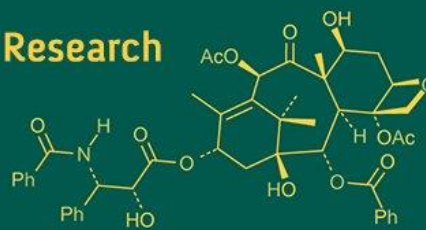


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Hemato-biochemical responses in goat kids to fenugreek (*Trigonella foenum-graecum*) seed supplementation

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Abstract

This study explores the impact of fenugreek (*Trigonella foenum-graecum*) seed supplementation on the hemato-biochemical profile of goat kids. Twelve male goat kids, aged 4-5 months, were randomly divided into two groups: a control group (T₀) and a treatment group (T₁). The T₁ group received fenugreek seeds at 3% of their dry matter intake (DMI), while the T₀ group was fed a diet of concentrate and roughages. Over a 90-day period, blood samples were collected to evaluate various haematological and biochemical parameters. The analysis revealed that supplementation with fenugreek seeds did not significantly alter haemoglobin levels, packed cell volume, red blood cell count, white blood cell count, or differential leukocyte count between the two groups. However, a notable reduction in blood glucose levels was observed in the fenugreek-supplemented group ($P < 0.05$). The total protein, albumin, globulin, and albumin-globulin ratio remained similar across both groups. These results suggest that fenugreek seed supplementation can effectively lower blood glucose levels without adversely affecting other blood parameters. This indicates the potential of fenugreek as a beneficial feed additive to enhance the nutritional management of goat kids, supporting its use in improving metabolic health in livestock. Further research may be required to explore its broader impacts on growth and overall performance in different livestock settings.

Keywords: Feed, fenugreek, goat, glucose, haemato-biochemical

1. Introduction

Goats are highly adaptable animals that can thrive on marginal or uneven terrain, which is often unsuitable for other forms of livestock farming. According to the 2019 20th Livestock Census, India has a goat population of 148.88 million, with 7.42% found in Madhya Pradesh. Notably, the goat population has grown by 10.14% since the 2012 livestock census (BAHS, 2022) ^[1]. In Madhya Pradesh, goat farming is crucial for the livelihood security and economic stability of small-scale farmers and landless rural communities. However, one of the major challenges in raising small ruminants is the lack of adequate feed. To enhance growth, production, and reproduction in these animals, ongoing efforts are being made to develop new feed resources. Feed additives play a key role in improving nutrient absorption, palatability, and intake, ultimately leading to better growth and productivity. However, the use of antibiotics as feed additives has been prohibited in many countries due to concerns over antibiotic-resistant pathogens that affect both animals and humans. As a result, researchers are increasingly turning to natural alternatives, such as organic acids, probiotics, and herbal growth promoters, as safer options for enhancing livestock nutrition and performance.

Herbs can benefit livestock through enhanced feed intake, immune support, and antibacterial, antiviral, anti-inflammatory, and antioxidant properties. Fenugreek (*Trigonella foenum-graecum*), traditionally used in India, is rich in saponins (4-8%) and other bioactive compounds like alkaloids, mucilage, and volatile oils, which promote health and improve nutrient metabolism. Known for lowering blood sugar and offering therapeutic benefits such as anti-helminthic and antimicrobial effects, fenugreek also contains 4-hydroxy isoleucine, which stimulates insulin secretion and enhances glucose tolerance. Its seeds, rich in dietary protein and fatty acids, also act as appetite stimulants, aiding digestion and animal

performance (Xue *et al.*, 2007; Singh & Pandra, 2005; Khan *et al.*, 2009) [2-4]. Considering the beneficial properties of fenugreek and the limited research on its dietary use in livestock, the present study aims to evaluate the impact of fenugreek seed supplementation on the hemato-biochemical profile of goats. This investigation seeks to explore the potential of fenugreek as a viable feed additive for enhancing animal health and performance.

2. Materials and Methods

2.1 Experimental Kids

Twelve male kids of local goat breed of similar age (4-5 month) and body weight were selected for this study. All kids were apparently healthy, dewormed using Albendazole @ 2.5ml/10 kg body weight, vaccinated by Raksha-PPR @ 1ml per kid and maintained in intensive system of feeding consisting of mixture and roughage as per requirements ICAR (2013) [5]. All the experimental kids were housed in a well-ventilated shed with provision of individual feeding and watering. Strict management and hygienic practices were adopted throughout the experimental period. Clean drinking water was provided *ad libitum* twice a day at about 10:00 A.M. and 5:00 P.M. daily. The kids were offered gram straw, green (*Berseem*) and commercial concentrate mixture (Sudana) to meet their nutrient requirements as per ICAR (2013) [5] recommendation for a body weight gain of 50 g/day. All the experimental kids were fed on a basal diet comprised of concentrate mixture, green and gram straw. Weighed amount of concentrate mixture was provided at 10:00 A.M. daily to meet their whole CP and major part of TDN requirement. Gram straw was provided *ad libitum* after complete consumption of concentrate mixture. The amount of concentrate mixture required by each kid was adjusted at every fifteen days interval based on their body weights.

2.2 Plan and Design of experiments

Fresh fenugreek seeds were procured from the market and sun-dried for three days. Twelve goat kids were randomly divided into two groups: a control group (T_0) and a treatment group (T_1), with six animals in each group. The T_1 group was supplemented with fenugreek (*Trigonella foenum-graecum*) seeds at 3% of their dry matter intake (DMI), while the T_0 group received only concentrate and roughages. At the end of the experiment, blood samples were collected for hematological analysis.

2.3 Blood analysis

2.3.1 Collection of blood and separation of serum

About 3 ml blood was collected from each kid through jugular vein puncture in the morning (before watering and feeding) after 90 days of feeding trial. Out of 3 ml blood, 1ml was collected into clean and dry test tube and kept in slanting position for 6-8 hours, 2 ml of blood was taken in another clean and dry vials containing anticoagulant for estimation of haematological parameters. After separation of serum, it was collected in clean and dry serum tubes and stored at -20°C for further analysis (Jain, 1986) [5].

2.3.2 Estimation of haematological parameters

2.3.2.1 Haemoglobin

Estimation of Hemoglobin by SAHLI's method. The principle of sahli's method or Acid hematin is quite easy that when the blood is added to N/10 Hydrochloric acid

(HCL), the hemoglobin present in RBCs is converted to acid hematin which is a dark brown colored compound.

2.3.2.2 Packed cell volume (PCV)

PCV was expressed as percentage (Jain, 1986) [5] Wintrobe tubes were filled with blood up to the ten marks and centrifuged at 3000 rpm for 30 min.

2.3.2.3 Red Blood Count (RBC)

Red Blood cells were counted by using a Hemocytometer or Neubauers chamber. For this, the blood specimen is diluted (usually in 1:200 ratio) with the help of RBC diluting fluid (commonly the Hayems Fluid) which preserve and fix the red blood cells. The Hayems fluid is isotonic to the red blood cells and does not cause any damage to it. After diluting the specimen, the content is charged on Hemocytometer/Neubauers chamber and the cells are counted in the areas specific for RBC count.

2.3.2.4 Total leukocyte count (TLC)

Whole blood is diluted with a 3% acetic acid solution, which hemolyzes mature erythrocytes and facilitates leukocyte counting. The standard dilution for leukocyte counts is 1:20. This dilution is prepared using the leukocyte Unopette system. The dilution is mixed well and incubated to permit lysis of the erythrocytes. Following the incubation period, the dilution is mounted on a hemacytometer. The cells are allowed to settle and then are counted in specific areas of the hemacytometer chamber under the microscope. The numbers of leukocytes are calculated per μL ($\times 10^9/\text{L}$) of blood.

2.3.2.5 Differential Leucocyte Count (DLC)

2.3.2.5.1 Leishman staining

Dry the thin blood smear in air. Cover the smear with a known quantity of the stain (normally, 8-10 drops) and allow to remain for 1 minute. Add double the quantity of neutral distilled water and mix it well with the stain by means of blowing air through a pipette and allowed to remain for 10-15 minutes. Washed off the stain quickly and allowed the slide to dry in a vertical position and then examined under oil immersion objective.

2.3.2.5.2 Staining procedure

Air dried film is placed on the staining rack and flooded with Leishman stain Allowed it to act for 30 seconds to one minute for fixation The stain was diluted nearly by twice of its original volume with distilled water or buffer. The pH of the buffer should be 7.2 for mammalian smear and 6.8 for avian blood Blow the solution until it forms a uniform mixture Allow it to act for 20 minutes Wash carefully with tap water dry it and examine first at low power and then at oil immersion, especially at t fringed edge and border.

2.3.3 Estimation of Blood Biochemical constituents

All the blood biochemical parameters were estimated by using diagnostic kits manufactured by Transasia Bio-medicals Ltd., Nalagarh Road, Village Malpur, Baddi, Dist. Solan, and (HP). In Technical Collaboration with: ERBA diagnostics Mannheim GmbH.

2.3.3.1 Serum glucose

Glucose in the sample is oxidised to yield gluconic acid and hydrogen peroxide in the presence of glucose oxidase. The

enzyme peroxidase catalyses the oxidative coupling of 4-aminoantipyrine with phenol to yield a coloured quinonimine complex, with absorbance proportional to the concentration of glucose in sample.

23.3.2 Total protein

The peptide bond of protein reacts with copper II ions in alkaline solution to form blue-violet complex (biuret reaction). Each copper ion complexing with 5 or 6 peptide bonds. Tartarate is added as a stabilizer whilst iodide is used to prevent auto-reduction of the alkaline copper complex. The colour formed is proportional to the protein concentration and is measured at 546 nm (520-560nm).

23.3.3 Albumin

Albumin binds with bromocresol green (BCG) at pH 4.2 causing a shift in absorbance of the yellow BCG dye. The blue-green colour formed is proportional to the concentration of albumin present, when measured photo metrically between 580-630 nm with maximum absorbance at 625 nm.

23.3.4 Serum globulin

It was calculated by subtracting serum albumin from total protein and expressed as g/dl.

23.3.5 Albumin and Globulin Ratio

It was calculated by dividing serum albumin by serum globulin.

2.4 Statistical analysis

The data were analyzed statistically using an independent t-test, following the method of Snedecor and Cochran (1994). The independent t-test was employed to compare differences between the groups and evaluate period means for various parameters, utilizing SPSS 20.0 software for the analysis.

3. Results and discussions

The data on haemato-biochemical profile of experimental kids estimated at the end of experiment and presented in Table 01. The average Hb value was 9.38 ± 0.23 and 9.60 ± 0.20 in groups T_0 and T_1 , respectively and was statistically similar. The average PCV (%) values were 28.10 ± 0.72 and 28.75 ± 0.62 in control and treatment groups, respectively and did not differ. The average RBC (million mm^3) values were 10.43 ± 0.35 and 10.83 ± 0.41 in group T_0 and T_1 , respectively and were similar between the groups. The average WBC ($10^3/\text{cu/mm}$) values were 8.70 ± 0.17 and 8.75 ± 0.18 in control and treatment groups, respectively and was statistically similar ($p > 0.05$). The average Neutrophils (%) values were 58.15 ± 0.27 and 58.27 ± 0.38 in control and treatment groups, respectively and did not differ. The average Eosinophils (%) values were 1.76 ± 0.22 and 1.90 ± 0.16 in control and treatment groups, respectively and were similar. The average Monocytes (%) values were 1.60 ± 0.41 and 1.66 ± 0.20 in control and treatment groups, respectively and did not differ. The average Lymphocytes (%) values were 40.52 ± 0.73 and 40.60 ± 0.79 in control and treatment groups, respectively and were statistically similar. The average Basophils (%) values were 0.09 ± 0.04 and 0.10 ± 0.16 in control and treatment groups, respectively and did not differ.

Total average total protein (g/dl) values were 5.74 ± 0.12 and 5.93 ± 0.05 in groups T_0 and T_1 , respectively and were similar. The glucose level is an indicator of the normal physiological condition and well-being of animals. The average glucose (mg/dl) values were 59.59 ± 3.12 and 56.49 ± 1.35 in control and treatment groups, respectively and lower ($P < 0.05$) in treatment group. Total average albumin (g/dl) values were 2.71 ± 0.05 and 2.83 ± 0.10 in groups T_0 and T_1 , respectively and were similar. The average globulin (g/dl) values were 3.03 ± 0.10 and 3.10 ± 0.10 in control and treatment groups, respectively and was statistically similar. Total average A: G ratio values were 0.89 ± 0.03 and 0.91 ± 0.06 g/dl in groups T_0 and T_1 , respectively and did not differ between the groups.

To ascertain the effect of fenugreek seeds supplementation on physiological status of health, routine haemato-biochemical studies (hemoglobin, packed cell volume, blood glucose and total serum protein) were made in goat kids. In the present study, supplementation of fenugreek seeds @ 3% of dry matter intake resulted in decrease ($p < 0.05$) in blood glucose concentration but, there was no effect on concentration of, serum total protein, serum albumin, serum globulin, albumin-globulin ratio, TLC, DLC, RBCs, PCV and haemoglobin.

The findings of the present study with respect to blood glucose is in agreement with the findings of Alamer and Basiouni (2005) [8] who reported that supplementation of 60 g per day fenugreek seeds in goats exhibited lower ($p < 0.05$) plasma levels of glucose compared to control group. Similarly, Ahmed and Al-Janabi (2012) [9] reported that supplementation of 4% and 6% fenugreek seeds powder in lactating Damascus crossbred goats resulted in lowered ($p < 0.05$) blood glucose but increased serum total protein level as compared to control group. Babekir (2015) [10] also reported that increasing fenugreek level to 15% in Nubian Goats lead to a decreasing trend in blood glucose level. Al-Sherwany (2015) [11] also reported that addition of fenugreek seeds to basal ration at the level 0.6 and 1.2g/kg live body weight in Hamdani ewes resulted in no change in globulin concentrations as compared to the control group.

The decrease in blood glucose concentration in the fenugreek supplemented groups may be due to the fact that fenugreek seeds contain 50% pectin that forms a colloid suspension when hydrated which decreases the rate of gastric emptying and inhibition of glucose transporter leading to slow carbohydrate absorption (Al-Habori and Raman, 2002) [12]. However, Bordia *et al.* (1997) [13] reported that fenugreek seeds contain chemicals that slow down the time that food takes to go through the intestinal tract leading to slow absorption of sugars and blood sugar levels may not rise as high or fluctuate as much as usual. The low blood glucose concentration due to fenugreek supplementation may also be due to the presence of an amino acid called 4 hydroxy iso leucine in fenugreek seeds which appears to act on pancreatic beta cells to increase the body's production of insulin. Higher amounts of insulin production may decrease the sugar that stays in the blood (Schryver, 2002) [14]. The findings of Raju *et al.* (2001) [15] and Devi *et al.* (2003) [16] that the ability of fenugreek seeds to modulate key glucose metabolizing enzymes such as hexokinase (glycolysis), glucose-6-phosphatase or fructose-1,6-bisphosphatase (gluconeogenesis) also might be considered as a possible mechanism. Further, Abbas *et al.* (2012) [17] who observed the effect of dietary fenugreek

seeds on Saidi lambs and reported that supplementation of dietary fenugreek seeds at the rate of 3% of body weight had no increase in serum total protein. Similarly Al-Sherwany (2015) [11] also observed no effect of feeding fenugreek seeds on the total serum protein in hamdani ewes. In similar line, Al-Wazeer (2017) [18] studied effect of supplementing graded level of fenugreek seeds (0, 2.5, 5, 7.5 gm/head/day) on serum metabolites in Awassi lambs and they observed that the no difference ($p>0.05$) among serum total protein. Motamedi and Taklimi (2014) [19] in laying hens and Yatoo *et al.* (2012) [20] in broilers reported no decrease ($p>0.05$) in serum protein content while Patel *et al.* (2014) [21] and Abbas (2010) [22] in broilers reported no increase ($p>0.05$) in serum protein content upon inclusion of fenugreek seed in the diets. The present study substantiates the results obtained by, Patel *et al.* (2014) [21] and Abbas (2010) [22] reported no difference in serum albumin content of broilers fed diets containing fenugreek seed at 3g/kg. Motamedi and Taklimi (2014) [19] in laying hens and Yatoo *et al.* (2012) [20] in broilers reported no decrease ($p>0.05$) in serum albumin content upon inclusion of fenugreek seed in the diets. Motamedi and Taklimi (2014) [19] in laying hens and Patel *et al.* (2014) [21] in broilers reported no increase in serum globulin content upon inclusion of fenugreek seed in the diets. On the other hand, Yatoo *et al.* (2012) [20] reported no decrease in serum globulin content of broilers when fed diets containing fenugreek seed.

The present findings are in contrast to the findings of Abo El-Nor *et al.* (2007) [23] wherein they reported that supplementation of 200 g fenugreek seeds resulted in increase ($p<0.05$) in blood glucose, total protein and albumin. Abbas *et al.* (2012) [17] reported that supplementation of dietary fenugreek seeds at the rate of 3% of body weight exhibited elevation trend of serum total protein and blood glucose concentrations. Ishtiyak *et al.* (2013) [24] reported that supplementation of raw fenugreek seeds at 3% of dry matter intake resulted in increase ($p<0.05$) in total serum protein and serum albumin but there was no difference in serum globulin. El-Ashry *et al.* (2006) [25] who found that the mean values of blood metabolites, serum, total protein, globulin and albumin/globulin ratio were higher in treated calves with medicinal herbs than control.

From the results of the present findings, it may be concluded that the blood biochemical parameters were not influenced by supplementation of fenugreek seeds except blood glucose level and supplementation did not have any adverse effects on the health of goat kids.

Table 1: Haemato-biochemical parameters of experimental kids

Attributes	T ₀ (Control)	T ₁ (Treatment)	p-value
Haemoglobin (g/dl)	9.38±0.23	9.60±0.20	0.69
PCV (%)	28.10±0.72	28.75±0.62	0.68
RBC (million/mm ³)	10.43±0.35	10.83±0.41	0.72
WBC (10 ³ /mm ³)	8.70±0.17	8.75±0.18	0.96
DLC (%)			
Neutrophils	58.15±0.27	58.27±0.38	0.12
Eosinophils	1.76±0.22	1.90±0.16	0.38
Monocytes	1.60±0.41	1.66±0.20	0.17
Lymphocytes	40.52±0.73	40.60±0.79	0.10
Basophils	0.09±0.04	0.10±0.16	0.25
Total Protein (g/dl)	5.74±0.12	5.93±0.05	0.03
Glucose (mg/dl)	59.59±3.12	56.49±1.35	0.04
Albumin (g/dl)	2.71±0.05	2.83±0.10	0.10
Globulin (g/dl)	3.03±0.10	3.10±0.10	0.78
A:G ratio	0.89±0.03	0.91±0.06	0.07

4. Conclusion

Supplementing the diet of goat kids with fenugreek seeds at 3% of dry matter intake resulted in a significant reduction in blood glucose levels ($P<0.05$), indicating a positive impact on glucose metabolism. However, no significant changes were observed in other hematological parameters, such as hemoglobin, packed cell volume, red and white blood cell counts, or differential leukocyte counts. Similarly, the total protein, albumin, globulin levels, and the albumin-globulin ratio remained unaffected by fenugreek supplementation. These findings suggest that fenugreek seeds can be a beneficial feed additive for managing blood glucose levels in goat kids without adverse effects on overall blood health. The results support the potential of fenugreek as a natural alternative to enhance livestock nutrition. Further research is needed to explore its long-term effects and broader benefits on animal health and performance.

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