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Evaluation of nutritive traits and *in vitro* production of gas for Alfalfa hays and Fenugreek (*Trigonella foenum-graecum*) on feeding ruminants

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Abstract

The aim of the current study was to determine the value-nutritive of two species of forage, *Trigonella foenum-graecum* (TFG) and Alfalfa (ALF), grow in Iran by the use of chemical composition (CP, OM, EE, NDF, ADF, ADF, NFC, GE), *in vitro* gas production, digestibility (DMD, OMD) and Metabolizable Energy (ME) contents. The Legume forages were collected from the farms around Karaj in the middle of summer, and fenugreek without flowers was chopped and sun-dried. For the gas test, the microbe inoculums were collected from four fistulated Taleshi male cows in Iran as a rumen-mixed. Forge samples were incubated with rumen fluid, and measurements were conducted at; (2, 4, 6, 8, 12, 24, 48, 72, and 96) hours to determine the production of gas. Significant differences were found between all fractions of chemical composition contents of experimental forages ($p<0.01$). The production of gas at the exact times of incubation that the constants' (b, c, and a+b) were higher -significantly ($p<0.01$)- in ALF hay. The DMD, OMD, and ME (MJ/Kg DM) for TFG and ALF hays were calculated (66.96, 75.59%, 21.15, 66.96, 75.59%, and 21.15), respectively ($p<0.01$). Finally, the value-nutritive of TFG was higher than that of ALF because more CP, OMD, and ME were used in feeding ruminants and lost cost feeding.

Keywords: Fenugreek, gas production, nutritive value, metabolizable energy, digestibility

Introduction

Belonged to the family (*Fabaceae*), the annual legume Fenugreek (*Trigonella foenum-graecum*) originated in the Mediterranean region**.** Fenugreek, as an additive, has many nutritional and medicinal values for humans and animals, and this trigonelline is a valuable alkaloid with practical therapeutic effects, especially for diabetes**.** Iranian fenugreek of *Zanjani* or *Borazjani* type is the best type for cultivation and forage production. This plant has a high nutritional value and contains valuable substances such as calcium, phosphorus, iron, carotene, vitamin C, and crud protein. Some different species of fenugreek have been introduced as a forage plant with good nutritional quality for ruminants, which, unlike alfalfa, does not cause bloating in livestock. In addition to that, it has natural steroid compounds used to increase livestock growth (Mir *et al.*, 1997; Mir *et al.*, 1998) [11, 12]. In some regions, traditional ranchers were aware of the fattening *potential* of fenugreek, and they added fenugreek to their fodder before selling it (Azad, 2000) [5]. Fenugreek is cultivated as a spice in India and most Mediterranean regions. The Latin name of this plant is "Foenum-graecum," which means "Greek forage," identifying the utilized of it as a forage (Acharya *et al.*, 2006) [1]. As reported, the fenugreek reduces the growth of the rumen protozoal population by up to 50% (Goel *et al.*, 2008a) [7]. Also, previous studies have shown that several saponin-containing plants inhibited the growth of ruminal protozoa (Diaz *et al.*, 1994; Newbold *et al.*, 1997) [6, 13]. Fenugreek saponins can improve fermentation efficiency (Goel *et al.*, 2008b; Klita, 1996) [8, 10]. There is not enough scientific information about the effect of adding fenugreek range forage on ruminant nutrition. This forage is produced in summer more than human consumption and is discarded in large fenugreek cultivation farms as well as Fruits and Vegetable Markets. Considering the high nutritional value of this forage and the lack of scientific information about the nutritional value of this additive in Iran, this study was conducted **.**Therefore, this study aimed to do a nutritional evaluation of the Iranian fenugreek in the diet of ruminants**.**

Material and Methods

Forages: In the middle of summer, fenugreek (*Trigonella foenum-graecum*) was collected from the farms around Karaj, Iran. Fenugreek forages without flowers were harvested, sun-dried, and transferred to the laboratory of the Animal Science Research Institute. Then, samples were ground and screened (1mm) for the next tests (chemical analysis & *in vitro* gas production).

Chemical analysis

By drying the samples at 105^0c (in 24 hours), dry matter (DM) was determined. Ash was estimated by using a muffle furnace for 8 hours at 550^oc. Nitrogen (N) content was measured by the Kjeldahl method (AOAC, 2005) [4]. Crude

Protein (CP) was calculated as 6.25×N. The Natural Detergent Fiber (NDF), Acid Detergent Fiber (ADF), Acid Detergent Lignin(ADL), and Crude Fiber(CF) were determined by the Fibertec system with modifications according to Van Soest (1967) [22]; sulfite was omitted from the NDF analysis. Ether Extract (EE) was measured using a Soxhlet extractor. -Non-Fiber Carbohydrate (NFC) and Organic Matter (OM) were calculated as (100-CP-EE-Ash-NDF) and (100-Ash), respectively. In an adiabatic bomb calorimeter, gross energy (GE) was determined.

Animals' management: The rumen fluid was collected from four mature fistulated *Taleshi* male cows (Safaei *et al.*, 2007) [20] of Iran (age= 4 years; weighted = 415 kg), and animals were fed daily twice, with (DM_{Intake}= 8 kg/day) diet contained:

Table 1: Tap water available ad libitum

Component	Composition (%)
Hay	70%
Alfalfa Hay	70%
Wheat Straw	30%
Concentrate	30%
Barley Meal	35%
Soybean Meal (SBM)	17%
Whole Cottonseed	25%
Wheat Bran	20%
Calcium Carbonate ($CaCO_3$)	1%
Minerals and Vitamins	2% TMR (NRC, 2007) [16].

Production of gas -*In vitro*

In the lab of the Institute of Animal Science Research\Karaj-Iran, the measurements of the production of gas -*In vitro*- were carried out. Fermentation of FTG samples was carried out with rumen liquor collected from 4 fistulated *Taleshi* male cows of Iran (Menke & Steingass, 1988) [15]. The inoculum was formulated as outlined by Menk and Steingass (1988). It was made by combining rumen liquor with artificial saliva at a ratio of 1:4 (v/v). For a total volume of 1L, the artificial saliva included a solution of buffer (237 ml), solution of main element (237 ml), solution of trace element (0.12 ml), solution of resazurin (1. 22 ml) (which was resazurin "100 mg" dissolved in distilled water "100 ml"), and 49. 5 ml of a reduction solution (this was freshly prepared separately and comprised 2 ml of 1 N NaOH, Na_2S-$7H_2O$ (285 mg), and distilled water (47. 5 ml) to make 1 L of saliva, topped up with distilled water (475 ml). The buffer solution contained $NaHCO_3$ at 35 g and NH_4HCO_3 at 4 g. The solution of main element contained Na_2HPO_4 at 5. 70g, KH_2PO_4 at 6. 20 g, and Mg SO_4-$7H_2O$ at 0. 60 g the solution of trace element included $CaCl_2$-$2H_2O$ at 13. 20 g, $MnCl_2$-$4H_2O$ at 10. 00 g, $CoCl_2$-$6H_2O$ at 1. 00 g, and $FeCl_2$-$6H_2O$ at 0. 80 g, diluted to 100 ml with distilled water. After the reduction process was completed (indicated by the decoloration of resazurin upon adding the reduction solution), the rumen liquor was added to the medium. All procedures were conducted with continuous CO2 reflux.

Around 200 mg of ground TFG samples were measured into 100 ml glass syringes. A volume of 30 ml of the fluid buffer mixture was placed into the 100 ml glass syringes. The glass syringes, which contained the TFG samples and rumen fluid buffer mixture, were kept at an incubation temperature of 39°C. After 30 minutes of incubation, each syringe was shaken gently. The production of gas has been measured at the incubation times 2, 4, 6, 8, 12, 24, 48, 72, and 96 h. In triplicate, treatment samples were incubated, in addition to three blank syringes, which only contained a rumen liquor buffer mixture. By subtracting the volume of blanks' produced gas, the production of gas for FTG samples was determined (Menke & Steingass, 1988) [15]. The data of the production of gas was fitted to the model given by Ørskov and McDonald (1979) [17] by the software Fit Curve (new-way).

$$Y = b\ (1\text{-}e^{-ct})$$

Where is the gas production from the immediate soluble fraction (ml);

b = the gas production from the immediately insoluble fraction (ml);

c = the gas production rate constant for the insoluble fraction ($ml.h^{-1}$);

a + b = the potential gas production (ml);

t = the incubation time (h), and Y is the gas production at time t.

The contents of ME (MJ kg^{-1} DM) of TFG samples were calculated by using the following equation given by Menke and Steingass (1988) [15]:

ME (MJ kg-1 DM) = 2.20 + 0.136 GP + 0.057 CP

Where GP = the 24-hour production of gas (ml 200^{-1} mg), and CP = the crude protein (%).

The digestibility of Dry Matter of TFG samples was calculated by using the following equation given by Menke and Steingass (1988) [15]:

DMD (%) = 15 + 10.1 (a+b) + 623(c) + 0.51 CP

Where a + b =potential gas production (ml);

c = the constant gas production rate for the insoluble fraction ($ml.h^{-1}$);

CP =crude protein from the TFG sample (%).

The Digestibility of organic matter of TFG samples was calculated by using the following equation given by Menke and Steingass (1988) [15]:

OMD (%) = 14.88 + 0.889 GP + 0.45 CP + 0.0651 XA (4)

Where GP = the 24-hour net gas production (ml 200-1 mg DM), CP = the crude protein from the TFG sample (%), and XA = the ash content from the TFG sample (%).

Short-chain fatty acid (SCFA) was calculated using the equation of Makkar (1995) and Menke *et al.* (1997) [14];

SCFA (mmol) = 0.0222GP - 0.00425 (5)

Where GP = the 24-h production of gas (ml g^{-1} DM).

NE (MJ/kg DM) = 0.115*GP + 0.0054*CP + 0.014*EE - 0.0054*CA – 0.36

Where GP =the 24-hour production of gas (ml/200mg DM), CP=crude protein, EE=ether extract, and CA=crude ash (%DM).

Statistical analysis

The treatments were TFG and ALF, with three replications in randomization, which were designed entirely. By using the software Statistical Analysis Systems of Virgin 9.1proc, all data were analyzed. GLM and a, b, and c (parameters of the gas test) were obtained by Fit Curve (new-way) software (Ørskov & McDonald, 1979; R, 2015) [17].

Results and Discussion

The chemical compositions of the two forage species (TFG, ALF) are presented in Table 1 and Fig. 1. The fenugreek hay was significantly ($p<0.01$) higher in CP, Ash, EE, NFC, and GE than ALF, n but NDF, ADF, ADL, and OM of TFG were less than ALF hay ($p<0.01$).

Table 1: The composition (%) and GE (Kcal/Kg) of forages.

Treat	CP	NDF	ADF	ADL	Ash	EE	NFC	OM	GE
TFG	22.22	27.62	21.58	3.81	16.50	7.60	29.85	83.50	5008.33
ALF	14.37	53.64	38.74	6.45	9.60	1.30	21.09	90.58	4247.67
SEM	0.24	0.16	0.12	0.13	0.09	0.04	0.25	0.08	17.82
Sig	**	**	**	**	**	**	**	**	**

The chemical compositions of TFG and ALF hays used in this experiment were consistent with findings by Kamlak *et al.* (2005) [9].

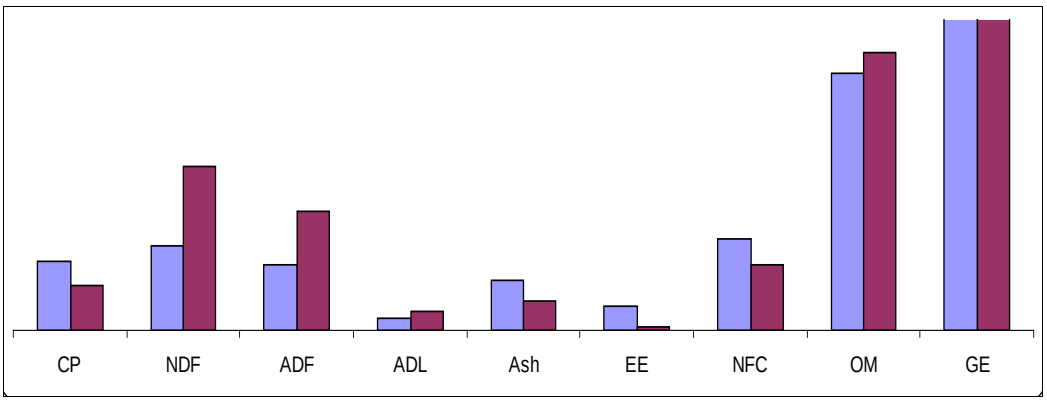

Fig 1: Chemical composition of samples

Table 2 and Fig. 2 show gas production data during the fermentation period. The cumulative volume of gas production increased with increasing incubation time.

Table 2: Gas production volume (ml) in incubation times (h)

Treat	2	4	6	8	12	24	48	72	96
TFG	4.89	8.41	15.49	21.62	28.70	46.21	54.29	56.81	57.73
ALF	8.14	18.66	26.67	30.30	36.04	42.74	47.63	49.92	50.34
SEM	0.12	0.24	0.27	0.43	0.39	0.29	0.17	0.15	0.17
Sig	**	**	**	**	**	**	**	**	**

Even though the availability of several models for estimating the kinetic production of gas, but, the method of Ørskov and McDonald (1979) [17] has been chosen, because of the relationship of its parameters with the intake, the digestibility, and the degradation characteristics of the forages. With those reported by Kamlak *et al.* (2005) [9], cumulative gas production and estimated parameters in ALF hay were comparable.

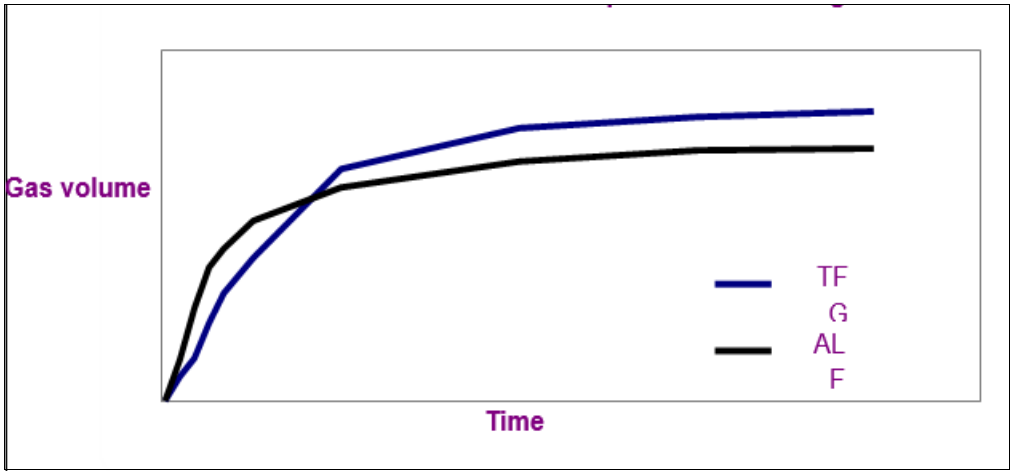

Fig 2: Gas production of forages

Table 3: Parameter gas production, Digestibility, Short chine fatty acid and Energy (ME, NE)

Treat	b	a + b	c	DMD	OMD	SCFA	ME	NE
TFG	59.94	58.00	0.06	66.95	75.59	11.10	21.15	5.92
ALF	48.89	48.64	0.12	58.85	65.92	10.28	16.20	5.19
SEM	0.17	0.15	0.002	0.22	0.24	0.07	0.12	0.02
Sig	**	**	**	**	**	**	**	**

The estimated parameters (b and a + b) of TFG hay, therefore, were higher -significantly (p<0.01)- than those of ALF, but c of TFG was lower (p<0.01). The DMD, OMD, ME, and NE contents of TFG hay were higher -significantly (p<0.01)- than those of ALF hay.

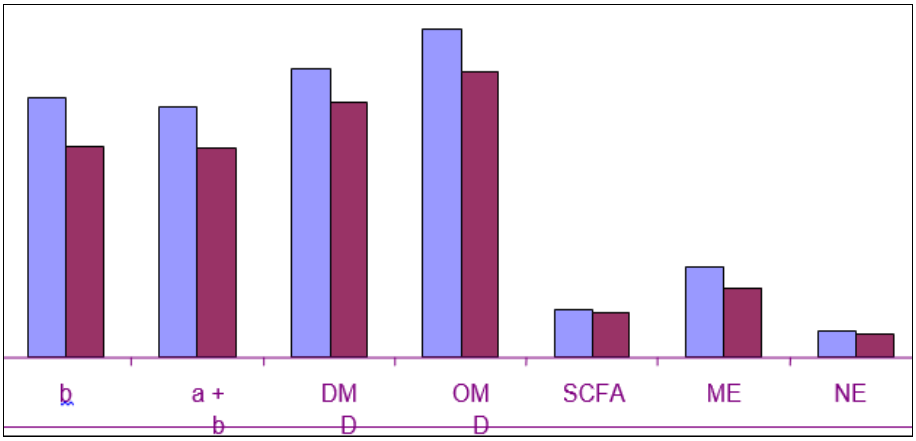

Fig 3: Parameters gas test energy fraction and digestibility

Goel *et al.* (2008a) [7] tested the fenugreek seeds effect and their aqueous and alcoholic/aqueous extracts on the production of methane and other fermentation products in all-forage diets as well as forage and concentrate mixtures in the rumen. The researchers concluded that supplementing fenugreek seeds with all-forage diets or concentrate-forage mix diets, both induced the degradability of OM in the rumen and reduced methane production per decomposed material. Therefore, it has been suggested that this supplement may improve ruminal fermentation efficiency. In Mir *et al.* (1997) [11] study, total volatile fatty acids, ruminal pH, and ruminal ammonia concentration were not significantly different in castrated calves fed fenugreek silage and alfalfa silage. The *in vitro* gas production rate for fenugreek silage was significantly higher than that for alfalfa silage (17.4 vs. 12.6% per hour). However, the final gas production after 72 hours was no different for the two silages (22.3 and 21.6ml /100 g of DM for fenugreek silage and alfalfa-silage, respectively. The availability of estrogenic compounds in the seeds of fenugreek has long been known (Acharya *et al.*, 2006; Acharya *et al.*, 2008) [1, 2]. Estrogens of foreign origin generally increase the storage of lean tissue in castrated ruminants and reduce subcutaneous and intramuscular fat. Phytoestrogens have been shown to have beneficial effects in stimulating growth and increasing growth rate in the diet of ruminants and non-ruminants (Trenkle & Borroughs, 1978) [21]. Petit *et al.* (1995) [18] reported that fenugreek seed steroid saponins increase feed intake and weight gain in mice. Mir *et al.* (1998) [12] investigated the effect of dietary feeding of fenugreek silage and alfalfa silage with barley supplement (at three levels of 0, 15, and 30%) balanced by adding soybean meal for the same nitrogen level on the growth performance of male calves. In this study, feed intake, mean daily weight gain, and feed intake in fenugreek treatment were similar to those in alfalfa treatment. The researchers suggested that the nutritional value of fenugreek silage in ruminant growth was comparable to that of alfalfa silage . On the other hand, Al-Amer and Basiouni (2005) [3] reported that growth hormone levels in goats treated with 60 g of fenugreek seeds per day for 7 weeks were higher than the control treatment.

Conclusion

Finally, the value of TFG hay -nutritionally- was higher than that of ALF hay, because, it contained more CP, OMD, and ME that were used to feed ruminants and reduce lost cost feeding.

Acknowledgments

The Animal Science Research Institute carried out this research, and the authors are grateful to the institute's laboratories in Karaj, Iran.

Novelty statement

Many studies have been conducted on the fenugreek plant as a forage for ruminants. However, this study evaluates fenugreek as a forage harvested from Karaj farms and compares it with alfalfa in terms of nutritive value and feeding cost.

References

1. Acharya SN, Thomas JE, Basu SK. Fenugreek: an “old world” crop for the “New World.” Biodiversity. 2006;7:27-30.
2. Acharya SN, Thomas JE, Basu SK. Fenugreek, an alternative crop for semiarid regions of North America. Crop Sci. 2008;48:841-853.

3. Al-Amer MA, Basiouni GF. Feeding effects of fenugreek seeds (*Trigonella foenum-graecum* L.) on lactation performance, some plasma constituents and growth hormone level in goats. Pakistan J Biol Sci. 2005;8:1553-1556.
4. AOAC. International Official Methods of Analysis, XXI. Gaithersburg, MD: AOAC International; 2005.
5. Azad M. Your health, your beauty. 100 planets, 1000 usages. Tehran: Nahid Publications; 2000. p. 292.
6. Diaz A, Avendan OM, Escobar A. Evaluation of *Spinadus saponaria* as a defaunating agent and its effects on different ruminal digestion parameters. Livestock Res Rural Dev. 1994;5:1-10.
7. Goel G, Makkar HPS, Becker K. Effects of *Sesbania sesban* and *Cardus pycnocephalus* leaves and fenugreek (*Trigonella foenum-graecum* L.) seeds and their extracts on partitioning of nutrients from roughage and concentrate-based feeds to methane. Anim Feed Sci Technol. 2008;147:72-89.
8. Goel G, Makkar HPS, Becker K. Changes in microbial community structure, methanogenesis, and rumen fermentation in response to saponin-rich fractions from different plant materials. J Appl Microbiol. 2008;105:770-777.
9. Kamlak A, Canbolanao B, Gubruz K, Ozay O. Comparison of *in vitro* gas production technique with in situ nylon bag technique to estimate dry matter degradation. Czech J Anim Sci. 2005;50(2):60-67.
10. Klita PT, Mathison GW, Fenton TW, Hardin RT. Effects of alfalfa root saponins on digestive function in sheep. J Anim Sci. 1996;74:1147-1156.
11. Mir Z, Acharya SN, Mir PS, Taylor WG, Zaman MS, Mears GJ, Goonewardene LA. Nutritive composition, *in vitro* gas production, and digestibility of fenugreek (*Trigonella foenum-graecum*) and alfalfa forages. Can J Anim Sci. 1997;77:119-124.
12. Mir Z, Mir PS, Acharya SN, Zaman MS, Taylor WG, Mears GJ, McAllister TA, Goonewardene LA. Comparison of alfalfa and fenugreek (*Trigonella foenum-graecum*) silages supplemented with barley grain on performance of growing steers. Can J Anim Sci. 1998;78:343-349.
13. Newbold CJ, Hassan El SM, Wang J, Ortega ME, Wallace RJ. Influence of foliage from African multipurpose trees on activity of rumen protozoa and bacteria. Br J Nutr. 1997;78:237-249.
14. Makkar HPS. Applications of the *in vitro* gas method in the evaluation of feed resources and enhancement of nutritional value of tannin-rich tree/browse leaves and agro-industrial by-products. Animal Production and Health Section, International Atomic Energy Agency, Vienna; 1995.
15. Menke KH, Steingass H. Estimation of energetic feed value obtained from chemical analysis and *in vitro* production using rumen fluid. J Anim Res Dev. 1988;28:7-55.
16. National Research Council. Nutrient requirements of small ruminants: sheep, goats, cervids, and New World camelids. 6th ed. Washington, DC: National Academy Press; c2007.
17. Ørskov ER, McDonald I. The estimation of protein degradability in the rumen from incubation measurements weighed according to rate of passage. J Agric Sci. 1979;92:499-512.
18. Petit PR, Sauvaire YD, Buys DMH, Leconte OM, Baissac YG, Ponsin GR, Ribes GR. Steroid saponins from fenugreek seeds: Extraction, purification, and pharmacological investigation on feeding behavior and plasma cholesterol. Steroids. 1995;60:674-680.
19. R statistical software. R 3.2.0 [Internet]. The R Journal. 2015;5(1). Available from: www.r-project.org
20. Safaei AM, Fazaeli H, Zahedifar M, Mansouri H, Mirhadi SA. The developmental process of rumen fistulation in Iranian native ruminants. The 58th annual meeting of the European Association for Animal Production (EAAP). Dublin, Ireland; 2007.
21. Trenkle A, Burroughs. Physiological effects of estrogens in animal feeds with emphasis on growing of ruminants. Nutrition and drug interactions. New York: Academic Press; 1978. p. 577-611.
22. Van Soest PJ. New chemical procedures for evaluation forages. J Anim Sci. 1967;23:838-847.