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In vitro Evaluation of Alfalfa Substitution with Fenugreek (*Trigonella foenum graegum*) Hay in a High Concentrate Ration

Research Article

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ABSTRACT

This study was conducted to evaluate the possibility of alfalfa replacement with fenugreek hay for ruminants, using *in vitro* gas production parameters and some of rumen fermentation characteristics. For this propose, five different total mixed rations were formulated to meet nutrient requirements of 35 kg growing lambs with gradually replacement of alfalfa in control diet (T1) with different levels of fenugreek hay (25, 50, 75 and 100%, respectively T 2, T3, T4 and T5). *In vitro* gas production (mL/200 mg DM) was determined at 2, 4, 6, 8, 12, 24, 48, 72 and 96 hours of incubation. Ammonia-N concentration (mg/dL) and pH of rumen liquor was determined immediately after removal. Gas production was increased with increasing levels of fenugreek (P<0.05). Significant increases of gas production from insoluble fraction (b) were observed with increasing levels of fenugreek hay (P<0.05). A same trend was observed for potential gas production (a+b, P<0.05). Also, the constant rate of gas production (c) of T2, T3 and T4 were significantly higher than control, but the differences between T3, T4 and T5 were not significant. Replacement of fenugreek at 25 and 75 percent significantly increased final pH compared to control (100% alfalfa) (P<0.05), but the differences between T3, T5 and control and between T3, T4 and T5 were not significant. Short chain fatty acids production (mmol/200 mg DM) was significantly greater in treatments with 50% or more fenugreek substitution (P<0.05). Organic matter digestibility of treatments containing 50% or more fenugreek hay (T3, T4 and T5) were significantly higher than control (P<0.05). Similarly, estimated metabolisable energy content of these treatments were higher than control (P<0.05). According to these results, it can be concluded that fenugreek can provide highly valuable forage for ruminants with comparable feeding values with alfalfa, and alfalfa can be replaced completely in ration with fenugreek hay without any problem.

KEY WORDS alfalfa, fenugreek, gas production.

INTRODUCTION

Legume forages are an important source of high-quality feed in dairy nutrition. In most of the regions of Iran, alfalfa (*Medicago sativa*) is the primary forage legume on dairy operations. However, there is increased interest in diversification of forage in cropping rotations. Annual legumes have many of the same benefits as alfalfa, such as nitrogen fixation and high feed quality, but increase crop rotation flexibility as they occupy cropping land for only one growing season (Mir *et al.* 1997). Fenugreek (*Trigonella foenum graecum*) is a single-cut, annual legume that is commonly cultivated in Asia and Africa as vegetable for human consumption. It is probably better known as a spice and medicinal plant. Recently, interest in cultivating fenugreek in temperate climates such as western Canada has increased, because its seeds contain a steroidal sapogenin, known as diosgenin, which is used to manufacturing some commercial steroids, contraceptive steroids and corticosterone by pharmaceutical companies (Montgomery, 2009). However, it has been used since ancient times as forage, as it can be understood from the word "fenugreek" that actually means "Greek hay" (Montgomery, 2009). Interest has extended to the use of fenugreek as a forage because of the high and sustained quality of the plant (Acharya *et al.* 2008; Basu *et al.* 2008), in addition to nitrogen fixation, and drought- and frost tolerance (Acharya *et al.* 2008; McCormick *et al.* 2006). Recently, it has been evaluated as an plausible alternative forage for ruminants in several studies (Mir *e.al.*1993; Mustafa *et al.* 1996; Acharya *et al.* 2008; Montgomery, 2009) and new forage-type fenugreek cultivars also, have recently developed at Agriculture and Agri-Food Canada (AAFC) research station (Prasad, 2011). Fenugreek can be harvested for hay or silage. Several researches have shown that fenugreek quality does not deteriorate with age (Mir *et al.* 1997; Abdouli *et al.* 2012). Also, Mustafa *et al.* (1996) concluded that fenugreek hay nutrient content was similar to that of late cut alfalfa hay. Feeding trails conducted in Canada reported a similar average daily gain and dry matter intake when steers were fed alfalfa or fenugreek silage (Mir *et al.* 1998). The main objective of this study was to establish whether fenugreek may be a suitable alternative to alfalfa for use in ruminant rations and to evaluate effect of their different levels of replacement on rumen fermentation using *in vitro* degradation parameters and some of rumen fermentation characteristics.

MATERIALS AND METHODS

Experimental materials and design

The substrates examined in this experiment were five different TMR rations (Table 1) formulated to meet nutrient requirements of 35 kg growing lambs (NRC, 1985). The forage portion of control diet (T1) was consisted of wheat straw and alfalfa hay. In other treatments, alfalfa hay was substituted with different levels of fenugreek hay (25%, 50%, 75% and 100%, respectively T 2, T3, T4 and T5). Calibrated glass syringes (Model Fortuna, Haberle Labortechnik, Germany) were used as incubating media to assess *in vitro* degradation parameters of experimental diets. Every three syringes were considered to one treatment.

In vitro gas production study

The gas production study was conducted according to Hohenheim gas production method (Menke and Steingass, 1988). About 200 mg of dried substrates were milled to pass 1 mm sieves and accurately weighed into glass syringes (150 mL volume, 200 mm length, 32 mm inner diagonal) fitted with vaselinated pistons. Syringes then were filled with 30 mL medium consisting of 10 mL filtered fresh rumen fluid and 20 mL buffer solution as described by Menke and Steingass (1988). Rumen fluid was collected from two rumen fistulated Sistani cows (540 kg average body weight) which were fed on a maintenance diet (consisted of wheat straw, alfalfa and concentrate). Filled syringes were placed in a 39 ˚C incubator (WTB Binder-workservice WTB Binder labortechnik GmbH, Bersstr. 14, 78532 Turttlingen) equipped with a rotor. The gas production (mL/200 mg DM) was determined at 2, 4, 6, 8, 12, 24, 48, 72 and 96 h of incubation. All samples were incubated in triplicate with three syringes containing only rumen fluid-buffer mixture (blank). The net gas productions for samples were determined by subtracting the volume of gas produced in the blanks.

Calculation of degradation parameters

Gas production data were fitted using non-linear regression of SAS (2002) to the equation:

$$p= a + b\,(1-e^{-c\,(t-Lt)})$$

Where:
p: represents the *in vitro* gas production (mL) at time t.
a + b: the potential gas production.
c: the fractional rate of gas production per hour.
Lt: represents a lag phase before gas production commenced.

The metabolizable energy content (ME) and organic matter digestibility (OMD) of samples were calculated using equations of Menke and Steingass (1988) as:

ME (MJ/kg DM)= 2.20 + 0.136GP + 0.057 CP
OMD (%)= 14.88 + 0.889 GP + 0.45 CP + 0.0651 XA

Where:
ME: metabolizable energy.
OMD: organic matter digestibility.
GP: 24 h gas production (mL/200 mg DM).
CP: crude protein (%).
XA; ash content (%).

Short chain fatty acid (SCFA) production was calculated using the equation of Makkar (2004), Where, GP is 24 hour net gas production (mL/200 mg DM):

SCFA (mmol)= 0.0222 GP - 0.00425

Table 1 Ingredients and proximate composition of TMR rations containing 100% alfalfa (treatment 1, as control) and 25%, 50%, 75% and 100% replacement of alfalfa with fenugreek hay (T 2, T3, T4 and T5, respectively)

	Rations (%)				
Ingredients	**T1**	**T2**	**T3**	**T4**	**T5**
Alfalfa hay	16	12	8	4	0
Fenugreek hay	0	4	8	12	16
Wheat straw	10	10	10	10	10
Barley grain	35	35	35	35	35
Wheat bran	15.7	15.7	15.7	15.7	15.7
Cotton seed meal	16.1	16.1	16.1	16.1	16.1
Beet pulp	5.3	5.3	5.3	5.3	5.3
Calcium carbonate	1	1	1	1	1
Vitamin-mineral supplement	0.6	0.6	0.6	0.6	0.6
Salt	0.3	0.3	0.3	0.3	0.3
Chemical composition					
Crud protein, (% of DM)	16.58	16.55	16.51	16.47	16.44
Natural detergent fiber, (% of DM)	35.33	35.22	35.11	35.01	34.90
Acid detergent fiber, (% of DM)	19.56	19.53	19.50	19.47	19.44
Ash, (% of DM)	6	5.94	5.88	5.82	5.76
Metabolizable energy, Mcal/kg	2.63	2.64	2.65	2.66	2.67
Calcium, (% of DM)	0.73	0.76	0.78	0.81	0.83
Phosphorous, (% of DM)	0.54	0.55	0.55	0.56	0.56

DM: dry matter.

Measurement of pH and ammonia

The pH of rumen liquor was determined immediately after removal using a pH meter (pH Meter CG 804, SCHOTT GERATE). In order to determination of ammonia nitrogen concentration, after completion of incubation period rumen fluid of each syringe was filtered through 2 layer chees cloth and 5 ml of them were mixed with 1 mL 0.2 N HCl and stored at -20 ˚C untile analysis. Ammonia-N concentration (mg/dL) was determined by steam distilation in boric acid and titration with diluted hydrocholoric acid (0.1 N).

Statistical analysis

Experimental data were analyzed using General Linear Model procedure of SAS (2002). One way completely randomized design was used to analyze the dependent variables including cumulative gas production at different times and gas production derived parameters a, b, (a+b), c, ODM, ME, SCFA, and ammonia-N and pH data. Means of variables were compared among treatments usining Duncan's multiple range test.

RESULTS AND DISCUSSION

In vitro gas production parameters

Comparisons of means for gas production volumes (mL/200 mg DM) of all treatments in different incubation times are presented in Table 2 and their fitted curves are presented in Figure 1. The gas production was increased with increasing levels of fenugreek (P<0.05) and treatment with 75% replacement of fenugreek had the maximum cumulative gas production volume at all of measurement times, but its difference with treatment containing 100% fenugreek was not significant. Differences among treatments containing fenugreek hay were not significant at first 24 hour of incubation, but became greater as incubation time proceeded, except for T2 (Table 2).

Significant increases of gas production from insoluble fraction (b) were observed with increasing levels of fenugreek hay (P<0.05, Table 3). A same trend was observed for potential gas production (a+b, P<0.05, Table 3). Also, the gas production rate (c) of treatments containing 25%, 50% and 75% fenugreek was significantly higher than 100% alfalfa, but the differences between 50%, 75% and 100% fenugreek hay were not significant. The gas production of soluble fraction (a) of treatments containing 25 and 50% fenugreek were considerably lower than other treatments (Table 3), but the differences were not statistically significant. There was significant difference of incubation lag time among rations (P<0.05, Table 3). Lag time was greater for all rations containing fenugreek, but its trend was not consistent with fenugreek increase in rations. The greatest values were observed in rations containing 25 and 50% fenugreek and lag time of rations containing 75% and 100% fenugreek were significantly less than them.

Rumen fermentation parameters

The pH values of all treatments containing fenugreek were in normal range. Replacement of fenugreek instead of alfalfa at medium levels (25% and 75%) significantly increased final pH compared to control (100% alfalfa) (P<0.05, Table 4), but the differences between treatments of 50 and 100 percent fenugreek and control and between treatments of 50%, 75% and 100% fenugreek were not significant (Table 4). Ammonia-N concentrations were not significantly different between treatments.

Table 2 *In vitro* gas production volumes (mL/200 mg DM) of rations with 0%, 25%, 50%, 75% and 100% substitution of alfalfa (T1, T2, T3, T4 and T5, respectively) at different incubation times

Time (h)	2	4	6	8	12	24	48	72	96
Rations	Cumulative gas volume (mL/200 mg DM)								
T1	11.74[b]	25.04[c]	32.86[c]	40.54[c]	46.17[c]	56.50[c]	66.99[c]	70.28[c]	72.47[c]
T2	12.74[a]	26.57[b]	34.91[b]	42.77[b]	48.43[b]	58.65[b]	68.41[b]	70.29[c]	72.18[c]
T3	13.24[a]	27.57[a]	36.29[a]	44.55[a]	50.78[a]	61.53[a]	69.15[b]	74.56[b]	76.79[b]
T4	13.48[a]	28.22[a]	37.15[a]	45.77[a]	52.20[a]	63.01[a]	73.99[a]	77.28[a]	79.32[a]
T5	12.89[a]	27.68[a]	36.32[a]	44.82[a]	50.95[a]	61.80[a]	73.44[a]	76.74[a]	78.94[ab]

DM: dry matter.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).

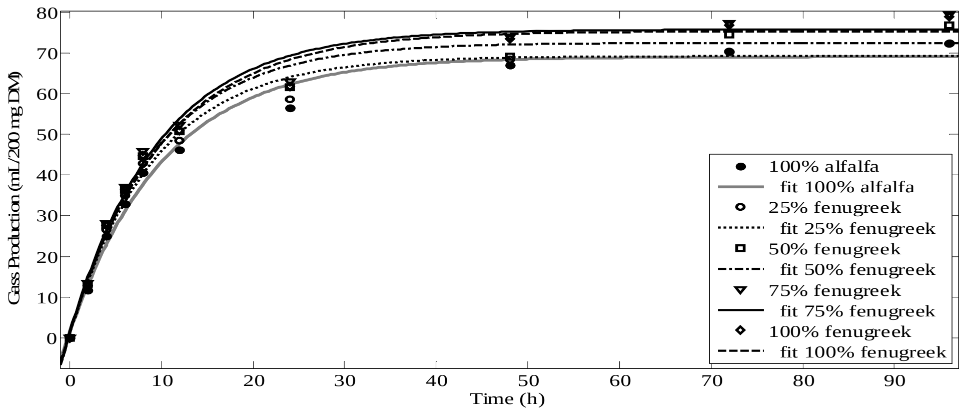

Figure 1 *In vitro* gas production profiles of rations with 0%, 25%, 50%, 75% and 100% substitution of alfalfa with fenugreek hay

Table 3 Gas production parameters means of rations containing different ratios of alfalfa: fenugreek hay

Gas production parameters	Alfalfa: fenugreek ratios				
	100: 0	75: 25	50: 50	25: 75	0: 100
a (mL)	1.533[a†]	1.507[a]	1.566[a]	1.535[a]	1.616[a]
b (mL)	67.480[c]	67.700[c]	70.890[b]	74.200[a]	73.680[a]
a + b (mL)	69.010[c]	69.207[c]	72.456[b]	75.735[a]	75.296[a]
c (mL/h)	0.096[c]	0.107[a]	0.106[ab]	0.102[ab]	0.098[bc]
Lt (h)	0.012[c]	0.066[a]	0.066[a]	0.029[b]	0.019[b]

a: gas production of soluble fractiona; b: gas production of insoluble fraction; a + b: the potential gas production; c: the fractional rate of gas production per hour and Lt: represents a lag phase before gas production commenced.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).

Table 4 Rumen fermentation characteristics of rations containing different ratios of alfalfa: fenugreek hay

Rumen fermentation characteristics	Alfalfa: fenugreek ratio				
	100: 0	75: 25	50: 50	25: 75	0: 100
pH	6.98[c†]	7.09[a]	7.06[abc]	7.08[ab]	7.01[b]
Ammonia-N (mg/dL)	7.62	4.50	8.64	5.80	8.49
ME (Mj/kg)	10.60[b]	10.77[b]	11.28[a]	11.48[a]	11.31[a]
OMD (%)	71.16[b]	72.28[b]	75.59[a]	76.89[a]	75.79[a]
SCFA (mmol/200 mg DM)	1.25[b]	1.30[b]	1.36[a]	1.39[a]	1.37[a]

ME: metabolizable energy; OMD: organic matter digestibility and SCFA: short chain fatty acids.
DM: dry matter.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).

Short chain fatty acids (SCFA) production (mmol/200 mg DM) was significantly greater in treatments with more than 50 percent fenugreek substitution (P<0.05, Table 4). Organic matter digestibility of treatments containing 50% or more fenugreek hay (T3, T4 and T5) were significantly higher than control (P<0.05, Table 4). Similarly, estimated ME content of these treatments were higher than control.

Results of this experiment showed that fenugreek hay has positive effect on cumulative gas production and gas production parameters *in vitro*. These results were in agreement with Mir *et al.* (1993), who had reported a greater (P<0.05) proportion of DM from fenugreek was degraded in the rumen, however, that characteristics of DM degradation in the rumen were similar for fenugreek and alfalfa hays. Effect of rations containing different ratios of fenugreek: alfalfa on pH was not consistent. Ammonia-N concentrations also showed no constant trend with increasing levels of fenugreek (Table 4). Mir *et al.* (1993), have reported that the degradation characteristics of CP in fenugreek and alfalfa hay were similar, except percentage of the degradable fraction which was greater (P<0.05) for fenugreek. In an *in situ* degradation experiment Mustafa *et al.* (1996) also showed that fenugreek hay had more soluble protein than alfalfa hay.

Despite the absence of significant differences between the two forages in degradation characteristics in their experiment, Mir *et al.* (1993) based on greater percentage of the degradable fraction and longer delay in initiation of CP degradation (P<0.05), have concluded that the affectivity of CP degradation of fenugreek hay is higher than alfalfa hay.

These researchers also proposed that the combined effects of DM and CP degradation characteristics of fenugreek may result in a more sustained release of nitrogen in the rumen and thus lead to a greater extend of forage digestion compared to alfalfa that may promote greater efficiency of feed utilization in ruminants. Based on these reports, it could be expected higher ammonia-N concentrations for treatments containing fenugreek hay, however, lack of difference in ammonia-N concentrations may be because of steroidal sapogenin compounds nature of fenugreek and their effect on microbial degradation of protein in rumen.

Anti-protozoal and ammonia-binding properties of steroidal sapogenins of different plants have reported in several studies (Hussain and Cheek, 1995; Wang *et al.* 2000; Hess *et al*. 2003).

Short chain fatty acids production was enhanced with 50%-100% substitution of alfalfa hay with fenugreek hay. This result was expectable according to increase of degradation potential (a+b) and constant rate of degradation (c) with increasing levels of fenugreek hay. The level of SCFA is an indicator of energy availability to the animal (Makkar, 2004). Since SCFA measurement is important for relating feed composition to production parameters and to net energy values of diets, prediction of SCFA from *in vitro* gas measurement will be increasingly important in developing countries where laboratories are seldom equipped with modern equipment to measure SCFA (Getachew *et al.* 2000). Highly significant positive effects of increasing fenugreek hay levels on estimated organic matter digestibility and metabolizable energy content of TMR rations were expectable from gas production results of these treatments. These results can be explained according to Mir *et al.* (1993) findings for effective rumen degradability (ERD) of fenugreek and alfalfa hays (65% and 58%, respectively). Greater values of ERD results more SCFA production and more organic matter digestibility, which in turn provides more metabolizable energy for animal.

CONCLUSION

According to these results, it can be concluded that fenugreek can provide highly valuable forage for ruminants with similar or higher feeding values than alfalfa, and alfalfa can be replaced completely in ration with fenugreek hay without any negative consequences. Cultivation of fenugreek as a new ruminants feed source will bring more benefits in other aspects as environmental pollution control and human health care. To approve some of these beneficial effects of fenugreek however, further *in situ* and *in vivo* researches have to be conducted to assess probable effects of fenugreek steroidal sapogenins (diosgenin) on rumen fermentation characteristics, reduction of methane production and enhancement of animal growth or meet quality.

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