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Trigonellafoenum-graecum SEEDS AS A FOOD SUPPLEMENT TO IMPROVE FOOD QUALITY IN THE TREATMENT OF MALNOURISHED RATS

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Abstract

Protein-Energy Malnutrition (PEM) is most prevalent in low and middle income countries were about 45% of child deaths were attributed to under-nutrition. *Trigonellafoenum-graecum* (fenugreek) is a plant of Fabaceae family whose seed is known for its important phytochemicals and its nutraceutical purposes. The aim of this study was to evaluate the effects of supplementing diets with fenugreek seeds has on food quality and hence the health of its consumer using rats. Forty (40) weaning wistar rats weighing between 30 and 50g were randomly grouped into treatment groups of ten (10) animals each. Group 1 was the control while all others were malnourished with low protein (4%) iso-caloric diet for four weeks thereafter, randomly grouped into group 2 fed with standard commercial feed, Groups 3 and 4 fed with 10g/kg and 20g/kg fenugreek seeds supplemented diets respectively for 4 weeks and Group 5 was untreated. There was significant ($p<0.05$) reduction in all heamatological parameters of malnourished untreated rats whereas, all treated groups were significant ($p<0.05$) increase after treatment with fenugreek supplemented diets. Moreover, the significant ($p<0.05$) increase in Alanine transaminase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) observed in the serum of untreated rats were significantly ($p<0.05$) reduced in all the treatment groups after treatment. Hence, fenugreek supplemented diets may improve hematological parameters of malnourished rats and it is not toxic to the liver thereby increasing the quality of food.

Keywords: fenugreek, protein-energy malnutrition, iso-caloric, supplement, diets, food quality

Introduction

Fenugreek is an angiosperm belonging to the family fabaccae. Fenugreek (*Trigonellafoenum-graecum*) seeds have gained attention due to their rich nutrient profile and potential health benefits. Fenugreek seeds are known for their high content of proteins, dietary fiber, vitamins, and essential minerals (Sarah *et al.,* 2024). Research indicates that fenugreek seeds contain approximately 23% protein, making them an excellent source of plant-based protein, 6-8% fat, and 25% dietary fiber (Bashir *et al.,* 2021). The carbohydrate content is primarily composed of soluble and insoluble fibers, which aid in digestion and contribute to satiety (Rathore *et al.,* 2018). Additionally, they are rich in essential amino acids and have a favorable fatty acid profile, contributing to their potential as a functional food (Zhang *et al.,* 2020). Fenugreek seeds are also rich in essential vitamins such as vitamin B6, folate, and vitamin C, which are vital for various metabolic functions (Bhandari *et al.,* 2020) and minerals like iron, magnesium, phosphorus, and calcium, contributing to the prevention of micronutrient deficiencies (Kumar *et al.,* 2016).

A crucial role is played by nutrition in the prevention of chronic diseases especially the diet-related diseases. Hence, the concept of regarding food as being "functional food" not only necessary for living but also as a source of mental and physical well-being, contributing to the prevention and reduction of risk factors for several diseases or enhancing certain physiological functions. Studies indicate that incorporating fenugreek into diets can enhance growth and improve metabolic health (Nagulapalli-Venkata *et al.,* 2017). Therefore, the aim of this study was to evaluate the effects of supplementing diets with fenugreek seeds on food quality and hence the health of its consumer using rats.

Materials and Methods

Materials

Experimental Animals

40 weaning wistar rats weighing 30-40g were obtained from the Animal holding unit, University of Ilorin and transported to the animal house of the biochemistry unit of Al-Hikmah University, Ilorin.

They were acclimatized for seven days in metabolic cages under good laboratory condition. They were acclimatized using growers mash feed (top feed) and clean water *ad libitum*. Proper sanitation was maintained in the animal house to ensure a healthy and clean environment. The animals were weighed using triple beam weighing balance.

Feed Material

Feed was constituted according to the method used by Lambe and Bewaji (2022). Corn starch, soya beans, soybean oil, sucrose (Golden Penny refined sugar) were obtained from Mandate Market, Ilorin, Kwara State. Corn bran was gotten from Mende, Maryland, Ikeja, Lagos state. Fenugreek seed was gotten from Ogbomosho, Oyo state. Identification and identification of the fenugreek was done at the University of Ilorin Herbarium where voucher number was deposited under UILH/001/1799/1024.

DL-methionine and Vitamin-Mineral mix (Miavit GmbH Germany) were purchased from Olufunmilayo Farms Limited, Offa Garage Road, Ilorin. The components of the formulated meals are as shown in Table 1.

Table 1: Components of the Control and Test Diets

Diet composition	Control diet	Low isocaloric Diet	10g fenugreek diet	20g fenugreek Diet
Corn starch	516	100	496	506
Fibre	40	400	40	40
Sugar	100	366	100	100
Soybean	250	40	25	25
Oil	40	40	40	40
Dl ethionine	4	4	4	4
Vitamix	50	50	50	50
Fenugreek	-	-	10	20

Methods

Proximate Analysis

For the proximate analysis of the feed samples, Association of Official Analytical Chemists recommended methods (AOAC, 1990), 18th edition was used to measure the level of crude protein, ash, moisture content and fat. All analysis was done thrice and the mean of the results was obtained and reported.

Animal Grouping and Induction of Malnutrition

Ten of the rats belonging to the control group were not malnourished at all while others were induced with malnutrition using the method of Lambe and Bewaji (2017) by feeding the rats with 4% low protein iso-caloric diet for duration of 4 weeks. The weights of the rats were taken weekly using an analytical weighing balance. At the end of the four (4) weeks of induction, the rats that were induced with malnutrition were regrouped randomly into 4 groups. Table 2 shows Animal Grouping and their Treatment:

Table 2: Animal Grouping and Treatment

Groups	Treatment Administration (g/kg body weight)
Control	Rats that were given regular diet
Standard	Malnourished that were given Commercial Feed
10g/kg fenugreek	Malnourished rats that were treated with 25g/kg supplemented diet
20g/kg fenugreek	Malnourished rats that were treated with 50g/kg supplemented diet

The grouping is as follows:

Anthropometric Measurements

The weights of the rats were measured every week throughout the experimental period with 0.01 precision Kern weighing balances.

Collection of Blood and Preparation of Homogenates

The rats were sacrificed by cervical dislocation and blood was collected by jugular puncture. Blood samples were collected into plain and some into EDTA coated sample bottles (to prevent clotting) for serum and haematological analysis respectively. Serum was thereafter prepared by centrifuging the blood samples at 3000 rpm for 5 minutes.

Biochemical Assay

Activity of aspartate transaminase (AST) and alanine transaminase (ALT) in the serum and liver homogenate were determined following the method reported by Reitman and Frankel (1957) as modified by Schmidt and Schmidt (1963) which is based on the enzymatic conversion of substrates by ALT and AST, leading to the formation of colored products. The intensity of the color formed is directly proportional to the enzyme activity, which can be quantitatively measured using a spectrophotometer.

Haematological Analysis: The haematological parameters analyzed include, red blood cell count, white blood cell count, blood haemoglobin, full blood count. These were analyzed using automated haematological analyzer, which uses the impedance principle to count and size cells. This principle involves measuring changes in electrical impedance as the cells pass through a small aperture in the instrument.

Statistical Analysis

Data were analyzed using one-way ANOVA and differences were considered significant when $P < 0.05$. Values presented are mean ± SEM. Graph pad prism for windows was used to analyze the data.

Results

Proximate Composition of Formulated Feed

The proximate analysis shows that the % moisture, % ash, % fat, % fiber, %protein of the control diet, standard diet, 10%and 20% fenugreek seed-supplemented diet have no significant difference ($p \geq 0.05$). Whereas, the % moisture of the low protein iso-caloric diet is significantly higher ($p<0.05$) in comparison to the control diet and the %ash, % fat, % fiber and % protein of the low protein iso-caloric diet are significantly lower ($p<0.05$) in comparison to the control diet. The % carbohydrates of all the feeds have no significant difference ($p \geq 0.05$) (Table 3).

Table 3: Proximate Analysis of Formulated Feeds

	CONTROL	UNTREATED	STANDARD	1.0% Fenugreek	2.0% Fenugreek
WBC×10^9/L	5.30±0.50[a]	4.00±0.58[b]	4.33±0.57[a]	4.80±0.58[a]	5.65±0.38[a]
NEU (%)	67.00±2.80[a]	56.00±2.31[b]	62.75±2.36[a]	65.50±3.93[a]	74.00±1.16[a]
LYMPH (%)	33.00±2.78[a]	44.00±2.31[b]	37.25±2.36[a]	34.50±2.75[a]	26.00±0.95[a]
RBC×10^{12}/L	5.20±0.03[a]	4.06±0.35[b]	4.92±0.15[a]	5.42±0.05[a]	5.59±0.08[a]
HB (g/dl)	9.63±1.37[a]	7.20±0.65[b]	9.84±0.44[a]	10.67±0.58[a]	11.01±0.19[a]
PCV (%)	30.00±0.00[a]	22.00±1.03[b]	33.25±2.78[a]	33.00±0.41[a]	34.00±0.82[a]
MCV (fl)	61.22±3.33[a]	42.68±0.04[b]	58.99±0.01[a]	62.30±1.75[a]	63.48±2.03[a]
MCH (Pg)	20.05±0.41[a]	15.40±0.58[b]	20.00±0.00[a]	20.50±0.50[a]	20.70±0.75[a]
MCHC (g/d)	33.33±0.40[a]	30.28±2.88[a]	29.46±0.47[a]	32.20±0.01[a]	36.35±0.25[a]

Values with different superscript across the same row are significantly different ($p < 0.05$)

Effects of Fenugreek Supplemented Diet on the Anthropometric Parameters of Malnourished Rats

Low protein iso-caloric diets produced significant *($p<0.05$*) weight reduction in the experimental rats (Figure 1). However, after feeding the animals with the treatment feeds, all the anthropometric parameters were significantly increased with no difference (*$p\geq0.05$*) in those fed control diets, standard diets, 10% and 20% supplemented diets (Figure 1 and 2).

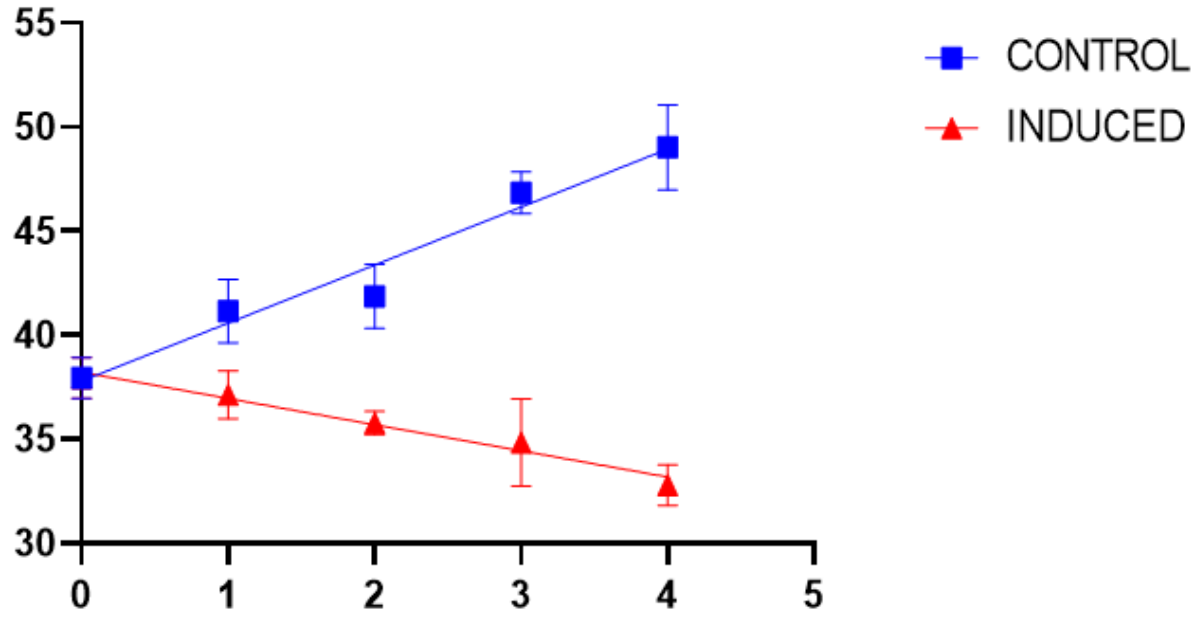

Figure1: Mean weight of rats in the first four weeks of malnutrition induction

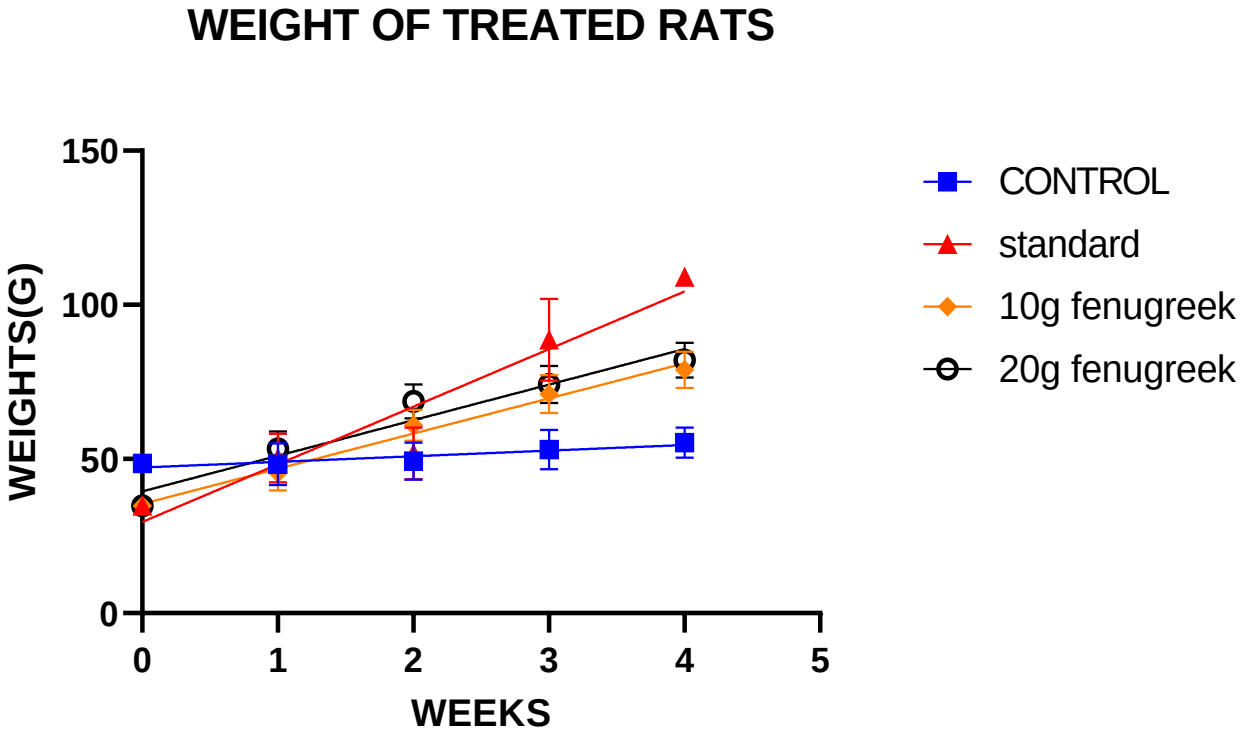

Figure 2: Mean Weight of Rats after Treatment

Effects of Fenugreek Seed Supplements on the Liver Function Indices of Malnourished Rats

Serum concentration of ALT, AST and ALP was significantly (P< 0.05) increased in experimental rats following feeding with low protein iso-caloric diets. This concentration however increased significantly after feeding the malnourished animals with the formulated treatment feeds, with the most significant increase in the group treated with 20% fenugreek supplemented diet. (Figure 3 and 4)

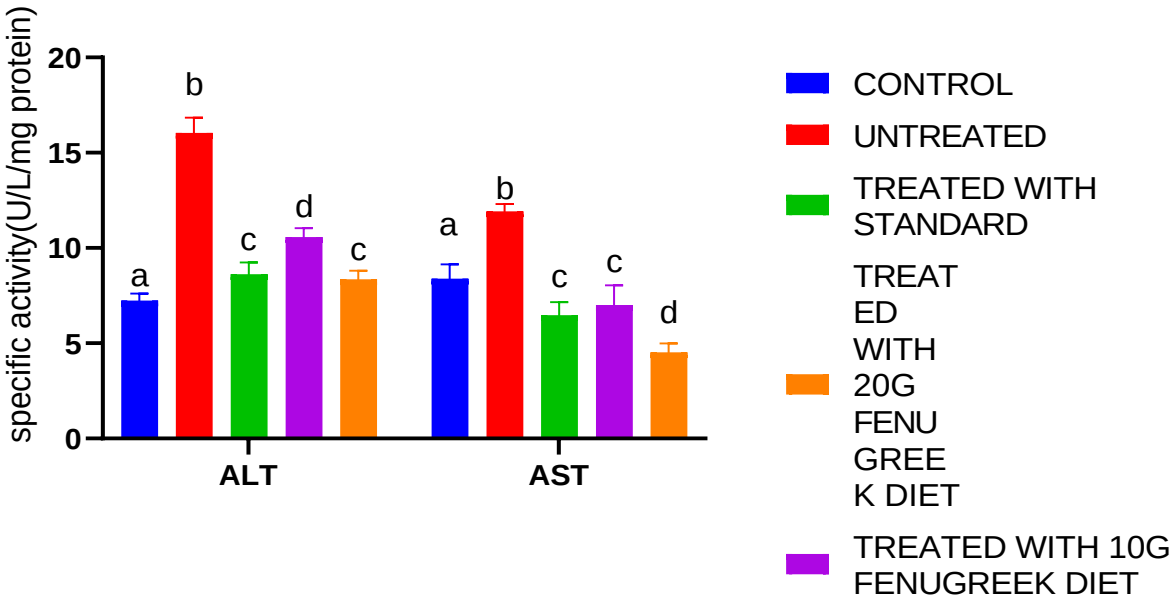

Figure 3:

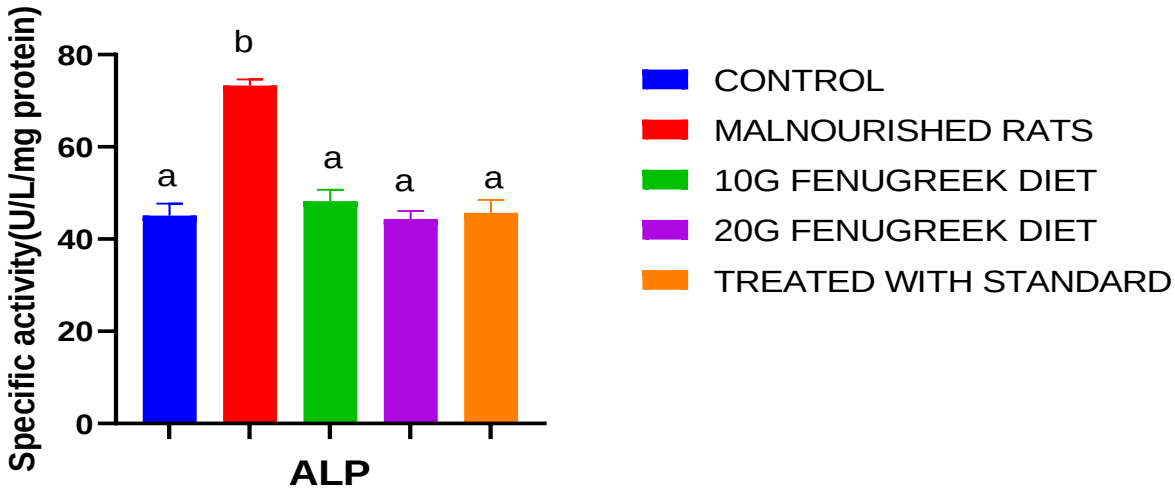

Figure 4:

Hematological Parameters

Table 4 shows the result of the heamatological evaluation of the cmtrol and treated rats. Following the significant reduction in the number of WBC in the blood of untreated animals, there was significant increase in those of standard and treatment groups with no significant difference with the control. The same trend was observed with all other heamatological parameters except MCHC where there was no significant difference within all the experimental groups.

Table 4: Heamatological Parameters of Malnourished and Treated Rats

	CONTROL	UNTREATED	STANDARD	1.0% Fenugreek	2.0% Fenugreek
WBC×10^9/L	5.30±0.50[a]	4.00±0.58[b]	4.33±0.57[a]	4.80±0.58[a]	5.65±0.38[a]
NEU (%)	67.00±2.80[a]	56.00±2.31[b]	62.75±2.36[a]	65.50±3.93[a]	74.00±1.16[a]
LYMPH (%)	33.00±2.78[a]	44.00±2.31[b]	37.25±2.36[a]	34.50±2.75[a]	26.00±0.95[a]
RBC×10^{12}/L	5.20±0.03[a]	4.06±0.35[b]	4.92±0.15[a]	5.42±0.05[a]	5.59±0.08[a]
HB (g/dl)	9.63±1.37[a]	7.20±0.65[b]	9.84±0.44[a]	10.67±0.58[a]	11.01±0.19[a]
PCV (%)	30.00±0.00[a]	22.00±1.03[b]	33.25±2.78[a]	33.00±0.41[a]	34.00±0.82[a]
MCV (fl)	61.22±3.33[a]	42.68±0.04[b]	58.99±0.01[a]	62.30±1.75[a]	63.48±2.03[a]
MCH (Pg)	20.05±0.41[a]	15.40±0.58[b]	20.00±0.00[a]	20.50±0.50[a]	20.70±0.75[a]
MCHC (g/d)	33.33±0.40[a]	30.28±2.88[a]	29.46±0.47[a]	32.20±0.01[a]	36.35±0.25[a]

Discussion

Fenugreek seed has been reported to be rich in important phytochemicals which are responsible for its various actions. The result of the proximate analysis carried out on the fenugreek supplemented feed is similar to the one reported by Sahelian (2004) who claimed that fenugreek seed contains high carbohydrates (mucilaginous fibre and galactomannan) and proteins which were reported to be 20-30% high in tryptophan and lysine, free amino acids such as 4- hydroxyisoleucine, arginine, lysine and histidine. Abdel-Azeem (2006) also reported similar results for crude protein, moisture and ash contents. Hence, fenugreek seed supplemented diet can be used as a nutritional supplement to improve the quality of a food due to their lysine content and as a substitute for soybean.

The reduction in the weights of the rats following the induction of the animals with malnutrition and it showed a significant increase in their weights after they were fed with the 10% and 20% supplemented diet. This is in tandem with the reports that show that fenugreek seed helps with weight gain.

Similar to the reports of Kumar *et al.,* (2018) who explored the therapeutic and nutritional effects of fenugreek seeds in addressing malnutrition and Albasha, *et al.* (2014), which indicated the ability of fenugreek to prevent pathological changes in rat hepatocytes against various causes of toxicity. The reduction in ALT and AST levels in the serum of rats fed fenugreek supplemented diets after induction with malnutrition, suggests a decrease in liver damage and inflammation, which is further supported by the significant increase in the AST and ALT in the liver of these rats. These indicates improved liver synthetic function and a potential reduction in liver fibrosis (Lee *et al.*, 2018).

In addition, it was observed that *Trigonella foenum-graecum* seed improved hematological parameters of malnourished rats including its ability to increase the number of White Blood Cell (WBC) and Packed Cell Volume (PCV), improving blood immunity and preventing anemia. This can be supported by a study by Abdel-Hamid (2019) which reported this may be because fenugreek seed is rich in protein, essential amino acids, iron, ascorbate, and folate content. Doshi *et al.,* (2012) reported that fenugreek seed improved the hemoglobin level in females of childbearing age.

These positive effects observed in the fenugreek-supplemented diets could be attributed to the presence of bioactive compounds like flavanoids, saponins and fiber which have shown anti-inflammatory, antioxidant and hepatoprotective activities (Suresh *et al*., 2018).

Conclusion

Conclusively, fenugreek seed- supplemented diet improves biochemical parameters and is safe for consumption.

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