and bursa lymphocyte number ($p<0.05$). **Conclusion:** The results demonstrated that GM could be used at 5% of broiler diet with β-mannanase supplementation without adverse effects on growth performance and blood biochemistry.

Key words: β-mannanase, guar meal, broiler, growth parameters, immunity

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Guar meal is the residual part of guar plant (*Cyamopsis tetragonoloba*) after production of guar gum. It is considered as a relatively inexpensive protein source for poultry. It is composed of two parts; germ fraction with high protein content and hull fraction with low protein content. The crude protein level of guar meal vary from 35-47.5% according to fraction type[1]. Moreover, around 88% of the nitrogen content of guar meal is true protein, with arginine content nearly twice that of soybean meal which makes guar meal economically useful as a partial replacement for soybean meal in poultry diets[2,3].

The use of guar meal at high levels in poultry diets has been limited due to its adverse effects which include diarrhea, decreasing growth rate and increasing mortality[4,5]. These negative effects were prominent in younger birds than older birds[4]. These adverse effects might be attributed to the presence of some anti-nutritional compounds in guar meal such as guar gum, trypsin inhibitors, saponins and some unknown toxic substances[6].

Gaur meal contains 13-18% guar gum[7], which is residual galactomannans gum. β-galactomannans also referred to as β-mannans are linear polysaccharides with repeating units of β-1-4 mannose with β-1-6 glucose or galactose attached to the β-mannan backbone[8]. They are highly viscous, water soluble and heat resistant compounds[9]. β-mannans are considered powerful anti-nutritional factors for monogastric animals[10], increasing digesta viscosity and decreasing nutrient digestibility in broiler chicken[11]. They trigger the innate immune response and use of energy which would otherwise be available for growth[12]. In addition, β-mannans reduce glucose absorption and insulin secretion[13]. Different studies[3,12,14] have illustrated that the inclusion of 2-4% β-mannans in broiler feed severely retards growth, FCR and feed efficiency.

β-mannanase is a fermentation product of *Bacillus lentus* that degrades β-mannans in broiler feed. The enzyme cleaves randomly within the 1,4-β-D-mannan main chain of galactomannan, galactoglucomannan and mannan[15].

The use of a β-mannanase has been shown to improve feed: gain and reduce water: feed ratio, leading to drier fecal output[12]. β-mannanase supplementation to the broiler's diet resulted in decreased intestinal viscosity and increased growth and feed efficiency[3]. Supplementing a corn/soybean-based diet with β-mannanase has been shown to improve both growth and feed efficiency by 3%[16].

Therefore, the objective of this study was to investigate the effect of partial replacing soybean meal with 5% guar meal with or without β-mannanase supplementation on growth performance, carcass traits, serum biochemical and immunological parameters of broiler chickens.

MATERIALS AND METHODS

All procedures and protocols follow the Research Ethics committee of faculty of veterinary medicine, Cairo University.

Enzyme characteristics: The granular form of β-mannanase enzyme (Hemicell HT; Elanco Animal Health) was used in this study. Endo 1,4-β-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 temperature of 40°C.

Guar meal characteristics: Guar Korma Meal (Kar Agro Company, India) is a by-product of guar gum industry consisting of the guar germ material is called guar meal 50%. Nutrient compositions of guar Korma meal used are shown in Table 1.

Experimental design: The trial was conducted at research center of Department of Poultry Disease, Faculty of Veterinary Medicine, Cairo University. A total of 496 one-day-old male broiler chicks (Ross 308) were obtained from Al-Wady Company, Egypt. Chicks with an average body weight of 47.53±0.08 g were randomly divided into 4 treatment groups with 4 replicates of 31chicks per replicate. Birds were housed in an open house system on wood shavings with a constant lighting program (23 h on - 1 h off) during the whole experimental period (5 weeks). Birds were provided continuously with clean drinking water. All birds were kept under standard hygienic conditions and subjected to prophylactic vaccination program against viral diseases. The

Table 1: Nutrient compositions of guar Korma meal (Kar Agro Company, India)

Items	Chemical analysis (as fed basis)
ME (kcal kg^{-1})	3,191
Moisture (%)	8.00
CP (%)	48.00
CF (%)	4.05
EE (%)	7.08
NFE (%)	28.54
Total phosphorus (%)	0.74
Available phosphorus (%)	0.23
Ca (%)	1.12
L-lysine (%)	2.17
Methionine (%)	0.57

ME: Metabolizable energy, CP: Crude protein, CF: Crude fiber, EE: Ether extract, NFE: Nitrogen free extract

Table 2: Diet composition and chemical analysis

	Starter (0-10 days)		Grower (11-24 days)		Finisher (25-35 days)	
Items	SBM*	GM**	SBM	GM	SBM	GM
Ingredients (g/100 g)						
Yellow corn	51.46	53.01	54.69	56.41	60.36	61.98
SBM (45.5%)	37.34	31.92	33.40	27.87	26.06	20.61
Corn gluten meal	3.00	3.00	3.20	3.20	4.70	4.70
Guar meal (48%)	0.00	5.00	0.00	5.00	0.00	5.00
DL-Methionine	0.17	0.18	0.13	0.14	0.10	0.11
L-Lysine	0.21	0.27	0.16	0.22	0.23	0.30
Soy oil	3.80	2.69	4.71	3.55	4.90	3.76
Mono calcium phosphate	1.60	1.63	1.45	1.46	1.28	1.29
Broiler premix#	0.30	0.30	0.30	0.30	0.30	0.30
Choline chloride	0.10	0.10	0.10	0.10	0.10	0.10
Lime stone	1.59	1.47	1.44	1.33	1.57	1.45
Sodium chloride	0.35	0.35	0.30	0.30	0.30	0.30
Sodium bicarbonate	0.08	0.08	0.12	0.12	0.10	0.10
Total	100.00	100.00	100.00	100.00	100.00	100.00
Calculated analysis (%)						
ME (kcal kg^{-1})	3,000	3,000	3,100	3,100	3,200	3,200
CP	23.00	23.00	21.50	21.50	19.60	19.60
CF	3.78	3.64	3.58	3.43	3.21	3.06
Lysine	1.44	1.44	1.29	1.29	1.16	1.16
Methionine	0.56	0.56	0.51	0.51	0.47	0.47
Ca	0.96	0.96	0.87	0.87	0.87	0.87
Total P	0.75	0.75	0.72	0.72	0.69	0.69
Available P	0.48	0.48	0.44	0.44	0.39	0.39
Chemical analysis (%) (as fed basis)						
CP	23.02	23.01	21.72	21.63	19.84	19.78
CF	3.85	3.71	3.60	3.48	3.26	3.09
Ca	0.97	0.98	0.88	0.86	0.89	0.88
Total P	0.77	0.76	0.74	0.74	0.71	0.70

*SBM: Corn-soybean meal-based experimental diet, **GM: Corn-soyabean based diet containing 5% guar meal, ME: Metabolizable energy, CP: Crude protein, CF: Crude fiber, #Broiler premix (per kg of diet), Vitamin A: 15,000 IU, Vitamin D_3: 1,500 IU, Vitamin E: 20 mg, Vitamin K_3: 5 mg, Vitamin B_1: 3 mg, Vitamin B_2: 6 mg, Niacin: 25 mg, Vitamin B_6: 5 mg, Vitamin B_{12}: 0.03 mg, Folic acid: 1 mg, D-biotin: 0.05 mg, Ca-pantothenate: 12 mg, Carophyll-yellow: 25 mg, Choline chloride: 400 mg, Mn: 80 mg, Fe: 60 mg, Zn: 60 mg, Cu: 5 mg, Co: 0.2 mg, I: 1 mg and Se: 0.15 mg

experimental design was a 2×2 factorial arrangement of 2 levels of guar meal (0 and 5%) and 2 levels of β-mannanase (0 and 0.03%). The experimental diets were formulated according to the breed nutrition specifications[17] and offered to the birds for starter (0-10 days), grower (11-24 days) and finisher (25-35 days) periods. Calculations and chemical analysis of different diets were performed according to AOAC International[18]. Diet composition and chemical analysis are shown in Table 2. Birds in different experimental groups were individually weighed at the start of the experiment then weekly till the end of the experimental period. Then body weight gain (BWG), feed intake (FI) and FCR were calculated.

Blood samples and carcass traits: At the end of the experimental period, 3 birds per replicate of experimental groups were randomly taken and slaughtered, scalded at 55-65°C, de-feathered, eviscerated and washed with tap water. The breast meat was cut from the remaining upper back and rib cage of the carcass, washed, cooled in ice water tank for 2 h, dried for 10 min. The dressing yield (%), breast muscle Yield (BMY%) were calculated according to El-Banna *et al.*[19]. The intestine, gizzard, liver, heart, spleen, bursa and thymus were dissected and weighed and weights relative to dressed carcass weight (g/100 g) were calculated.

Blood samples were taken during slaughter. Non-haemolyzed sera were separated by centrifugation at 1,500×g for 15 min at 4°C, stored in deep freezer at -20°C until analysis to determine serum alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), uric acid and creatinine using commercial kits (Biomaria, France). Blood glucose level was measured directly after separation of sera using commercial kits (Biomaria, France).

Histomorphological examination: Tissue specimens from small intestine (ileum), thymus and bursa were collected and fixed in 10% neutral buffered formalin. The fixed tissue specimens were processed and embedded in paraffin wax,

sectioned at 4 mm and then stained with hematoxylin and eosin[20]. Histo-quantitative studies were performed by measuring the villus height, crypt depth and villus to crypt ratio using an Olympus light microscope (Olympus, Japan) and image analysis software as described by Iji *et al.*[21]. Histo-quantitative studies were also performed by counting the number of thymus and bursa lymphocytes according to morphometric method of Culjkovic *et al.*[22]. The test areas of thymus and bursa were 3 random fields under light microscope, lymphocytes in these fields were counted and then the mean values were calculated for each sample.

Statistical analysis: The experiment was conducted using a 2×2 factorial arrangement of 2 levels of guar meal (0 and 5%) and 2 levels of β-mannanase (0 and 0.03%).

Statistical analysis was performed using the GLM procedure of SAS[23]. The differences between the treatment means were analyzed using Tukey's HSD test with a significance level of $p<0.05$[24].

RESULTS

Performance and carcass characteristics: The effect of guar meal with or without β-mannanase on growth performance and carcass characteristics of broiler chickens were summarized in Table 3 and 4. Data showed that partial replacement of SBM by 5% GM negatively ($p<0.05$) affected BWG, FCR, DW, breast weight and thigh weight. β-mannanase supplementation to GM diet significantly ($p<0.05$) improved BWG, DW, breast weight and thigh weight and decreased FCR in comparing with birds fed GM without enzyme. Feed intake of birds fed 5% guar meal with or without β-mannanase supplementation was numerically increased when compared with those fed SBM.

Relative weights (g/100 g dressed weight) of visceral organs (intestine, gizzard, liver and heart) were detailed at Table 5. The data showed that the intestine relative weight was significantly ($p<0.05$) increased by using GM. β-mannanase supplementation to GM diet significantly decreased intestine relative weight in comparing with birds fed GM without enzyme. The relative weight of gizzard, liver and heart did not significantly ($p>0.05$) affected by using GM with or without β-mannanase.

Histomorphological image of ileum: Data regarding histomorphological parameters at the end of the experiment for the different replicates of experimental groups were presented in Table 6. Results showed a significant ($p<0.05$) decrease in villus height and villus to crypt ratio of ileum of birds fed 5% GM when compared to those fed SBM diet. β-mannanase supplementation of GM diet significantly ($p<0.05$) increased villus height and improved villus to crypt ratio in comparing with birds fed GM without enzyme.

Table 3: Growth performance parameters

Items		Body weight (g) Initial	Body weight (g) Final	Total weight gain (g)	Total feed intake per chick (g)	FCR (g/g)
Treatments						
Diet	*Enzyme (0.03%)					
SBM	-	47.65[a]	1,917.86[ab]	1,870.21[ab]	3,199.57[a]	1.71[b]
GM	-	47.45[a]	1,837.05[c]	1,789.59[c]	3,321.31[a]	1.86[a]
SBM	+	47.60[a]	1,943.89[a]	1,896.29[a]	3,195.29[a]	1.69[b]
GM	+	47.40[a]	1,909.17[b]	1,861.77[b]	3,224.88[a]	1.73[b]
SEM		0.0829	10.842	10.828	19.267	0.019
Main effects of diet						
SBM		47.62[a]	1,930.88[a]	1,883.25[a]	3,197.43[b]	1.79[b]
GM		47.42[a]	1,873.11[b]	1,825.68[b]	3,273.10[a]	1.69[a]
Main effects of enzyme						
0		47.55[a]	1,877.46[b]	1,829.91[b]	3,260.44[a]	1.78[a]
0.03%		47.50[a]	1,926.53[a]	1,879.03[a]	3,210.09[a]	1.71[b]
SEM		0.119	11.373	11.375	23.216	0.017
p-value						
Diet		0.227	<0.001	<0.001	0.033	<0.001
Enzyme		0.781	<0.001	0.001	0.136	0.003
Diet×enzyme		1.000	0.014	0.014	0.169	0.031

[a,b]Means within a column with different superscripts are significantly different ($p<0.05$), *Hemicell HT, a β-mannanase-based enzyme commercial product, SBM: Corn-soybean meal-based diet, GM: Corn-soyabean based diet containing 5% guar meal, SEM: Standard error of mean

Table 4: Carcass traits of different experimental groups at the end of the experiment

Items		Live weight (g)	Dressed weight (g)	Dressing (%)	Breast weight (g)	Breast muscle yield (%)	Thigh weight (g)	Thigh muscle yield (%)
Treatments								
Diet	*Enzyme (0.03%)							
SBM	-	2,275.62[a]	1,536.87[a]	67.54	562.50[a]	36.60	747.50[a]	48.63
GM	-	2,186.75[b]	1,451.25[b]	66.36	483.12[b]	33.29	668.75[b]	46.08
SBM	+	2,303.75[a]	1,561.87[a]	67.79	595.62[a]	38.13	774.37[a]	49.57
GM	+	2,266.87[a]	1,531.87[a]	67.58	560.62[a]	36.59	740.00[a]	48.31
SEM		12.146	11.490		10.367		9.477	
Main effects of diet								
SBM		2,289.69[a]	1,549.38[a]		579.06[a]		760.93[a]	
GM		2,226.81[b]	1,491.56[b]		521.87[b]		704.37[b]	
Main effects of enzyme								
0		2,231.19[b]	1,494.06[b]		522.81[b]		708.12[b]	
0.03%		2,285.31[a]	1,546.88[a]		578.12[a]		757.18[a]	
SEM		14.026	13.287		10.984		9.765	
p-value								
Diet		0.003	0.004		0.001		<0.001	
Enzyme		0.010	0.008		0.001		0.001	
Diet×enzyme		0.195	0.142		0.157		0.109	

[a,b] Means within a column with different superscripts are significantly different (p<0.05), *Hemicell HT, a β-mannanase-based enzyme commercial product, SBM: Corn-soybean meal-based diet, GM: Corn-soyabean based diet containing 5% guar meal, SEM: Standard error of mean

Table 5: Internal organs relative weights (g/100 g dressed weight) of different experimental groups at the end of the experiment

		Relative weight (g/100 g dressed weight)			
Items		Intestine	Liver	Gizzard	Heart
Treatments					
Diet	*Enzyme (0.03%)				
SBM	-	7.544[b]	4.4612[a]	3.3866[a]	0.7311[a]
GM	-	10.055[a]	4.0690[a]	3.4824[a]	0.7428[a]
SBM	+	7.619[b]	4.2034[a]	3.7979[a]	0.5791[a]
GM	+	7.891[b]	4.3932[a]	3.5440[a]	0.7190[a]
SEM		0.302	0.128	0.090	0.028
Main effects of diet					
SBM		7.581[b]	4.332[a]	3.592[a]	0.655[a]
GM		8.973[a]	4.231[a]	3.513[a]	0.731[a]
Main effects of enzyme					
0		8.799[a]	4.265[a]	3.434[a]	0.737[a]
0.03%		7.755[b]	4.298[a]	3.671[a]	0.649[a]
SEM		0.378	0.188	0.128	0.039
p-value					
Diet		0.010	0.705	0.665	0.179
Enzyme		0.046	0.901	0.201	0.122
Diet×enzyme		0.033	0.281	0.341	0.254

[a,b] Means within a column with different superscripts are significantly different (p<0.05), *Hemicell HT, a β-mannanase-based enzyme commercial product, SBM: Corn-soybean meal-based diet, GM: Corn-soyabean based diet containing 5% guar meal, SEM: Standard error of mean

Immune response: Lymphoid organs relative weights were detailed in Table 7. The results showed a significant (p<0.05) increase in lymphoid organs relative weights as a result of dietary inclusion of guar meal at 5% in comparing with birds fed SBM. Moreover, β-mannanase supplementation to both SBM and GM diet significantly (p<0.05) increased lymphoid organs relative weights in comparing with the non supplemented groups.

The immune system of birds is constituted of several cells and soluble factors that must act together to produce a protective immune response[25]. Results presented in Table 8 showed that partial replacement of SBM by 5% guar meal significantly (p<0.05) increased lymphocyte number of thymus and bursa in comparing with birds fed SBM. Moreover, β-mannanase supplementation to both SBM and GM diet significantly (p<0.05) increased lymphocyte number of thymus and bursa in comparing with the non-supplemented groups.

Serum parameters: Results of serum parameters at the end of the experiment were detailed in Table 9. The results showed that serum concentration of glucose tended to be higher in

Table 6: Villi height, crypt depth and villus: Crypt ratio of ileum of different experimental groups at the end of the experiment

Items		Villus height (μm)	Crypt depth (μm)	Villus: Crypt ratio
Treatments				
Diet	*Enzyme (0.03%)			
SBM	-	708.93[a]	147.42[a]	4.82[a]
GM	-	567.33[b]	157.28[a]	3.61[b]
SBM	+	713.60[a]	149.09[a]	4.78[a]
GM	+	689.42[a]	152.34[a]	4.53[a]
SEM		18.269	1.821	0.152
Main effects of diet				
SBM		711.26[a]	148.26[a]	4.80[a]
GM		628.37[b]	154.81[a]	3.07[b]
Main effects of enzyme				
0		638.13[b]	152.35[a]	4.21[b]
0.03%		701.51[a]	150.72[a]	4.66[a]
SEM		14.528	2.360	0.124
p-value				
Diet		<0.001	0.085	<0.001
Enzyme		<0.001	0.638	0.001
Diet×enzyme		<0.001	0.352	<0.001

[a,b] Means within a column with different superscripts are significantly different (p<0.05), *Hemicell HT, a β-mannanase-based enzyme commercial product, SBM: Corn-soybean meal-based diet, GM: Corn-soyabean based diet containing 5% guar meal, SEM: Standard error of means

Table 7: Lymphoid organs relative weights (g/100 g dressed weight) of different experimental groups at the end of the experiment

Items		Relative weight (g/100 g dressed weight)		
		Spleen	Bursa	Thymus
Treatments				
Diet	*Enzyme (0.03%)			
SBM[2]	-	0.186[c]	0.197[c]	0.054[d]
GM[3]	-	0.250[ab]	0.275[b]	0.077[b]
SBM	+	0.237[b]	0.257[b]	0.064[c]
GM	+	0.320[a]	0.324[a]	0.088[a]
SEM[4]		0.013	0.010	0.003
Main effects of diet				
SBM		0.212[b]	0.227[b]	0.059[b]
GM		0.285[a]	0.299[a]	0.082[a]
Main effects of enzyme				
0		0.218[b]	0.235[b]	0.066[b]
0.03%		0.278[a]	0.290[a]	0.075[a]
SEM		0.014	0.009	0.002
p-value				
Diet		0.001	<0.001	<0.001
Enzyme		0.004	<0.001	0.004
Diet×enzyme		0.620	0.663	0.941

[a,b,c] Means within a column with different superscripts are significantly different (p<0.05), *Hemicell HT, a β-mannanase-based enzyme commercial product, SBM: Corn-soybean meal- based diet, GM: Corn-soyabean based diet containing 5% guar meal, SEM: Standard error of mean

β-mannanase supplemented groups. Dietary inclusion of guar meal at 5% with or without β-mannanase supplementation had no significant (p>0.05) effect on serum alkaline phosphatase, AST, ALT, uric acid and creatinine.

Table 8: Thymus and bursa lymphocyte number of different experimental groups at the end of the experiment

Items		Number of lymphocyte	
		Thymus lymphocyte count	Bursa lymphocyte count
Treatments			
Diet	*Enzyme (0.03%)		
SBM	-	933.33[c]	810.00[d]
GM	-	1110.00[b]	1076.67[c]
SBM	+	1196.67[b]	1466.67[b]
GM	+	1906.67[a]	2120.00[a]
SEM		113.112	150.095
Main effects of diet			
SBM		1065.00[b]	1138.33[b]
GM		1508.33[a]	1598.33[a]
Main effects of enzyme			
0		1021.67[b]	943.33[b]
0.03%		1551.67[a]	1793.33[a]
SEM		68.925	56.350
p-value			
Diet		<0.001	<0.001
Enzyme		<0.001	<0.001
Diet×enzyme		<0.001	0.005

[a,b,c] Means within a column with different superscripts are significantly different (p<0.05), *Hemicell HT, a β-mannanase-based enzyme commercial product, SBM: Corn-soybean meal-based diet, GM: Corn-soyabean based diet containing 5% guar meal, SEM: Standard error of mean

DISCUSSION

Results of the present study about BWG and FCR are consistent with the finding of Kamran *et al.*[26] who found that the addition of 5% guar meal to broiler diets reduced final body weight and feed efficiency. Similarly, Nasrala *et al.*[27] stated that using guar meal at levels more than 2.5% adversely affect weight gain, feed conversion and nutrient digestibility. The same findings were recorded by Lee *et al.*[28].

Regarding to the effect of β-mannanase supplementation of GM diet on BWG and FCR. The results are compatible with the results of Daskiran *et al.*[12] who reported an improvement in body weight and feed conversion ratio of broilers fed with β-mannanase supplemented diet containing various levels of β-mannan from guar gum. Lee *et al.*[28] and Nasrala *et al.*[27] concluded that guar meal can be used up to 5% with β-mannanase in broiler diet without adverse effect on chick performance.

The improved BWG and FCR by β-mannanase supplementation of GM diet may be attributed to the breakdown of the β-mannans, thereby increase energy availability, reduce intestinal viscosity and better nutrient absorption[29]. Moreover, the improvement in villus height and villus/crypt ratio of ileum may be a contributing factor.

Table 9: Serum biochemical parameters of different experimental groups at the end of the experiment

Items		Alkaline phosphatase (mg dL^{-1})	Creatinine (mg dL^{-1})	Uric acid (mg dL^{-1})	AST (U L^{-1})	ALT (U L^{-1})	Glucose (mg dL^{-1})
Treatments							
Diet	*Enzyme (0.03%)						
SBM[2]	-	64.75	0.36	3.62	37.45	22.25	221.17^{b}
GM[3]	-	70.50	0.44	4.16	45.91	25.37	220.79^{b}
SBM	+	65.00	0.44	3.79	39.54	26.50	242.96^{ab}
GM	+	64.50	0.42	3.89	36.15	31.00	254.82^{a}
SEM[4]		6.61	0.041	0.380	3.937	2.906	4.722
Main effects of diet							
SBM		64.87	0.42	3.83	43.49	21.87	232.06^{a}
GM		67.50	0.44	3.91	43.53	22.44	237.81^{a}
Main effects of enzyme							
0		67.62	0.42	3.89	44.18	22.56	220.97^{b}
0.03%		64.75	0.43	3.84	42.84	21.75	248.89^{a}
SEM		6.425	0.480	0.394	4.709	0.202	4.135
p-value							
Diet		0.282	0.307	0.480	0.986	0.505	0.493
Enzyme		0.241	0.206	0.636	0.496	0.340	0.002
Diet×enzyme		0.205	0.055	0.854	0.262	0.723	0.015

AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ^{a,b} Means within a column with different superscripts are significantly different ($p<0.05$), *Hemicell HT, a β-mannanase-based enzyme commercial product, SBM: Corn-soybean meal-based diet, GM: Corn-soyabean based diet containing 5% guar meal, SEM: Standard error of mean

Absorptive surface of the villi plays an important role in the final phase of nutrient assimilation[30]. The maturation of the small intestine is essential to optimize broiler growth and digestion, absorption rates are directly correlated to cell proliferation and differentiation rates, the higher the villi and their density, the larger is the area of surface for digestion and absorption[31].

Regarding feed intake, the results were agreed with that of Mishra *et al.*[32] who stated that using 5% guar meal in broiler diet with or without β-mannanase supplementation leading to increase feed intake when compared with the SBM fed groups. Similarly, Nasrala *et al.*[27] found an increase in feed intake with increasing the level of GM in broiler diets. Increased feed intake by using GM may be attributed to decrease nutrient digestibility by β-mannans[11], thus increases feed intake to compensate the low nutrient digestibility.

Results of the present study about carcass traits are consistent with the result of Hajati[33] who found that enzyme supplementation improved carcass percentage and thigh percentage. Similarly, Nasrala *et al.*[27] recorded an improvement in dressing percentage when diets containing GM were supplemented with β-mannanase. In contrast, Mishra *et al.*[32] recorded a non significant difference in dressed weight by dietary inclusion of 5% guar meal to broiler diet with or without β-mannanase supplementation.

The effect of GM on intestinal relative weight may be attributed to increase cell-proliferation rate by β-mannans as discussed by Chegeni *et al.*[34]. Moreover, β-mannans are water soluble compounds which increase intestinal viscosity and reduce the ability of the gut to mix intestinal content[9,35]. The results are agreed with that of Chegeni *et al.*[34] who found an increase in relative weight of the duodenum, jejunum and ileum when Canola Meal (CM) was included in broiler diets.

Results of the present study about relative weight of gizzard, liver and heart are agreed with Mishra *et al.*[32] who found that the weight of liver and giblet did not significantly affected by inclusion of GM to broiler diet with or without β-mannanase. Hajati[33] stated that the percentage of heart, liver, gizzard did not significantly affected by enzyme supplementation. Similarly, Zaib ur Rehman *et al.*[29] reported a non significant difference in gizzard and liver percentage in birds fed standard diet, low energy diet or low energy diet supplemented with β-mannanase.

β-mannanase improved villus height and crypt depth of ileum, this improvement may be due to the degradation of β-mannans by the enzyme to mannan-oligosaccharides (MOS). Baurhoo *et al.*[36] recorded an improvement in villi height, goblet cell number with addition of mannan oligosaccharide to broiler diets. The findings are agreed with that of Adibmoradi and Mehri[37] who found that duodenal villus height and crypt depth was increased by the addition of β-mannanase to corn-soybean-based diets in a dose dependent manner.

Results of the present study regarding the effect of guar meal on broiler immunity confirmed the findings of Che *et al.*[38] who stated that mannan-containing products are able to modulate immune response in animals. Also, Gharaei *et al.*[39] found an improvement in immune response of broiler chickens with dietary inclusion of 3 and 6% guar meal with and without β-mannanase supplementation.

Zou *et al.*[40] found that β-mannanase supplementation to broiler diet at 0, 0.025, 0.05 and 0.075% resulted in a significant increase in the relative weights of spleen and bursa in comparing with the control group. Also, it found that significant increase of T-lymphocyte proliferation in 6 weeks-old broiler with 0.05% enzyme supplementation. This may be attributed to the degradation of β-mannans by β-mannanase with liberation of mannan-oligosaccharides (MOS). Spring *et al.*[41] described MOS as pre-biotic. Shashidhara and Devegowda[42] suggested that the gut associated lymphoid tissue (GALT) can recognize microbes through unique recognizing molecule called pathogen associated molecular patterns (PAMP). The MOS have the same PAMP of some pathogens thus they can bind to pattern-recognizing receptors on the defense cells of GALT and in turn activate innate immune defenses[43,44].

The effect of β-mannanase supplementation on glucose level may be attributed to the degradation of β-mannans by the enzyme. β-mannans are considered as anti-nutritional factor in mono-gastric species they inhibit postprandial insulin secretion in human[45] and interfere with glucose metabolism and insulin secretion rates in swine[13]. The results are in line with Azarfar[46] who recorded an increase in plasma glucose level with β-mannanase supplementation at 0.5 and 1 g kg^{-1} to broiler diet.

The results of serum alkaline phosphatase, ALT, AST, uric acid and creatinine indicate that both guar meal and β-mannanase supplementation had no effect on liver and kidney functions. The same were recorded by Shahbazi[47] who stated that the level of serum uric acid was not affected by dietary inclusion of guar meal and β-mannanase supplementation to laying hens.

This study discovered the beneficial effect of β-mannanase enzyme supplementation to broiler diet containing GM in improving broiler performance and immunity. This study will help the researcher to uncover the critical area of using inexpensive protein source like GM in broiler diet with maintaining production level. Thus, a new theory on using GM as a partial replacement for soybean meal in poultry diet with β-mannanase supplementation for decreasing feed cost with maintain the performance parameters may be arrived.

CONCLUSION

From the results, it could be concluded that GM could be applied at 5% in broiler diets with β-mannanase supplementation maintaining the same broiler performance parameters and with improve bird immune response.

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