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Effect of Feeding Fermented Guar Meal *Vis-À-Vis* Toasted Guar Meal with or without Enzyme Supplementation on Immune Response, Caeca Micro Flora Status and Blood Biochemical Parameters of Broiler Quails

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ABSTRACT

A five weeks (0 to 5 weeks of age) feeding trial was conducted as per CRD design involving six dietary inclusion levels (0, 7.5 and 15%) of toasted guar meal (TGM) with or without enzyme and two levels of (7.5 and 15.0%) fermented toasted guar meal (FTGM) in a standard broiler quail diet as per NRC (1994). Each dietary treatment was replicated four times having 15 chicks in each replication. The study envisaged that incorporation of 7.5% TGM without enzyme supplementation and 15 % TGM with enzyme supplementation or 15% FTGM proved beneficial for immune competence and gut health. Supplementation of 15% TGM with or without enzyme supplementation and 15% FTGM found to be most effective in reduction of serum cholesterol and glucose level.

Key words: *Broiler quail diet, immune response, caeca micro flora status and blood biochemical parameters*

INTRODUCTION

Guar meal (GM) is the by product of guar seed which is obtained after the mechanical separation of endosperm from both hull and germ of guar seed. The crude protein contents of germ, hull and endosperm (gum) are 45, 35, 5-6% and they contribute 44, 21, 29-35% of the guar bean respectively[6]. These fractions contain residual gum in different concentrations. Guar gum is a highly viscous galactomannan polysaccharide. Guar gum is composed of 65% mannose and 35% galactose. According to Verma and McNab[25], GM contains various antinutritional factors which include gum 6-18%, saponin 9-14%, hydrocyanic acid 5-20 mg /100gm and variable proportion of trypsin inhibitor, haemagglutinin and tannins. Various treatments had been used for alleviation of deleterious factors including heat treatment[17], water and alcohol treatment[19], hot water and acid treatment[13], cooking[18], toasting and steam pelleting[25], enzyme supplementation[3] and solid substrate fermentation using *Aspergillus niger*[23]. Effect of feeding fermented guar meal *vis-à-vis* toasted guar meal with or without enzyme supplementation on immune response, caeca micro flora status and blood biochemical parameters of broiler quails has been done in this study.

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MATERIALS AND METHODS

Experimental design

Biological experiment was undertaken in a completely randomized design (CRD) with day old quail chicks (n = 480) assigned to eight dietary treatments (i.e. one control + 7 test diets) in such a way that each treatment had 4 replicates of 15 quail chicks, each accommodating 60 chicks per treatment. The layout of experimental diets is shown in the table 1.

Experimental diets

Prior to diet formulation, all the ingredients were procured at one time and analyzed for proximate analysis[1], calcium[22] and phosphorus[1] contents. Eight experimental diets were prepared by incorporating TGM at 0, 7.5% and 15% levels (Diet D1, D2, D3); enzyme supplemented TGM at 0, 7.5% and 15% level (Diet D4, D5, D6) and fermented TGM at 7.5 % and 15% levels (Diet D7 and D8) respectively (Table 1). All diets were kept isocaloric and isonitrogenous in nature while CP was maintained at 24% and ME 2900 kcal /kg of feed in broiler quail diet as per NRC (1994). Optimum conditions for better growth of *Aspergilus niger* on TGM and economic fermentation were found to be with TGM and water ratio of 50:50 and *Aspergillus niger* spores inoculation at the rate of one lac spores/kg of substrate at 37^{o}C incubation for a period of 60 hrs during the experiment. Ingredients composition of experimental diets (%) has been given in the table no.2.

Caeca microbial status

On 35th day, two chicks per replicate per dietary treatment were sacrificed by cervical dislocation and caeca contents were collected in sterile vials for evaluation of total microbial load colonization. One gram caeca content was weighed and dissolved in 9 ml sterile normal saline solution (NSS). Volume of 0.5 ml from 10^4 dilutions was taken in sterile Petri dish and 15-20 ml of sterile nutrient agar media was poured in each Petri dish. It was mixed gently and allowed to stand until the media solidify. Then plates were incubated in BOD incubator at 37^{o}C for 24 hrs and total numbers of colonies were counted by a colony counter (ICMSF, 1978). Total numbers of colonies were calculated as following formula:

cfu / gm = Total No. of colony counted x Dilution factor/ Volume of aliquot taken

Blood biochemical parameters

At the end of the feeding experiment blood samples from two birds per replicate per dietary treatment were randomly collected into sterile glass test tubes without addition of any anticoagulant. Test tubes containing the blood were kept in slanted position at room temperature for two hours to facilitate separation of serum. Serum was separated by centrifugation at 3000 rpm for 10 minutes and serum was decanted into ependorff tubes, then stored at -20^{o}C for estimation of total protein by modified biuret end point assay[8]; albumin by bromocresol green end point assay[27], glucose by GOD-POD end point assay[12] and cholesterol by Wybenga *et al* [26].

Immunological Assay

- Humoral immune (HI) response

Humoral immune response estimated by method of Siegel and Gross[21] by assaying the immune response to sheep red blood cells (SRBCs).The reciprocal of highest dilution which show clear agglutination was the end titer. Titers were expressed as log $_2$.

- Cell mediated immune response

The *in vivo* cell mediated immune response to PHA-P was evaluated by the method of Cheng and Lamont[5]. Phytohaemagglutinin type P is used in form of mucopolysaccharide from the red kidney bean, *Phaseolus vulgaris.* PHA-P provokes responses, influenced by subpopulation of T-helper and T-suppressor

cells. Good responder to PHA-P means a higher general level of cellular immunity influencing T-cell mechanisms restricting or preventing lymphoma formation. Quails of 21 days have been taken to evaluate response of PHA-P. Foot index (FI) was calculated as following formula:

FI (mm) = (Post inj. – Pre inj.) – (Post PBS – Pre PBS)

RESULTS AND DISCUSSION

Results of immune response, caecal microbial status and blood biochemical parameters have been presented in the table 3 and 4.

Serum Glucose

The hypoglycemic effect of guar gum was observed in diets containing 7.5 and 15% TGM without enzyme (D2 and D3 group), wherein serum glucose level was reduced by 2.50 and 3.23%, which was significantly (P<0.05) lower as compared to control and other groups (table 4). Our results are in line with Jenkins *et al*[11]., and Morgan *et al*[14]., who reported that dietary guar gum can be effectively used in the reduction of blood glucose, increase in plasma insulin and plasma gastric inhibitory polypeptide (GIP) in human. The hypoglycemic effect of guar gum was due to gum, lecithin like interaction with intestinal mucosa, changes in intestinal micro flora, or interaction with gut hormones and increased viscosity of digesta. However, contrary to this, Bhutia[3], Daskiran *et al*[7]., and Patel *et al*[16]., reported that neither guar nor enzyme supplementation had an impact on serum glucose level.

Serum Cholesterol

Diets containing TGM at the levels 7.5 and 15% with or without enzyme supplementation reduced serum cholesterol level by 4.80% and 8.73%, respectively as compared to control. However, there was no significant (P>0.05) in serum cholesterol content in groups fed diet containing 7.5% TGM with or without enzyme supplementation and both FTGM supplemented (7.5 and 15%) groups (table 4). Changes in several blood biochemical parameters were observed when guar gum was incorporated into the diets. It lowers total plasma cholesterol in fasting rats[4] and in human with type II hyperlipidemia[15]. According to Patel *et al*[16]., and Ray *et al*[20]., addition of 2% guar gum to a corn diet reduced liver fat and serum cholesterol in chicks. [8]reported that 5 and 10%TGM with or without enzyme supplementation reduced cholesterol significantly (P>0.05) as compared to 0%TGM.

Serum Protein

No significant (P>0.05) differences in serum total protein, albumin (A), globulin (G) and A/G between different dietary treatments could be observed (table 4). Our results are in line with Patel *et al*[16]., and Bhutia[3] who reported serum protein, albumin, globulin and A/G ratio contents to be unaffected by the feeding of guar meal in chicks and broiler quails.

Humoral immune response

The humoral immune response as measured by SRBC haemagglutination (HA) antibody titre (log2) ranged from 4.25±0.24 in control (D1) group to 6.25±0.25 in 7.5% FTGM (D7) group. Haemagglutination (HA) titer values were also significantly (P < 0.05) higher in FTGM 7.5 and 15% (D7 and D8 groups) as compared to other dietary treatments. The HA titre values were also significantly (P < 0.05) higher in 7.5 and 15% TGM with enzyme (D5 and D6) group as compared to control (D1) group, whereas in other treatment groups the values remained comparable to control (table3).

Cell mediated immune response

Cell mediated immune response (CMI) as measured by foot pad index (mm) ranged from 0.37±0.02 in 15% TGM without enzyme (D3) group to 0.40±0.01 in 15% FTGM (D8) group. No significant (P >0.05) difference could be observed in foot pad index due to FTGM and

TGM with or without enzyme supplementation (table 3). Information on effect of feeding diets containing various levels of FTGM and TGM with or without enzyme supplementation on immune response of broiler quail is scanty in literature. [8]observed that the titer values were tended to increase at 14th days post immunization and it did not differ significantly among different dietary groups on the basis of inclusion of TGM and enzyme. The result of leukocyte migration inhibition test (LMIT) on 10, 20 and 30th day of post immunization in quails revealed that the levels of TGM showed no significant difference among different dietary groups on cell mediated immune response. Present results are in line with Bhutia[3] in CMI immune response did not differ significantly (P>0.05). No data is available in literature regarding effect of FTGM on immune response. However, in our study humoral immune response (HI) was significantly (P>0.05) higher in all dietary treatment groups. Possible reason of improved immune response in TGM or FTGM diet is that gum residue of guar meal may act as prebiotic and prevent colonization of pathogens by competitive exclusion.

Caeca Microbial Count

Caeca microbial count was significantly (P<0.05) higher in control (D1) as compared to other dietary treatments. Enzyme supplemented groups along with TGM (D4, D5, D6) significantly (P<0.05) reduced caeca microbial count as compared to their respective non-enzyme supplemented groups (D1, D2 and D3). FTGM at 7.5 and 15% (D7 and D8) and 15%TGM (D6) with enzyme level reduced caeca microbes significantly (P<0.05) as compared to control and other dietary groups (table 3). The present study are in line with Bailey *et al*[2]., Bhutia[3], Ishihara *et al*[10]., and Gujral[9]. Bailey *et al*[2]., reported that chicken fed fructo-oligo-saccarides (FOS) had four fold reduction in the level of *Salmonella* present in the caeca. Ishihara *et al*[10]., who reported that partially hydrolyzed guar gum prevented colonization of *Salmonella enteriditis* in young and laying hen. Gujral[9] reported that the fructo-oligo-saccharides (FOS) and mannan oligo saccharides (MOS) reduced significantly (P<0.05) caecal colonization of *Salmonella typhimurium* upon challenge with live bacteria on third day of age in broiler quails. Bhutia[3] reported that TGM with or without enzyme supplementation significantly (P<0.05) reduced caeca microbial count as compared to control.

Table 1: Layout of experimental diets

Experiment diet	Level of guar meal (%)	Treatment
D-1	0	-
D-2	7.5	-
D-3	15	-
D-4	0	Multienzyme
D-5	7.5	Multienzyme
D-6	15	Multienzyme
D-7	7.5	FTGM
D-8	15	FTGM

Table 2: Ingredients composition of experimental diets (%)

FEED	D1	D2	D3	D4	D5	D6	D7	D8
MAIZE	59.12	57.2	55.2	59.12	57.2	55.2	60.52	61.2
SBM	33.5	27	20.5	33.5	27	20.5	24.23	15.2
FISH MEAL	5	5	5	5	5	5	5	5
TGM	-	7.5	15	-	7.5	15	-	-
FTGM	-	-	-	-	-	-	7.5	15
ANI.FAT	-	0.83	1.73	-	0.83	1.73	0.2	0.78
M.MIX(ISI)	1	1	1	1	1	1	1	1
LIMESTONE	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
DCP	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
DL-MET.	0.02	0.02	0.06	0.02	0.02	0.06	0.05	0.08
LYSINE	-	-	0.15	-	-	0.15	0.14	0.28
SALT	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
TM PREMIX*1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
VIT.PREMIX*2	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.25
B COMPLEX*3	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
CHOLIN CHL.	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
TOXIN BINDER	0.04	0.04	0.04	0.04	0.04	0.04	0.02	0.02
MULTIENZYME	0	0	0	0.05	0.05	0.05	0	0

TM.PREMIX*1(10g/100Kg):FeSO4.7H2O=8g;ZnSO4.7H2O=10g;MnSO4.H2O=10g;CuSO4.5H20=1g;KI=30mg.VIT PREMIX*2(A,B2D3K) each gram contains:VitA-82500 IU;Vit B2-50mg;VIT.D3-12000 IU and Vit.K-10 mg. B Complex*3 each gram contains;Vit B1=8 mg;Vit.B 6=16 mg;Vit. B12=12 mg;Niacin=120 mg;Cal.Pantothenate=80mg;Vit E50%=160mg;L-Lysine=10 mg and DL-Methionine=10mg Multienzyme@ 50gm/100Kg of feed(.05%) contains= alpha amylase 7×10^5,protease 3×10^6,cellulase 6×10^6,beta glucanase 7×10^5,pectinase 7×10^4,phytase 4×10^5 and xylanase10×10^6 and lipase 5×10^3 units per kg

Table 3: Effect of different levels of FTGM and TGM with or without enzymes on immune response and caecal microbial status in quails

Group	TGM (%)	Treatment	Humoral immune response (log 2)	Cell mediated immune response (foot index in mm)	Ceca microbes total plate count (cfu / g)
D-1	0	—	4.25^{a}±0.24	0.39±0.01	140.12^{e}±2.59
D-2	7.5	—	4.62^{ab}±0. 26	0.38±0.01	94.37^{d}±2.26
D-3	15	—	5.12^{bc}±0.22	0.37±0.02	78.62^{bc}±2.32
D-4	0	Multienzyme	4.62^{ab}±0.24	0.40±0.02	99.37^{d}±2.31
D-5	7.5	Multienzyme	5.25^{c}±0.25	0.40±0.02	81.25^{c}±1.54
D-6	15	Multienzyme	5.12^{bc}±0.23	0.39±0.18	73.62^{ab}±1.61
D-7	7.5	FTGM	6.25^{e}±0.25	0.39±0.01	70.12^{a}±2.43
D-8	15	FTGM	5.75^{d}±0.24	0.40±0.01	69.00^{a}±1.71

Means bearing different superscripts in a column differ significantly (P <0.05)

Table 4: Effect of different levels of FTGM and TGM with or without enzymes on serum parameters in quails

Group	Level of TGM (%)	Treatment	Glucose (g/dl)	Cholesterol (mg/dl)	Total Protein (g/dl)	Albumin (A)	Globulin (G)	A/G
D-1	0	—	224.75^{c}±1.46	216.31^{c}±1.35	3.19±0.02	1.23±0.00	1.96±0.02	0.63±0.01
D-2	7.5	—	219.25^{ab}±0.94	206.40^{b}±1.43	3.14±0.01	1.24±0.01	1.90±0.01	0.66±0.01
D-3	15	—	217.50^{a}±1.03	197.43^{a}±0.94	3.20±0.04	1.25±0.01	1.95±0.04	0.65±0.02
D-4	0	Multienzyme	225.37^{c}±1.51	216.19^{c}±1.28	3.22±0.04	1.24±0.01	1.97±0.04	0.63±0.01
D-5	7.5	Multienzyme	223.50^{bc}±2.35	206.83^{b}±0.81	3.14±0.02	1.23±0.01	1.92±0.02	0.64±0.01
D-6	15	Multienzyme	222.87^{bc}±1.43	199.13^{a}±0.37	3.14±0.02	1.24±0.01	1.90±0.02	0.66±0.01
D-7	7.5	FTGM	224.62^{bc}±1.56	206.72^{b}±1.03	3.17±0.02	1.25±0.00	1.92±0.02	0.65±0.01
D-8	15	FTGM	222.87^{bc}±2.66	205.01^{b}±1.35	3.18±0.02	1.24±0.01	1.94±0.01	0.64±0.00

Means bearing different superscripts in a column differ significantly (P <0.05)

CONCLUSION

The study envisaged that incorporation of 7.5% toasted guar meal (TGM) without enzyme supplementation or 15 % TGM with enzyme supplementation or 15% fermented TGM proved beneficial for immune competence & gut health. Supplementation of 15% TGM with or without enzyme supplementation and 15% fermented TGM were found to be most effective in reduction of serum cholesterol and glucose level. Beneficial effect of enzyme supplementation can be substituted with fungal fermentation of TGM with better humoral immune response and gut health. Thus, it is concluded that toasted guar meal(TGM) is potential alternate and economical protein source at the inclusion level of 7.5% TGM without enzyme supplementation or 15% TGM with enzyme supplementation or 15% fermented TGM is beneficial for immune competence & gut health along with reduces serum cholesterol and glucose level.

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