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Effect of Dietary Supplementation of Fenugreek Seed (*Trigonella Foenum-Graecum* L.) Powder on Body weight, Blood-biochemical Parameters and Immunity in Broilers

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ABSTRACT

A six-week study was carried out to investigate the effect of dietary supplementation of Fenugreek seeds (Trigonella foenum-graecum L.) powder (FSP) on serological and immunological parameters in broiler chickens. One hundred and forty four day-old chicks were randomly divided into six dietary treatment groups having 24 birds in each group and three replications of 8 birds each. Supplementation with FSP was done at the rate of 0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0% for T1 to T6. The result of the study revealed that there was a significant ($p<0.05$) decrease in serum total cholesterol and LDL-cholesterol concentrations in the broilers fed diets supplemented with FSP at 1.5% or above. HDL-concentrations were found to be improved significantly ($p<0.05$) upon FSP supplementation at 1.0% and above. Blood glucose concentration was reduced significantly ($p<0.05$) as the supplementation of FSP was increased from 1.0% to 3.0%. Expression of genes controlling antibacterial and anti-viral activities revealed that relative mRNA expression of TLR-2 and TLR-7 of broilers was found to be enhanced upon feeding FSP. But a significant down regulation was seen in case of relative mRNA expression of TLR-4 receptors in FSP supplemented groups. Serum triglycerides, SGPT and SGOT concentrations remained unaffected. It was inferred from the study that FSP fenugreek supplementation in the diets of broiler birds have substantial improvement in the serum biochemical parameters along with showing immunological activities.

Key words: Fenugreek Seed Powder, Broiler, Cholesterol, Blood biochemical and Immunity

INTRODUCTION

The consumption pattern in our society is shifted from red meat (beef, mutton, lamb, pork) to white meat (broiler meat) due to high saturated fat and cholesterol content of the red meat (Daniel *et al.,* 2011). A regular consumption of red meat has been shown to be directly associated with cardiac disease, high blood pressure, arteriosclerosis, stroke, diabetes, obesity and earlier death. Many medicinal and aromatic plants have been used for broilers to increase dietary energy utilization and improve performance efficiency. A large number of plant-derived products are becoming important, due to their antioxidant effects, and in particular, their potential prophylactic benefit in poultry production, as the use of most antibiotic growth promoters as feed additives has been banned by many countries around the world. Fenugreek (*Trigonella foenum-graecum* L.) is an annual legume and widely cultivated in India. Fenugreek has been widely used not only as a constituent in traditional foods, but also as a natural herbal remedy in its leaf and seed forms. Human and animal studies show that fenugreek seeds have various physiological effects, including anti-diabetic, anti-inflammatory, anti-cancer, and immunomodulatory effects (Basch *et al.,* 2003; Srinivasan, 2005; Kawabata *et al.,* 2011; Thomas *et al.,* 2011). Fenugreek seeds are also rich in phenolic compounds, especially flavonoids, which have extensively antioxidant capacity (Kaviarasan *et al.,* 2004). It has also been reported that fenugreek seed have hypolipidemic effects due to high amount of galactomannan, and lipotropic effect due to mainly high choline amount. The aim of the present study was to analyze the effect of fenugreek seed powder supplementation at different level on serum parameters and investigating the relationship between inclusion level of fenugreek seed and expression of genes associated with antibacterial or antiviral activities in broiler birds.

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MATERIALS AND METHODS

This study was conducted with a total of 144 day-old as broiler chickens at poultry shed of Department of Animal Nutrition, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar for a period of 42 days to examine the effect dietary supplementation of fenugreek seed powder on serological and immunological parameter. A total of 144 day-old experimental broilers were weighed, identified by wing bands and randomly distributed to 6 treatment groups. Each treatment was divided into 3 replicates having 8 birds in each. The ration was formulated as per the BIS (2007) Specification. The control group (T1) was fed a basal diet having no fenugreek seed powder. While the rations of broilers under treatment group T2, T3, T4, T5 and T6 was supplemented with fenugreek seed powder (FSP) at the rate of 1.0, 1.5, 2.0, 2.5 and 3.0%, respectively. The ingredient and chemical composition of diets fed during different stages of growth and fenugreek seed powder have been presented in table 1. Fenugreek seed powder contained 28.4% CP, 9.30% CF, 7.14% EE and 3.28% total ash.

Table 1. Ingredient and analyzed chemical composition of basal diet fed during different phases of broiler production

Attributes	Pre starter	Starter	Finisher
Ingredient composition* ((kg /100 of feed)			
Maize	55	56	57.20
Soybean meal	20	14	14.4
Ground nut cake	12	16	14.4
Fish meal	7	7	6
Vegetable oil	4	5	6
Mineral mixture	2	2	2
Chemical composition**			
Moisture (%)	9.83	10.05	9.97
% DM basis			
Crude Protein (%)	23.12	22.03	20.42
Crude Fiber (%)	2.50	2.59	2.56
Ether Extract (%)	6.32	6.64	8.24
Total Ash (%)	6.08	6.18	6.65
ME (kcal/kg)	3013	3102	3210

*Feed additive mixture was added @ 410 g/100kg of control ration and 310 g/100 kg feed in the rest of treatment rations during different growth phases of broilers. Feed additive mixture was composed of Vitamin Mixture-I-10 g, Vitamin mixture-II-20 g, Coccidiostat-50 g, Choline chloride-100 g, Antibiotic, oxytetracycline (only in T1) 100g, Lysine-50 g and DL-methionine-80 g.

** Each value is mean of three observations.

For proper brooding of chicks, sufficient heat and light was provided by using electric bulbs in each treatment for first three weeks of age. Temperature of brooding was 950F for first week. A weekly reduction of 50F was done till brooder temperature reached to 850F by third week of age. Afterword sufficient artificial light was provided during night hours throughout the experimental period. Birds were vaccinated against F1 strain of Newcastle disease on 3rd day and Infectious Bursal Disease on 14th day through intranasal route. Fresh and clean drinking water was provided ad-libitum. Body weight of the birds was recorded at the end of pre-starter (day 7), starter (day 21) and finisher phase (day 42). All the precautionary measures against diseases were taken throughout the

experimental period of six weeks. At the end of the feeding trial (6th week), blood samples were collected from five broiler per treatment and thus a total of 30 samples were analyzed. About 2 ml of blood was collected from each bird via brachial wing vein puncture using sterilized syringes. Serum parameters were determined by auto analyzer using commercial kits. Serum samples were analyzed for different serum variables like total cholesterol, glucose, triglyceride, SGOT, SGPT high density lipoproteins (HDL) and low-density lipoproteins (LDL). To study the effect of dietary FSP on expression of genes related to antibacterial and anti-viral activities, extracted RNA from fresh blood sample was used for cDNA preparation and for the analysis of temporal expression profile of different genes, real-time PCR was carried out using Step one plus real-time PCR system and the average CT (Threshold cycle) value obtained for the TLRs 2, 4 and 7 (target) gene was normalized (endogenous control). The data obtained were subjected to comparative CT method for the analysis of the expression levels of targeted TLR gene and an endogenous control.

STATISTICAL ANALYSIS

Data was analyzed statistically as described by Snedecor and Cochran (1994). Analysis of variance was used to study the differences among treatment means and they were compared by using Duncan's Multiple Range Test (DMRT) as modified by Kramer (1956).

RESULTS AND DISCUSSION

Body weight of the broiler birds at the start of the feeding trial was similar ($p>0.05$). At the end of pre-starter phase, group T3 had significantly ($p<0.05$) higher body weight as compared to the control and other groups followed by group T4 while at the end of starter phase, group T4 and T5 had statistically ($p<0.05$) higher body weight followed by group T3. At the completion of the trial the overall body weight of birds under group T3 and T4 was significantly ($p<0.05$) higher than control group and numerically higher than other treatment groups (Table 2) Similar results were also confirmed by Yatoo *el al.* (2012).

Table 2. Body weight of the broiler birds at the end of different growth periods

Treatments	Average body weight (g)			
	Initial BW (g)	Pre-starter phase (g)	Starter phase (g)	Finisher phase (g)
T_1	44.37±1.57	96.16±0.66^{b}	501.3±9.72^{a}	2130±1.09^{a}
T_2	44.83±0.75	96.39±0.55^{b}	577.8±5.66^{ab}	2441±4.19^{b}
T_3	44.64±0.58	106.2±0.53^{d}	669.3±3.69^{c}	2457±8.53^{bc}
T_4	44.73±1.12	98.15±0.35^{c}	713.6±8.48^{cd}	2552±13.47^{b}
T_5	44.89±1.77	98.17±1.17^{c}	762.9±8.84^{d}	2478±1.54^{bc}
T_6	44.17±0.78	90.51±2.67^{a}	636.1±3.09^{bc}	2379±8.14^{bc}

*Mean values bearing different superscript within a column differ significantly ($p<0.05$)

There was no statistical variation in serum triglycerides concentrations among various groups. Serum total cholesterol decreased significantly ($p<0.05$) in fenugreek seed powder supplemented groups at the level of 2% and above (Table 3). Anti-cholesteremic effect of fenugreek seed may be due to presence of saponins which either compete with cholesterol at binding sites or interfere with cholesterol biosynthesis in the liver. The results are in agreement with the study of Abbas and Ahmed 2010; Abdel-Rasoul and Yousif, 2003 and El-Ghamry *et al.,* 2002 in broilers. Abdul-Rahman, (2012), Safaei *et al.* (2013) and Mamoun *et al.* (2014) have also observed cholesterol and glucose lowering effects of fenugreek seeds in broilers.

Table 3. Mean values of biochemical parameters of broilers fed different diets

Treatment	Triglycerides (mg/dl)	Total cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	SGOT (IU/L)	SGPT (IU/L)	Glucose (mg/dl)
T_1	96.33±4.61	143.0±2.64^{d}	74.44±2.70^{a}	63.50±1.30^{d}	225.0±10.10	9.40±0.60	230.0±3.70^{d}
T_2	88.33±10.20	140.0±1.70^{d}	74.44±1.70^{a}	52.60±0.80^{d}	220.3±23.10	8.60±0.20	226.3±2.80^{d}
T_3	90.67±8.22	139.0±2.90^{d}	80.06±2.70^{b}	51.30±1.40^{d}	222.0±30.70	8.30±2.10	215.4±2.20^{c}
T_4	83.33±4.12	137.0±2.90^{c}	82.03±1.40^{bc}	49.10±1.40^{c}	210.0±23.40	7.70±0.90	203.3±3.30^{b}
T_5	84.08±6.00	136.0±2.30^{a}	85.48±1.90^{c}	47.00±1.10^{a}	198.9±29.50	7.30±1.20	182.5±1.50 ^{a}
T_6	78.33±1.40	132.0±2.40^{b}	89.03±1.60^{bc}	40.30±1.20^{b}	197.7±19.70	6.90±1.30	177.5±3.20^{a}

*Mean values bearing different superscripts in a column differ significantly ($p<0.05$).

Serum HDL was increased significantly ($p<0.05$) in the broilers fed fenugreek seed powder at or above 1.5% in comparison to control group. LDL-cholesterol decreased ($p<0.05$) on fenugreek seed powder supplementation. In harmony of these results, Mullaicharam *et al.* (2013) also reported that there was a significant suppression in the human serum total cholesterol, triglycerides and LDL cholesterol by fenugreek supplementation.

The mean values of blood biochemical parameters were within normal range. Supplementation of fenugreek seed powder at the level of 1.5% or above in the diets of broilers resulted in significant drop ($p<0.05$) in the blood glucose concentration. The significant fall in blood glucose might be due to increased plasma insulin by a direct stimulatory effect on B-cells. Earlier studies by Sauvaire *et al.* (1998) and Schryver (2002) have suggested that abundant 4-hydroxyisoleucine amino acid in fenugreek has a stimulatory effect on the pancreas to release insulin. Safaei *et al.* (2013) and Mamoun *et al.* (2014) have also reported similar effects.

Dietary supplementation of fenugreek seed powder had no effect ($p<0.05$) on the liver function indicators i.e., Serum glutamic oxaloacetic transaminase (SGOT) and Serum Glutamic pyruvic Transferase (SGPT). In contrast to the current finding, Nakhla *et al.* (1991) reported that SGOT activity was elevated by fenugreek seed saponins diets in boiler chicks while Ali and Ismail (2012) reported that inclusion of fenugreek seeds in the diet of broiler chicken had no significant ($P>0.05$) effect on the liver enzymes. Hepatoprotective role of fenugreek seeds has also been stated by Meghwal and Goswami (2012).

A comparison was made in between the treatment groups T2, T3, T4, T5 and T6 and control group for relative mRNA expression study of TLR2, TLR4 and TLR7 (Table 4).

Table 4. Relative quantification expression analysis of the toll like receptors (TLR 2, TLR 4 and TLR 7) with the reference to the endogenous reference gene ß actin

Sample Name	Target Name	C_T Mean	C_T SD	ΔC_T Mean	$\Delta\Delta C_T$	RQ
T_1	TLR 2	19.54	0.74	2.24	0	1
T_2		18.50	0.34	1.84	-0.4	1.31
T_3		18.77	0.12	1.48	-0.76	1.69
T_4		17.80	0.13	1.28	-0.96	1.94
T_5		18.23	0.77	1.08	-1.16	2.23
T_6		17.54	1.26	1.02	-1.22	2.32
T_1	TLR 4	20.43	0.74	3.12	0	1
T_2		19.45	0.29	2.79	-0.33	1.25
T_3		20.78	0.24	3.48	0.35	0.78
T_4		20.05	0.15	3.52	0.39	0.76
T_5		21.50	0.62	4.34	1.21	0.432
T_6		14.31	0.81	2.21	1.34	0.4
T_1	TLR 7	26.20	0.26	8.90	0	1
T_2		26.59	0.07	9.93	1.03	0.49
T_3		27.53	0.29	10.29	1.33	0.39
T_4		28.04	0.22	11.51	2.61	0.16
T_5		25.13	1.80	7.97	-0.92	1.89
T_6		22.31	0.65	5.78	-3.11	1.07
T_1	ß actin	17.30	0.13	-	-	-
T_2		16.66	0.25	-	-	-
T_3		17.29	5.29	-	-	-
T_4		16.52	0.16	-	-	-
T_5		17.15	6.09	-	-	-
T_6		16.52	0.32	-	-	-

The treatment group supplemented with 3% FSP (T6) had highest RQ value showing maximum expression of TLR2. On the other hand, treatment group supplemented with 2.5% FSP (T5) had highest RQ value showing maximum expression of TLR7. While, in case of TLR4 mRNA expression study, a significant down regulation of RQ value was obtained with the increasing level of supplementation of FSP in broilers' diet. TLR-2 denotes presence of Gram-positive bacteria that are beneficial for the health of the gut e.g., Lactobacillus. Therefore, up regulation of the TLR-2 RQ values in FSP supplemented groups enhanced the population of Lactobacillus bacteria in the gut microflora which is better for competitive exclusion of harmful gut microorganisms. Augmented TLR signaling denotes up-regulation of cell-mediated immunity, improvement of T-cell homing to

mesenteric lymph nodes (Corthésy *et al.,* 2007). TLR-4 depicts the presence of Gram-negative bacteria in the gut microflora e.g., E. coli. The down regulation of TLR-4 RQ values in FSP supplemented groups indicates an elevated anti-bacterial activity against these harmful bacteria. TLR-7 represents antiviral activity of the immune system, thus, up regulation of TLR-7 RQ values for FSP fed groups signifies a boosted immune system response against different viruses. In vitro study by Qureshi *et al*. (2015) also reported antibacterial activity of Fenugreek seed in which they observed a 2.1 mm of zone of inhibition for the concentration of 0.05 mg/ml of extract against E. coli on the Mueller Hinton agar. Dash et al. (2011) also concluded also demonstrated the antibacterial activity of methanolic extract of fenugreek against E. coli due to flavonoids, saponins and phenols present in it. In a similar study, Gao et al. (2008) also reported that probiotics bacteria augment toll-like receptor (TLR) signaling and regulate local mucosal cell-mediated immune responses in broilers.

CONCLUSION

Based on the findings of the study, it was concluded that dietary supplementation of fenugreek seeds powder modulates the cholesterol profile by improving HDL cholesterol and lowering LDL cholesterol and lowering blood glucose. Therefore, it was inferred that fenugreek seed powder can be supplemented at the level of 1.5% or above for its beneficial effects on broiler production.

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