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SHORT COMMUNICATION

Rumen fermentation and diet degradability in sheep fed sugarcane (*Saccharum officinarum*) silage supplemented with *Tithonia diversifolia* or alfalfa (*Medicago sativa*) and rice polishing

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ABSTRACT. The objective of this study was to evaluate ruminal fermentation (i.e. pH, ammonia, and volatile fatty acid concentrations) and *in situ* degradability of diets in sheep fed sugarcane silage (SCS) supplemented with *Tithonia diversifolia* (Td) or alfalfa (*Medicago sativa*) hay (A), with or without rice (*Oriza sativa*) polishing (RP) as an energy source. Four Blackbelly sheep (35 kg average body weight) with rumen cannula were used. The experimental diets were (g/kg of dry matter): Diet 1) SCS (686) + Td (294), Diet 2) SCS (460) + Td (226) + RP (294), Diet 3) SCS (637) + A (343), and Diet 4) SCS (441) + A (245) + RP (294). The remainder (20 g/kg of dry matter) was composed by minerals supplement and salt. Samples of diets were incubated into rumen for 3, 6, 9, 12, 24, 30, 36, 48, and 72 h to determine *in situ* degradability. Data were analysed with a linear mixed model. The lamb, period and lamb nested in period*diet were considered as a random variable. The inclusion of RP improved the degradability of diets and ammonia production in the rumen. The acetic, propionic, and butyric acid concentrations (mmol/100 mL) in rumen increased (P<0.03) when diets with alfalfa and RP were provided. The degradability of diets based on sugarcane silage supplemented with either alfalfa or *Tithonia diversifolia* was improved (P<0.05) with the rice polishing inclusion, with no difference (P>0.05) between these forages. In conclusion, energy supplementation, not necessarily from starch, is important to improve rumen fermentation and degradability of diets based on sugarcane silage.

Key words: ammonia, energy, forage, protein.

INTRODUCTION

Sugarcane (*Saccharum officinarum*) is an important forage in the tropics because of its great biomass and energy production and low cost per ton of dry matter. Moreover, its harvest coincides with the dry season, and its conservation by ensiling could be an important contribution towards improving animal production in tropical regions (Santos da Silva *et al* 2014). Sugarcane silage could be an alternative forage for use at times when the quality and growth of grasses are poor (Montañez-Valdez *et al* 2013). Sugarcane silage contains a high concentration of soluble carbohydrates (Pedroso *et al* 2005, Sousa *et al* 2017), but low protein quality and low digestibility of fibre. Digestibility and intake of forage poor in nitrogen (N) can be improved by supplementation with *Tithonia diversifolia* (Td) (Wambui *et al* 2006[a]), which is a shrub native to Mexico and widely distributed in the humid tropics and sub-humid areas of Central and South America (Jama *et al* 2000). This shrub has been shown to undergo significant leaf production (Wuambui *et al* 2006[b]) and vigorous regrowth after cutting (Ramírez-Rivera *et al* 2010) and has elevated crude protein content (~20%) as well (Kayuki and Wortmann 2001, Wuambui *et al* 2006[a]). The soluble protein content of its foliage accounts for 40% of its total protein content (Ramirez-Rivera *et al* 2010), and the potential utilisation of this soluble protein by cattle can be increased by the soluble carbohydrates of sugarcane silage.

The synchrony of rumen degradable protein (RDP) and fermentable metabolisable energy (FME) concentration in animal diets influence microbial protein yield and animal productivity (López-Soto *et al* 2014). Therefore, the importance of using by-products that increase the energy available to the silages-based diets enriched with high-protein forages is relevant. Rice polish is a by-product of the rice milling industry and is derived from the outer layers of the rice caryopsis during milling. It consists of pericarp, seed coat, nucleus, aleurone layer, germ and part of the sub-aleurone layer of starchy endosperm (Juliano 1988). For the above, rice polish contains similar metabolisable energy compared with that of grains such as corn or sorghum (NRC 2007, Hossain *et al* 2012), and have a lower price than those grains; thus, rice polish may be used as an energy supplement in diets based on silages combined with high-protein forages. However, the energy of this by-product comes from a different source compared with other by-products (i.e. molasses) because of its elevated protein and lipid content (NRC 2007, Salinas-Chavira *et al* 2008).

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To our knowledge, there are no published studies that directly assess the rice polishing as an energy source of silage-based diets enriched with high protein forage sources. Hence, the aim of the study was to determine if diets based on sugarcane silage supplemented with *Tithonia diversifolia* (Td) or *Medicago sativa* (alfalfa, A), as a source of protein, and with rice polishing (RP) as a source of fat-derived energy, could improve the ruminal degradation and fermentation. Hypothetically rice polishing used as an energy supplement in diets based on sugar cane silage combined with high-protein forages would improve rumen fermentation and degradability because of complementation with rumen degradable protein and fermentable metabolisable energy of these diets.

MATERIAL AND METHODS

Experimental trials were conducted at the Universidad Autónoma de Nayarit (UAN) in Compostela, Nayarit, Mexico (21°17'46'' N, 104°54'00'' W, and 880 masl) where the predominant climatic conditions correspond to sub-humid tropical type, with a mean temperature of 22 °C, rainfall mainly in the summer, and total annual precipitation of 1,000 mm.

Animal management procedures were conducted following the guidelines officially-approved, NOM (*Norma Oficial Mexicana*), for animal use and care (NOM-051-ZOO-1995: humanitarian care of animals during mobilisation of animals; NOM-062-ZOO-1999: technical specifications for the care and use of laboratory animals).

ANIMALS AND TREATMENTS

Four uncastrated Blackbelly rams (35 ± 1.2 kg body weight, BW) fitted with cannulas in the rumen were used in a 4 × 4 Latin square design with 21-d periods for adaptation and sampling. The animals were housed in individual pens of 60 × 180 cm with steel mesh flooring and individual feeders and drinkers. The sheep were treated for internal and external parasites with ivermectin (200 µg/kg BW; SC).

Four experimental diets were formulated to meet the nutritional requirements for growing sheep (147 g/kg CP and 3.2 Mcal/kg DE) according to the NRC (1985) and were randomly assigned to each animal. The diets were isocaloric and isoproteic, and were based on sugarcane silage supplemented with either *Tithonia diversifolia* or alfalfa, with or without rice polishing. The *Tithonia diversifolia* forage was harvested at the UAN 60 d after the last cut, chopped to obtain 2-3 cm particle size and sun dried for 72 h and turned every 24 h. Alfalfa hay and rice polishing were purchased at a commercial establishment. Sugarcane silage was prepared using the entire plant (harvested 24 m after sowing), chopped up into 3-5 cm pieces. An inoculum prepared with sugarcane molasses (10%), urea (0.5%), chicken manure (5%), yoghurt (1%), and water (83.5%) was added to the fresh forage at a level of 3% (Reyes-Gutierrez *et al* 2012). Also, urea (1.0%), ammonium sulfate (0.1%), and diammonium phosphate (0.25%) were added by spraying it on each layer of sugarcane during ensiling. The forage was compacted with 4 passes of the Massey Ferguson tractor of 3.5 tons of weight. Once compacted, the forage was covered with plastic and a layer of 10-15 cm of dust on top until silage was used three months later.

The experiment lasted 84 d divided into four experimental periods of 21 d each, consisting of first 14 d for diet adaptation, followed by 7 d for rumen fluid sample collection and determination of the kinetics of disappearance of dry matter. During the adaptation period, the feed was offered in amounts 110% of that consumed the previous day, so that sheep had *ad libitum* access to feed. During the sample collection period, sheep received 90% of the amount of feed previously consumed *ad libitum* to avoid refusals. Each animal received a different experimental diet in each period. On the fifteenth day of each period, rumen content samples (approximately 100 mL) was obtained from each lamb at 0, 3, 6, 9, and 12 h after feeding via the ruminal cannula. Ruminal samples were manually taken from different sites of the rumen. Ruminal content pH was immediately determined on fresh samples with a portable pH meter (Horiba, Ltd.). Following pH determination, a sample of rumen fluid was obtained through filtering rumen content sample with four layers of cheesecloth. Two mL of freshly prepared 25% (w/v) meta-phosphoric acid was added to 8 mL of strained ruminal fluid. Samples were centrifuged (17,000 × g for 10 min) and the supernatant fluid was stored at –20 °C for VFA analysis (Erwin *et al* 1969). For ammonia nitrogen (NH_3-N) analysis, 10 mL of strained ruminal fluid was acidified with 0.5 mL of 6 N HCl and was stored at –20 °C for N-NH_3 determination (Godeau *et al* 1987).

On the 18th day of each period, the ruminal degradation of diets was determined using nylon bags (Diammod bar®) with rounded corners. They were 10 × 15 cm in size with a pore size of 50-µm. Diet samples were oven-dried at 60 °C for 48 h, and were ground to pass through a 2 mm screen (Wiley® mill). Five grams of diet sample were placed in each bag. Two bags per diet, animal and time were prepared. Bags with sample were placed into the rumen tying them to a chain with a polyester cord at 3, 6, 9, 12, 24, 30, 36, 48, 56, and 72 h post-feeding. After retrieval, of all bags at the same time were manually rinsed under running water until the wash water was clear, dried in a forced-air oven at 65°C for 48 h and then weighed. The same procedure was applied to bags without rumen incubation (0 h). Rumen dry matter (DM) disappearance was estimated using a model of first-order kinetics proposed by Waldo *et al* (1972). The NEWWAY software (Rowett Research Institute) was used to fit data obtained from the DM degradation estimates to the equation $Y = a + b (1 – e^{ct})$ for kinetic degradation (Orskov and McDonald, 1979), where Y is the DM degradation at time t, a is the

soluble fraction, b is the potentially degradable insoluble fraction, c is the rate of degradation, and t is the time.

STATISTICAL ANALYSIS

Experimental data (ruminal pH, NH_3-N, VFA and pH) were analyzed using a linear mixed model for repeated measurements (SAS 2007, version 9.1). The sheep, period and the interaction sheep*period*diet was considered as a random variable. The hour of sampling was considered as a repeated measurement. The model adjusted was as described below.

$$Y_{ijkl} = \mu + D_i + T_j + (D*T)_k + (D*T2)_l + (D*T3)_m + (S/D*T)_n + \varepsilon_{ijklmn}$$

Where μ is the overall mean, Dk is the effect of the ith diet, Tj is the effect of the lth time after feeding (0, 3, 6, 9 and 12 h), $(D*T)_k$ is the effect of the kth interaction, $(D*T2)_l$ is the effect of the lth interaction of diet and quadratic time, $(D*T3)_m$ is the effect of the mth interaction of diet and cubic time, $(S/D*T)_n$ is the random effect of the animal nested in diet * period of sampling, and εijklmn is the residual term. Time after feeding was considered as a repeated measurement with an autoregressive covariance structure. When a significant difference was detected, treatment means were compared with the Tukey test (P<0.05).

RESULTS AND DISCUSSION

The soluble fraction was similar (P>0.05) among the experimental diets. The potential of degradation and the percentage of insoluble but potentially degradable fraction was increased (P<0.05) by the inclusion of rice polishing, with no difference (P>0.05) between the diet with *Tithonia diversifolia* and alfalfa (table 1). The potential of degradation (a + b) of diets without rice polishing in this study was around those observed (57.2%) by Montañez-Valdez *et al* (2013) for ensiled sugarcane without supplementation, but were up to 74% greater with the addition of rice polishing. This finding can be explained by the greater insoluble but degradable fraction of diets with rice polishing. Although the *in situ* degradation of diets has been shown to be improved with *Tithonia diversifolia* inclusion (Premaratne *et al* 1997, Naranjo and Cuartas 2011), according to these results the benefits of protein provided by forages, such as those used in the present study, can be useful if energy is available from ingredients such as rice polishing.

The ruminal pH values declined (P<0.05) at 3 h after feeding with all diets. In addition, differences were detected at 6 and 9 h in which values with Diet 4 were lower (P<0.05) compared to Diet 1. The pH decline at 3 h post-feeding on all experimental diets reflects the time at which fermentation occurred (figure 1). The highest pH values observed for the diet with *Tithonia diversifolia* without rice polishing may be explained its NDF content (53%), which implies slower digestion. The average pH range (6.14-6.88) of the rumen observed on all experimental diets was within the normal range (6-7) for microbial fibre digestion and corresponds to that observed for forage-based diets with high percentages of cellulose and hemicellulose (Krause *et al* 2002).

Ruminal NH_3-N concentration increased (P<0.05) at 3 h with Diet 1 and Diet 4, and decreased (P<0.05) at 9 h compared to 3 h post-feeding with rice polishing addition (figure 2). Therefore, the rice polishing addition increased the production and absorption of NH_3-N. Vega *et al* (2019) observed greater intake, retention and absorption of N with rice polishing addition. The NH_3-N increase after 3 h post-feeding agrees with a study by Nolan (1993). After the peak, however, NH_3-N concentration decreased with all diets, which is similar to findings of Benedeti *et al* (2014), who observed lower concentrations of NH_3-N 10 h after feeding. However, the concentration decline was quite pronounced in diets with rice polishing, perhaps because the higher energy availability of these diets led to NH_3-N

Table 1. *In situ* kinetics of dry matter digestibility (± SEM) of the experimental diets.

Item	RP, %/h †	Diet 1	Diet 2	Diet 3	Diet 4
a ¶		20.7±4.2	26.4±2	24.9±6.4	20.2±8.1
b ¶¶		31.5±3.3^b	52.5±4.7^a	35.6±6.6^b	54.1±4.1^a
c §		0.06±0.03^a	0.05±0.01^a	0.032±0.02^b	0.05±0.01^b
Zero time, %		12.4±6.4	16.95±4.8	13.9±7.3	10.1±7.3
PD, % Þ		52.10±4.5^b	78.9±3.8^a	60.5±8.1^b	74.3±8.03^a
ED, % ÞÞ	2	44.4±5.2^b	70.2±2.3^a	45.6±1.5^b	68.8±7.8^a
ED, % ÞÞ	5	38.2±3.1^b	58.1±1.4^a	37.4±0.7^b	57.2±7.8^a
ED, % ÞÞ	8	34.4±3.1^b	51.5±1.03^a	34.2±1.1^b	47.2±7.8^a

^{a,b}Means with different letter in a row are statistically different (Tukey, P<0.05), SEM: standard error of the mean, Diet 1: sugar cane silage + *Tithonia diversifolia*, Diet 2: sugar cane silage + *Tithonia diversifolia* + rice polishing, Diet 3: sugar cane silage + alfalfa, Diet 4: sugar cane silage + alfalfa + rice polishing. † Rate of passage. ¶ Soluble fraction (%). ¶¶ Potentially degradable insoluble fraction (%). §Rate of degradation (h^{-1}). ÞPotential degradability. ÞÞ Effective degradability.

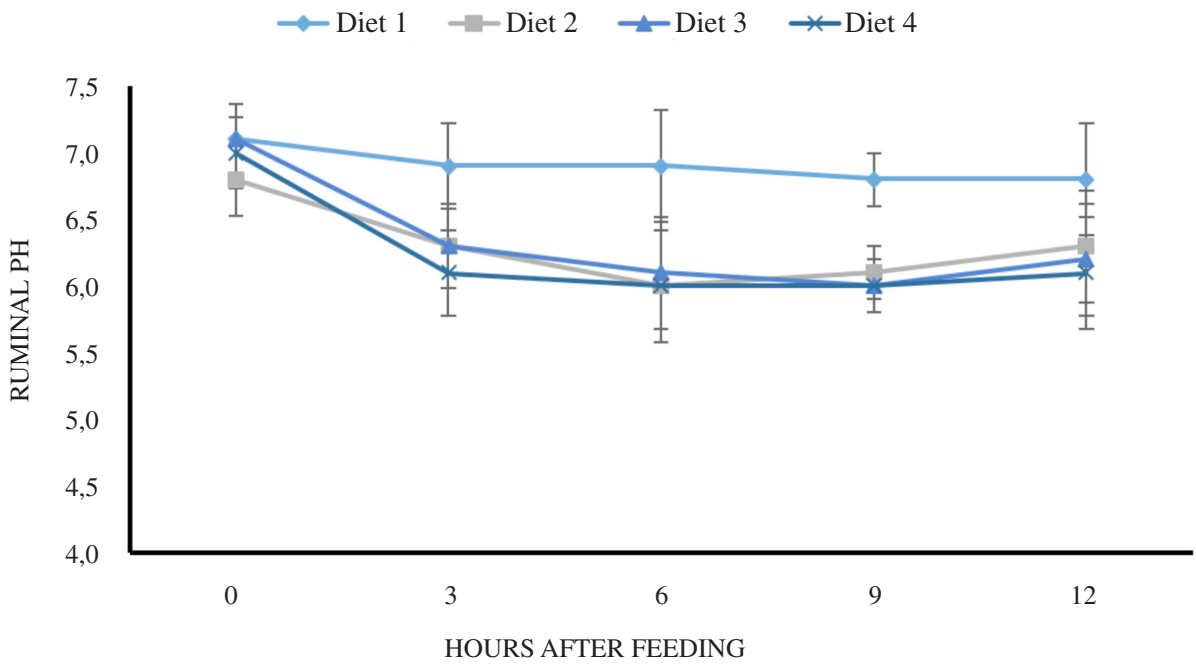

Figure 1. Ruminal pH of animals fed with sugarcane silage supplemented with *Tithonia diversifolia* or alfalfa, with or without rice polishing at different times (hours after feeding). Diet 1: sugar cane silage + *Tithonia diversifolia*, Diet 2: sugar cane silage + *Tithonia diversifolia*+rice polishing, Diet 3: sugar cane silage + alfalfa, Diet 4: sugar cane silage + alfalfa + rice polishing.

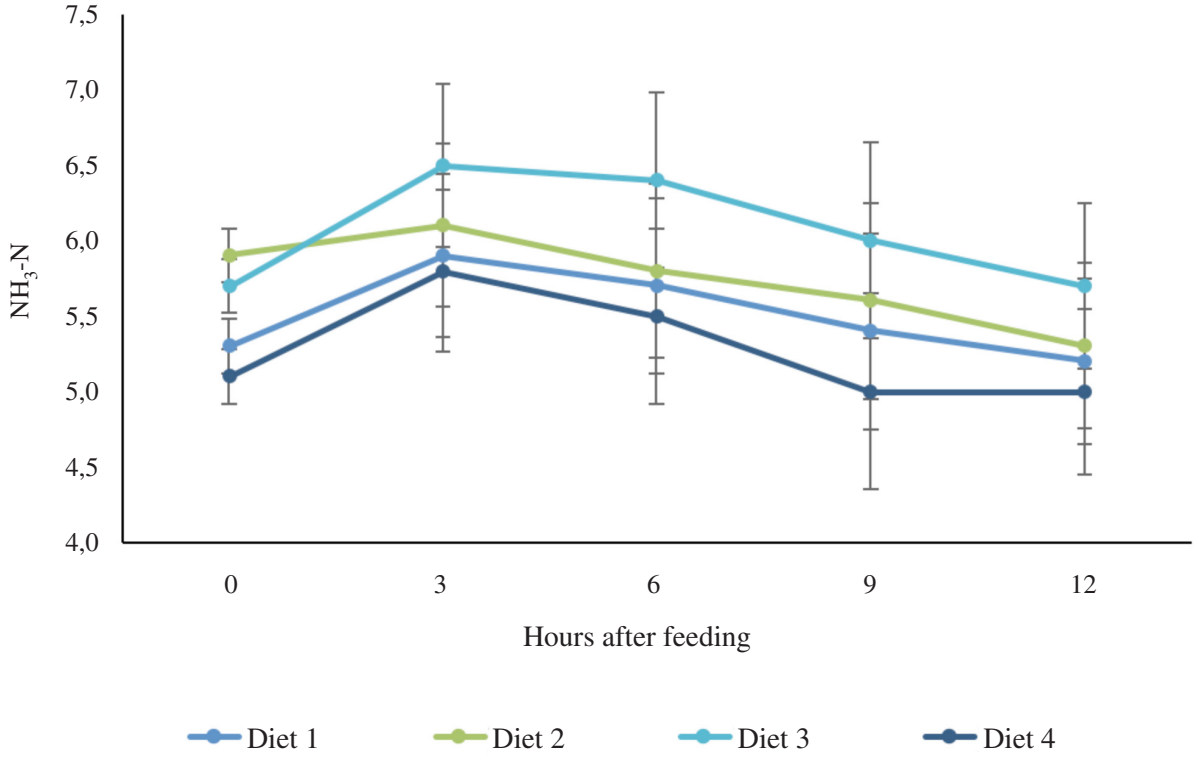

Figure 2. Ammonia-N (mg/100 mL) concentrations in the rumen of animals fed with sugarcane silage supplemented with *Tithonia diversifolia* or alfalfa, with or without rice polishing at different times (hours after feeding). Diet 1: sugar cane silage + *Tithonia diversifolia*, Diet 2: sugar cane silage + *Tithonia diversifolia* + rice polishing, Diet 3: sugar cane silage + alfalfa, Diet 4: sugar cane silage + alfalfa + rice polishing.

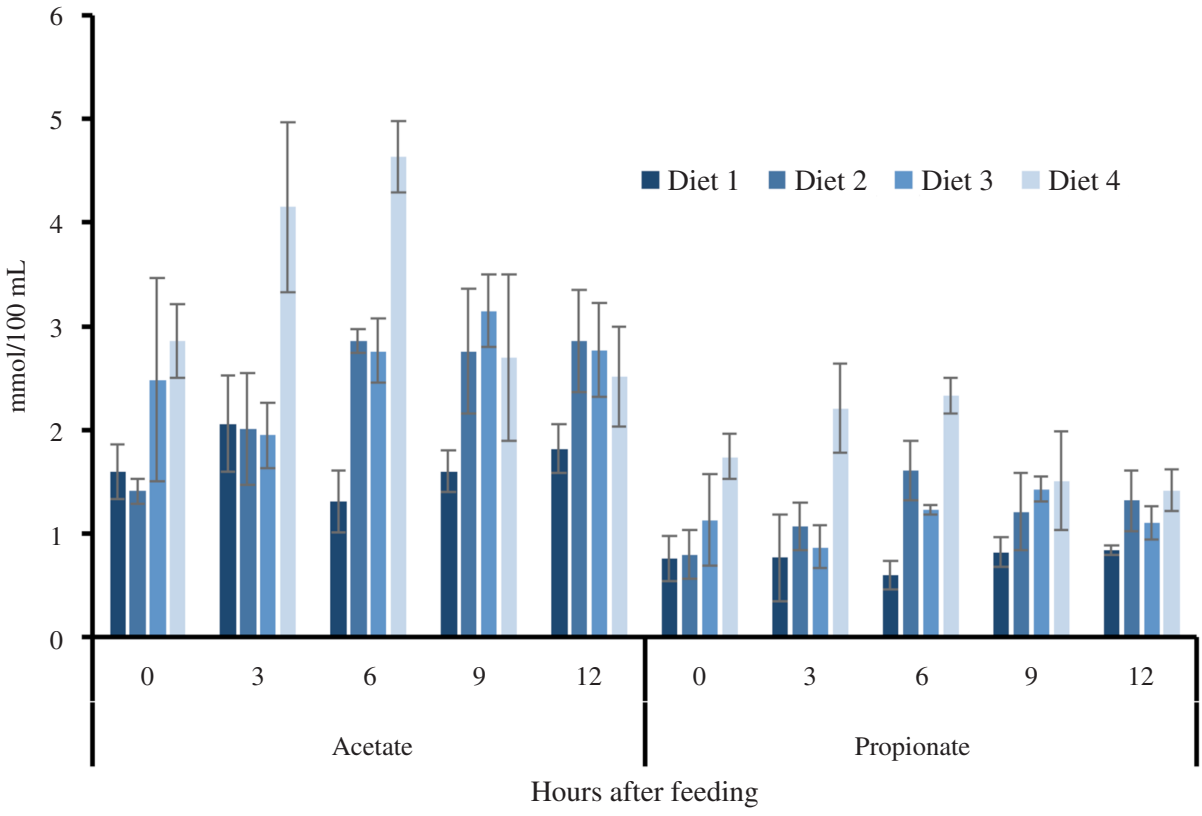

Figure 3. Acetate and propionate (mmol/100 mL) concentrations of animals (n = 4) fed with sugarcane silage supplemented with *Tithonia diversifolia* or alfalfa, with or without rice polishing at different times (hours after feeding). Diet 1: sugar cane silage + *Tithonia diversifolia*, Diet 2: sugar cane silage + *Tithonia diversifolia* + rice polishing, Diet 3: sugar cane silage + alfalfa, Diet 4: sugar cane silage + alfalfa + rice polishing.

assimilation by rumen microorganisms (Benedeti *et al* 2014). Similarly, Bailey *et al* (2012) observed an increase in ruminal NH_3-N between 2 and 10 h after high levels of casein supplementation to steers consuming low-quality forage (5.8% CP and 71% NDF), but not in those steers with lower levels of casein and glucose supplementation.

Rice polishing and alfalfa (Diet 4) addition increased (P<0.05) ruminal concentration of acetate, propionate, butyrate and valerate and decreased isovalerate at 3 and 12 h post-feeding, respectively. Inclusion of rice polishing in diets increased (P<0.05) the concentration of acetate, propionate, butyrate, and isovalerate between 3 and 9 h post-feeding.

Fibrous carbohydrates in diets elevate acetate production (Lins *et al* 2016), but this requires elevated energy and protein as well. Starch content of diets enhances the development of propionate-producing bacteria and, consequently, propionate production at the expense of acetate (France and Siddons 1993). Bailey *et al* (2012) observed greater ruminal acetate concentration with casein and protein supplementation. The lowest concentration of isovaleric acid at 12 h (figure 4) observed in this study with the diet of alfalfa supplemented with rice polishing may have been due to the utilisation of this acid by rumen bacteria to improve fermentation, which was reflected in the greatest concentration of acetate at this point.

Protein may be important as a source of VFA when diets are rich in rumen-degradable protein (France and Siddons 1993). Protein fermentation could generate high amounts of branched-chain fatty acids (Dijkstra 1994). France and Siddons (1993) showed that increases in valerate, isovalerate, and isobutyrate stimulated cellulose digestion. VFA concentration is positively related to ruminal NH_3-N (Wanapat and Pimpa 1999). Adequate NH_3-N concentration for ruminal microorganism growth with no effect on VFA production may indicate energy deficiency or asynchrony of the availability of N and energy, or both.

In conclusion, rumen fermentation and degradability of sugarcane silage-diets with *Tithonia diversifolia* or alfalfa were enhanced by the inclusion of rice polishing. Rice polishing improved the utilisation of the NH_3-N. Therefore, energy, not exclusively from starch, is important to take advantage of the forage protein to improve rumen fermentation of diets based on sugarcane silage.

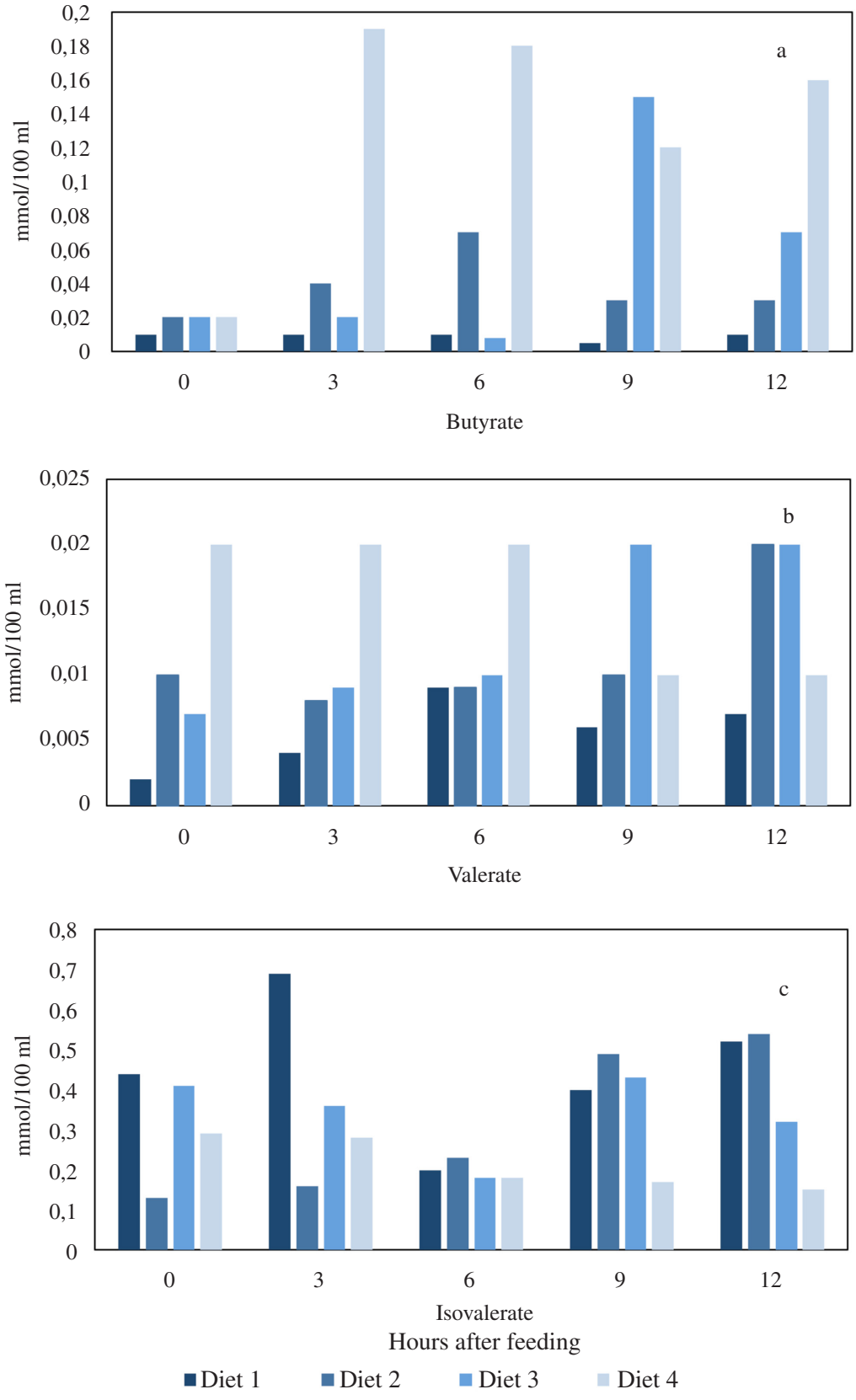

Figure 4. Butyric (a), valeric (b) and isovaleric (c) acids (mmol/100 mL) concentrations in sheep (n = 4) fed with sugarcane silage supplemented with *Tithonia diversifolia* or alfalfa, with or without rice polishing at different times (hours after feeding). Diet 1: sugar cane silage + *Tithonia diversifolia*, Diet 2: Sugar cane silage + *Tithonia diversifolia* + rice polishing, Diet 3: Sugar cane silage + alfalfa, Diet 4: Sugar cane silage + alfalfa + rice polishing.

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