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Effect of Different Feeding Method on Methane and Carbon Dioxide Emissions Milk Yield and Composition of Lactating Awassi Sheep

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Abstract: This study was performed to evaluate the effect of different feeding systems (choice feeding and conventional system) on performance and emission of carbon dioxide (CO_2) and enteric methane (CH_4) in dairy Awassi ewes. One chamber was equipped with gas analyzers to measure CH_4 and CO_2 for 23 h day^{-1}. In total, 16 ewes were used. The ewes were divided into two groups: the Free Choice (FC) group received feed ingredients separately and the Total Mixed Ration (TMR) group received a standard mixed concentrate: forage diet in a ratio of 60:40. The results showed no significant differences between treatments in performance parameters. However, the results of CH_4 and CO_2 measurement indicated significant differences between groups in the amounts of CH_4 and CO_2 produced per kg dry matter intake. The ewes in the FC group produced less CH_4 per animal than the ewes that received the TMR system. In ewes on the FC system, the level of propionate was greatly increased relative to the total VFA components. There were no significant differences in ruminal pH and acetate level between treatments. The results indicate that the FC system may be a potential mitigating effect on enteric emission of CH_4 and CO_2.

Key words: Global warming, diet selection, methane, carbon dioxide emission, respiration chamber

INTRODUCTION

Ruminants typically lose 2-12% of feed gross energy as emissions of methane (Johnson *et al*., 1993). Nutritionists have focused on finding methods to mitigate methane emission because of its inefficiency on account of feed conversion ratio and the role of methane in global warming. In the ruminant nutrition, decreased production of CH_4 can represent an improvement in feed efficiency. Thus, mitigating CH_4 losses from ruminants has both long-term enviroumental and short-term economic benefits. Carbon dioxide and methane are the main greenhouse gases related to animal nutrition and methane has greater global warming potential than carbon dioxide (IPCC, 1996). The mean volume of methane produced by a dairy ewe each year is 17.8 m^3, which is similar to that of a dairy goat (Vermorel, 1997).

The one source of CH_4 is hydrogen, which is produced from fermentative reactions in ruminal fluid; excess hydrogen must be utilized in order to decrease the production of methane. Improvements in ruminal fermentation that favor propionic acid may also allow a decrease in methane production, because propionic acid contains more hydrogen than other Volatile Fatty Acids (VFA). Some feed

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additives (oils, methane inhibitors, ionophores, organic acids, etc.) have been shown to decrease CH_4 emissions (Johnson and Johnson, 1995; McGinn *et al*., 2004). Based on these results, addition of oil to the diet can be used to decrease CH_4 emissions, but the total energy intake must not be increased because of its negative effects on usage of fiber. Given that prophylactic usage of antibiotics is not permitted in Europe farming systems, they have been limited their usage in farming. In any event, the effect of ionophore antibiotics on emission of CH_4 is short lived (Johnson and Johnson, 1995). The pattern of ruminal fermentation may be changed by the use of different feeding strategies that encourage effective utilization of the feed ingredients. Feeding systems that allow choice may provide one mechanism by which ruminants can alter consumption time, meal size, meal length and meal order to weight the balance of ruminal fermentation in favor of the propionic acid ratio (Askar *et al*., 2006). In addition, a higher content of fibrous carbohydrates (NDF) in diets of low feeding value has been shown by Moe and Tyrell (1979) to increase the methane emitted per unit digested. An increase in the concentrate ratio may also improve the nutritional status of sheep by standardizing consumption of energy, protein and mineral and will also give an opportunity to increase the ratio of propionic to acetic acid. But there is a risky of metabolic disorders in Standard Mixed Ratios (SMR) having high concentrate: forage. Ruminants can learn the physiological consequences of ingestion of particular foods and can recognize the feed ingredients offered as free choice according to their post-ingestive effects (Provenza, 1995; Yurtseven and Görgülü, 2004). Thus ruminants can consume high concentrate feed without suffering from metabolic disorders (Fedele *et al*., 2002; Yurtseven and Görgülü, 2004). The aim of the present study was to evaluate the effect of two different feeding systems (a free choice feeding system and a conventional diet) as a factor to mitigate enteric emissions of CH_4 and CO_2 in Awassi dairy ewes.

MATERIALS AND METHODS

Animals and Diets

The study was carried out at the Sanliurfa Harran University in the Southeast Anatolian Region of Turkey, which lies on longitude 38°46′ E and latitude 37°08′ N. The mean temperature and relative humidity for the experimental period (from the end of May through Jnne) were 32.12°C and 25.3%. The experiment was conducted with a total of 16 Awassi dairy ewes in their second and third lactation, each with a single lamb. The ewes were allocated after weaning of their lambs to two experimental groups (free choice feeding-FC and total mixed ratio-TMR), with eight replicates comprising one ewe in a 2.25 m^2 (1.5×1.5) pen. The ewes were assigned to each treatment according to their live weight (FC:51.75±7.6 kg; TMR: 52.17±18.2 kg) and milk yield (FC: 802.5±242.8 g day^{-1}; TMR: 804.75±310.5 g day^{-1}) at the begiuning of the experiment. Each pen contained a 15 L bucket for fresh water, which was available during the entire experimental period (training and testing). The feed fed by free choice was divided into six equal parts so that the feed ingredients were presented separately, but *ad libitum*. The experiment, including a two-week preliminary period, lasted for a total of 4 weeks plus two days. During the second week of the preliminary period, the ewes of the Free Choice (FC) feeding group were given the opportunity again to experience the ingredients that were subsequently given as choices. During this training period, lucerne straw was available at all times and the other ingredients (corn, barley, soybean meal (SBM), cottonseed meal (CSM) and wheat bran) were offered alternately every six days for a period of 14 days. Each feed ingredient, except alfalfa hay, was mixed with 1.33% limestone, 1% salt and 0.11% vitamin-mineral mixture in order to ensure adequate intake of micronutrients and also to prevent any possible effect of micronutrients on selection of feed ingredients. The Total Mixed Ration (TMR) group received componnd feed supplied as a concentrate and lucerne hay, made from hay by chopping to lengths of 1-2 cm, as roughage were used in the experiment. The composition and nutrient contents of the TMR diet, based on 60:40 concentrate:

forage. The metabolizable energy content of the diets was calculated based on the values published by National Research Council (1985). Each of feed ingredients given to FC groups and TMR was added daily but residual feed checking control was done manually by first and second half of the experiment to prevent any stress changing feeding behavior especially in FC groups. Live weight changes of both groups were measured at the beginning and end of experiment in order to see any effect of feeding system on live weight.

Methane and Carbon Dioxide Measurement: Respiration Chamber

During the second 14 days of the data collection period, the ewes were taken into one chamber one by one with own manger. In experiment, all of the animals (16) in each treatment group were used and CH_4 measurements were made using a ventilated respiration chamber. The respiration chamber formed a closed system in which the fresh intake airflow, based on a continuous system, was controlled. The calculation of the volume of methane emissions was based on the concentration measured in the airflows into and out of the chamber. The chamber was 2.5×3.9×3.7 m wide, tall and deep, respectively. The walls and windows of the chamber were insulated to prevent risk of gas leakage. During the experiment, no significant difference was seen between the outside and inside barometric pressure, which would have encouraged leakage of gas (941-952 MB). Ventilation of the chamber was provided by means of individual fresh air intake and chamber exhaust ducts. The ducts were 10 cm diameter and 30 cm long. Fresh intake and exhaust air (90 m^3 h^{-1} = 0.25 m^3 sec^{-1}) was fed directly into the duct, which contained a pair of fans of the same turning velocity (31.8 m sec^{-1}). The chamber was equipped with a dedicated infrared gas analyzer to detect CH_4 and CO_2. The ewes were left with their feed trough in the chamber for 23 h, from early morning (08:30) until the following day (07:30) in order to measure differences according as time and feeding behavior between the emissions during day and night. The concentration (ppm, on a volume basis) of CH_4 in the intake and exhaust ducts of chamber was measured by pumping a sample of the air stream in each duct through the infrared gas analyzer. The level of CH_4 and CO_2 emission generated from the chamber (FCH_4, g sec^{-1}) was calculated via data loggers for each 3-min period from the concentrations in the fresh air intake and the chamber exhaust (C_i and C_e, respectively; ppm) (McGinn *et al*., 2004).

$$CH_4 = \left[C_e M_a \frac{P}{RT} V_e \frac{T}{P} A\right] - \left[C_i M_a \frac{P}{RT} V_i \frac{T}{P} A\right]$$

where, V_e and V_i are the mean air velocity (m sec^{-1}) in the fresh air and the chamber exhaust, MW is the molecular weight of CH_4 (16 g moL^{-1}) or CO_2 (14 g moL^{-1}), P is barometric Pressure (Pa), R is the universal gas constant (8.31 J moL^{-1} deg K^{-1}), T is the temperature of the air stream (°K) and A is the cross-sectional area of the duct (0.0250 m^2).

On the following day the ewes were removed from the chamber and the air inside the chamber was ventilated for one hour. Manure and urine were removed and the chamber floor was prepared for new gas measurements the following day. Thereby, gas emissions from animals from different groups (FC vs. TMR) were analyzed in the different time but during the experiment, the temperature and barometric pressure inside and outside the chamber were recorded for each 3 min period by data loggers. The concentrations of CH_4 and CO_2 were calculated after adjustment (P/RT) for the temperature, humidity and barometric pressure. The values shown also include emissions of gas from the manure.

Ruminal Fermentation Measurement

Ruminal pH was measured once for four animals per group 32th day after the experiment, in order to prevent changes in their feeding behavior and diet selection caused by stress. A rubber tube was inserted into the rumen via the esophagus 5 h after the feeding and a sample of the ruminal contents

(250 mL) were removed using a manual pump. After sampling per content, rubber tube was wiped out again and again to prevent any contamination with saliva. The pH was measured immediately using a pH meter. The rumiual contents were filtered through four layers of cheesecloth. After filtration, the filtrate was centrifuged at 3000 x g for 15 min according to the method of Erwin *et al*. (1961). Five milliliters of the filtrate was combined with 1 mL of 25% (w/v) metaphosphoric acid and stored frozen (-25°C) until VFA analysis.

Chemical and Statistical Analysis

The compositions of the feed ingredients were determined by the method of AOAC (1998). The Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) were analyzed based on the method of Van Soest *et al.* (1991) using an ANKOM fiber analyzer. The metabolizable energy content of the diets was calculated based on the tabulated values published by National Research Council (1985). Milk yield and feed intake were determined every 2 weeks. The animals were milked by hand during the morning and milk samples were analyzed for milk fat, total solid, ash, milk protein, Non Protein Nitrogen (NPN) and casein nitrogen using a FOSS Milco Scan FT120 (Denmark). The composition of TMR's and the diet selected by the ewes were compared by one sample t-test for independent samples of FC and TMR in the Compare Means procedure of SPSS (SPSS, 2006). Independent sample t-test was also carried out to determine the significance of the effect of the treatments on the other performance parameter and cumulative standardized fluxes of CO_2 and CH_4 according to temperature and barometric pressure.

RESULTS

The components of the ration selected by the ewes are shown in Table 1 for the total experimental period because there were no significant differences between the first and the second half of the

Table 1: Compositions of the diets selected by Awassi ewes offered different grain and protein sources vs. TMR ration (DM basis)

	Treatments			
Items	FC	TMR[A]	SEM	Effect
Ingredient (%)				
Corn	28.38	13.56	4.96	0.04
Barley	19.97	20.00	6.17	0.99
Wheat bran	8.56	16.00	1.96	0.03
Soybean meal	2.85	5.00	2.45	0.44
Cottonseed meal	18.82	3.00	2.73	0.01
Alfalfa hay	18.96	40.00	3.37	0.01
Trace mineral-vitamin premix[B]	0.08	0.11	---	NP
Salt	0.80	1.00	---	NP
Limestone	1.07	1.33	---	NP
Chemical composition				
Dry matter (%)	93.25	90.90	0.009	0.001
Metabolisable energy (Mcal kg^{-1})	2.57	2.38	0.033	0.01
Digestible energy (Mcal k g^{-1})	3.13	2.93	0.04	0.01
Crnde protein (percentage of dry matter)	15.54	15.63	0.78	0.91
Crnde fiber (percentage of dry matter)	12.70	12.65	0.56	0.92
ADF (percentage of dry matter)	19.22	22.69	1.04	0.04
NDF (percentage of dry matter)	28.66	34.31	1.65	0.04
Ca (percentage of dry matter)*	0.71	1.12	---	NP**
P (percentage of dry matter)*	0.48	0.47	---	NP**
Ether extract (percentage of DM)	3.60	2.50	0.18	0.009

[A]Calculation based on the values published by NRC (1985); [B]Each kg vitamin-mineral premix provides vit. A 8.000.000 IU; vit. D_3 1.000.000 IU; vit. E 30.000 mg; Mn 50.000 mg; Fe 50.000 mg; Cu 10.000 mg; Co 150 mg; I 800 mg and Se 150 mg; *There is a significant difference between the two groups ($p<0.05$); **NP: Statistical analysis not performed because each feed ingredient in FC, except alfalfa hay, was mixed with limestone, salt and vitamin-mineral mixture in order to prevent any possible effect of micronutrients on selection of feed ingredients experiment. The results showed that diet selection, with respect to consumption of feed ingredients and nutrient intake was different for the FC and the TMR rations. The ewes receiving feed ingredients as free choice selected a ration that contained more maize (FC: 28.38; TMR: 13.56%) and cottonseed meal (FC: 18.82; TMR: 3%) but less wheat bran (FC: 8.56; TMR: 16%) and lucerne hay (FC: 18.96; TMR: 40%) compared with the TMR group ($p<0.05$). The differences for barley and soybean meal were not statistically significant ($p>0.05$). The different composition of the ration in the two groups affected the nutrient composition of the diet. The FC group selected a ration with a higher content of dry matter (93.3 vs. 90.9%), metabolizable energy (2.57 vs. 2.38 Mcal kg^{-1}) and ether extract (3.6 vs. 2.5%). Given that the ewes in the FC group preferred a ration with a lower content of lucerne hay, this group consumed a ration with a lower ADF (19.22 vs. 22.69%) and NDF (28.66 vs. 34.31%). However, there were no differences between the two groups in the protein and crude fiber content of the diet. The ewes in the FC group consumed more dry matter, metabolizable energy, digestible energy, crude protein and crude fat than the ewes in the TMR group (Table 2, $p<0.05$).

The feeding method had no significant effects on milk yield, 4% fat corrected milk yield or milk components ($p>0.05$). However, the efficiency of milk production deteriorated in the FC group and the ewes in the FC group consumed more dry matter per kg milk production than those that received the TMR (FC 4.16 vs. TMR 2.55). Ruminal pH values were not affected by the treatments (Table 3). Although, there was no difference in the proportion of acetate between the treatments, the proportion of propionate and butyrate and the acetate:propionate ratio differed ($p<0.05$). Ewes fed separate feed ingredients (the FC group) had higher propionic acid concentrations than those in the TMR group. Consequently, the acetate:propionate ratio averaged 3.46 for the FC treatment compared with 4.93 for the TMR treatment ($p<0.05$). The CH_4 and CO_2 emissions per animal were not significantly different, but tended to be lower in the FC group ($p>0.05$); however the emission value per kg dry matter intake decreased significantly in the FC group (FC 10.65 vs. TMR 18.12 g DMI^{-1}).

Table 2: Dry matter and nutrient intakes and milk yield and composition of Awassi ewes fed using different methods

	Treatments			
Items	FC	TMR	SEM	Effect
DMI (kg day^{-1})	3.10	2.16	0.04	0.04
ME intake (kg day^{-1})	7.99	5.36	0.16	0.001
DE intake (kg day^{-1})	9.75	6.62	0.18	0.01
CP intake (g day^{-1})	483.11	352.31	19.07	0.01
ADF intake (kg day^{-1})	0.59	0.51	0.03	0.06
NDF intake (kg day^{-1})	0.89	0.77	0.04	0.09
Ether extract (kg day^{-1})	0.11	0.05	0.02	0.001
Milk yield (g day^{-1})	809.71	881.50	124.54	0.69
FCM (g day^{-1})	1115.00	1200.00	182.28	0.75
Milk fat yield (g L^{-1})	52.83	56.62	8.96	0.78
Milk protein yield (g L^{-1})	44.52	51.41	6.58	0.48
MPE (DMI milk $yield^{-1}$)	4.16	2.55	0.48	0.04
LWC (g day^{-1})	179.16	183.33	82.72	0.99
Rumen pH	5.70	5.50	0.12	0.29
Milk composition				
TS (%)	17.67	17.59	0.46	0.88
Protein (%)	3.51	3.75	0.16	0.32
Fat (%)	6.61	6.35	0.39	0.66
Lactose (%)	4.60	4.44	0.12	0.38
Nitrogen fraction				
Total N (g L^{-1})	5.50	5.88	0.19	0.24
Protein N (g L^{-1})	4.67	5.07	0.18	0.18
NPN (g L^{-1})	0.82	0.80	0.70	0.82
Casein N (g L^{-1})	4.39	4.59	0.18	0.46
Whey N (g L^{-1})	0.28	0.48	0.003	0.02

DMI: Dry matter intake SEM: Standard error of the mean,. ME: Metabolizable energy, DE: Digestible energy, CP: Crude protein, FCM: 4% fat corrected milk, MPE: Milk production efficiency, LWC: Live weight change, TS: Total solids, SNPN: Nonprotein nitrogen

Table 3: CO_2 and CH_4 emissions and ruminal fermentation variables for Awassi ewes offered different grain and protein sources vs. TMR diet

	Treatments			
Items	FC	TMR	SEM	Effect
Daily gas production				
CH_4 (g/day/sheep)	33.13	38.54	4.46	0.43
CH_4 (g DMI^{-1})	0.65	18.12	2.09	0.04
CH_4 (% DE intake)	4.42	7.69	0.88	0.04
CO_2 (g day^{-1})	1120.14	1288.41	75.75	0.16
CO_2 (g DMI^{-1})	360.36	596.68	26.85	0.002
VFA				
Acetate (%)	68.34	70.71	0.59	0.13
Propionate (%)	19.77	14.33	0.56	0.03
Butyrate (%)	6.88	9.95	0.16	0.01
Acetate:Propinate	3.46	4.93	0.16	0.02

DMI: Dry matter intake, VFA: Volatile fatty acids, SEM: Standard error of the mean

DISCUSSION

Diet Preference and Performance

The ewes in the FC group selected a diet that contained a higher concentrate ration than that received by the TMR group. There were important differences between groups in the energy, ADF, NDF and ether extract of the diets, but not in the level of protein and crude fiber. The diets consumed by the ewes in the FC group contained less lucerne hay than the TMR diet, thus their diet had lower bulk and higher dry matter content than the TMR diet. The changes in diet preferences in FC group according to the TMR (Table 1) diet in the present study could be also a reflection of the freedom in feed intake and feeding behavior of the sheep with enviroumental conditions. The ambient temperature and relative humidity were higher (42.9 vs. 21.7°C and 29.1 vs. 23.4%) in the second half of the experiment. It is well documented that ruminants increase their intake of concentrate and reduce their intake of roughage to minimize the heat increment in their bodies under high enviroumental temperature, when concentrate and roughage are available as free choices (Fedele *et al*., 2002; Yurtseven and Görgülü, 2004, 2007). Yurtseven and Görgülü (2004) suggested that goats having free access to feed ingredient tended to decrease forage intake as the temperature was in progress. This could be also explained by the changes in feeding behavior (the order of consumption of feed ingredients, the interval between meals, the meal size and meal length, etc.) of the ewes that had free access to feed ingredients (Abijaoude *et al*., 2000; Görgülü *et al*., 2003). In this experiment, no metabolic disorder derived from feeding of a high concentrate ration was seen in the FC group during the experiment. The results also showed that the ewes in the FC group consumed a larger amount of corn and CSM and less wheat bran and lucerne hay than those that received the TMR diet. The present study also showed that the ewes in the FC group consumed more corn than barley, probably because of the high fiber content and lower energy level of barley. The FC ewes ate a diet with lower ADF and NDF content because they consumed a larger amount of grain and cottonseed meal during the experiment compared with the TMR group. Small ruminants have a lower capacity to digest forage than larger ruminants. Their gastrointestinal size and capacity may also limit the intake and utilization of a TMR, because this diet contains more forage.

The experimental data showed that the protein deficiency in the FC group that originated from consumption of less lucerne hay was compensated for by the increased consumption of CSM. Although, SBM and CSM have similar contents of rumen undegradable protein, when SBM was presented as a choice with CSM, the ewes consumed almost 6.6 times more CSM than SBM. This is probably, a reflection of the high protein content of SBM (51.94% CP) and the ewes fed FC could meet their protein requirements by consuming less SBM than CSM (21.4% CP). The high protein content of SBM may have also induced negative post-ingestive feedback based on high protein (Provenza, 1995) and thus it was consumed less by ewes in the FC group. The ewes given free access to feed ingredients had higher daily dry matter intake (3.1 vs. 2.16), therefore, they consumed higher levels of nutrients (ME, CP, ADF, NDF and ether extract) compared with the ewes that received the TMR (Table 2). It is concluded that the feeding behavior of ewes consuming an alternative fiber source to lucerne hay, such as CSM, is a reflection of resistance to the potential risk of acidosis. These results suggest that when given a choice of feeds, ruminants alter their diet selection to attempt to attenuate acidosis (Keunen *et al*., 2002).

Milk yield and the components of milk, especially the N fraction, were not affected significantly by the treatments. The results are attributed to high variation in terms milk yield among groups and some ewes have dried early than target date. There was no important difference in milk NPN content between the FC and TMR groups. It was revealed that the ewes in the FC group selected a synchronized diet from among the different energy and nitrogen sources. Görgülü *et al*. (2008) reported that does given free access only to SBM with barley produced higher milk NPN, although all does had similar protein intake. This probably resulted from inefficient use of ammonia nitrogen released from the highly degradable soybean protein used in their experiment. However, in the present study, the sheep used alternative energy and protein sources (corn and CSM) to provide a synchronized diet (Table 2).

Methane Emission and VFA Concentrations

A diet selection that leads to substantial reductions in CH_4 emissions may maintain an optimal rumen ecosystem. Although, the reductions in CH_4 and CO_2 were small on the basis of an individual animal and were not statistically significant, the decrease in energy lost as CH_4 was significant and was the resnlt of higher feed intake in the FC group compared with the TMR group. Johnson *et al*. (1993) reported that, as the daily amount of feed eaten by any given animal increases, the percentage of the dietary gross energy lost as methane decreases per unit of intake. However, in our study, the FC system had a significant effect on the rumiual content of VFA and clearly decreased the acetate:propionate ratio. The ewes in the FC group consumed larger amounts of concentrate, which contained readily fermentable components such as starch. Daily CH_4 emissions per kg dry matter intake were decreased significantly in the FC group. The differences between the two groups are attributed to the differences in composition of the diets. This agrees with another report in which the net production of VFA was highly correlated with diet composition (Castillejos *et al*., 2005). Moe and Tyrell (1979) found fermentation of soluble carbohydrate to be less methanogenic than that of cell wall carbohydrates. On the other hand, the reduction in ruminal fiber intake in the FC group probably accounted. Although, ruminal fiber digestion was not measured for a large proportion of the reduction in CH_4 emissions. Although, ruminal fiber digestion was not measured directly in this study, other variables (for example the acetate:propionate ratio) indicated a considerable reduction in ruminal fiber digestion. These data suggest that the FC system had a potential mitigating effect on enteric methane emissions in dairy ewes. These resnlts also indicated that there was a relationship between dry matter intake and emission of methane. The values of daily methane emission showed a similar trend with time and followed the feeding and digestive processes. For example, after each feed the emissions of CO_2 and CH_4 increased sharply and then slowly decreased overnight to the lowest values at 08:30 h, just before the morning feed (Fig. 1).

Although, emission of CH_4 reflects the fermentation activity of the digestive tract, the output of CO_2 is primarily an indicator of the respiratory activity of the ewes. For this reason, in the experiment, the level of CH_4 emission was higher than that of CO_2 (Fig. 2). A similar diurnal pattern of gas emission was observed by Kirchgessner *et al*. (1991). In these trials also, CH_4 emissions increased after feeding and then declined until the next fresh feed was distributed.

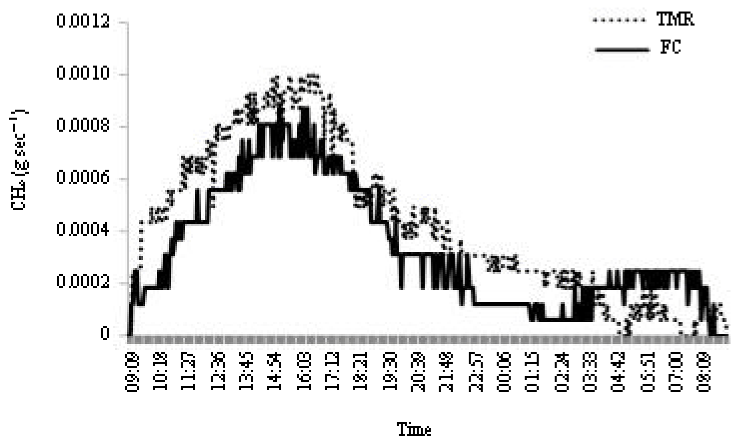

Fig. 1: Diurnal CH_4 (g sec^{-1}) emissions profiles from ewes receiving FC and TMR diets recorded over the experimental period (this figure based on data of 8 animals/treatment)

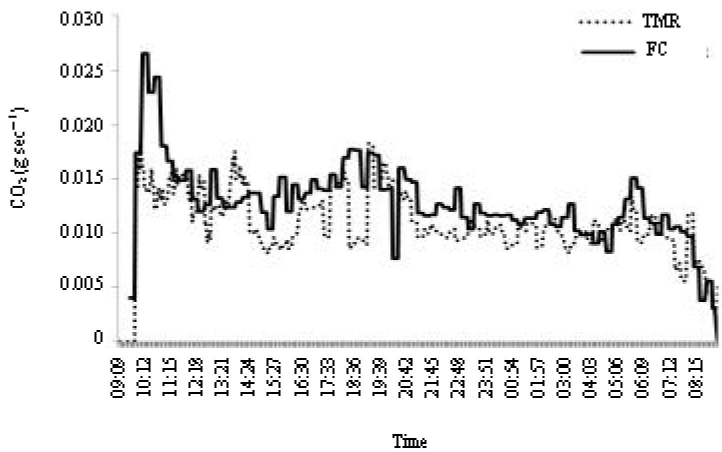

Fig. 2: Diurnal CO_2 (g sec^{-1}) emission profiles from ewes receiving FC and TMR diets recorded over experimental period (this figure based on data of 8 animals/treatment)

Figure 1 shows a clear pattern throughout experiment CH_4 emission reaching a peak at or around sunset and then falling through the night to a minimum at dawn. Feeding behavior was not assessed in the present studies. However, the pattern of CH_4 emission is certainly compatible with the feeding behavior: from sunrise, as feeding activity increased so did CH_4 emission, with the accumulation of ingesta in the rumen. Later, after sunset, feeding gave way to rumination or idling and emissions fell as the content of the rumen was reduced. Lockyer (1997) also found peaks of CH_4 emission during daylight periods in grazing sheep. Present study has shown that CH_4 emissions from sheep may be linked to diurnal patterns of feeding behavior and to changing levels of dry matter intake.

The ecosystem of the rumen microbial flora was not investigated in this study because the experiment focused especially on the measurement of methane emissions. However, it is well known that cellulolytic microbes tend to diminish in the presence of competitive substrates such as starch. The results showed that there was an important relationship between ruminal VFA and ruminal production of methane. The decreased production of methane by ewes that received separate feed ingredients may be related to an increased rumen turnover ratio and digestibility of organic matter in the FC group. In addition, the CSM may have affected the dry matter intake and so ruminal turnover ratio for ewes in the FC group may have been affected positively. Redman *et al*. (1980) reported increased digestion of organic matter in the reticulorumen of steers fed oaten chaff, as a result of protein supplementation. Voluntary intake of low quality forages may be enhanced by ruminal escape of supplemental protein and its subsequent metabolic effects on voluntary intake (Kempton *et al*., 1977). The methanogenic bacteria are the most sensitive to changes in the rumen enviroument and are affected by many dietary factors. Given that the methanogens are the principal utilizers of hydrogen, their welfare affects rumiual and enteric metabolism and carbon balance. Increased rumen turnover may also restrict methanogens through competition for sufficient generation time. Reduction of methanogenesis tends to promote hydrogen production and shifts the carbon balance toward propionate (Van Soest, 1994). Ruminal pH values were not affected by the treatments in the current study, although the ewes in the FC group selected a diet with a lower forage:concentrate ratio (21.4/78.6). Rumiual pH value was too low in both group and results indicated the effects of feeding system on ruminal pH to be minimal. This result may have been concluded that high concentrate ratio in FC and high ground (1-2 cm) forage in TMR. On the other hand, the rumen of a grain-fed dairy cow may show a reduction in cellulose digestion without any change in pH (Murphy, 1989) and this occurs even if the rumiual pH rises to 6. Total cellulolytic activity may be as high in the grain-fed animal as in the forage-fed animal.

Given that there is very limited information on the effects of FC systems on methane emission and rumen VFA content, the results of this study are important and indicate that FC systems have the potential to decrease methane production in the rumen. The factors that influence methanogenic bacteria include those that create a less favorable rumen enviroument for methanogenesis through an increased rate of passage and rate of digestion, depression of rumination and depression of rumen pH. In the FC system, these conditions may favor propionate-producing bacteria over acetate producers, making less hydrogen available to methanogens (Van Soest, 1994), as seen this experiment. Results also indicated that sheep having free access to feed ingredient tended to decrease forage intake in hot climate. Similar experiments in winter tide will improve the knowledge of sheep's behavior and requirements and provide some useful information on how to modulate rations and methane emission. Additionally the experiment using low-roughage based TMR with free choice method will be required to prove that FC systems have a potential to decrease CH_4 production in ewes.

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