



Impact of Diet Supplementation with Fenugreek Seeds on Digestibility and Some Rumen and Blood Parameters in Lambs

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Abstract: This study was designed in two parts to assess the impact of fenugreek seeds (FS) supplementation on lambs' nutrient digestibility and some parameters of rumen and blood. Four lambs were used during the first part of the study by Latin square design, and the lambs were individually fed a concentrate on the level of 3% of live body weight. The first referred to as control (C) had no FS; the second, third, and fourth groups (T1, T2, and T3) were treated by FS at rates of 5, 10, and 15%, successively. Each lamb gradually received feed through a preliminary interval for 10 days and subsequent 5 days for sampling, and then every lamb was switched to another treated diet. In the second part of the experiment, in Sacco degradability of the rations and FS was measured by incubating samples in the rumen of two cannulated ram lambs for 1, 2, 4, and 6 hours. The findings show that adding FS did not change the ruminal pH, ammonia-nitrogen concentration (NH₃-N), or nutrient digestibility coefficients. Serum glucose (Glu) levels were significantly ($P < 0.05$) reduced by fenugreek seeds, while other blood biochemicals were unaffected. After the feed samples were incubated in the rumen for one hour, there was a notable interaction between time and FS level that increased the Sacco degradability of the FS-treated diets. Six hours post-incubation in the rumen, supplementing FS to the lambs' feed at up to 10% showed a significant elevation in the Sacco DM degradability. In light of the current results, it is concluded that supplementing fenugreek seeds to the lamb's ration has not influenced nutrient digestibility and ruminal fermentation.

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1. Introduction

Fenugreek (*Trigonella foenum-graecum* L.) from the Leguminosae family is one of the oldest known therapeutic herbs and is utilized in ayurvedic solutions. It is considered to be a multipurpose edit for its nourishment and non-food employments such as herb, zest, and grub, and its nutraceutical, pharmaceutical, and therapeutic properties (Ahmad et al., 2016). It is well accredited for its different therapeutic excellencies as a metabolic modifier, antioxidant, and anti-inflammatory (Wani and Kumar, 2018). Moreover, some other organic compounds are found in FS including the saponins (Rao and Sharma, 1987) and alkaloids (Mishkinsky et al., 1974) which actively affect the nutritional properties

of feed. It is demonstrated that Fenugreek might be used as a feed additives with beneficial effects on the intake of feed and rumen and blood parameters (Abu EL-Kassim et al., 2018). It has been shown that supplementing the diet with FS increases the digestibility extent of organic matter, dry matter, and crude protein of goats (Abou-Elenin et al., 2016), with no effects on rumen pH and rumen ammonia nitrogen concentration (Al-Wazeer, 2017), or reducing ammonia nitrogen concentration Ibidhi and Ben Salem, (2022). No effects of feeding FS were found in blood glucose, cholesterol, and total protein levels in lactating goats (Al-Shaikh et al., 1999) and total proteins in dairy cows (Maher and Nadya, 2013). In contrast, El-Tarabany et al. (2018) found that feeding dairy goats on a diet including FS led to a significant drop in blood glucose concentration. Since studies with the impact of feeding FS on digestibility, rumen fermentation, and blood metabolites in ruminants have shown controversial results, hence, the current study aimed to evaluate the impact of supplementing the diet of Karadi lambs with FS on whole tract digestibility, rumen fermentation, *in Sacco* degradability and blood biochemical parameters.

2. Material and Methods

The study was performed at the animal production farm of the College of Agricultural Engineering Sciences / Duhok University. In part one of the study, four ewe lambs of one year old and 34 ± 1 kg live weight in a Latin square design were fed on four rations, the first was control (C) consisted mostly of wheat bran, barley, soybean meal, and wheat straw, the same ration was supplemented by fenugreek seeds at levels of 5 (T1), 10 (T2), and 15% (T3) as shown in Table 1. Lambs were fed for 10 days as a preliminary period of one of the experimental rations to be accustomed to the diet and then moved, and held in metabolic cages, daily feed intake was recorded and fecal samples were collected for five successive days before the morning feeding, weighed and 25% of feces kept for analysis. Feed and feces samples were analyzed chemically in triplicates for DM, OM, CP, and EE according to the methods of (Thiex et al., 2012). On the 15th day, rumen fluid samples were withdrawn by suction through a stomach tube pre and two hours post-morning feeding, strained through two folds of cheesecloth, and pH registered immediately (Ceyhan and Erdoğan, 2025). Ammonia-nitrogen concentration was measured using a spectrophotometer (Mustafa et al., 2022), according to the method of Broderick and Kang (1980). Blood samples also were drawn on the 15th day via jugular venipuncture, centrifuged, serum separated, and kept at -20 °C for upcoming analysis. Total proteins, albumin, triglyceride, cholesterol, and urea levels in serum were determined calorimetrically using standard kits (Biolabo SA Laboratory Reagent and Products, France). Serum glucose was determined calorimetrically using standard kits (Olasmatec Laboratory Product, UK). The hormones T3 and T4 in serum were measured using the Vidas apparatus which depended on Enzyme Linked Fluorescent Assay.

Two ram lambs of Karadi breed weighing 32 kg equipped with rumen cannula were saved in the second part of the study to determine the *in Sacco* degradability of dry matter and crude protein for the experimental rations via polyester bags technique (Örün and Erdoğan, 2022), according to the method shown by Paine et al. (1982). The degradability of the experimental rations was measured after 1, 2, 4, and 6 hours of incubation time in triplicates while the cannulated rams were managed to consume the control diet.

2.1. Statistical analysis

The data of the digestibility, rumen fermentation, and blood metabolites were subjected to statistical analysis using Latin square design via SAS program software (SAS, 2001), and the difference among the means was determined using Duncan's multiple test range. The data of the degradability were analyzed for repeated measures analysis of difference using the SAS program (SAS, 2001).

3. Results and Discussion

The feed ingredients and the chemical composition of the experimental diets and FS are shown in Table 1. The crude protein content of FS seeds was 26% therefore, to prepare isonitrogenous diets, soybean meal was added to the control diet.

Table 1. Showed the ingredients and composition of the experimental rations

Ingredient (%)	C	T1	T2	T3		Chemical composition (%)				
						C	T1	T2	T3	FS
Barley grain	50	50	50	50	DM	95.40	95.30	95.81	95.73	94.67
Wheat bran	35	35	30	25	OM	94.23	93.72	95.21	95.26	97.27
Soybean meal	5	0	0	0	CP	14.50	14.28	14.17	14.84	26.0
FS	0	5	10	15	EE	3.36	3.08	3.49	3.38	2.56
Wheat straw	8	8	8	8	CF	9.26	9.30	9.35	9.40	8.50
Vitamins-minerals	1	1	1	1	Ash	5.77	6.28	4.79	4.74	2.73
Salt	1	1	1	1	NFE	62.51	62.36	64.01	63.37	54.88

The values are expressed as means of triplicate analysis. Control 0 FS, T1 5% FS, T2 10% FS, T3 15% FS.
Chemical analysis was carried out (on the basis of dry matter) at the nutrition lab. Animal Production Department.
¹NFE%= 100- (Moisture + Ash + EE + CP + CF contents).

3.1. Nutrient digestibility

The results of digestibility are presented in Table 2. Supplementing diets with FS had no significant effect on DM and OM digestibility. These results were in agreement with those reported by Al-Wazeer, (2017) and Smit (2014), who noted feeding FS, did not affect the digestibility of DM and OM. In contrast, Elmnan et al. (2013) noted that the DM and OM digestibility were significantly higher in Nubian goats fed FS levels of up to 15% as compared to the control diet. However, the result of Abo El-Nor et al. (2010) showed that nutrients digestibility for DM and OM was significantly improved by adding fenugreek seed meal.

The results revealed that supplementing different FS levels in this study had no significant effect on CP digestibility. Similarly, some studies have reported that the supplementation of fenugreek seed has no effect on CP digestibility (Smit, 2014; Al-Wazeer, 2017). The differences in the findings of the current study may be due to the difference in animal species as goats are more efficient in digesting plant active compounds than sheep (Hidayet and Mustafa, 2020). In contrast, Elmnan et al. (2013) found that the digestibility of CP was significantly higher ($P<0.05$) in animals fed on 10 % and 15 % fenugreek seeds than those fed on 5% and control diet.

There was a numerical decrease in the digestibility of EE as the level of FS increased in the treatment groups (Table 2), and these results disagree with that of Abou-Elenin et al. (2016), who noted that digestibility for EE was significantly ($P<0.05$) increased by using 30 g of FS than of those not receiving FS. It is obvious in this study that feeding 15% FS lowered EE digestibility by about 20%, which disagrees with those found significant improvement in EE digestibility when they fed FS to lactating buffalos (Abo El-Nor et al., 2010). Such differences in the effect of FS on digestibility coefficients may be related to the effect of FS level used in the diet which led to different amounts of available saponins that stimulate the anaerobic fermentation and nutrients utilization (Abo El-Nor et al., 2010).

Table 2. Effect of different levels of fenugreek seeds on nutrient digestibility

Treatment	Nutrient digestibility (%)			
	DM	OM	CP	EE
Control	66.18±1.56	69.05±1.35	71.99±3.04	72.30±1.52
T1 5% FS	66.29±1.46	70.22±1.58	73.49±4.07	66.75±4.75
T2 10% FS	66.48±2.19	69.06±1.83	72.09±3.05	61.20±5.98
T3 15% FS	66.06±3.16	68.79±3.16	70.49±2.55	58.20±3.60

Values are presented as means± SE.

3.2. Rumen parameters

The pH values of the rumen liquor before feeding were 6.65, 7.03, 6.85, and 6.80 for control, T1, T2, and T3, respectively, the change was only significant ($P<0.05$) between control and those animals fed on T1, while after feeding, the differences were not significant and pH values ranged from

6.64 to 6.78 (Table 3). Such findings are in accordance with results obtained by Kumar et al. (2016) and Abo-Donia et al. (2005). Also, it was reported by (Abu El-Kassim et al., 2018) that pregnant ewes consuming a diet supplemented by FS had not shown significant fluctuations in the levels of volatile fatty acids in the rumen. It is well known that rumen liquor pH usually decreases after feeding due to the fermentation and formation of volatile fatty acids. In this study, the pH was not affected by FS levels after feeding, and the reason is not clear, but maybe be due to the absence of the reactions of FS on volatile fatty acids in the rumen and/or due to the lower rate of DM digestibility of the rations used in this experiment.

The results in Table 3 present that feeding different rates of FS had no significant effect on $\text{NH}_3\text{-N}$ concentration. The values ranged between 5.75 to 6.46 mmol dL^{-1} and 4.63 to 6.96 mmol dL^{-1} before and after feeding, respectively. A slight decrease was noted in $\text{NH}_3\text{-N}$ concentration after feeding, this finding could be explained as noted by Newbold et al. (2015) by the fact that the supply of FS had an anti-protozoal influence which could be correlated with an enhancement in microbial synthesis or may be due to its utilization by the microorganisms and/or to the low-rate degradability of FS protein content. Ibidhi and Salem (2022) noted that the addition of FS to concentrate feeds significantly ($P<0.05$) decreased rumen $\text{NH}_3\text{-N}$ concentration. On the other hand, Santoso et al. (2007b) noted that the ($\text{NH}_3\text{-N}$) proportion in rumen is mainly under the effect of both protein degradation and synthesis of microbial protein. The current results conform with the published by Al-Wazeer (2017). However, contrary to our results, an elevation in ruminal $\text{NH}_3\text{-N}$ was shown by Elmnan et al. (2013), with an increasing FS ratio in the diet.

Table 3. The Effect of dietary levels of fenugreek seeds on rumen pH and ammonia-nitrogen

Treatment	pH		$\text{NH}_3\text{-N}$ concentration (mmol dL^{-1})	
	Before feeding	After feeding (2hrs.)	Before feeding	After feeding (2hrs.)
Control	6.65±0.13b	6.64±0.19	5.75±0.91	6.96±0.77
T1 5% FS	7.03±0.12a	6.66±0.27	6.46±0.72	5.51±0.68
T2 10% FS	6.86±0.13ab	6.74±0.06	6.09±0.86	4.63±1.49
T3 15% FS	6.80±0.13ab	6.78±0.10	6.01±0.59	5.0±0.18

Values are presented as means± SE. Different letters in the same column means significant difference ($P<0.05$).

3.3. Blood metabolites

In the present experiment, results linked to blood biochemical parameters of lambs fed different rates of FS are demonstrated in Table 4. Feeding lambs on a diet supplanted with 5% FS significantly ($p<0.05$) decreased serum glucose content as compared with those fed on control or FS 10%, but not with those fed FS 15%. The effect of feeding FS on glucose levels in the current study supports the findings reported by Yetem (2015), Hasin et al. (2019), and Mustafa and Yateem (2019), who observed that feeding FS to goats led to a significant decrease in blood glucose, or lactating Murrah buffaloes (Kirar et al., 2018, Kirar et al., 2020). In contrast to these findings, several workers have noted a non-significant response of glucose concentration in sheep (Al-Sherwany, 2015; Kobeisy et al., 2017), lambs (Ibidhi and Salem, 2022), and goats (Al-Shaikh et al., 1999, Çayiroğlu et al., 2023). A possible mechanism for decreasing blood glucose concentration in this study may be due to the capacity of fenugreek seeds to adjust important enzymes involved in glucose metabolism such as hexokinase (in glycolysis), glucose-6-phosphatase or fructose- 1,6-bisphosphatase (in gluconeogenesis) as reported by Raju et al. (2001) and Devi et al. (2003).

There were no significant effects of feeding 5, 10, and 15% FS on serum total protein, albumin, and globulin (Table 4). A similar trend was reported in sheep by Salem et al. (2004), in goats by Al-Shaikh et al. (1999), and buffaloes by Kirar et al. (2018), and Kirar et al. (2020). In contrast to current achievement, some workers found a significant increase (Abu EL-Kassim et al., 2018, Saleh and

Alssadi, 2022) or a reduction (El-Tarabany et al., 2018) in seral total proteins and albumin in sheep and goats respectively.

Serum cholesterol was not significantly affected by supplemented treatments compared with control (Table 4). The results are in line with that of other authors who found no effect of FS on sheep serum cholesterol (Al-Sherwany, 2015; Abu EL-Kassim et al., 2018), and on goats, (Al-Shaikh et al., 1999) and on buffaloes (Kirar et al., 2018 and 2020). however, Akbağ et al., (2022) noted a significant drop in serum cholesterol content of goats fed a diet including fenugreek seed. In the current study, supplementation of FS had no significant impact on serum triglycerides (Table 4) which complies with the reports of Kobeisy et al. (2017), but is contrary to the findings shown by Yetem (2015), and Akbağ et al. (2022), who noticed that serum triglyceride content were significantly ($p<0.01$) reduced upon consuming FS by goats. While Çayiroğlu et al. (2023), observed an increase in serum triglyceride of goat fed FS.

Table 4. Effect of different levels of fenugreek seeds on some blood biochemical characteristics

Item	Control	T1 5% FS	T2 10% FS	T3 15% FS
Glucose (mg/dl)	92.07 \pm 4.31a	66.10 \pm 4.78b	101.04 \pm 6.09a	85.20 \pm 4.13ab
Cholesterol (mg/dl)	69.38 \pm 3.59	66.73 \pm 2.54	71.53 \pm 1.92	78.79 \pm 6.73
Triglycerides mg/dl)	16.52 \pm 5.04	16.52 \pm 5.76	21.63 \pm 3.53	21.46 \pm 4.24
Total protein (g/dl)	5.95 \pm 0.26	5.67 \pm 0.29	6.48 \pm 0.51	6.57 \pm 0.42
Albumin (g/dl)	3.03 \pm 0.06	2.78 \pm 0.25	3.30 \pm 0.18	3.22 \pm 0.30
Globulin (g/dl)	2.9 \pm 0.20	3.07 \pm 0.22	3.18 \pm 0.38	3.0 \pm 0.33
Urea (mg/dl)	26.21 \pm 2.75	31.24 \pm 2.09	25.53 \pm 2.75	32.29 \pm 1.86
T3 hormone (nmol/l)	2.37 \pm 0.36	3.29 \pm 0.23	2.91 \pm 0.35	2.39 \pm 0.31
T4 hormone (nmol/l)	76.34 \pm 4.51	84.93 \pm 8.33	84.55 \pm 5.80	83.68 \pm 8.33

Values are presented as means \pm SE. Different letters in the same row means significant difference ($P<0.05$).

There was no significant dissimilarity in serum urea levels in lambs fed the treatment diets as shown in Table 4. The result obtained herein is with those noticed in dairy goats by Abou-Elenin et al. (2016), Hamadani ewes by Al-Sherwany (2015), and lambs by Ibidhi and Salem (2022). In contrast to our finding, Yetem (2015), reported that blood urea level was significantly reduced in the fenugreek-treated goats.

Feeding different levels of dietary FS had no significant effects on triiodothyronine and thyroxine hormone levels (Table 4). El-Tarabany et al. (2018) reported that adding fenugreek seeds caused a significant drop in serum triiodothyronine hormone while increasing thyroxine hormone concentration. Moreover, Komáromyová et al. (2021) observed that the concentration of blood triiodothyronine and thyroxine decreased significantly in lambs fed diet supplemented with dry medicinal plants.

3.4. Dry matter and crude protein degradability

The DM degradability of incubated samples of control and experimental diets is shown in Figure 1. There was a significant ($P<0.05$) impact of incubation time in the rumen on the degradability of DM and CP, where samples of T2 and T3 (38.84 \pm 0.13% and 36.14 \pm 0.14% respectively) showed a significant ($P<0.05$) rise in DM degradability comparing to that of control samples (31.70 \pm 0.55%). While DM degradability of T1 (32.60 \pm 0.20%) was significantly ($P<0.05$) under that of T2 only (Figure 1). Furthermore, there was a significant ($P<0.05$) impact of interaction between time and level on DM degradability as samples of control diet showed a significant ($P<0.05$) reduction after one hour of incubation time (20.32 \pm 0.55%) as compared to other treated diets, while T2 showed a significantly ($P<0.05$) higher DM degradability (55.33 \pm 0.001) after six hours of incubation time in rumen as compared to other diets. The findings are in accordance with those of Kumar et al. (2016). In the current

study, the increase in DM degradability of T2 and T3 may be due to the action of saponins in FS (Makkar et al., 1998). It is shown that the extent of the effect of saponins on rumen bacteria is depending on the species of bacteria, it may support the growth of some species, while other species may remain unaffected, thus offering a selective manipulation of degradability (Wang et al., 2000). Low dietary levels of saponins may increase bacterial cell membrane permeability, which leads to an enhancement of nutrients in bacterial cells (Sen et al., 1998). In addition, it has been concluded that the increased number of rumen bacteria following consumption of saponin-containing plants could be related to the inhibitory effect of saponin on the rumen protozoa (Newbold et al., 2015).

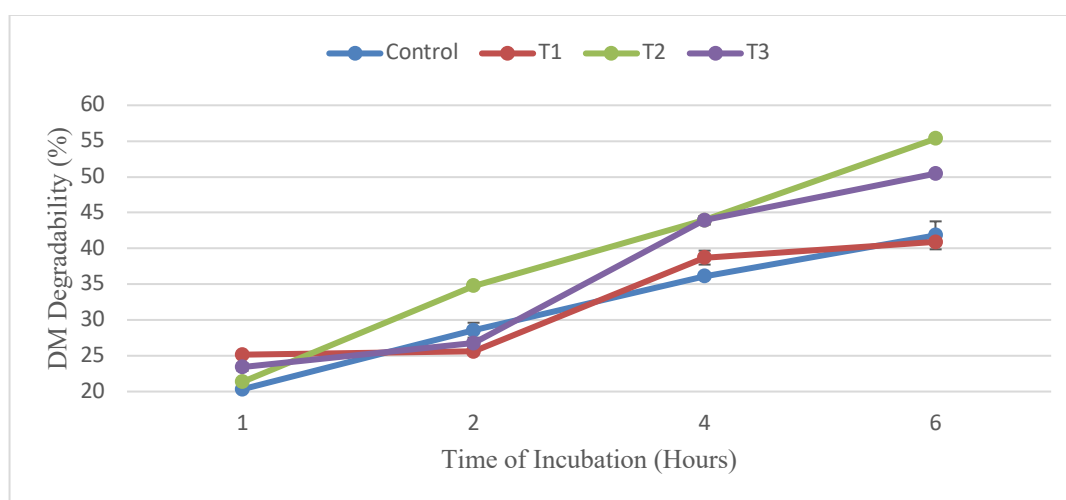


Figure 1. Dry matter degradability of the experimental diets.

There was no impact of treatments on CP degradability of the experimental diets in the rumen. While a significant ($P<0.05$) impact of interaction between level and time was seen on the mean of CP degradability where control diet showed a significant ($P<0.05$) drop in degradability ($38.90\pm0.41\%$) after one hour of incubation time as compared to the other diets, while T2 exhibited a significant ($P<0.05$) elevation in CP degradability ($77.92\pm0.001\%$) as compared to all other treatments (Figure 2). The absence of the effect of treatments on CP degradability might result from the increased microbial synthesis upon FS supplementation which contains saponins that support microbial protein formation (Newbold et al., 1997; Makkar et al., 1998). A significant amount of turnover is formed for microbial protein in the rumen of ruminants due to the uptake and digestion of bacteria, archaea, and fungal zoospores by protozoa, and this decreases the utilization efficiency of protein (Wallace and McPherson, 1987). Many negative effects caused by defaunation might dissipate as the number of bacteria and fungi increases and fulfill the space occupied by protozoa previously Williams and Coleman (1997), and this may enhance the viability of microbial protein formation and protein inflow into the intestine and improve the nitrogen retention in ruminants (Santoso et al., 2007a).

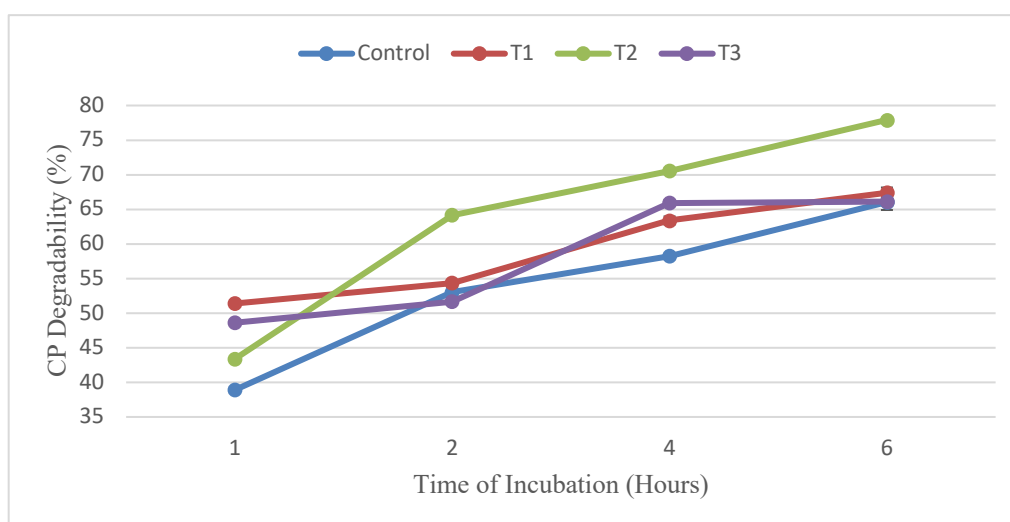


Figure 2. Crude protein degradability of the experimental diets.

4. Conclusion

From the results obtained in the current investigation, it could be concluded that supplementing diet with FS at different levels to lambs did not affect the digestibility coefficients, pH, and $\text{NH}_3\text{-N}$. All blood biochemicals were not affected except glucose and cholesterol. Supplementation of FS affects Sacco degradability upon interaction with incubation time. Further research about Sacco and Situ's degradability of fenugreek-included feeds is recommended.

Ethical Statement

Ethical approval for this study was obtained from the University of Duhok Ethics Committee vide number AEC28072023.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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Author Contributions

The research idea was formulated by authors 1 and 2. The experiment was conducted by author1, with supervision from author 2. The manuscript was written by authors 1 and 2.

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