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Profiling of Rumen Fermentation and Microbial Population Changes in Goats Fed with Napier Grass Supplemented with Whole Corn Plant Silage

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ABSTRACT

The effects of different inclusion level of whole corn plant silage to Napier grass were observed in determining rumen fermentation and microbial population in goats. Fifteen male Boer cross goats around six months old of approximately 18.54±1.83 kg of b.wt., were used as experimental animals. The goats were assigned into five groups with three goats per treatment group. The five treatment groups consisted of different proportions of Napier Grass (G) and whole corn plant silage (CS)-G/CS, (T1) 100/0, (T2) 75/25, (T3) 50/50, (T4) 25/75 and (T5) 0/100, respectively. The mean concentrations of rumen NH_3-N (mg dL^{-1}) were not significant differences among the treatments, although T4 and T5 were slightly increased compared to other treatments. The total VFA production in the rumen fluid of the goat was not significantly different among the treatments, however; highest molar proportion of propionic acid and lowest proportion of acetic acid was observed in goat fed with T5 diets. Although the total bacteria population of rumen content was not significantly different among the dietary treatments, the population of *R. albus*, *R. flavefaciens* and *F. succinogen* showed significantly ($p<0.05$) among the treatments. The lowest population of methanogen and protozoa were detected in the rumen of goats fed T5 diet compared with other treatments. Thus, the animals fed with T5 diet showed the highest proportion of propionic acid and the lowest number of methanogen and protozoa population in the rumen.

Key words: Whole corn plant silage, napier grass, rumen fermentation, cellulolytic bacteria, protozoa, methanogenic archea

INTRODUCTION

The use of corn silage as a green forage for ruminant feed has increased rapidly (Hafez *et al*., 2012) due to high yielding properties, relatively high energy content, palatability and easy to incorporated with total mixed ration (Cherney *et al*., 2004; Kononoff *et al*., 2003). Starch, in the kernels of whole corn plant silage, optimizes the growth of rumen microbial population and influences the rate of microbial protein synthesis, nitrogen utilization and production of Volatile Fatty Acids (VFA) (Jalc *et al*., 2009). On the other hand, relatively high level of starch and fermentable carbohydrate in the corn silage may lead to rumen acidosis and may suppress

cellulolytic bacteria population in the rumen. According to Lettat *et al.* (2013), corn silage contains higher amounts of starch, which make it an interesting means to reduce methanogenic archea production as compared with alfalfa silage. It is well known that starch fermentation in the rumen favors propionate production at the expense of acetate and decreases ruminal pH, which reduces hydrogen availability and inhibits the activity of rumen methanogens (Martin *et al*., 2010; Hook *et al*., 2011). Rumen protozoal numbers are also often decreased in ruminants fed high-starch diets, which also reduces the transfer of hydrogen from protozoa to methanogens (Morgavi *et al*., 2012). In the ruminant, the production of methane reduces the energy availability of the animals. It is estimated between 2-12% of the gross energy in the feed is lost as a methane production (Johnson and Johnson, 1995). Thus, dietary manipulation to improve efficiency of nutrient utilization as well as to decrease the impact of ruminant production on the environment is an important goal for ruminant nutritionists. According to Lettat *et al.* (2013), increasing the corn silage in the diet of dairy cow increased propionate concentration but decreased ruminal pH, methane production and concentration of acetate and butyrate compared to alfalfa silage. De Campos *et al.* (2002) described that *in vitro* rumen fermentation profiles of 50:50 sugar cane/corn silage diets were higher compared to those of other combination. However, these investigations are more focused on inclusion of corn silage to other types of forage on the rumen parameter observation. There is very limited information on the effects of the inclusion of whole corn plant silage to Napier grass on rumen parameter in goats. Therefore, the objective of the current study was to evaluate the effect of different inclusion level of whole corn plant silage to Napier grass on rumen fermentation parameter and microbial population in the goats.

MATERIALS AND METHODS

Experimental diets and animals: The Suwan corn forage was planted at the Field 2, Department of Animal Science, Universiti Putra Malaysia and harvested at the 1/ 3 milk line stage of maturity around 90 days. The harvested whole corn plants were chopped around 2 cm particle length using mechanical chopper and were ensiled for 2 months before given to the animals. Napier grass was harvested from ruminant farm which was established at the Field 2, Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia. The Napier grass was harvested around 2-2 .5 months maturity and it was harvested daily in the morning. Similarly, it was chopped mechanically around 2 cm long before fed to the animals.

Fifteen Boer crossbred male goats around six months old of approximately 18.54±1.83 kg of body weights were used in this study. These animals were housed in the metabolic crate and randomly assigned into five treatment groups with three goats per treatment in a completely randomized design. The animals in the respective groups were fed *ad- libitum* twice daily around 9.00 am and 5.00 pm with a treatment, diet consist of different proportion of Napier Grass (G) and whole corn plant silage (CS)-G: CS, (T1) 100:0, (T2) 75:25, (T3) 50:50, (T4) 25:75 and (T5) 0:100. The experimental diets given to the animals were formulated based on 60:40 of roughages to concentrate ratio based on DM basis. The Napier grass and whole corn plant silage are considered as roughages whereas commercial goat pellets was used as a concentrate requirement. After 21 days of adaptation period, samples of rumen fluid were collected using a stomach tube after 2 h of feeding.

Determination of pH, fermentation acids of whole corn plant silage: The values of pH and the concentration of fermentation acids were measured from the whole corn plant silage samples.

The pH of each sample was determined in triplicate using 25 g wet ensilage that was mixed with 100 mL of distilled water. After hydration for 30 sec using a blender, the filtrate was filtered through Whatman filter paper (No. 1) to obtain the extracts. Immediately after extraction, the pH was measured using a pH meter (BP 3001, Benchtop pH meter) according to Filya (2003).

Immediately, after the pH measurement has completed, the remaining filtrate was preserved with 2-3 drops of 5% sulfuric acid and kept frozen at -20°C pending for fermentation acid analysis. Lactic acid and VFA concentration in the silage extract were determined by using gas chromatography (Agilent 69890N Series Gas Chromatography System from Agilent 97 Technologies, USA) equipped with a flame ionization detector.

pH, VFA and NH_3-N determination of rumen fluid: The pH of the rumen fluid sample was measured immediately after collecting from the each goat. Subsequently, approximately 15 mL of rumen fluid sample was transfer to a sterile container for determination of rumen NH_3-N, VFA and microbial population and stored at -20°C until further processing. The NH_3-N was determined by using a spectrophotometer (modified from Parsons *et al*., 1984). The VFA concentrations in the rumen fluid were determined by using gas chromatography.

Chemical analysis of experimental diets: The percentage of DM, OM and CP of feed samples were analyzed according to the procedure of AOAC (1990), while the percentage of NDF, ADF and ADL were determined according to Van Soest *et al.* (1991).

DNA extraction and Real-time PCR of rumen fluid sample: Total bacterial DNA was extracted using the QIAamp® DNA mini stool kit (Quagen, Inc., Valencia, CA, USA) according to the manufacturer's protocol with a few modifications. Briefly, 1.4 mL of buffer ASL was added to 350 μL of rumen sample and was vortexed continuously for 1 min. After that, the sample was incubated at 95°C for 5 min. The sample was then vortexed again for 15 sec before centrifuged at 15,000 g for 1 min to separate pellet of the cell debris and other suspended particles present in the sample. Approximately, 1.2 mL of supernatant was taken out and then transferred into the new 2 mL micro centrifuge tube before the pellet was discarded.

Afterwards, one inhibit EX tablet was added to each sample and immediately and continuously vortexes until the tablet was completely dissolved. The sample was then incubated at room temperature for 1 min to allow inhibitors to absorb to the inhibit EX matrix, before centrifuging again at 60,000 g for 3 min to let the pellet inhibitors bound to inhibit EX. All of the supernatant was transferred into a new 1.5 mL micro-centrifuge tube and re-centrifuged for another 3 min to remove any residual inhibitor. Approximately 350 μL of the supernatant was added into the 1.5 mL micro centrifuge tube which was already contained 15 μL of proteinase K followed by adding of 200 μL of AL buffer, vortexed and incubated at 70°C for 10 min. Approximately 200 μL of 100% ethanol was added to the proteinase digested sample. This sample was then added to the QIAamp spin column, which was placed in a 2 mL collection tube and centrifuged at 15, 000 g for 1 min to bind the DNA. The column was washed with 500 μL of buffer AW1 and AW2, respectively. The spin column was centrifuged at 15, 000 g to remove residual buffer AW2 and the DNA was later eluted using 50 μL of buffer AE. The extracted DNA was stored at -20°C until use.

The quantification of rumen microbial populations, methanogenic archea and protozoa of goats were carried out based on the standard curve method in Real-Time PCR. The standard curves were constructed using number of copies of the 16S rRNA gene plotted against quantification cycle (Cq)

obtained from 10-fold serial dilutions of PCR products from a pure culture of each rumen microorganism group. In order to prepare the standard curves, the DNA was extracted from the pure culture of each targeted rumen microorganisms (total bacteria populations, *Ruminococcus albus*, *Ruminococcus flavefaciens*, *Fibrobacter succinogens*, methanogen and total protozoa population). Conventional PCR was used to amplify bacterial DNA. The PCR products of the targeted rumen microorganisms were run in 1% agarose gel and specific bands were purified using the mEGAquick-spin™ purification kit (iNtRON Biotechnology, Korea). Purity and concentration of 16S rRNA gene in each sample was measured using a Nanodrop ND-1000 spectrophotometer (Implen NanoPhotometer™, Germany). The copy number of the 16S-rRNA gene per ml of elution buffer was calculated using the following formula that is available online in Genomics and Sequencing Center web-based calculator developed by University of Rhode Island (URI):

$$\text{Number of copies} = \frac{\text{The amount of DNA } (\mu g\ mL^{-1})\ 6.022 \times 1023}{\text{Length of (bp)} \times 109 \times 650} \times 100$$

Since the efficiency of amplification among primers and templates may be variable, the amplification Efficiency (E) of each primer-template combination was determined based on the slope value of the linear regression of each standard curve calculated by the following equation:

$$E\ (\%) = [10^{(-1/\text{slope})} - 1] \times 100$$

In this equation, E is 100% if a 10-fold dilution of DNA template results in a Cq difference of 3.32.

Real-time PCR was performed using a BioRad CFX96 Real-time PCR system (BioRad, USA) equipped with optical grade plates. Primers used in the quantification of different rumen microorganism populations are shown in Table 1. The real-time PCR reaction was performed in a total volume of 25 μL using the Maxima SYBR Green q PCR Master Mix (Fermentas, USA). Each reaction consisted of 12.5 μL of 2×SYBR Green Master Mix, 1 μL of 10 μM forward primer, 1 μL of 10 μM reverse primer, 2 μL of DNA samples and 8.5 μL of nuclease-free water. Each sample was assayed with triplicate reactions. Negative controls without a DNA template were run with every assay to assess overall specificity. The following reaction conditions were applied to each well: initial 5 min incubation at 94°C and 40 cycles of denaturation at 94°C for 20 sec, annealing

Table 1: PCR primers for real time-PCR assay

Target species	Forward/reverse	Primer sequence (5'-3')	Annealing temperature (°C)	References
Total bacteria	F	CGGCAACGAGCGCAACCC	55	Koike and Kobayashi (2001)
	R	CCATTGTAGCACGTGTGTAGCC		
Ruminococcus albus	F	CCCTAA AAGCAG TCTTAGTTCG	55	Koike and Kobayashi (2001)
	R	CCTCCTTGCGGTTAGAACA		
Ruminococcus flavefaciens	F	TCTGGAAACGGATGGTA	60	Koike and Kobayashi (2001)
	R	CCTTTAAGACAGGAGTTTACAA		
Fibrobacter succinogens	F	GTTCGGAATTACTGGGCGTAAA	55	Lane (1991)
	R	CGCCTGCCCCTGAACTATC		
Methanogen	F	TTCGGTGGATCDCARAGRGC	58	Denman and McSweeney (2006)
	R	GBARGTCGWAWCCGTAGAATC		
Protozoa	F	CTTGCCCCTCYAATCGTWCT	55	Sylvester *et al.* (2004)
	R	GCTTTCGWTGGTAGTGTATT		

(temperature for different primers are described in Table 1 for 30 sec and extending at 72°C for 20 sec. To confirm the specificity of amplification, melting curve analysis was carried out after the last cycle of each amplification.

Statistical analysis: The experimental data from feeding trial study were analyzed by one way analysis of variance (ANOVA) using the General Linear Model (GLM) program of SAS (package version 9.2) and differences between treatment means was compared using Duncan's multiple range tests.

RESULTS AND DISCUSSION

In the present study, the whole corn plant silage was considered well fermented as demonstrated by a data that show low pH, high concentration of lactic acid and acceptable level of VFA contents. Fermentation analysis of the whole corn plant silage and chemical composition of feedstuffs of experimental diets is presented in Table 2-4. All values except DM were expressed in DM basis.

Measuring of rumen pH is the best indicator that reflects ruminal health. The mean value of rumen pH in the present study was not affected by dietary treatments (Table 5). According to Sung *et al.* (2007), the pH level is one of the most important factors in the rumen environment because fibrolytic bacteria are very sensitive towards pH changes. The pH values of all diets were between pH 6.2-6.4. It was probably due to the suitable particle length and sufficient content of fiber in the diets that promotes chewing and rumination process in the rumen. Particle length or fiber content of the diet can be manipulated to increase the chewing time and, consequently, to increase saliva production. The saliva serves as a buffering capacity within the rumen and it is related with level of pH. Muia *et al.* (2000) reported that the values of pH were in the range of

Table 2: Fermentation analysis of whole corn plant silage

Fermentation acids (%)	Values
pH	3.70
Lactic acid	73.03
Acetic acid	9.31
Propionic acid	0.72
Butyric acid	0.12

Table 3: Chemical composition of feedstuffs

Items	DM (%)	OM (%)	CP (%)	NDF (%)	ADF (%)
Napier grass	23.4	97.1	8.0	74.8	42.6
Whole corn plant silage	23.3	96.1	8.1	58.6	35.1
Commercial pellet	89.2	91.0	15.4	37.0	8.3

DM: Dry matter, OM: Organic matter, NDF: Neutral detergent fiber, CP: Crude protein, ADF: Acid detergent fiber

Table 4: Chemical composition of experimental diets

	Grass (%): Whole corn plant silage (%)				
Items	T1	T2	T3	T4	T5
DM (%)	95.60	95.50	95.00	94.90	94.50
OM (%)	95.70	95.50	95.70	95.20	95.40
CP (%)	10.60	10.60	10.90	10.70	10.80
NDF (%)	59.20	56.80	54.70	52.50	49.50
ADF (%)	23.50	22.30	21.00	19.90	19.20
ADL (%)	8.30	7.80	7.70	6.30	6.11
ME(MJ/kg DM)	9.47	10.06	11.04	10.73	11.65

Calculated value ME (MJ/kg DM) = 0.016 DOMD (g digestible organic matter kg^{-1} DM (AFRC 1998), T1: Napier grass: Corn silage (100: 0), T2; Napier grass: Corn silage (75: 25), T3: Napier grass: Corn silage (50: 50), T4; Napier grass: Corn silage (75: 25), T5: Napier grass: Corn silage (0: 100), DM: Dry matter, OM: Organic matter, NDF: Neutral detergent fiber, CP: Crude protein, ADF: Acid detergent fiber

Table 5: Effect of dietary treatments on fermentation profiles in rumen fluid of goats

	Treatment						
Items	T1	T2	T3	T4	T5	SEM	P
pH	6.37	6.27	6.30	6.23	6.27	0.15	NS
NH_3N (mg dL^{-1})	17.6	17.50	17.50	17.80	17.80	0.45	NS
Total VFA (mM L^{-1})	55.7^{a}	55.70^{a}	56.10^{a}	55.90^{a}	56.40^{a}	1.21	NS
Molar proportion (%)							
Acetic acid	60.5^{a}	61.50^{a}	60.50^{a}	55.80^{b}	48.60^{c}	1.27	*
Propionic acid	25.3^{c}	25.80^{c}	29.40^{b}	30.30^{b}	34.00^{a}	0.64	*
Butyric acid	17.8^{c}	17.60^{c}	18.30^{b}	18.30^{b}	22.80^{a}	0.54	*
Isobutyric acid	0.6^{b}	3.00^{a}	3.30^{a}	0.90^{b}	1.00^{b}	0.61	*
Isovaleric acid	0.9^{bc}	1.00^{bc}	0.80^{bc}	1.50^{b}	2.20^{a}	0.73	*
Valeric acid	0.7^{b}	1.00^{b}	2.00^{a}	2.20^{a}	2.00^{a}	0.39	*
Caproic acid	ND	0.40	ND	1.80	1.80	NA	NA
Minor VFA	2.1^{c}	5.30^{ab}	6.20^{a}	7.00^{a}	7.00^{a}	0.80	*
Acetic/propionic	2.4^{a}	2.40^{a}	2.10^{ab}	1.80^{ab}	1.40^{c}	0.35	*

*Significantly different at 5% level ($p<0.05$), **Significantly different at 1% level ($p<0.01$), [a, b and c]Means with different superscript in each row differed significantly, T1: Napier grass: Corn silage (100: 0), T2; Napier grass: Corn silage (75: 25), T3: Napier grass: Corn silage (50: 50), T4; Napier grass: Corn silage (75: 25), T5: Napier grass: Corn silage (0: 100)

6.3-7.0 and Erdman (1988) stated that the value of between 6.0-7.0, which was considered optimal for the activity of rumen microbial population for their growth and VFA absorption (Dijkstra *et al*., 1993).

The mean concentrations of rumen NH_3-N (mg dL^{-1}) are presented in Table 5. There were not significant difference among the treatments, although T4 and T5 were slightly increased compared to other treatments. The concentration of NH_3-N has been used as a qualitative reference to detect the microbial activity in fibrous carbohydrates in the rumen (Detmann *et al*., 2009). A ruminal fluid NH_3-N concentration of at least 5 mg dL^{-1} is generally accepted as the minimum value of adequate microbial protein production (NRC., 1985). According to Leng (1990), the level of NH_3- N within the range of 10-20 mg dL^{-1} is required to optimize digestion of fibrous feed for rumen microorganisms. In the present study, ruminal fluid NH_3- N concentrations of animals in all diets exceeded 5 mg dL^{-1}, indicating microbial protein production was not limited. The average ruminal NH_3-N values for goats in the different treatment groups were between 17.4-18.00 mg dL^{-1}. It indicates the level of NH_3-N obtained in all dietary treatments were sufficient for optimum rumen fermentation and microbial growth.

The total VFA production (mM L^{-1}) in the rumen fluid of the goat was not affected by dietary treatments. However, the molar proportions of fermentative acids were significantly difference among the treatments ($p<0.05$). A high proportion of acetic acid was detected in T1, T2 and T3, whereas the lowest proportion was observed in T5. The molar proportion of propionic and butyric acid were higher in the goat fed with T5 diet than those of other diets (Table 5). According to Firkins *et al.* (2006), the proportion of VFA produced in the rumen depends largely on the composition of diets consumed by the ruminant in particular the fractions contained in the feed. The production of VFA in the rumen is affected by numerous factors such as substrate composition and availability of specific types of rumen microbes to degrade the received diets (Dijkstra, 1994). In the present study, the molar proportion of acetic acid was high, whereas the level of propionic acid was lower in goats fed with higher levels of Napier grass diets with the exceptions of T3. Similarly, Fenner *et al.* (1969) reported that a complete substitution of hay with corn silage did not change not only rumen pH but also the concentration of total VFA in the rumen fluid; however, the proportion of acetic acid is decreased; whereas the proportions of propionic acid increased. This is

probably due to high degradation of fiber component in the diet, which was fermented by cellulolytic bacteria in animals fed with high level of Napier grass (T1) because of high content of fiber than fermentable carbohydrate.

The differences in dietary treatments in the present study on the molar proportion of acetate and propionate were also reflected differently. High fiber diet has resulted in greater proportions of acetate, whereas diet with high starch content has resulted in greater proportions of propionate. This study was in agreement with Kariuki *et al.* (2001) who reported that a high proportion of acetate and low proportion of propionate were observed in Napier grass diet. Similarly, Firkins *et al.* (2006) reported that high degradation of fiber in the diets will produce a high proportion of acetic acid and low amount of propionic acid in the rumen. On the other hand, as the content of starch and sugar level was increased in the diet, the proportion of acetic acid decreased; resulting in the increased of propionic acid concentration (Leek, 1993). Kariuki *et al.* (2001) and Widiawati and Thalib (2009) have reported that if the level of propionic acid is increased, the energy of the ruminant ration will be more efficiently used. The shift of acetic to propionic acid ratio in favour of propionic acid has reduced the energy losses that occur during cellular metabolism. Likewise, Potter *et al.* (1976) reported that a higher proportion of propionic acid was energetically more efficient for beef cattle production.

Moreover, Kung (2010) stated that ingested lactic acid from the silage gives a positive effect to animal due to the conversion of the lactic acid into propionic acid in the rumen by propionibacteria (*Selenomonas ruminantium, Mesasphaera elsdenii*). Similarly, Gill *et al.* (1986) also reported that ruminal propionic acid was produced from lactic acid in the rumen and could be also derived from the remaining water soluble carbohydrate in silage after feeding. Absorption of propionic acid from the rumen wall will be converted to glucose by the liver of animals. Propionate is an important precursor for gluconeogenesis and increased propionate concentrations in the rumen has resulted in increased of glucose synthesis. Increased glucose concentrations provide more energy to the animals, thereby, increasing production response. In addition, Weinberg *et al.* (2004) stated that LAB from silage has a potential role as probiotic which beneficially affects the host animal by improving its intestinal microbial balance. The ratio of acetate to propionate was decreased according to inclusion of whole corn plant silage to Napier grass diets (Table 5). Takahashi *et al.* (2005) reported that the molar proportion of propionic acid tended to increase, leading to the ratio of acetic acid to propionic acid decreased.

In the rumen butyric acid also is primarily used as a source of energy for the host (McDonald *et al*., 2002). In the present study, high level of butyric acid was observed when whole corn plant silage diets are given in high quantity to the goats. Leedle *et al.* (1995) stated that the proportion of butyric acid has significantly increased when the diet is changed from a low energy diet with a high energy diet. The greater population of protozoa was observed in rumen fluid of cattle when proportion of concentrate to fiber-based diet was increased (Dennis *et al*., 1983). In the present study, high level of butyric acid in high inclusion level of whole corn plant silage diet probably could be related to population of protozoa because rumen protozoa produce butyric acid as their metabolic end-product (Carberry *et al*., 2012). However, the population of protozoa was detected low in the rumen of goats fed with T5 diets compared to T3 diets. It might be due to high concentration of propionic acid in T5 diets. Carberry *et al.* (2012) reported that the populations of protozoa have a negative relationship with the level of propionate, whereas, positive relationship appears when butyric acid is present.

The proportion of minor VFA (Branched chain VFA): Isobutyric, iso-valeric and valeric; medium chain VFA: caproic) were comparable among the dietary treatments. In the present study, a high proportion of minor VFA was observed in T3, T4 and T5, whereas the lowest proportion was detected in T1. Fenner *et al.* (1969) reported that high level substitution of corn silage in hay diet has decreased the level of acetate, whereas levels of propionic, iso-valeric, valeric and caproric acid have recorded increased. This could be probably due to degradation of starch in the corn silage diets by one of the most active proteolytic bacteria species *Bacteroides amylophilus* that possess amylolytic activity. In addition, Wallace *et al.* (1999) described that proteolytic bacteria; *Eubacterium pyruvovorans* produced valerate and caprorate during their growth. Our data are consistent with Noziere *et al.* (2014) whose reported that high starch diets had reduced shift in the proportion of VFA with low acetate and a relatively high propionate, isovletate, valerate and caprorate were detected. Likewise, Fenner *et al.* (1969) reported that, the inclusion of a high proportion of corn silage in hay diet has demonstrated in increased iso-valeric, valeric and caproic acid proportion in the rumen. Tedeschi *et al.* (2000) reported that the proportion of minor VFA mostly originates from specific dietary protein or recycling of bacterial protein by ruminal oxidative deamination and decarboxylation of valine, leucine and isoleucine. Those are needed as essential growth factors for the several bacterial species, mostly cellulolytic bacteria because *R. albus*, *R. flavefaciens* and *F. succinogen* requires minor VFA for their growth (Feng, 2004). According to Moharrery (2004), the minor VFA can increase apparent DM digestibility and microbial growth and improve microbial functions and enzyme activities in the rumen of sheep because those minor VFA are used to synthesize branched chain amino acid by rumen microorganisms (Allison *et al*., 1962). According to Bentley *et al.* (1955), iso-butyric acid or other valeric and caproic acids had improved cellulolytic activity of cellulolytic bacteria in the *in vitro* fermentation (Bentley *et al*., 1955).

It has been well documented that bacterial populations in the rumen at a given time, largely determine the extent and rate of fiber degradation (Khampa *et al*., 2006). Diet is one of the main factors influencing the rumen microbial populations and specifically the milieu of substrate derived from microbial fermentation of ingested feed (Carberry *et al*., 2012). The effects of dietary treatment on different types of rumen microbial population of goats are reported in Table 6. In the present study, the mean concentration of total bacteria population in the rumen fluid of goats was not affected by dietary treatments. However, the populations of cellulolytic bacteria species were statistically lower in goat fed with T5 diet compared to other diets. This was probably due to the low amount of fiber content and high availability of readily fermentable carbohydrates in the 100% whole corn plant silage diet (T5). Fiber degradability mainly depends on the accessibility of rumen microbes which may be influenced by the morphological structure and chemical composition of the

Table 6: Effect of dietary treatments on microbial population (copies mL^{-1}) in rumen of goats

	Treatment (Log_{10} cell mL^{-1})						
Species	T1	T2	T3	T4	T5	SEM	p
Total bacteria	10.0^{a}	9.9^{a}	10.0^{a}	10.1^{a}	10.2^{a}	0.21	NS
F. succinogen	6.3^{a}	5.8^{ab}	6.2^{a}	6.1^{a}	5.6^{b}	0.11	*
R. albus	6.4^{a}	6.2^{ab}	6.5^{a}	6.4^{a}	6.2^{ab}	0.15	*
R. flavefaciens	7.3^{a}	7.0^{ab}	7.4^{a}	7.1^{ab}	7.0^{ab}	0.18	*
Methanogen	4.9^{ab}	4.9^{ab}	5.3^{a}	4.8^{ab}	4.7^{b}	0.07	*
Protozoa	6.9^{ab}	7.0^{ab}	7.23^{a}	6.9^{ab}	6.8^{ab}	0.18	*

*Significantly different at 5% level ($p<0.05$), **Significantly different at 1% level ($p<0.01$), $^{a, b \text{ and } c}$Means with different superscript in each row differed significantly, T1: Napier grass: Corn silage (100: 0), T2; Napier grass: Corn silage (75: 25), T3; Napier grass: Corn silage (50: 50), T4; Napier grass: Corn silage (75: 25), T5: Napier grass: Corn silage (0: 100)

fibrous tissue (Ngwe *et al*., 2012). The predominant rumen cellulolytic bacteria are *F. succinogen, R. albus* and *R. flavefaciens* (Hobson and Stewart, 1997) and they possess a greater ability to degrade fiber than other cellulolytic bacteria species (Carberry *et al*., 2012). In the present study, high numbers of *R. flavefaciens* was detected in all dietary treatments compared to other two cellulolytic bacteria. It is probably due to the constituents of structural carbohydrate in the diets degraded by specific types of cellulolytic bacteria. According to Collings and Yokoyama (1980), hemicellulose was degraded by *R. flavefaciens* and *F. succinogen* with the same pattern of degradation; however, *R. flavefaciens* degraded slightly more effective in wheat straw, corn silage and kentucky bluegrass degradation, whereas, *F. succinogen* degrades more effectively in hemicellulose degradation of alfalfa. Hespell *et al.* (1997) reported that carbohydrate fermentation and end fermentation profiles of *R. albus* and *R. flavefaciens* are almost similar, however, only *R. flavefaciens* produces succinate as one of its major fermentation end-products. According to Lettat (2011), the effect of diets on the growth of cellulolytic bacteria varies due to changes of pH, the capacity of the different species to adapt to dietary changes and the interactions among different bacterial species.

In the present study, the population of *R. flavefaciens, R. albus* and *F. succinogen* were comparable among the dietary treatments. High numbers of predominant fiber degrading bacteria was detected in T1 and T3, while the lowest was were observed in T5. This might be due to low fiber content of the diets in T5 due to low structural carbohydrate content in the whole corn plant silage than those of other diets. This is why; less population of cellulolytic bacteria was required to break down the hemicellulose and a cellulose fraction of the whole corn plant silage diets (T5). Carberry *et al.* (2012) reported that a forage-based diet is dominated by cellulose and hemicellulose, which favors the proliferation of cellulolytic bacteria. However, the population of cellulolytic bacteria in T3 was almost the same as T1. It might be due to interactive effect of their physical and chemical constituents of combined diets. This study was in contrast with Khafipour *et al.* (2009) and Hook *et al.* (2011); who reported that the inclusion of readily fermentable carbohydrate to the forage based diets decreases the population of bacteria. Nevertheless, Lettat *et al.* (2013) stated that the number of *R. albus* and *F. succinogen* were not affected after replacing alfalfa silage with corn silage in the rumen of dairy cows. In the present study, the number of the total bacteria population was not significant among the dietary treatments, although the number of cellulolytic bacteria population was low in the whole corn plant silage diet. The reduction of the cellulolytic bacteria could be substituted by increased of amylolytic bacterial numbers due to availability of more readily fermentable carbohydrates (i.e., starch) in whole corn plant silage diet.

The population of methanogens in the present study is highly affected by types of the diets given. High fiber diet generally produced more hydrogen gas due to cellulolytic bacteria produces hydrogenfor their fermentation end-products. Hydrogen does not accumulate in the rumen because it is immediately utilized by hydrogen utilizing archea (methanogen) that present in the complex rumen microbial ecosystem (Bunglavan, 2014). Lange *et al.* (2005) reported that the symbiotic relation of hydrophobic methanogens with hydrogen producers is usually realized by attachment or by floc formation. In the present study, the highest methanogen was detected in T3, followed by T1, T2 and T4 and the lowest was observed in the rumen of goats fed with T5 diets. It might be due to high production of propionic acid in T5 diets that reduces the uses of hydrogen availability by rumen methanogen and inhibits the activity of their population (Martin *et al*., 2010; Hook *et al*., 2011). Similarly, animals fed with high fiber diets resulted in more production of acetic acid together with high production of hydrogen and leading to an increase number of methanogenic

archaea (Adeyosoye *et al*., 2010). A positive relation of starch-based diets (grains) and methane production has also been stated by Beauchemin and McGinn (2005). An increased proportion of starch in the diet changes in proportion of rumen VFA in such a way that less acetic and more propionic acid is formed and the supply of hydrogen for methanogenesis is rather limited (Iqbal *et al*., 2008).

In the present study, high level of acetic acid and low level of propionic acid were detected in T1 and T2, however, less population of methanogen was observed in those diets. It was probably due to the maturity stage of the Napier grass and the present of suppressing compound such as oxalate which contained in Napier grass. According to Surendra and Khanal (2014), feeding of early harvested Napier grass in subtropical regions might facilitate more methane yields due to low concentration of lignin in the younger harvested biomass, which resulted in less recalcitrance in the biological degradation of hemicellulose and cellulose into the simple sugar and finally to the end product of methane. The degree of polymerization and crystallization of hemicellulose and cellulose has been increased with maturity due to the increased compactness of the hydrogen bonds inherent in the structure of the forage (Cherubini, 2010). According to Rahman *et al*. (2009), Napier grass contained high level of oxalate and their concentration was declined gradually with the maturity stage of the plant. Moreover, the amount of methane produced was directly related to the amount of oxalate degradation and this is why high amount of methane production was observed in animal fed with young Napier grass because Napier grass at early maturity stage contained high levels of oxalate (Dawson *et al*., 1980). In the present study, the use of Napier grass to feed the animals was considered at maturity stage and this is why; low proportion of methanogen has been observed in T1 and T2.

The population of rumen protozoa was affected by dietary treatments in the current experiment. The highest protozoa population was observed in T3 diets compared to other dietary treatments. Carberry *et al.* (2012) reported that the number of protozoa population was decreased in cows fed with 100% corn silage compared with cows fed the 0% corn silage and 50% corn silage diets. Dennis *et al.* (1983) reported that the number of rumen protozoa population was increased when the level of starch was incorporated into the diets. However, the rumen protozoa engulf the starch particle and attacked amylolytic bacteria, thus regulates the rate of starch fermentation in the rumen (Carberry *et al*., 2012). In the present study, low protozoa population detected in whole corn plant silage diets might be due to a toxic effect of high level propionic acid in the diets (Lettat, 2011). This study is agreement with Carberry *et al.* (2012) who reported that an increase of corn silage proportion in the diet (i.e., increasing starch supply) has reduced the cellulolytic bacteria population but increased the number of total bacteria and amylolytic bacteria species which favoured the production of propionic acid. Similarly, Brown *et al.* (2006) also stated that, low number of protozoa population in high starch level diets would be expected to enhance the growth of amylolytic bacteria and rapid production of organic acid. Feeding 100% of whole corn plant silage diet in the present study has decreased the number of protozoa, which is consistent with the reduction of methanogenic archaea population. The rumen protozoa are important hydrogen producers and reduction of these microbes, affects the transfer of hydrogen between the protozoa and the methanogens (Martin *et al*., 2010; Morgavi *et al*., 2012).

CONCLUSION

The inclusion of whole corn plant silage in the basal diet of Napier grass of goat diets showed a high molar proportion of propionic acid and low proportion of acetic acid, although the value of

ruminal pH, the concentration of ammonia-N and total VFA concentration were not significantly different. The highest concentration of minor VFA was observed in high inclusion level of whole corn plant silage diets. Although the number of the total bacteria population was not affected by dietary treatments, low number of cellulolytic bacteria population was detected in T5. However, lowest populations of methanogen and protozoa were observed in goats fed with T5 diets compared to those of other dietary treatments. Based on the information observed in the current study, it can be concluded that 100% whole corn plant silage can be used as a sole diet without compromising the production of VFA and the total bacterial population, whereas the highest proportion of propionic acid and lowest number of methanogen and protozoa population in the rumen.

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