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Rumen Fermentation Pattern in Lambs Fed Maize (*Zea mays*) Grain Sprouts to Substitute Conventional Green Fodder and Compounded Feed Mixture

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ABSTRACT

To investigate the effect of feeding maize (*Zea mays*) grain sprouts (MGS) on rumen fermentation characteristics in lambs, a feeding trial was conducted for 120 days. Eighteen healthy male lambs of comparable age (local Mandya crossbreed, 3-4 months) and body weight (Av.12 ± 0.2 kg) were randomly allotted in a complete randomized design (CRD) to three dietary groups of six each. The MGS were produced in a polyhouse and harvested on 10th day. The experimental diets were : Control (T1) group lambs were fed with diet comprising conventional green fodder (CGF), finger millet straw (FMS) and compounded feed mixture (CFM) at 50:10:40 ratio, respectively on dry matter (DM) basis (without maize grain sprouts). In treatment group two (T2), proportion of CGF and CFM was reduced by 50% and substituted with 45% MGS on DM basis (CGF, FMS, CFM and MGS on total DM basis was in 25:10:20:45 ratio, respectively). In treatment group three (T3), CGF was completely replaced with MGS (80%) and CFM was reduced to 25% of control (CGF, FMS, CFM and MGS on total DM basis was in 0:10:10:80 ratio, respectively). After 3 months of feeding CH_4 emission measurements using SF_6 tracer technique was conducted over a period of 10-days successively, so as to get at least 6 days of reading for each animal and simultaneously digestion trial was conducted to assess the intake and digestibility of nutrients. The representative rumen liquor samples at 0, 4 and 8 h post feeding were collected at the end of experiment from three animals of each group to record pH, protozoal count, nitrogen fractions (Total nitrogen, TCA nitrogen, Ammonia nitrogen), TVFA and individual volatile fatty acids (Acetate, Butyrate, Propionate, Valerate and Isovalerate). Results showed significantly lower ($P<0.01$) DMI (g/d) in T2 and T3 groups (486.5 & 354.3) as compared to control (687.8) respectively. Rumen liquor nitrogen parameters indicated higher concentration of total nitrogen and similar ammonia nitrogen and TCA precipitable nitrogen in MGS fed groups as compared to control. Significantly lower concentrations of TVFA and acetate were found in T3 group as compared to control and T2. Production of methane (g/d) did not differ significantly among the groups. However, when expressed (g/kg) per unit intake of DM and digested nutrients, methane production was significantly ($P<0.01$) increased in MGS fed groups as compared to control group. It can be concluded that replacement of compounded feed mixture or conventional green fodder by inclusion of maize grain sprouts in the diet of growing lambs was not beneficial in terms of improving the rumen fermentation and methane reduction.

KEYWORDS: Lamb, Maize grain sprouts, Methane emission, Rumen fermentation, SF_6 method

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INTRODUCTION

Availability of quality feed and fodder throughout the year is the major constraint in sustainable livestock production in India (Birthal and Jha, 2005; Earagariyanna et al., 2017). Thus, it is obvious to look for alternative feed resources that are sustainable and available around the year. In addition, it must be environment friendly as enteric fermentation in ruminants is reported to produce about 2/3rd of total anthropogenic emissions of methane (CH_4) (Bhatta et al., 2007; Malik et al., 2012) and rumen accounts for about 87% of the total digestive tract CH_4 production (Murray et al., 1976; Torrent and Johnson, 1994; Immig, 1996). The

technology of growing fodder without soil and sprinkling water quickly is called hydroponics (Bakshi et al., 2017). Among the alternative newer feed resources for green fodder cultivation, the hydroponic technique is promising, as it is water efficient; require less land and is immune to weather conditions (Naik et al., 2011; Naik et al., 2012; Naik et al., 2014; Bakshi et al., 2018; Gunasekaran et al., 2019). The sprouting process leads to increased enzyme activity, bioconversion of nutrients from one form to another, leading to increased protein, vitamins and fibre compared to the seed per se (Chavan and Kadam, 1989). Maize grain sprout has a different nutrient composition than maize grain in terms of higher CP and fibre; conventional green fodder has higher fibre and less protein as compared to MGS (Chethan et al., 2021). Feeds with high fibre and less digestible nutrients like roughages produce more methane (Moss et al., 2000). Thus, with these beneficial changes in nutritive value, maize grain sprouts could have a good potential in improving livestock performance and reducing enteric methane emissions. The present study was therefore aimed to study the effect of replacement of concentrate mixture or conventional green fodder by including maize grain sprouts in the diet of growing lambs on rumen fermentation pattern and methane emission.

MATERIALS AND METHODS

Animals, housing, experimental design and feeding of animals

The feeding trial was conducted at the small ruminants unit of livestock experimental station, National Institute of Animal Nutrition and Physiology, during October – February 2019- 2020. The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC). Eighteen healthy male lambs of comparable age (Mandya crossbreed, 3-4 month) and average body weight of 12 ± 0.2 kg were purchased and randomly allotted based on body weight in a complete randomized design (CRD) to three dietary groups of 6 each. Both the lamb and treatment were randomly allotted to three similar groups of control (T1), T2 and T3. All the experimental lambs were housed in a well ventilated clean shed with adequate spacing for individual feeding and were dewormed orally at first day of experiment (Albendazole, 10mg/kg body weight). The first one month was considered as a preliminary period and the animals were acclimatized to new diet. All the lambs were maintained under identical conditions of feeding and management throughout the experimental period. The experimental groups were control (T1) in which lambs were fed with diet comprising conventional green fodder (CGF), dry fodder and CFM (maize grain 43.5 parts, wheat bran 26.0 parts, groundnut cake 30.0 parts and common salt 0.5 parts) at 50:10:40 ratio on DM basis. In treatment group two (T2), proportion of CGF and CFM was reduced by 50% and replaced with MGS (comprising 45% of total DM) on DM basis. In treatment group three (T3), CGF was completely replaced and CFM was reduced to 25% of control with MGS (comprising 80% of total DM) on DM basis. Practically all the three diets were made isocaloric and isonitrogenous. All the experimental animals of different groups were fed with additional 10 gram of mineral mixture daily. Weighed quantity of compounded feed mixture (CFM), conventionally grown green fodder (CGF) consisting of green maize and sorghum (*Sorghum bicolor*) fodder at a ratio of 50:50, Hydroponic maize grain sprouts (MGS) and finger millet straw were offered to all the experimental animals during the forenoon and afternoon periods and the residue left in the next day morning was weighed to record the actual intake of offered feed. All the lambs were provided with *ad libitum* clean drinking water. The harvested mats of sprouts was exposed to air ventilation for one day in order to be drier, then weighed and chopped to small pieces before feeding to the animals. During this feeding trial of four month duration, individual data on quantities of CFM, conventionally grown green fodder, MGS and finger millet straw offered daily were recorded. The daily allowance of CFM, CGF, MGS and finger millet straw were periodically revised taking into consideration the change in body weight of lambs.The lambs were fed to meet nutrient requirement as per Indian standard (ICAR, 2013).

Digestion trial, sample collection and analysis

After three months of feeding trial a digestibility trial coinciding with methane measurement was conducted for 6 days to assess the impact of feeding hydroponic maize grain sprouts in lamb. The lambs had free access of drinking water throughout 24 h and feed residues were collected every day at 9:30 AM from the individual animal during the trial and dried in hot air oven (Forced convection hot air oven, USA) at 60 C for 48 h. Dried samples of feed offered, residue and faeces were pooled for the entire period and ground samples were stored in air tight polythene bags. Daily intake through MGS, CFM, FMS and conventional green fodder was recorded. Faecal sampling was done every day at 9.00 AM. The total quantity of faeces voided by each animal during 24 h was recorded individually. The daily output of dung (pellets) from each lamb was crushed and mixed for sub sampling. The pooled and ground dung samples of individual lamb were subjected to proximate analysis (AOAC, 2005). For dry matter, a representative sample of daily faeces voided by each animal was taken in previously weighed moisture cup and dried overnight in hot air oven at 100±5°C. Apparent digestibility coefficient of nutrients was determined by assessing the nutrient composition in both consumed feed and faeces voided.

Collection and analysis of rumen liquor

The rumen fluid was collected from the lambs with the help of a stomach tube attached to a suction pump. The rumen liquor samples were collected at 0, 4 and 8 h post feeding from three lambs of each group at the end of experiment. First few drops of the rumen liquor were discarded to prevent the saliva contamination and approximately, 50 ml of rumen liquor collected in a glass container from each animal was preserved in ice and brought to the laboratory for further processing immediately. Thereafter, the rumen liquor was strained through four layers of muslin cloth and pH was recorded. The pH of strained rumen liquor (SRL) was determined by using a digital pH meter immediately after collection. Each sample was divided into two equal subsets. The first subset was centrifuged and the supernatant of strained rumen liquor (SRL) sample (25 ml) from each animal was poured into a bottle having 1:4 H_2SO_4 (to preserve SRL) for estimation of N fractions and TVFA. The second subset was processed for the enumeration of protozoa. For counting of rumen protozoa equal amount of rumen liquor and formal methyl green were mixed and kept overnight for proper staining. Glycerol (30%) was used for further dilution. The number of protozoa was counted under the microscope (10×) in a known volume of sample and the total number was calculated (Kamra et al., 1991). Nitrogen fractions in the rumen liquor (SRL) *Viz.,* total-N by Micro Kjeldhal procedure (AOAC, 2005), TCA priciptible-N (Lowry et al., 1951) and ammonia- N (Weatherburn, 1967) were analyzed. Total volatile fatty acids and individual volatile fatty acids were analyzed by Gas Chromatography (Agilent; Model 7890A GC System) using Flame Ionization Detector, programmable temperature vaporizer injector and capillary column (Agilent J&W DB-WAX GC Column 40 m × 0.18 mm × 0.18 µm). The concentrations of the individual VFA in the rumen liquor samples were determined by recording the area of both the VFA mixed standards and as well as the sample and expressed as mmol/L (Soren and Rao, 2015).

Enteric methane emission measurement by sulphur hexafluoride (SF_6) tracer technique

After three months of feeding period, the sulfur hexafluoride (SF_6) tracer technique of Johnson et al. (1994) and Berndt et al. (2014) was used to measure *in vivo* enteric methane emission. SF_6 trial was conducted for 10 days, and a minimum of six days successful gas sample collections from each lambs fed control and MGS based diets were ensured for quantifying the methane emission The lambs were initially accustomed to the gas collection apparatus and daily handling during the acclimatization period (one week). About 650-750 mg SF_6 gas (99.9% purity; Chemix specialties gases, Bengaluru) was filled in 32 mm long brass permeation tubes which were placed at 39ÚC in incubator (HCIS, Bengaluru) for the calibration of

its release rates. The permeation tubes were monitored and weighed everyday to determine the daily SF_6 release rate (mg/d) and after achieving a constant release rate of 2.8-4.3 mg/d, the tubes were placed in the rumen to serve as source of tracer gas during methane emission quantification. Different components *viz*. halter, connectors, capillary tube, teflon tube, air filter etc. were assembled following the standard guidelines (Berndt et al., 2014).

The exhaled gas from the nose and mouth was drawn into pre evacuated polyvinyl chloride (PVC) canisters [initial pressure measured with a Mano meter (Model: Keller,Leo2/1to 3 bar/81021,0.1%)] through stainless steel capillary tubing fitted to a halter for a complete feeding cycle of 24 h. Background air samples were collected daily using similar collection apparatus. After collection of gas samples, the final pressure of canister was measured before pressurizing with nitrogen and recording the dilution pressure.

A gas chromatograph (GC 2010 plus, Shimadzu, Japan), fitted with electron capture detector (ECD) and flame ionization detector (FID) was used for quantifying the SF_6 and CH_4 concentration, respectively. Following conditions were maintained in GC for the SF_6 analysis: inlet temperature 100°C, column temperature 40°C, detector temperature 250°C, airflow rate of 400 ml/min, hydrogen flow rate 40 ml/min, nitrogen flow rate 30 ml/min; whilst for methane analysis following conditions were uphold: inlet temperature 100°C, column temperature 60°C, detector temperature 150°C, airflow rate 400 ml/min, hydrogen flow rate 40 ml/min, nitrogen flow rate 30 ml/min. Before sample analysis, standards of SF_6 and CH_4 were injected in to gas chromatograph. Thereafter, gas samples from the animals were drawn in hamilton syringe and injected in to gas chromatograph. The peak area of standards and samples (CH_4 and SF_6) was measured and used for calculating methane emissions (g/d) using following formula:

$$QCH_4 = QSF_6 \frac{[CH_4]}{[SF_6]}$$

Where, QCH_4 is daily CH_4 emission in mole/animal/d; QSF_6 is daily release rate of SF_6 from the permeation tube; CH_4/SF_6 is the mixing ratio of CH_4 and SF_6 in collected breath samples.

STATISTICAL ANALYSIS

The data obtained in the present study were subjected to analysis of variance (ANOVA) to compare the means of each treatment by using the procedures described by Snedecor and Cochran (1989) using Statistical Package for Social Sciences (SPSS, 2009, version 18.0 Chicago, USA).

RESULTS AND DISCUSSION

Animals were fed compounded feed mixture, finger millet straw, conventional green fodder and maize grain sprouts to meet entire CP and greater part of TDN requirement to gain 100g per day (ICAR, 2013). Chemical composition of feed ingredients used in the experiment is given in Table 1.

Table 1. Chemical composition of experimental feedstuffs (% DM basis)

Attribute	Maize Grain	CMF	MGS	SGF	FMS	CFM
Dry matter	92.5	32.4	16.5	33.6	92.9	92.0
Organic matter	98.6	94.5	97.1	88.5	91.8	94.4
Crude protein	8.50	7.81	13.2	8.10	4.20	20.0
Ether extract	2.44	1.93	4.4	1.80	0.90	5.10
Crude fibre	2.43	27.6	10.0	33.2	36.8	4.50
Total ash	1.42	5.49	2.89	11.5	8.18	5.56
NDF	13.3	52.8	33.0	63.8	74.1	27.4
ADF	3.42	25.7	15.5	36.2	38.7	14.4

*Each value is the average of three observations. CMF: Conventionally Maize Fodder; MGS: Maize Grain Sprouts; SGF: Sorghum Green Fodder; FMS: Finger Millet Straw; CFM: Compounded Feed Mixture.

Dry matter intake and nutrient digestibility

The vaules of total DMI, DMD and OMD for T1, T2 and T3 groups were presented in Table 5**.** The total DMI (g per day) for T1, T2 and T3 groups was 687.8, 486.5 and 354.3, respectively. The DMI as a per cent of body weight was 3.42, 3.17 and 2.40, respectively. The DMI (g) per kg metabolic body weight for treatments T1, T2 & T3 was 72.5, 64.8 and 46.8, respectively. There was significant ($P<0.01$) difference in total DMI (as g per day, per cent of body weight and g per kg metabolic body weight) among the treatment groups. There was significantly ($P<0.01$) lower DMI in T3 group. The MGS intake (g per day) for T2 and T3 was 183.7 and 220.3, respectively. The intake of MGS as a per cent of body weight was 1.04 and 1.53, respectively. The present values observed are similar with other workers who reported lower DMI of the animals fed hydroponic sprouts (Fazaeli et al., 2011; Naik et al., 2016), which might be due to higher water content of the sprout fodder leading to lower DM intake. The present findings showed lower DM intake due to inclusion of fresh maize grain sprouts replacing conventional green fodder which contained higher moisture content contributing to bulk. The mean apparent digestibility (per cent) of DM and OM for T1, T2 and T3 treatment groups during the digestion trial was 67.6, 65.0 and 71.1 and 70.3, 69.7 and 74.0, respectively. The per cent DMD and OMD were significantly ($P<0.05$) higher in group T3 as compared to group T2 or T1, which might be due to the lower feed intake and lower crude fiber content and easily digestible nutrients in MGS. Reddy et al. (1988) also observed significant increase in the digestibility (%) of DM and OM and concluded that the increase in the digestibility of the nutrients may be due to the tenderness of the fodder due to its early stage of growth.

Rumen liquor parameters

pH

The values of rumen liquor pH measured at an interval of 0, 4 and 8h post feeding the experimental diets in groups T1, T2 and T3 are presented in Table 2. The difference among the three treatment groups for rumen liquor pH collected at 0, 4 and 8h post feeding was statistically non-significant. Comparisons between the time intervals within the treatment group were statistically significant. In the present findings rumen pH decreased after four hours post feeding compared to zero hour values and returned to original status after eight hours post feeding in all the experimental groups. In agreement with the present findings Raeisi et al. (2018) and Helal (2018) observed that, there was no effect on rumen pH by feeding hydroponic barley sprouts. However, between time period of zero and four hours post feeding, Helal (2018) found decrease in pH which was similar to present findings. Similarly Rupal et al. (2020) found significant ($P<0.01$) effect on time of sampling post feeding hydroponic maize fodder in Rathi calves where in ruminal pH significantly decreased from 0 h to a minimum level at 3 h post feeding and after that there was increasing trend in pH was noticed and values at 24 h were close to the 0 h values. Between treatment groups there was no significant difference and concluded that the ruminal pH was unaffected by hydroponics maize fodder. On the contrary Dung et al. (2010) reported lower rumen pH value obtained in lambs fed diet containing hydroponic barley fodder as compared to control diets. Similar results were also reported by Fayed (2011). Farghaly et al. (2019) reported lower pH values in rumen of rams fed barley sprouts alone or with concentrate mixture. Sprout fodder contain higher amounts of easily digestible carbohydrates, less lignified crude fibre which increases activity of starch utilizing ruminal microorganisms resulting in reduction of pH. However, in the present findings there was no difference among the groups which might be due to balance of fibre and starch digestion resulting in optimum rumen microbial fermentation. The rumen pH values were within the normal range of 6.0 to 7.0 where rumen micro flora functions effectively. Any increase or decrease in ruminal pH beyond this optimum level would cause disturbance in normal rumen micro flora and fauna which was not observed in the present study.

Table 2. Rumen liquor pH and protozoal count at 0, 4 and 8 hour post feeding in experimental lambs

Group/ Period	0 Hour	4 Hour	8 Hour	SEM	P Value
pH					
T1	7.02[a]	6.42[b]	6.31[b]	0.22	0.000
T2	6.74[a]	6.19[b]	6.08[b]	0.20	0.000
T3	6.83[a]	6.38[b]	6.46[b]	0.14	0.000
SEM	0.043	0.048	0.065		
P value	0.000	0.000	0.000		
Protozoa count (10^5/ml)					
T1	2.27[b]	5.21[aq]	4.27[a]	0.87	0.000
T2	2.41[b]	5.21[aq]	4.27[a]	0.83	0.000
T3	2.33[b]	6.45[ap]	4.38[a]	1.19	0.000
SEM	0.025	0.25	0.076		
P value	0.000	0.000	0.000		

T1: Control diet; T2: 45% MGS group; T3: 80% MGS group;
Means with different superscript in a row (a, b) and column (p, q) differ significantly (P<0.01)

Protozoal count

The mean values for protozoal number in rumen liquor collected at interval of 0, 4 and 8h post feeding the experimental diets in groups T1, T2 and T3 are given in Table 2. Treatment wise no specific trend was observed but from period of 4 to 8 h post feeding maximum fermentation was noticed. In the present study protozoa number was within the normal range of 10^4 to 10^6 / ml. There was no significant difference between the experimental groups in protozoa number except in group T3 at four hour post feeding found significantly higher number of protozoa compared to control. This indicates T3 group fed 80% MGS had higher easily digestible starch and other nutrients. Between the time periods followed similar trend as pH values. Wherein four hours after feeding there was increase in protozoa number and at eight hours after feeding different diets showed declining trend which was non significant among the groups. Farghaly et al. (2019) observed the higher (30%) total rumen protozoal count in barley sprouts fed group than other treatments and opined that, improvement in protozoal count could be attributed to the sprouted barley composition such as enzymes, grass juice factor and high energy that may enhance the growth of rumen microorganisms. Though, higher protozoal count has a negative effect by contributing to methane production, it also has beneficial effects like maintaining optimal pH in high energy rich diet and they contribute up to 50% of the bio-mass in the rumen which are highly beneficial.

Nitrogen fractions in rumen liquor

The average values of nitrogen fractions in rumen liquor *viz.,* total nitrogen, ammonia nitrogen and TCA perceptible nitrogen (mg / 100 ml) at 0, 4 and 8 hour post feeding the diets in control and experimental groups are furnished in Table 3. Total nitrogen concentrations were significantly higher in MGS fed groups T3 and T2 as compared to control T1 especially at 8 h post feeding. When compared between time periods of all the groups after feeding experimental diets, there was significant increase in TN which continued even upto 8 hours post feeding. Ammonia nitrogen concentrations when compared among the groups, there was significant increase in all the groups at respective time periods of 0, 4 and 8h collection and among T3, T2 and T1 groups, it was comparable. When compared between time

periods there was significant increase 4 hours post feeding and thereafter remained similar or decreased until 8 hours after feeding the experimental diets. TCA precipitable nitrogen concentrations when compared between the groups, there was significant increase in T3 group at 0 hour collection as compared to control. At 4 and 8 hour observation there was no significant difference among the treatment groups. When compared between time periods, there was significant increase after feeding at 4 hours and thereafter remained similar or decreased until 8 hours after feeding the experimental diets. Overall higher concentration of total nitrogen and similar ammonia nitrogen and TCA precipitable nitrogen was observed in MGS fed groups compared to control. Higher total nitrogen helps in increasing microbial growth which in turn contributes to higher microbial protein yield to the host animal which is a desirable feature.

Similar to our findings, Helal (2018) found no significant difference in ammonia concentration between the barley sprout supplemented group and control group. When compared between 0 and 4 hour after feeding there was significant increase in ammonia concentration in respective time periods. As against the present findings, Dung et al. (2010) reported higher ammonia concentration in fresh hydroponic barley fodder fed groups. In the present study no such increase was found indicating similar level of protein degradability and better utilization due to synchronization of energy and protein release. While Rupal et al. (2020) reported that the values of total rumen nitrogen, TCA-precipitable nitrogen and non protein nitrogen were significantly ($P<0.01$) higher in hydroponics maize fodder fed groups as compared to control group. Across the time period within the group, the total rumen nitrogen, TCA-precipitable nitrogen and non protein nitrogen showed significantly increasing trend across period of sampling with maximum concentration at 3 h post feeding and later decreased up to 24 h post feeding. Similar results of no difference in ammonia concentration were reported by Raeisi et al. (2018). Non-significant difference in ruminal ammonia concentration was also reported by Farghaly et al. (2019) when barley hydroponic fodder was fed alone or along with concentrate mixture. The MGS contains highly digestible CP and non starch carbohydrates. The CF present is less lignified and well utilized by ruminal microorganisms in rumen. Higher concentrations at 4 h post feeding in the present study are mainly due to higher rumen microbial activity which indicates availability of abundant substrates. Hence, utilization of nutrients in MGS compares well with the nutrient utilization of diet in control group.

Table 3. Nitrogen fractions (mg /100 ml) in rumen liquor at 0, 4 and 8 hour post feeding in control and experimental groups

Group\period	0 Hour	4 Hour	8 Hour	SEM	P Value
	Total nitrogen				
T1	47.6^{a}	61.8^{aq}	128^{bq}	24.6	0.000
T2	47.3^{c}	86.4^{bq}	235^{ap}	57.1	0.000
T3	60.9^{ca}	125^{bp}	235^{ap}	50.8	0.000
SEM	5.33	9.77	22.29		
P value	0.000	0.000	0.000		
	Ammonia nitrogen (NH_3-N)				
T1	16.5^{c}	33.3^{a}	29.1^{a}	5.06	0.000
T2	16.3^{c}	33.5^{b}	30.9^{a}	5.35	0.000
T3	20.9^{b}	29.9^{a}	23.4^{b}	2.69	0.000
SEM	1.83	3.15	3.11		
P value	0.000	0.000	0.000		

	TCA precipitable nitrogen				
T1	15.8^{bq}	52.6^{a}	47.1^{a}	38.5	0.000
T2	28.7^{bq}	50.8^{a}	46.9^{a}	42.1	0.000
T3	47.9^{ap}	49.9^{a}	43.1^{a}	46.9	0.000
SEM	4.50	4.69	3.89		
P value	0.000	0.000	0.000		

T1: Control diet; T2: 45% MGS group; T3: 80% MGS group
SEM: Standard error of means

Means with different superscript in a row (a,b) and column (p,q) differ significantly (P<0.01)

Volatile fatty acids

The values of total volatile fatty acid and individual VFA's *Viz.,* acetate, propionate, butyrate, iso-butyrate, valarate and iso-valarate (mmol/L) in rumen liquor of control and experimental groups at 0, 4 and 8 h of feeding the diets are presented in Table 4. There was significant (P<0.01) difference among treatment groups in TVFA concentration and within the group between time intervals there was significant (P<0.01) increase in TVFA concentration after feeding of experimental diets. Significantly lower concentrations were found in T3 80% MGS fed group to substitute CGF and CFM, respectively. The concentration of TVFA was comparable between T1 and T2 groups. Similarly lower concentrations of acetate were found in T3 group as compared to control and T2. The propionate concentration in T3 group was comparable with control and T2. The results indicated lower (P<0.01) total volatile fatty acid in group T3 which was fed 80% MGS (dry matter basis) as the sole source of fodder. As high fibre content in the diet promotes acetate production, lower intake of fibre fractions and tenderness of the MGS has resulted in lower TVFA and acetate. Contrary to present findings Fayed (2011) and Dung et al. (2010) found higher TVFA in sheep fed barley sprouts than control. In their study, comparison was made between barley grain and sprouts fed groups, where grain had lesser fibre content than sprouts. Rupal et al. (2020) also reported that the TVFA values were found to be significantly (P<0.01) higher in all the hydroponics maize fodder fed groups as compared to control group. Upon comparison between time periods of at 0, 3, 6, 12 and 24 h post feeding initially showed a continuous rising trend with peak at 6 h post feeding. Thereafter, TVFA showed decline trend up to 24 h and opined that the observed trend is due to availability of higher energy and organic matter in hydroponic maize fodder which contributed for higher concentrations of TVFA. Similar results of increase in TVFA and propionate was reported by Farghaly et al. (2019). Concentrations of TVFA and individual VFAs showed increasing trend after feeding experimental diets and reached its peak 4 hours post feeding and then showed declining trend at 8 hours post feeding. Butyrate concentrations in MGS fed groups were significantly lower compared to control and almost a similar trend was observed for iso-butyrate, valerate and iso-valerate among the experimental groups.

Table 4. Total and individual volatile fatty acid fractions (mmol/L) in rumen liquor of control and experimental groups at 0, 4 and 8 hour post feeding

Group\period	0 Hour	4 Hour	8 Hour	SEM	P value
		Total volatile fatty acid			
T1	53.3^{bp}	86.1^{ap}	80.6^{ap}	10.1	0.001
T2	51.8^{bp}	74.5^{ap}	75.0^{ap}	7.66	0.001
T3	36.8^{cq}	61.8^{aq}	56.0^{bq}	7.57	0.001
SEM	3.38	3.48	4.29		
P value	0.000	0.000	0.000		
		Acetate			
T1	35.4^{bp}	53.3^{ap}	51.5^{ap}	5.70	0.000
T2	32.8^{bp}	43.3^{ap}	45.0^{ap}	3.81	0.000
T3	22.8^{bq}	36.8^{aq}	33.9^{aq}	4.26	0.000
SEM	2.61	2.30	3.09		
P value	0.000	0.000	0.000		
		Propionate			
T1	9.66^{bpr}	22.5^{ap}	19.0^{ap}	3.84	0.039
T2	11.9^{bp}	19.8^{ap}	18.7^{ap}	2.48	0.039
T3	8.63^{bqr}	15.8^{aq}	14.6^{aq}	2.21	0.039
SEM	0.72	1.12	1.08		
P value	0.000	0.000	0.000		
		Butyrate			
T1	5.67^{ap}	8.36^{ap}	8.38^{ap}	0.90	0.002
T2	5.04^{ap}	8.80^{ap}	9.18^{ap}	1.32	0.002
T3	3.61^{aq}	7.21^{aq}	5.89^{aq}	1.05	0.002
SEM	0.72	1.12	1.08		
P value	0.000	0.000	0.000		
		Iso-Butyrate			
T1	0.90^{ap}	0.52^{bq}	0.57^{bp}	0.12	0.000
T2	0.60^{aq}	0.56^{aq}	0.46^{bq}	0.04	0.000
T3	0.62^{aq}	0.66^{ap}	0.44^{bq}	0.07	0.000
SEM	0.05	0.05	0.03		
P value	0.000	0.000	0.000		
Group\period	0 Hour	4 Hour	8 Hour	SEM	P value
		Valerate			
T1	0.40^{bp}	0.80^{ap}	0.38^{bq}	0.14	0.000
T2	0.40^{bp}	0.87^{ap}	0.75^{ap}	0.14	0.000
T3	0.27^{bq}	0.61^{aq}	0.55^{ap}	0.11	0.000
SEM	0.05	0.05	0.03		
P value	0.000	0.000	0.000		
		Iso-valerate			
T1	1.22^{ap}	0.61^{bq}	0.69^{bq}	0.19	0.000
T2	1.03^{ap}	1.13^{ap}	0.86^{bp}	0.08	0.000
T3	0.84^{aq}	0.80^{ap}	0.65^{bq}	0.06	0.000
SEM	0.09	0.11	0.08		
P value	0.013	0.013	0.013		

T1: Control diet; T2: 45% MGS group; T3: 80% MGS group

SEM: Standard error of means

Means with different superscript in a row (a,b) and column (p,q) differ significantly ($P<0.01$)

Methane production

The average values of methane (CH_4) g/d produced, CH_4 g/kg DMI, CH_4 g/kg DMD and CH_4 g/kg OMD during the feeding trial in groups T1, T2, and T3 are presented in Table 5. There was significant increase in methane production when expressed (g/kg) per unit intake of DM and digested nutrients in MGS fed groups. Daily methane emission from average Indian cattle, buffalo, sheep and goat is 76.64, 97.01, 11.63 and 10.14 g/day/head, respectively (Singh, 2001). Thirumalaisamy et al. (2020) reported methane emission of 22.02 g/day/head in sheep fed control diets. There was no significant difference in methane production per day among different dietary treatment groups. As MGS contained higher digestible nutrients and less fibre might be the reason for less methane production but non-significant. Type of carbohydrate regulates the rumen pH and methanogen population. As plant matures the proportion of structural carbohydrates increases and CH_4 emission in the rumen is a result of fiber digestion; thus, a 25% increase of non-structural carbohydrate in the diet can reduce the methane production by 20% (Moss et al., 2000). Highly digestible feeds contain less structural carbohydrate than dry roughages, and the effect of increasing the proportion of such feeds in the diet results in increasing ruminal VFA concentrations is well documented. With increase in the proportion of propionate and a decrease in the proportion of acetate and this has impact on methane production (Moss et al., 2000). But in the present study lower methane production was not associated with higher volatile fatty acid production. As MGS has faster passage (Dung et al., 2010) rate in the rumen thus less time was available for microbial fermentation. Hence, further research on methane production upon feeding MGS is required. Methane production in MGS fed group T3 when expressed as g/kg nutrient intake, there was significantly ($P<0.01$) higher methane production. This might be due to lower DMI and associated lesser nutrient digested. Increase in protozoal numbers observed might also to some extent contributed to increase in methane production. Similar to present findings Hafla et al. (2014) reported that, supplementation of either sprouted barley or barley grain did not affect methane output in a dual flow continuous culture fermenters system.

Table 5. Methane emission in experimental groups of lambs during feeding trial184

Parameter	T1	T2	T3	SEM	P value
Methane (g/d)	26.8	22.0	23.1	1.07	0.156
CH_4 (g/kg DMI)	39.0^b	45.2^b	65.4^a	2.99	0.000
CH_4 (g/kg DMD)	83.9^a	68.7^b	91.6^a	3.90	0.000
CH_4 (g/kg OMD)	60.2^c	74.4^b	93.1^a	4.15	0.000
DMI (g/d)	687.8^a	486.5^b	354.3^c	34.8	0.000
DM digested (g/d)	465.3^a	319.9^b	253.1^c	23.5	0.000
OM digested (g/d)	446.0^a	295.4^b	248.8^c	20.4	0.000

T1: Control diet; T2: 45% MGS group; T3: 80% MGS group
SEM: Standard error of means
Mean values in a row bearing different superscripts (a,b,c) differ significantly ($P<0.01$)

CONCLUSION

Rumen fermentation parameters were not improved upon feeding MGS and methane (g/d) production per day in different dietary treatment groups was comparable. However, methane production per unit of digestible nutrient intake in lambs fed 80% MGS was significantly higher as compared to control group. It is deduced that, the replacement of compounded feed mixture or

conventional green fodder by inclusion of maize grain sprout in the diet of growing lambs was not beneficial in terms of improving the rumen fermentation and methane reduction.

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