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Clinical study to evaluate fenugreek (*Trigonella foenum-graecum* L.) seed and galactomannan depleted fenugreek residue on blood glucose level in broiler breeder hens

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Abstract
A study was undertaken to evaluate the blood glucose level of broiler breeder hens supplemented with fenugreek seed and Galactomannan Depleted Fenugreek Residue (GDFR). The evaluation was based on series of biological trial and chemical analyses. In a broiler breeder trial, 4320 numbers of thirty three weeks old Vencobb 400-Y strain were distributed randomly to nine treatments and each treatment had five replicates and each replicate had 96 birds. Supplementation of fenugreek seed or GDFR up to 4% in broiler breeder decreased the blood glucose level. The blood glucose level was decreased at all level of fenugreek seed supplementation and when GDFR was supplemented 2% and above.

Keywords: broiler breeder hens, fenugreek seed, GDFR, blood glucose

Introduction
Poultry scientists today are challenged to find out new alternative growth promoters possessing "gut health promotion, improving nutrient utilization and enhance immunity with no deleterious effect on animal welfare, environment impact and provide a safe livestock product to the consumer and at the same time the material should be from natural sources". There are products in nature like phytobiotic substances obtained from the plants and herbs having wide range of medicinal and growth promotional properties [1].

Medicinal and aromatic plants have been used for many years in human nutrition as spices and medical additives in animals to increase dietary energy utilization, improve the performance efficiency and as a new source of protein.

India, the spice bowl of the world with more than 50 varieties of spices being produced. The total production of spices in India is estimated at 5.8 million tonnes and it accounts for over 45 percent of the world spice trade by volume and value. Fenugreek, an important spice was produced to the tune of 1.279 lakh tonnes in the year 2010-11. Rajasthan accounts for 74% of the fenugreek seed produced in India [2].

The active therapeutic constituents of fenugreek seeds are 4-hydroxy isoleucine [3], lysine and L-tryptophan rich proteins, mucilaginous fibre (galactomannan) and other rare chemical constituents such as saponins, coumarin, fenugreekine, nicotinic acid, sapogenins, phytic acid, scopoletin and trigonelline, which are thought to account for many of its presumed therapeutic effects like inhibition of cholesterol absorption and lowering blood sugar level [4].

Galactomannan in fenugreek, due to its viscous property, is effective in inhibiting the intestinal glucose uptake and lower blood glucose [5], hence separation of galactomannan in fenugreek is undertaken at industrial level to produce anti-diabetic nutraceutical. The residue is designated as Galactomannan Depleted Fenugreek Residue (GDFR) and marketed as Parry Fenumax®. With the increased incidence of diabetes in India and the clamour for using natural drugs for diabetes, the growth of galactomannan separation from fenugreek is likely to increase resulting in more quantity of the GDFR available.

Fenugreek seed and GDFR contain 24 - 26% and 26 - 32% protein, respectively. Since both the products have high carbohydrate content, the expected energy value is presumed to be equivalent to cereals. In addition, these products possess nutraceutical value.

Pharmacological effect of fenugreek have been explored to identify the role for the plant in

diabetes management [6, 7] and in cardiovascular heath [8-10], indicating the presence of bioactive compounds in fenugreek which may responsible for its health benefits. Sharma *et al.* [11] evaluated the effect of fenugreek seeds on blood glucose and the serum lipid profile in insulin dependent (type 1) diabetic patients. Defatted fenugreek seed powder (100g) divided into two equal doses was incorporated into iso-caloric diets for 10 days during lunch and dinner. The fenugreek diet significantly reduced fasting blood sugar and improved the glucose tolerance test and LDL cholesterol and triglycerides were also significantly reduced. Kassaian *et al.* [12] carried study to evaluate the hypolipidemic and hypoglycemic effects of fenugreek seeds in types 2 diabetic patients, diabetic patients were placed on 10 g/day powdered fenugreek seeds mixed with yoghurt or soaked in hot water for 8 weeks. Findings showed that fasting blood sugar (FBS), Triglyceride (TG) and very low density lipoprotein cholesterol (VLDL.C) decreased significantly (25%, 30% and 30.6% respectively) after taking fenugreek seeds soaked in hot water, whereas there were no significantly changes in lab parameters in cases consumed it mixed with yogurt.

The soluble nature of galactomannan fiber from fenugreek has been linked to numerous human health benefits mainly in the reduction of plasma glucose levels which has an anti-diabetic effect[6,13]. Soluble dietary fiber (SDF) and protein of fenugreek can significantly improve glucose homeostasis in type 1 and type 2 diabetics and use of soluble fiber from fenugreek resulted inhibiting glucose absorption in the intestine [14]. Zargar *et al.* [15] found that patients taken orally 20gm of powdered fenugreek seed showed significantly drop in fasting sugar levels. Trigonelline, coumarin and nicotinic acid isolated from fenugreek seed and shown to be useful in diabetes [16].

The objective of the present study was to investigate the effect of different levels of the fenugreek seed and galactomannan depleted fenugreek residue (GDFR) on the blood glucose level in broiler breeder hens.

Methodology

The fenugreek seeds (FS) and galactomannan separated fenugreek seed designated as galactomannan depleted fenugreek residue (GDFR) were evaluated by chemical and biological methods. The chemical analyses were carried out at Department of Animal Nutrition, Veterinary College and Research Institute, Namakkal and the various biological experiments were conducted in M/s Kuppanna Poultry Farm, Muthur, Tiruppur District.

The fenugreek seed samples were collected from different areas of Tamilnadu. The sample used for conducting biological trial was procured locally. The samples were ground and used for analyses.

The samples of GDFR (Parry Fenumax®) and the test sample used in the biological trial was received from E.I.D. Parry (India) Limited, Parry Nutraceuticals Division, Chennai, Tamil Nadu.

Biological experiment

A feeding trial was conducted with 4320 numbers of thirty three weeks old Vencobb 400-Y broiler breeder birds. The birds were distributed randomly to nine treatments. Each treatment had five replicates and each replicate had ninety six birds. The treatments were diets containing 0, 1, 2, 3 and 4% inclusion level of fenugreek seed or GDFR. All the experimental diets were formulated to contain same levels of energy and protein. The ingredients and nutrient composition of the breeder layer mash used in the trial are presented in Table 2. The design of the experiment followed was completely randomized design.

Housing

The experiment was conducted in Mangalore tile roofing sheds. The birds were housed in 2 tier cages and reared from 33 weeks to 52 weeks following standard managemental practices. Throughout the experimental period, recommended standard amount of feed was provided daily and water was provided *ad libitum*.

The birds were vaccinated with live vaccine on 37th week against Ranikhet disease. On 38th week, killed vaccine against Ranikhet disease, Infectious bronchitis and Infectious bursal disease was carried out. On 49th week, Ranikhet and Infectious bursitis killed vaccination was done.

Maximum and minimum temperature in the shed were monitored daily by using maximum and minimum thermometer placed in the middle and two corners of the shed. By using a wet and dry bulb thermometer, the relative humidity percentage was recorded and presented in Table 1.

Blood samples

In addition at the end of each four weeks, blood was collected from ten birds per treatment (totally 90 birds) for the estimation of blood glucose. Blood glucose was estimated by instant blood glucose monitoring system (One touch, Life Scan Europe, Switzerland).

Statistical analyses

The data collected on various parameters were statistically analysed as per the method of Snedecor and Cochran[17] and the means of different experimental groups were tested for statistical significance by Duncan's multiple range test[18].

Results and Discussion

The effect of fenugreek seed and GDFR on blood glucose levels of broiler breeders are presented in Table 3. The blood glucose level was decreased in 3 and 4% fenugreek seed in the first 28 days assessment period, afterwards the blood glucose was decreased in all the fenugreek seed fed birds in second and third assessment periods and overall trial period (233.7 *vs* 214.90 to 220.18 mg/dL). The blood glucose level was decreased at all levels of GDFR in the first assessment period, 2% and above GDFR in the second assessment period, 2 and 4% GDFR groups in third assessment period. In overall five assessment periods the blood glucose level was decreased when GDFR was supplemented at 2% and above compared to the control (233.70 *vs* 217.32 to 218.30 mg/dL). This study concurred with the earlier works of Broca *et al.* [19], El-Ghamry *et al.* [20], Abaza [21], Safaa [22], Xue *et al.* [23] and Awadein *et al.* [24] and differed with the findings of Abbas [25] and Ali and Ismail [26].

Fibre rich meals are metabolized more slowly and nutrient absorption occurs over a longer period[27]. Fenugreek seed is an excellent source of fibre mainly comprising of galactomannans. It facilitates slow absorption of carbohydrates. The amino acid 4-hydroxyisoleucine present in fenugreek seed [19] stimulates the release of insulin thereby controlling blood sugar levels [28]. The hypoglycemic and hypolipidemic effects of fenugreek have mainly been attributed to its fiber, gum, saponin and amino acid content [29]. Hence, removal of galactomannan in GDFR also produce hypoglycemic activity.

Table 1: Temperature and relative humidity inside the experimental house

Environment	1-4 weeks	5-8 weeks	9-12 weeks	13-16 weeks	17-20 weeks	1-20 weeks
Broiler Breeder Experiment						
Temperature (°F)	81.39 ± 0.87	85.18 ± 0.41	85.64 ± 0.38	87.43 ± 0.28	88.07 ± 0.33	85.54 ± 1.16
Relative Humidity (%)	75.10 ± 0.57	76.11 ± 0.36	73.86 ± 0.27	74.00 ± 0.34	74.86 ± 0.37	74.79 ± 0.40

* Each value is a mean of twenty eight observations.

Table 2: Ingredients and nutrient composition (%) of broiler breeder diet (33 - 52 weeks of age)

Ingredients (%)	Fenugreek seed in the ration					GDFR in the ration			
	0%	1%	2%	3%	4%	1%	2%	3%	4%
Fenugreek Seed	0.0	1.0	2.0	3.0	4.0	0.0	0.0	0.0	0.0
GDFR	0.0	0.0	0.0	0.0	0.0	1.0	2.0	3.0	4.0
Maize	59.6	58.6	56.3	53.1	53.7	56.2	54.7	51.9	49.9
Bajra	3.9	4.1	4.3	7.7	5.9	4.6	6.0	8.0	9.5
Soyabean meal	19.9	19.1	18.2	17.9	17.4	18.4	17.9	17.2	16.5
Deoiled rice bran	6.0	7.0	9.3	8.4	8.9	10.0	9.5	10.0	10.0
Dicalcium phosphate	2.0	1.7	1.4	1.4	1.6	1.3	1.3	1.4	1.5
Shell grit	7.0	7.0	6.9	7.0	7.0	6.9	7.0	7.0	7.0
Lysine	0.06	0.10	0.05	0.04	0.02	0.05	0.03	0.01	0.05
Methionine	0.14	0.13	0.11	0.10	0.09	0.13	0.12	0.10	0.09
Salt	0.04	0.04	0.03	0.03	0.03	0.03	0.03	0.03	0.04
Additives and supplements	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

The calculated nutrient content (per cent) was crude protein - 15.5, calcium - 3, available phosphorus - 0.45, lysine - 0.8, methionine - 0.4, Metabolisable Energy (Kcal/kg) - 2730 in all the nine rations.

Additives and supplements (per 100 kg): Vitamin premix - 100g, trace mineral - 100g, toxin binder - 160g, liver stimulant - 100g, choline chloride (60%) - 150g, vitamin E (50%) - 10g, vitamin C - 20g, coccidiostat (salinomycin) - 200g, antioxidant (Endox) - 10g, oxytetracycline (10%) - 100g, phytase - 15g, NSP enzyme - 35 g.

Trace mineral mixture supplied per kg feed: Mn - 100 mg, Zn - 100 mg, Fe - 110 mg, I - 2.5mg, Cu - 20 mg and Se - 0.5mg.

Vitamins supplied per kg feed: Vitamin A - 15000 IU, vitamin D3 - 4000 IU, vitamin E - 100 mg, vitamin K - 4 mg, riboflavin - 20 mg, thiamine - 4.4 mg, pyridoxine - 6 mg, niacin - 66 mg, cyanocobalamine-0.03mg, calcium D pantothenate - 30 mg, folic acid - 4mg and vitamin C -100mg.

Table 3: Fenugreek seed and GDFR influence on blood glucose (mg/dl) in broiler breeders (33-52 weeks)

Treatment	4th week*	8th week*	12th week*	16th week	20th week	1-20 week*
Control	$228.8^{b} \pm 8.5$	$226.3^{c} \pm 4.1$	$240.3^{bc} \pm 3.1$	235.2 ± 3.6	237.9 ± 6.8	$233.70^{c} \pm 2.67$
1% FS	$217.0^{ab} \pm 4.3$	$209.9^{a} \pm 5.8$	$217.3^{a} \pm 5.1$	233.9 ± 5.3	219.2 ± 6.4	$219.46^{ab} \pm 3.94$
2% FS	$218.2^{ab} \pm 2.7$	$211.1^{a} \pm 2.7$	$214.3^{a} \pm 4.7$	212.0 ± 6.1	229.7 ± 6.5	$217.06^{ab} \pm 3.39$
3% FS	$210.4^{a} \pm 3.3$	$212.5^{a} \pm 3.4$	$221.4^{a} \pm 4.8$	226.2 ± 5.0	230.4 ± 6.5	$220.18^{ab} \pm 3.85$
4% FS	$203.7^{a} \pm 3.2$	$213.3^{ab} \pm 5.5$	$212.8^{a} \pm 5.3$	215.5 ± 7.2	229.2 ± 6.7	$214.90^{a} \pm 4.11$
1% GDFR	$211.7^{a} \pm 2.9$	$225.5^{bc} \pm 5.7$	$241.5^{c} \pm 4.7$	227.1 ± 8.2	233.1 ± 7.3	$227.78^{bc} \pm 4.90$
2% GDFR	$214.6^{ab} \pm 9.1$	$210.2^{a} \pm 4.7$	$216.2^{a} \pm 6.1$	229.2 ± 7.3	216.4 ± 5.9	$217.32^{ab} \pm 3.17$
3% GDFR	$213.0^{a} \pm 4.5$	$207.3^{a} \pm 5.0$	$226.4^{ab} \pm 6.8$	214.9 ± 5.6	229.9 ± 6.0	$218.30^{ab} \pm 4.25$
4% GDFR	$203.8^{a} \pm 3.1$	$207.7^{a} \pm 2.3$	$222.0^{a} \pm 4.4$	229.9 ± 11.1	224.1 ± 4.7	$217.50^{ab} \pm 5.01$
P value	0.05	0.05	0.05	0.17	0.46	0.05
CD value	14.69	12.89	14.57	--	--	11.39

Each value is a mean of ten observations.

Means with at least one common superscript in a column do not differ significantly *(P>0.05).

Conclusions

The blood glucose level was decreased in 3 and 4% fenugreek seed in the first 28 days assessment period, afterwards the blood glucose was decreased in all the fenugreek seed fed birds in second and third assessment periods and overall trial period (233.7 *vs* 214.90 to 220.18 mg/dL). The blood glucose level was decreased at all levels of GDFR in the first assessment period, 2% and above GDFR in the second assessment period, 2 and 4% GDFR groups in third assessment period. In overall five assessment periods the blood glucose level was decreased when GDFR was supplemented at 2% and above compared to the control (233.70 *vs* 217.32 to 218.30 mg/dL). The blood glucose level was decreased at all level of fenugreek seed supplementation and with GDFR supplementation at 2% and above. Hence supplementation of fenugreek seed or GDFR up to 4% to broiler breeders reduces the blood glucose level.

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