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Use of an *in vitro* Rumen Gas Production Technique to Evaluate the Nutritive Value of Five Forage to Concentrate Ratios

Research Article

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ABSTRACT

This study presents the chemical composition and *in vitro* fermentation of five diets with different forage (alfalfa) to concentrate (faba bean) (F:C) ratios, F0:C100, F25:C75, F50:C50, F75:C25, F100:C0 on a dry matter (DM) basis. Results indicate that the chemical composition in term of organic matter (OM), ether extract (EE), crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), neutral detergent insoluble protein (NDIP), acid detergent insoluble protein (ADIP) and total phenols (TPh), tannin (TT) and condensed tannin (TCT) were varies among five diets ($P<0.05$). After an initial gas test to evaluate 96 h gas production profiles of diets, the time to half maximal gas production was calculated and a second incubation was conducted with fermentation stopped at substrate specific half-time ($t_{1/2}$) and 24 h for each substrate. *In vitro* true DM degradability (*iv*TDDM), OM degradability (*iv*OMD) were increased ($P<0.01$) by addition proportions of concentrate in diets. Microbial mass (g/kg DM), metabolizable energy (ME) (MJ/kg DM), were greater in diets which those had been higher degradability. The efficiency of microbial production (PF) (mg/mL) were calculated for both substrate specific $t_{1/2}$ and 24 h and was not shown differences between experimental diets at 24 h but F100:C0 was lesser (1.17 mg/mL) at substrate specific $t_{1/2}$ ($P<0.01$). Gas produced from fermentable fraction (B) and the rate of gas production (c) were ($P<0.01$) greater in diets with grater concentrate ratio. Increasing the F:C ratio increased ruminal pH and N ammonia and affected concentrations of short-chain fatty acid (SCFA) ($P<0.01$). Amount of CH_4 emission from 13 to 17.16 g/kg DM and the great value was related to F100:C0.

KEY WORDS faba bean, *in vitro* gas production, methane, tannin.

INTRODUCTION

Researchers have been looking for alternative protein sources to replace soybean meal in animal nutrition (Volpelli *et al.* 2010). One of the possible protein sources successfully used in the feeding of ruminants and non-ruminants is faba beans, such as *Vicia faba* (Moschini *et al.* 2005; Volpelli *et al.* 2010). Despite the fact that faba bean is not rich in protein as soybean, it can be considered as a "dual purpose" feed, since it has greater starch contents, similar to barley (Masoero *et al.* 2006). Furthermore, the use of high levels of raw faba bean has no negative effects on palatability and digestibility in sheep (Liponi *et al.* 2009). On the other hand, faba bean is planted on 35000 hectares s in Iran where over 35% of those is located in Golestan Province. This makes Golestan the largest producer of faba bean in Iran (Sabaghpour, 1995). Due to the negative effects of ruminant methane production on the global warming, extensive efforts have been made to measure or/and reduce it (Steinfeld *et al.* 2006). Several of enteric methane production experiments are done measuring emission rates from animals in open circuit respirometers

and in strictly controlled environments (Murray *et al.* 1999). In contrast, *in vitro* techniques can be used, which are less expensive and time-consuming than *in vivo* trials. These techniques also allow one to preserve experimental conditions more precisely (Menke *et al.* 1979). The use of an *in vitro* gas technique in evaluating feedstuffs (through the measurement of variables like methane, microbial mass and short-chain fatty acids (SCFA) is a very effective and robust way of estimating energy loss from diets, microbial and feed nitrogen supply to ruminants (Anele *et al.* 2010). In the gas technique gases were produced directly as a result of fermentation containing CO_2 and CH_4 and the indirect gas produced from the buffering of SCFA (Blu¨mmel and Ørskov, 1993).

The objective of this study was to estimate differences in the nutritive value of five forage (alfalfa) to concentrate (faba bean) ratios based and to determine how diets differing in forage to content based on faba bean, would affect *in vitro* true degraded dry matter (DM), degraded organic matter, methane production, microbial mass, partitioning factor, short chain fatty acids, pH and N ammonia.

MATERIALS AND METHODS

Sample preparations and chemical analyses

Five diets with different forage (alfalfa) to concentrate (faba bean) (F:C) ratios, F0C100, F25C75, F50C50, F75C25, F100C0 on a dry matter (DM) basis were ground in mills with a 1-mm screen before being analyzed. The DM, OM, crude fat (ether extract), crude protein (CP, Kjeldahl N×6.25) and ash were determined according to the AOAC (2000). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined (Van Soest *et al.* 1991), and were corrected for residual acid insoluble ash; Neutral detergent fibre was assayed without sodium sulfite but with a heat-stable α-amylase due to the high levels of starch. Both aNDFom and ADFom were expressed without residual ash. Non-fibre carbohydrates (NFC) were calculated as: NFC=100 - % CP - % Ash - % EE - % NDF, All results were expressed on DM basis. Neutral detergent insoluble protein (NDIP) and acid detergent insoluble protein (ADIP) were measured as explained by Licitra *et al.* (1996). Total phenols and total tannins were determined using colorimetric assay with Folin-Ciocalteu phenol reagent according to Makkar (2003). Condensed tannins were consecutive extracted using the butanol-HCl method (Porter *et al.* 1986).

In vitro gas production measurement

The *in vitro* gas production measurement was done as described by Menke and Steingass (1988).

Rumen fluids were collected prior to feeding from three fistulated Dalagh sheep (≈50 kg body weight) fed a standard diet (500 g grass hay/500 g concentrate). Rumen fluid was poured into a prewarmed insulated container, and strained through sterile gauze. Then it was mixed well and continuous flow of CO_2 flushed rumen fluid. The buffered mineral solution was added to rumen fluid. The mixture was kept stirred under CO_2 in a water bath at 39 ˚C. Samples were weighed and 200 mg (basis DM) was added to each syringe. Buffered rumen fluid (30 mL) was dispensed into the syringes and those were immediately placed in a shaking water bath at 39 ˚C. Cumulative gas volume measurements of samples were read manually from the three replicates each at 0, 4, 6, 8, 12, 24, 48, 72 and 96 h of incubation.

Fermentation syringes without samples (blanks) were included to allow correction for gas produced directly from rumen fluid. After subtraction of gas production from blanks, data were fitted to exponential model Ørskov and McDonald (1979) equation:

$$y= B(1-exp-c\times[t - lag])$$

Where:
y: cumulative volume of gas produced at time ‘t’ (h).
B: asymptotic gas volume.
c: rate constant.
lag: time(h) between inoculation and commencement of gas production.

Halftime ($t_{1/2}$) production of the asymptotic gas volume (B; mL) was calculated as:

$$t_{1/2}= (\ln 2/c) + lag$$

Using the method of Menke *et al.* (1979), metabolizable energy:

$$ME \text{ in MJ}= 2.20 + 0.136\ GP + 0.057\ CP$$

And organic matter digestibility:

$$ivOMD\ \%= 14.88 + 0.889\ GP + 0.45\ CP + 0.0651\ XA$$

Where:
GP: total gas volume.
CP: crude protein.
XA: ash.

The SCFA production (mmol) was calculated using equation described by Getachew *et al.* (2000):

SCFA= 0.0239 GP - 0.0601

After ending of incubation at $t_{1/2}$, 24 h and 96 h, fermentation parameters such as pH and ammonia N of the fluid were determined (Hu *et al*. 2005).

In vitro truly degraded dry matter (*iv*TDDM)

In vitro truly degraded dry matter (*iv*TDDM) was determined using methods described by Anele *et al*. (2010). After the initial 96 h gas run, substrate specific $t_{1/2}$ was calculated and a second incubation of the samples was conducted to obtain degradability measures at substrate-specific $t_{1/2}$ and 24 h. The incubations were cut off at $t_{1/2}$ and 24 h and the volume of gas was recorded. In order to measure true substrate degradability of diets at $t_{1/2}$ and 24 h, refluxing the incubation residue with ND solution for 1 h with subsequent recovery of the truly undergirded substrate in dacron bags (3 cm×10 cm, 45 µm pore size) (Van Soest *et al*. 1991). *iv*TDDM= feed (DM) incubated − residue (DM) recovered in the crucibles/feed (DM) incubated.

Partitioning factor (PF, a measure of fermentation efficiency) was calculated as:

PF= *iv*TDDM (mg) / GP_{24} (mL)

Using the method of Blümmel *et al.* (1997) the *in vitro* microbial mass production can be calculated as:

Microbial mass (mg)= *iv*TDDM (mg) - (GP_{24} (mL)×2.2)

Where:
2.2: stoichiometrical factor according to the amounts (mg) of C, H and O required for production of 1 mmol of SCFA and associated 1 mL gas.

Methane measurement

For the measurement of methane, at the end of incubation ($t_{1/2}$ and 24 h), the clip was carefully unscrewed and 4 mL of 10 molar NaOH was introduced through the silicon tube into syringes.

So, CO_2 absorbed by the NaOH and piston of syringe dipped. The gas volume remaining in the syringe considered to be CH_4 (Demeyer *et al*. 1988).

Statistical analysis

Chemical composition and *in vitro* fermentation data were analyzed using the general linear models (GLM) procedure of SAS (1999). Data were analyzed within a completely randomized experimental design. Treatment means were separated using Duncan test at 0.05 and 0.01 probability levels.

RESULTS AND DISCUSSION

Chemical composition

As would be expected, there was different in chemical composition between five diets (Table 1). Organic matter content of faba bean seed (F0C100) were (P<0.05) greater than those of other diets. Diet with 75:25 ratio of concentrate to forage (F25C75) (P<0.05) greater in crude protein content except F50C50. The CP content of F0C100 (26.05) was lesser than of F25C75 (30.07). Ether extract varies (P<0.05) among diets. There was (P<0.05) differences in ADFom content among F0C100 and F100C0. The values of NDFom also differ (P<0.05), F0C100 and F25C75 seems to have greater values than others. The aNDFom content of diets ranged from 32.65% DM (F0C100) to 18.68% DM (F100C0), whereas that of ADFom ranged from 16.48% DM (F100C0) to 12.43% DM (F0C100). Results indicated a t variation in terms of protein fractionation and concentration addition linearly decreased the protein fractions NDIP and ADIP (P<0.05). Results showed that F0C100 and F25C75 have great levels of total phenol (TPh) compounds (P<0.05), 2.92 and 2.76% DM, respectively. After measuring the non-tannin phenols and subtract from total phenols, the total tannin (TT) was estimated which the treatments F0C100 and F25C75 had the great value and F100C0 had the less (P<0.05). Alfalfa hay (F100C0) had no condensed tannin (CT) and faba bean in levels 100% and 75% of diets had 0.34 and 0.31% DM.

In vitro gas production measurement

Gas production profiles of the experimental diets are shown in Figure 1, and the parameters of the gas production are presented in Table 3. Substrate specific $t_{1/2}$ was 9.56 h (F0C100), 9.06 h (F25C75), 8.76 h (F50C50), 8.52 h (F75C25) and 10.86 h (F100C0). The figure was plotted to show the trend of the incubation. A comparison of the diets showed that first diet (100% faba bean) produced the most gas (65 mL/200 mg DM) after 96 h and fifth diet (100% alfalfa hay) the least (26.91 mL/200 mg DM).

The maximum gas volume from fermentable fraction (B) was great for F0C100 and F25C75 and greater for F50C50 (P<0.01) than for F75C725 or F100C0. The maximum rate of gas production (c) was great for F50C50 and F75C25 (P<0.01), followed by F25C75 (P<0.01), and less for F0C100 and F100C0 (P<0.01). Hourly rumen ammonia-N concentration and pH are shown in Table 2. Reduction trend of the rumen ammonia-N levels and rumen pH on all the diets continued after $t_{1/2}$ h to 96 h. Increasing the concentrate component in the diet reduced rumen pH by around 0.3 pH units and caused a small but significant (P<0.01) increasing in SCFA molar.

Table 1 Ingredients and nutrient composition (% DM) of diets

Item	Treatment[1]					
	F0C100	F25C75	F50C50	F75C25	F100C0	SEM
Chemical composition, %						
Dry matter (DM)	92.85^{a}	92.72^{ab}	92.29^{b}	92.23^{b}	92.56^{ab}	0.06
Organic matter (OM)	97.06^{a}	94.31^{b}	92.54^{cb}	90.76^{cd}	88.92^{d}	0.28
Crude protein (CP)	26.05^{b}	30.07^{a}	28.35^{ab}	19.05^{c}	15.62^{d}	0.40
Neutral detergent fibre (aNDFom)	32.65^{a}	26.88^{ab}	23.44^{bc}	18.93^{c}	18.68^{c}	0.39
Acid detergent fibre (ADFom)	12.43^{c}	13.68^{bc}	14.54^{bc}	16.20^{a}	16.48^{a}	0.48
Ether extract (EE)	1.53^{b}	1.48^{b}	1.72^{ab}	1.79^{ab}	2.2^{a}	0.12
Non-fibre carbohydrates (NFC)	13.51^{b}	9.19^{b}	10.57^{b}	20.75^{a}	23.65^{a}	0.15
Neutral detergent insoluble protein (NDIP)	1.48^{b}	1.67^{b}	1.95^{b}	2.13^{b}	3.26^{a}	0.63
Acid detergent insoluble protein (ADIP)	0.43^{c}	0.80^{b}	1.73^{a}	1.79^{a}	1.66^{a}	0.25
Total phenols (TPh)	2.92^{a}	2.76^{ab}	2.27^{b}	1.69^{c}	0.87^{d}	0.18
Total tannins (TT)	1.97^{a}	1.80^{a}	1.48^{b}	0.66^{c}	0.18^{d}	0.30
Condensed tannins (CT)	0.34^{a}	0.31^{ab}	0.22^{b}	0.14^{cd}	0.00^{d}	0.08

[1] Diets with different forage to concentrate ratio.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

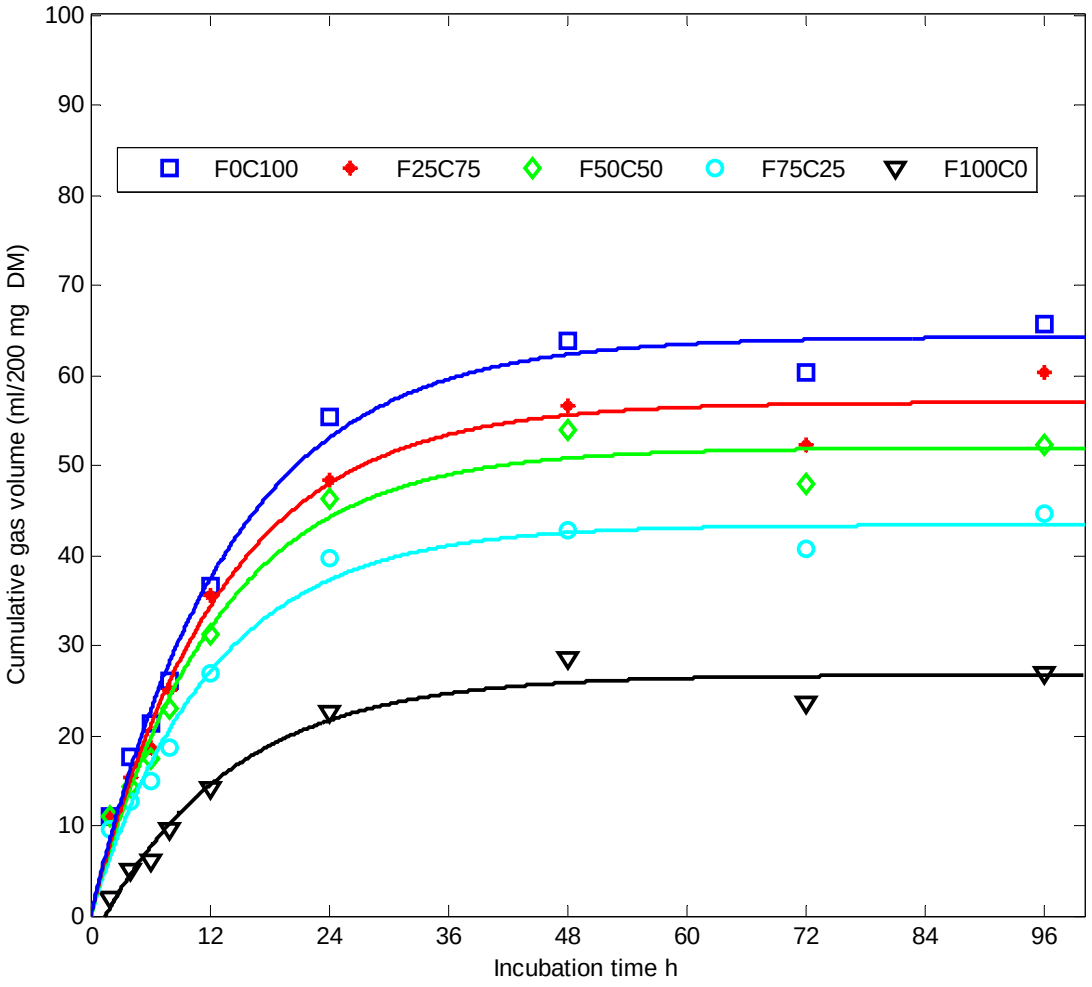

Figure 1 *In vitro* gas production profiles of the experimental diets, (F0C100 (100% concentrate); F25C75 (75% concentrate, 25% forage); F50C50 (50% concentrate, 50% forage); F75C25 (25% concentrate, 75% forage) and F100C0 (100% forage))

In this experiment, ammonia concentration in the fermenter fluid was increased (P<0.01) by supplementation with concentrate.

***In vitro* truly degraded dry matter (*iv*TDDM)**

After 24 h incubation, *iv*TDDM, *iv*DOM, microbial mass, ME, SCFA, PF of the experimental diets were observed in Table 3. Increasing concentrate ratio in the experimental diets led to greater *iv*TDDM and *iv*DOM coefficient. However, only F100C0 had significant difference with other treatments (P<0.01). Both *iv*TDDM and *iv*DOM coefficients at substrate specific $t_{1/2}$ followed similar trend observed at 24 h and were lesser in F100C0 (P<0.01). The PF values of the diets were calculated for both substrate specific $t_{1/2}$ and 24 h and were not shown significant differences between experimental diets at 24 h but F100C0 was grater (2.17 mg/ml) at substrate specific $t_{1/2}$ (P<0.01).

Methane measurement

The methane emission factors found in this study are showed in Figure 2. The CH_4 production from F25C75 (13 g/kg DM) was lesser (P<0.01). It was observed that the decreasing proportion of concentrate in diets generally produced more methane. However, F100C0 and F75C25 had not significant effect on methane production; also the same procedure was observed in three other diets means: F0C100, F25C75 and F50C50.

Chemical composition

In this study, the forage-to-concentrate ratios were F0C100, F25C75, F50C50, F75C25 and F100C0, for the alfalfa hay–faba bean. For F75:C25 and F100C0, the ADFom fraction is a large proportion of the NDFom, which indicates high content of cellulose, lignin and lesser levels of hemicellulose (Abdulrazak *et al.* 2000). Cell wall content of feeds is related to their organic matter digestibility and their nutritive value (Giger-Reverdin, 1995). Indeed, fibre content seems to have reduced fermentability of diets, whose fermentation parameters were consistently the less. Alfalfa (F100C0) had the great fibre content (NDFom, 18.68% DM and ADFom, 16.48% DM) among the substrates investigated. Utilization of alfalfa could not be limited by its fibre content. Tannins intervene in the definition of fibres by forming complex with proteins, and thus those are insoluble in the detergent solution (Makkar *et al.* 1995). Results demonstrated *iv*DOM and *iv*TDDM of diets with a greater ratio of forage were lesser. As can be seen in Figure 1, the gas production has been minus in these diets. There is a close relationship between the extent of potential NDF digestibility and the total volume of gas produced (Huhtanen *et al.* 2008). Archimede *et al.* (1995) indicated the use of concentrate in the ruminant diets causes the maximum utilization of energy, protein and other contents of feeds. The grain supplementation may reduce digestibility of forage of diet. The amount of this effect depends on the ratio of concentrate and its nature. However, a lack of effect of grain supplementation on NDF digestibility (Cerrillo *et al.* 1999) or even and increase on fibre digestibility (Molina Alcaide *et al.* 2000) have also been reported. The CP content of faba bean was 26.05%; this result is similar to results obtained by Volpelli *et al.* (2010) and Cazzato *et al.* (2012) who found 25.3 and 26.52%, respectively. The CP levels in the current study are within the range of 15.62-30.07% DM, which is adequate for maintenance and growth requirements of ruminants (NRC, 1996). However, optimum utilization of CP in diets is dependent on soluble phenolic compounds, such as tannins (Woodward and Reed, 1989). This fact has been reported that faba beans in the diet of non-ruminant animals depress the apparent digestibility of nitrogen compounds (Reddy *et al.* 1985). This harmful effect has been largely ascribed to the presence of condensed tannins in the majority of varieties of faba beans. The condensed tannins appear to be one of the most important antinutritional factors in faba beans, which it can interact and precipitate proteins. Also, the condensed tannins depressing the utilization of dietary protein either by interaction with proteins to form indigestible complexes and by formation of tannin-enzyme complexes resulting in an inhibition of digestive enzyme activity by similar processes (Ortiz *et al.* 1993). On the other hand, Makkar *et al.* (1997) suggested that the presence 2-3 percent of condensed tannins in the diet of ruminants can have beneficial effects. These useful results may be related to concentration, type and structure of tannins. Tannins can reduce proteolysis and loss proteins in the rumen. These functions resulted to increase the by-pass protein, efficiency of microbial protein synthesis and turn-over of urea.

***In vitro* gas production measurement**

Gas produced from fermentable fraction (B) was (P<0.05) greater in F0C100 may be due to their high content of CP and NDF (Khazaal *et al.* 1993). Therefore the greater values obtained for the B fraction in F0C100 and F25C75 will indicate a better nutrient availability for rumen microorganisms. As it was calculated F0C100 to F75C25 had the greater metabolizable energy (ME). The *in vitro* gas production technique has the potential to reflect the *in vivo* digestibility of feeds for ruminants. However, Prasad *et al.* (1994) indicated that the great predictive value for the *in vivo* digestibility of diets was obtained after 45 to 52 h of *in vitro* fermentation. Ruminal micro-organisms fermented Non-structural carbohydrates rapidly, both ruminal pH decline and also ruminal volatile fatty acids (VFA) patterns changed (Kalscheur *et al.* 1997).

Table 2 Effect of different concentration to forage ratio on parameters of gas production (defined by the equation: y= B(1−exp−c×[t − lag])), cumulative gas volume (mL/200 mg DM), ammonia N (mg/L) and pH

Treatment[1]	Parameters of gas production		Cumulative gas volume (mL/200 mg DM)			Ammonia N (mg/L)			pH		
	B[2]	c[3]	$t_{1/2}$	24 h	96 h	$t_{1/2}$	24 h	96 h	$t_{1/2}$	24 h	96 h
F0C100	64.27^{a}	0.072^{c}	30.34^{a}	55.33^{a}	65.58^{a}	101.4^{c}	97.0^{c}	85.9^{c}	6.41^{c}	6.38^{b}	6.32^{b}
F25C75	57.01^{ab}	0.076^{b}	27.67^{a}	48.33^{a}	60.25^{ab}	111.5^{c}	108.4^{c}	102.7^{b}	6.52^{b}	6.50^{ab}	6.48^{ab}
F50C50	51.94^{b}	0.079^{ab}	24.29^{a}	46.33^{a}	52.25^{bc}	153·6^{b}	147·1^{b}	138·5^{ab}	6.53^{b}	6.50^{ab}	6.50^{a}
F75C25	43.38^{c}	0.081^{a}	20.26^{a}	39.67^{ab}	44.58^{c}	164·7^{ab}	161·5^{a}	149·4^{ab}	6.65^{a}	6.60^{a}	6.56^{a}
F100C0	26.73^{d}	0.073^{c}	12.77^{b}	22.67^{b}	26.91^{d}	177·3^{a}	173·8^{a}	165·3^{a}	6.70^{a}	6.66^{a}	6.62^{a}
SEM	2.26	0.0012	2.17	2.80	1.78	4.71	4.16	3.63	0.28	0.46	0.34

[1] Diets with different forage to concentrate ratio.
[2] Gas produced from fermentable fraction (mL).
[3] Rate constant of gas production during incubation (mL h^{-1}).
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 3 *In vitro* truly degraded dry matter (*iv*TDDM); degraded organic matter (*iv*DOM) coefficient, microbial protein; efficiency of microbial production (PF) of the experimental diets

Item	Treatment[1]					
	F0C100	F25C75	F50C50	F75C25	F100C0	SEM
Twenty-four hour (24 h)						
*iv*TDDM	0.683^{a}	0.566^{ab}	0.511^{ab}	0.466^{ab}	0.328^{b}	0.02
*iv*DOM	75.99^{a}	69.97^{a}	71.09^{a}	59.32^{ab}	42.78^{b}	0.40
Microbial mass (g/kg DM)	561.59^{a}	464.73^{ab}	405.33^{ab}	379.39^{ab}	278.46^{b}	0.39
Metabolizable energy (ME) (MJ/kg DM)	11.21^{a}	10.39^{a}	10.21^{a}	8.68^{ab}	6.17^{b}	0.37
Short-chain fatty acids (SCFA) (µM)	1.22^{a}	1.02^{ab}	1.06^{ab}	0.87^{ab}	0.49^{b}	0.06
Efficiency of microbial production (PF) (mg/mL)	1.26^{a}	1.43^{a}	1.07^{a}	1.20^{a}	1.47^{a}	0.13
Half life ($t_{1/2}$)						
*iv*TDDM	0.558^{a}	0.461^{ab}	0.399^{abc}	0.371^{bc}	0.277^{c}	0.08
*iv*DOM	53.77^{a}	53.38^{a}	49.71^{ab}	42.06^{b}	33.98^{c}	0.33
Microbial mass (g/kg DM)	491.56^{a}	400.79^{ab}	345.72^{b}	327.09^{bc}	249.22^{c}	0.41
Metabolizable energy (ME) (MJ/kg DM)	7.81^{a}	7.67^{a}	7.11^{ab}	6.04^{ab}	4.82^{b}	0.26
Short-chain fatty acids (SCFA) (µM)	0.66^{a}	0.61^{a}	0.53^{b}	0.44^{b}	0.27^{c}	0.11
Efficiency of microbial production (PF) (mg/mL)	1.82^{b}	1.66^{b}	1.64^{b}	1.83^{b}	2.17^{a}	0.20

[1] Diets with different forage to concentrate ratio.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

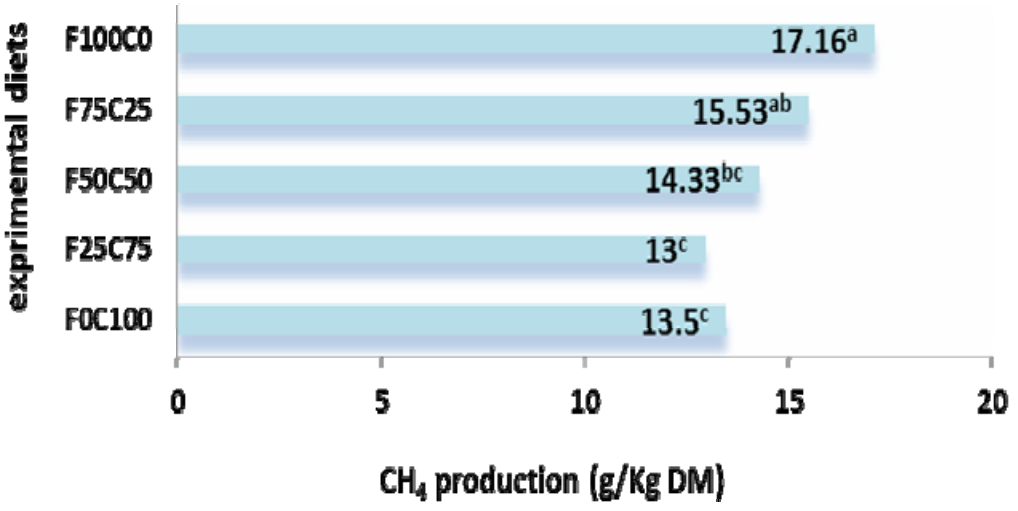

Figure 2 *In vitro* methane production profiles of the experimental diets (with different forage to concentrate ratio)
a, c and c: Means with no common superscript differ significantly (P<0.05)

When the concentrate in the diet increases, the size of the rumen pH decline is highly variable. In the present study, pH value decreased by increasing percentage of concentrate. However, this reduction was small and pH never went below 6.0. In some of the recent research works, it was reported that the reduction of rumen pH below 6·0 occurs when feeding a high proportion (70%) of concentrate in the diet (Carro *et al.* 2000). In contrast, Khorasani *et al.* (2001) and Ueda *et al.* (2003) indicated that the feeding of concentrate resulted in small drops of between 0 and 0·2 pH units. But in the own study used faba bean as its major dietary source, which its rapid fermentation pattern was similar to barley (Masoero *et al.* 2006). In some studies (Loor *et al.* 2003; Sackmann *et al.* 2003) where barley contributed as a major dietary supply of starch, it was shown to result in a greater decline in rumen pH. This small change in ruminal pH in the present study may be due to differences in the proportion of concentrate in the diet. Other factors which may explain this anomaly may include buffering capacity of the feeds, hence buffering capacity of the rumen, ruminal fluid sample (Lee *et al.* 2006). On the other, this small change in ruminal pH probably led to a lesser ruminal cellulolytic activity and as a result a small shift occurred in SCFA molar proportions (Ueda *et al.* 2003). In this study, incremental increase in concentrate led to a reduction in rumen ammonia concentration. Lee *et al.* (2006) demonstrated the feeding of readily available energy sources has decreased rumen ammonia concentrations. This reduction could be explained by a greater utilization of ammonia by the micro-organisms as a readily available energy source (Lee *et al.* 2003); or by reductions in the use of amino acids as an energy source by micro-organisms (Nocek and Russell, 1988). Optimization of microbial yield in the rumen depends largely on the availability of carbohydrates and nitrogen (N) in the rumen (Shabi *et al.* 1998).

In vitro truly degraded dry matter (*iv*TDDM)

Decreasing forage to concentrate ratio in the experimental diets led to greater *iv*TDDM and *iv*DOM coefficient but the greater *iv*TDDM and *iv*DOM were caused to greater microbial mass. The PF value for diets with more proportion of concentrate were lesser than F100C0 and this reason was supported the microbial mass production of those diets (Anele *et al.* 2010). In the ruminant nutrition to improve microbial efficiency, feed conversion into microbial biomass should be maximized because high microbial efficiency improves microbial protein supply to the small intestine and, proportionally, reduces fermentative gaseous carbon losses (Beever, 1993). The tannin content of faba bean in the concentrate section of experimental diets corroborated these results. Observations were showed that F0C100 and F25C75 had greater levels of TPh, TT and CT, but according to results of Makkar *et al.* (1997) amount of CT was low and seems it cannot influence on *iv*DOM and *iv*TDDM, but can have beneficial effects including tannins bind proteins to reduce loss proteins in the rumen. As previously mentioned this function can increase the by-pass protein. On the other hand, the gas volumes at all times of incubation increased with increasing proportions of concentrate to forage ratio in the mixtures and as Hess *et al.* (2006) had expressed the degradation of OM, NDF, ADF increased with legume containing tannin.

Methane measurement

Previous studies indicated, the optimum pH for methane production is 7.0-7.2, but the gas production can occur in the pH range of 6.6-7.6. However, beyond this range, the activity of fiber degraders reduces (Arglyle and Baldwin 1988; Dijkstra *et al.* 1992). Forage: concentrate ratio also influences the acetate: propionate ratio and methane emission decreases drastically from 6-12% (forage-based diet) to 2-3% when diet with the 90% concentrate predominates (Johnson and Johnson 1995).

Whitelaw *et al.* (1984) obtained same results and suggested that the feeding of a high concentrate: low roughage diet produce less methane as compared to low concentrate: high roughage diet. Benchaar *et al.* (2001) reported a decrease in the methane emission with inclusion legumes in the diet of ruminants. This lesser methane emission can be attributed to lesser portion of carbohydrates, faster passage rate, hence, shifting the fermentation pattern towards greater propionate production (Johnson and Johnson, 1995). Also, there are some indications that tannins in the diet reduce ruminal methane production (Waghorn *et al.* 2002). Tannins decrease the degradation of nutrients in the rumen which then may be degraded in the hindgut. This could have contributed to a lesser methane emission because hindgut fermentation resulted in a lesser methane production per unit of fermented nutrients.

CONCLUSION

Although the faba bean contains tannins, the results of the chemical composition and *in vitro* degradability study showed the rumen microbes can be effectively utilized to it and will supply the requirements of energy and amino acids to the ruminants. It was concluded that there were significant variations in chemical composition and gas production characteristics of five F:C ratios in this study. The results indicated that the addition of concentrate in diets increased nutritive value and limited the methane emission. If total condensed tannin was lesser than 2-3% DM, it cannot affect cumulative gas production and estimated parameters of feed.

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