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Research Article

In vitro Characteristics of Rumen Fermentation of Fattening Rations with Different Protein-energy Levels Fed to Bali Cattle

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Abstract

Objective: This study investigated *in vitro* characteristics of fermentation of fattening rations with different protein-energy levels fed to male Bali cattle. **Methodology:** Rations were composed of grass, *Gliricidia sepium*, corn meal and rice bran with different proportions of protein and energy. Ration T_1 was standard protein-standard energy/SS [(12.06% Crude Protein (CP) and 62.66% Total Digestible Nutrients (TDN)], T_2 was standard protein-high energy/SH (10.14% CP and 65.66% TDN), T_3 was high protein-standard energy/HS (14.79% CP and 63.66% TDN) and T_4 was high protein-high energy/HH (13% CP and 67.48% TDN). Data were analyzed by one-way analysis of variance. **Results:** Although, pH level of rumen fluid was similar for all treatments ($p>0.05$), digestibility of dry matter and organic matter in rations T_4 and T_3 was higher ($p<0.01$) than that for T_1 and T_2. For N-NH_3 (mg/100 mL), the yield of T_4 and T_3 was higher ($p<0.01$) than that for T_1 and T_2. Meanwhile, total VFA, acetic and propionic acids in T_2, T_3 and T_4 were higher ($p<0.01$) than for T_1 but butyric acid levels for T_4 were higher ($p<0.01$) than that for T_1, T_2 and T_3 and the acetic: propionic acid ratio of T_4 was lower ($p<0.01$) than that for T_1, T_2 and T_3. Microbial protein synthesis (mg mL^{-1}) for the T_1 ration was higher ($p<0.01$) than that of T_2, T_3 and T_4. **Conclusion:** Varying the protein and energy levels of rations fed to male Bali cattle did not affect rumen pH but the digestibility of dry matter and organic matter was unclear. Moreover, N-NH_3 utilization and VFA yield were not optimal for protein biosynthesis by microbes.

Key words: Rumen fermentation, fattening, Bali cattle, protein-energy, *in vitro*, smallholder beef cattle farming

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Protein and energy are two essential feed components that are needed to improve cattle productivity because feeds with high protein and high energy contents can accelerate fermentation rates and feed digestibility to increase nutrient intake by cattle. Low-quality feed, particularly feeds with low dry matter and organic matter digestibility, negatively affects feed consumption and metabolism in rumens, in turn can lead to inadequate protein and energy supplies for cattle. Feeds with higher digestibility are preferable given that some protein sources are positively correlated with degradation levels in the rumen[1]. In addition, feeds must also provide an adequate supply of nitrogen (ammonia) to support microorganism growth in the rumen. Cattle given high protein feed can metabolize the feed to produce high nitrogen-ammonia ($N\text{-}NH_3$) yields which is important for protein microbe synthesis when adequate digestible carbohydrates from Volatile Fatty Acids (VFA) are available. Inadequate energy causes $N\text{-}NH_3$ imbalances in cattle and in turn discharge of this compound from the body. It is therefore essential to achieve a balance between energy levels and protein digestibility in animal feeds.

In cattle rumens, microbial proteins contribute upto 40-80% of amino acids available for protein synthesis[2]. Accordingly, cattle production can be positively affected by improving protein microbe yield[3] through approaches such as modulating the availability of nitrogen precursors and energy derived from fermentation[4].

On smallholder farms with beef cattle livestock, feeds with adequate energy and protein levels are not always available to maintain maximum cattle performance throughout the year. Farmers often provide forage as the sole feed in fattening rations which has been shown to be ineffective in improving Bali cattle production. The energy value of a feed is primarily determined by the total proportion of Dry Matter (DM) intake or digestible energy and forage flow in the digestive tract. Thus, forage with higher digestibility provides more energy for cattle per unit DM consumed. Tahuk *et al.*[5] reported that male Bali cattle in a West Timor, East Nusa Tenggara feedlot consumed 7.079 kg $head^{-1}$ day^{-1} DM fattening forage to yield 0.321 kg $head^{-1}$ day^{-1} daily weight gain which translates to a 23.664 feed conversion ratio and 4.619% feed efficiency. This low performance likely correlates with low digestibility and energy levels in forage indicating that field legume proteins could not optimize cattle performance. Moreover, Buxton and Brasche[6] stated that forage as an energy supply for cattle is frequently a primary obstacle to herbivore productivity.

Considering the aforementioned circumstances, local feed with various protein and energy levels used by smallholder farms producing beef cattle is one alternative for resolving nutrient imbalances. *Gliricidia sepium* and natural grasses can be used as protein and structural carbohydrate sources, respectively. Furthermore, supplementation of this forage with highly digestible carbohydrates such as corn meal and rice bran would be expected to improve nutrient adequacy and balance for cattle, particularly by increasing energy levels that are known to increase animal growth, meat production and carcass weight[7].

This study examined male Bali cattle fattening rations with different protein and energy levels by taking *in vitro* measurements of rumen fermentation parameters such as pH, dry matter, organic matter digestibility, $N\text{-}NH_3$ yield, VFA yield and protein synthesis by microbes.

MATERIALS AND METHODS

Study location: *In vitro* study was conducted for 30 days at the laboratory of Animal Feed Technology, Department of Nutrition and Animal Feed, Faculty of Animal Science, Gadjah Mada University, Indonesia. The $N\text{-}NH_3$ analysis and microbe protein synthesis measurements were carried out at the Nutrition Biochemical laboratory, Animal Science Faculty, Gadjah Mada University, Indonesia and VFA analysis was conducted at the Biochemical Chemistry laboratory, Food and Nutrition Centre, Gadjah Mada University, Indonesia.

Research design and feed rations: Research materials were forage commonly used by local farmers, comprising natural grass and *Gliricidia sepium* leaves with additional corn meal and rice bran in the rations. Research equipment included laboratory apparatus for feed proximate analysis and Van Soest analysis Acid Detergent Fiber (ADF) and Neutral Detergent Fiber (NDF) fractions, analysis of VFA, $N\text{-}NH_3$ and protein synthesis by microbes) and a pH meter to measure ruminal fluid pH. Materials for *in vitro* analysis were fistular ruminal liquid from male Bali cattle, 39 °C McDougall solution pH 6.55-6.99, fresh 39°C ruminal fluid, 0.2% pepsin HCl, distilled water, saturated $HgCl_2$, saturated $NaCO_3$ solution, H_2SO_4 (0.005 N solution and boric acid with indicator), 5 N HCl solution, 15% H_2SO_4, 0.5 N NaOH and 0.1% phenolphthalein (PP) indicator solution.

Rations consisted of four materials: Natural Grass (NG), *Gliricidia sepium* (G), Corn Meal (CM) and Rice Bran (RB). Protein and energy levels of the rations were either those specified by standard cattle provisions or were varied to observe the effects on various parameters *in vitro*. The first

ration treatment (T_1) was prepared according to Kearl[8] for 200 kg male cattle with 0.75 kg Average Daily Gain (ADG) and contained standard protein-standard energy (SS) consisting of 5.6 kg DM, 3.7 kg (59.26%) Total Digestible Nutrients (TDN) and 577 g (11.56%) crude protein. The experimental rations were as follows; (T_2) standard protein-high energy (SH) with 12% crude protein and 70% TDN, (T_3) high protein-standard energy (HS) with 15% crude protein and 60% TDN and (T_4) high protein-high energy (HH) with 15% crude protein and 70% TDN. The chemical composition of the rations after proximate analysis changed slightly in protein and TDN proportion, wherein T_1 was 12.06 and 62.66%, T_2 was 10.14 and 65.66%, T_3 was 14.79 and 63.66% and T_4 was 13.04 and 67.48%, respectively. This post proximate analysis composition was subjected to *in vitro* evaluation.

Variables, research method and data collection: The variables analyzed in this study included ruminal fluid pH, digestibility (dry matter and organic matter), Volatile Fatty Acid (VFA) yield (including acetic, propionic and butyric acids), N-NH_3 yield and microbial protein synthesis.

To measure the initial pH of ruminal fluid, samples collected from male Bali cattle fistules were filtered through a three-layered cloth to avoid contamination with feed residue and then stored in a thermos warmed to approximately 37°C. The collected rumen fluid was transported to the laboratory, prepared and incubated in a water bath according to the method described by Tilley and Terry[9]. Data collection complied with the following procedures:

- Ruminal fluid pH was measured with a Hanna pH meter. Measurements were taken soon after collection in order to record the initial pH during the first 48 h and to monitor pH changes
- Feed digestibility (dry matter, organic matter and protein) was measured using the method described by Tilley and Terry[9]
- Volatile Fatty Acid (VFA) yield including acetic, propionic and butyric acid was determined by gas chromatography using the method described by Filipek and Dvorak[10]. Briefly, a Shimadzu GC8 (standard 1 μL) gas chromatograph equipped with a Flame Ionisation Detector (FID, detector temperature 240°C) and a 3 m GP 10% SP 1200/10% H_3P0_4 column running at 140°C was used for gas chromatography. The carrying gas was N_2 (nitrogen), the gas and hydrogen pressures were 1.8 and 0.8 kg cm^{-3}, respectively

Measurement procedure: Ruminal fluid taken with a stomach tube was centrifuged at 3,000 rpm for 25 min. Aliquots (0.2 mL) of the supernatant were stored in sealed Eppendorf tubes before 1 mL, 25% metaphosphoric acid was added. The mixture was centrifuged at 15,000 rpm for 15 min and 1 μL of the supernatant was injected for gas chromatography; a standard VFA solution was injected prior to injecting the sample:

- The N-NH3 yield was analyzed by spectrophotometry according to the method of Chaney and Marbach[11]. In brief, solution A (1 mL, Tungstat) with 2 mL ruminal liquid was mixed and combined with 1 mL cold solution B. This sample was frozen for ≤48 h before analysis. The samples were then centrifuged at 15,000 g for 10 min. Solutions C and D (2.5 mL) were then added to 20 mL supernatant and incubated in a 40°C water bath for 30 min until the solution turned blue. Samples were cooled and the absorbance values were determined at 630 nm
- Microbial protein synthesis in the rumen was measured spectrophotometrically based on the method by Lowry *et al.*[12]. Samples were first centrifuged at 3,000 g for 15 min and 1.5 mL of the resulting supernatant was further centrifuged at 10,000 g for 15 min. The supernatant was then removed and 0.5 mL distilled water was added to the precipitate. The mixture was centrifuged at 10,000 g for 15 min. The supernatant was removed, 1 mL distilled water was added and diluted as necessary. To determine protein content, 1 mL of the sample solution was mixed with 5 mL Lowry B reagent and incubated at room temperature for 10 min before 0.5 mL Lowry solution A was added. The solution was incubated for 30 min and the absorbance at 750 nm was determined

Statistical analysis: The data were collected using a one-way completely randomized design with four treatments and six replicates. The data were analyzed using SPSS software version 19 and one-way analysis of variance. When differences among treatments were suspected, the Duncan's Multiple Range Test (DMRT) was carried out[13].

RESULTS AND DISCUSSION

Acidity level: The acidity level (pH) of the ruminal liquid from animals given the four different protein-energy level rations did not differ significantly. Overall, the ruminal liquid pH was

relatively low due to the utilization of 19, 65, 2 and 43% of total digestible carbohydrate feed contained in T_1, T_2, T_3 and T_4, respectively. Although, the use of higher amounts of easily digestible carbohydrate (concentrate) would be expected to lower the rumen pH, there was non-significant decrease in pH among the four ration treatments because the forage used still contained high fiber which would neutralize decreases in pH. Even as the dry matter and organic matter digestibility increased, the rumen pH remained within the optimum range for bacterial growth and activity as well as for maximal cellulose digestion (6.4-6.8). Indeed, Erdman[14] stated that decreases in rumen pH in cattle could result from an acid-base imbalance and restricted metabolism rather than feed content.

Dry matter and organic matter digestibility: Dry matter and organic matter digestibility (%) of rations fed to beef cattle in this study was relatively high (~65.06-74.67%) and the digestibility of T_2 and T_4 which had higher TDN values was higher ($p<0.01$) than that of T_1 and T_3. The increase in Dry Matter Digestibility (DMD) and Organic Matter Digestibility (OMD) in rations T_2 and T_4 showed that rations with different protein and energy levels derived from easily digestible carbohydrates provided optimum energy (ATP) for rumen microbes to degrade feed. Corn meal also played an essential role in DMD and OMD as well as providing an adequate energy source for rumen microbe growth and activity. McDonald *et al.*[15] reported that highly soluble carbohydrates (non-structural) such as glucose, fructose and starch were optimal for digestion and metabolism and could be best used by several bacterial species in the rumen.

Rations T_1 and T_3 contained easily digestible carbohydrate contents of 19 and 2%, respectively and yielded relatively low DMD and OMD that were correlated with energy availability and low digestibility that in turn reduces the ability of rumen microbes to degrade feed. Carbohydrates are a main energy source for bacteria[16] although, they can also be used as a carbon frame for protein synthesis combined with ammonia. This study found that increases in DMD and OMD showed a linear relationship with the amount of digestible carbohydrate present in the rations as an energy source (Table 1).

Nitrogen-ammonia ($N\text{-}NH_3$) yield: The Nitrogen-ammonia ($N\text{-}NH_3$) yield (mg/100 mL) in the T_1 and T_2 rations which had lower crude protein contents was lower than that for T_3 and T_4 ($p<0.01$). These differences in $N\text{-}NH_3$ levels could be attributed both to the ration type and degradation rate. As mentioned above, rumen pH was not expected to negatively affect $N\text{-}NH_3$ production as the pH values seen here were within the range for optimal microbial growth and development as well as for feed degradation needed for protein synthesis.

The $N\text{-}NH_3$ yield in cattle fed the T_1 ration was relatively low compared to the other ration treatments because the $N\text{-}NH_3$ arising from degradation of proteins in the feed was supported by adequate energy levels from digestible carbohydrate that could be used for protein microbe synthesis. Accordingly, nitrogen and carbohydrate degradation were synchronized and positively impacted rumen $N\text{-}NH_3$ utilization.

In contrast, for ration treatment T_2 (standard protein levels with high energy levels) the $N\text{-}NH_3$ yield tended to increase which indicated that the $N\text{-}NH_3$ yield was not optimized despite an adequate supply of easily digestible carbohydrates. A lack of nitrogen-energy feed synchronization due to different feed degradation rates may also have contributed to

Table 1: Composition of chemicals, DMD and OMD (%) of fattening rations with different protein-energy levels fed to Bali cattle

	Feed ingredients			
Item	Natural grass	*Gliricidia sepium*	Corn meal	Rice bran
Dry matter (%)*	18.09	19.01	90.10	90.42
Organic matter (%)*	89.44	88.82	98.81	84.49
Crude protein (%)*	11.13	21.75	7.89	6.97
Crude fiber (%)*	28.99	12.35	1.82	17.37
Extract ether (%)*	2.44	2.93	1.44	2.03
Ash (%)*	14.68	11.19	1.19	8.26
NFE (%)**	33.20	38.12	87.66	65.37
Energy (Cal g^{-1})***	3379.89	3745.89	3854.94	3739.63
TDN (%)****	50.04	73.13	83.75	50.62
Digestibility (%)*				
Dry matter digestibilty	65.64	67.87	95.05	42.10
Organic matter digestibilty	63.15	62.75	93.31	44.58

*Analysis results from the Animal Feed Laboratory Technology, Faculty of Animal Science, Gadjah Mada University (2015), **NFE: [100–(Ash%+CF%+EE%+CP%)], ***Results Analysis of Laboratory Chemistry and Biochemistry, Study Center Food and Nutrition, Gadjah Mada University (2015), ****According to the equation Harris *et al.*[17] quoted in Hartadi *et al.*[18]

this condition. Corn meal and rice bran are easily digestible carbohydrate sources that have a higher degradation rate than feeds where *Gliricidia sepium* leaves are used as the nitrogen source. As such, when carbohydrate digestion reached optimum levels, nitrogen degradation rates for $N\text{-}NH_3$ production remained sub-optimal. The higher crude protein level in the T_3 ration (14.79%) contributed to the high $N\text{-}NH_3$ concentration but the energy (TDN) was 63.66% and the amount of easily digestible carbohydrate was 2%. This composition led to faster protein degradation from the feed but did not provide adequate energy from digestible carbohydrates. Consequently, the $N\text{-}NH_3$ derived from nitrogen feed degradation was not optimal for microbial protein synthesis. However, the complete amino acid content in *Gliricidia sepium* could stimulate microbe growth, so the ammonia yield was higher in animals fed rations T_1 and T_2. According to Nitis[19], *Gliricidia Sepium* leaves are a source of Rumen Degradable Protein (RDP) where 60.73% of the protein contained in the leaves could be degraded in the rumen into $N\text{-}NH_3$ that could be used for bacterial growth. These bacteria serve as sources for high quality protein while also improving crude fiber digestibility. Therefore, the high yield of $N\text{-}NH_3$ in this study was likely defined by the ration type and feed composition. Rations with higher protein and low energy would result in higher $N\text{-}NH_3$ accumulation because minimal amounts are used for protein microbe synthesis.

Ration treatment T_4 (high protein-high energy) resulted in higher production of $N\text{-}NH_3$ that is likely due to the absence of synchronization between energy use and protein degradation. Thus, using a high proportion of easily digestible carbohydrate sources to balance high nitrogen levels was not effective. Corn meal as an energy source is more soluble than *Gliricidia sepium* leaves, the protein source in forage. When carbohydrate degradation was optimum, nitrogen degradation decreased such that the use of $N\text{-}NH_3$ for protein microbe synthesis was not optimized which resulted in an accumulation of $N\text{-}NH_3$ in the rumen. Devant *et al.*[20] reported that such decreases in protein degradation were not solely due to changes in pH but instead correlated with the type of fermented substrate or predominant microbe population that arose in response to particular feed ratios.

In general this study showed that variations in the $N\text{-}NH_3$ concentration among the four treatments largely arose from different types of feed and the degradation rate. Decreases in carbohydrate-nitrogen degradation synchronization to produce $N\text{-}NH_3$ was a contributing factor to the low degradibility of crude protein that resulted from lowered microbe activity in the rumen. Although, Satter and Slyter[21] showed that 50 mg L^{-1}, $N\text{-}NH_3$ was adequate to support maximum growth of rumen bacteria, appropriate levels of $N\text{-}NH_3$ for rumen bacteria levels could be as low as 20 mg L^{-1} while, higher levels led to an excessive margin. The $N\text{-}NH_3$ yield was essentially optimal due to feed nitrogen degradation but also could have accumulated in the collecting syringe and was not absorbed during measurement due to the effect of buffer solutions containing $N\text{-}NH_3$.

Volatile Fatty Acid (VFA) yield: The molar amount of each VFA in the rumen can be determined by the amount of forage in the ration, dry matter intake and feeding method[22]. Total VFA of rations T_2, T_3 and T_4 was higher ($p<0.01$) than that for T_1 and the VFA yield increased concurrently with increased amounts of easily digestible carbohydrate. The VFA concentration in the rumen reflects feed fermentability and is the main energy source for ruminants and along with ammonia is a major component of microbial protein. As such, the VFA concentration is directly related to the degree to which the feed is fermentable[23].

The highest acetic acid level produced (mM) was observed in ration T_3 which had a higher forage proportion and was followed by T_2, T_4 and T_1, respectively ($p<0.01$). The VFA level also correlated with a higher proportion of crude fiber, Acid Detergent Fiber (ADF) and Neutral Detergent Fiber (NDF) in ration T_3 relative to the other rations (Table 2). Although, McDonald *et al.*[15] found that a high fiber content in forage resulted in higher production of acetic acid, the acetic acid values for rations T_1, T_2 and T_4 in this study did not reflect this trend treatment. Ration treatment T_1 had higher crude fiber, ADF and NDF than T_2 and T_4 but still had a higher proportion of acetic acid (Table 2, 3). The fatty acid proportion in ruminant digestion is determined by the type and composition of structural and non-structural carbohydrates as well as the amount of forage in the feed[24]. A high forage composition results in high acetic acid levels while, propionic acid levels increase when the concentration of degradable carbohydrate in the ration exceeds that of crude fiber[25].

The propionic acid yield (mM) of T_2, T_3 and T_4 was higher than that of T_1 ($p<0.01$) indicating that the increased amount of protein and energy in both T_2 (high energy-standard protein) and T_4 (high energy-high protein) positively affected propionic acid yield. Pilajun and Wanapat[26] reported that *in vitro* digestibility and VFA level, especially that of propionic acid increased along with substrate concentration. These increases are correlated with an adequate amount of degradable carbohydrate in the ration. A sufficient propionic yield is a beneficial component of cattle fattening rations because this acid increases the rate of body tissue synthesis. Accordingly, the propionic yield could be improved by

Table 2: *In vitro* chemical composition of fattening rations with different protein-energy levels fed to Bali cattle

	Treatment			
Item	T_1	T_2	T_3	T_4
Dry matter (%)*	90.20	90.74	89.21	88.93
Organic matter (%)*	87.20	90.26	86.17	89.44
Crude protein (%)*	12.06	10.14	14.79	13.04
Crude fiber (%)*	21.89	14.90	22.92	15.47
Extract ether (%)*	5.34	5.61	6.64	6.21
Ash (%)*	12.80	9.74	13.83	10.56
NFE (%)**	47.91	59.61	41.82	54.72
ADF (%)***	29.70	20.24	29.51	20.16
NDF (%)***	54.49	36.72	53.22	36.50
Energy (Cal g^{-1})****	3624.98	3996.76	3895.00	4086.47
TDN (%)	62.56	65.66	63.66	67.48

*Results analysis of Animal Feed Laboratory Technology, Faculty of Animal Science, Gadjah Mada University (2015), **NFE: [100−(Ash%+CF%+EE%+CP%)], ***Analysis results from the Forage Laboratory, Faculty of Animal Science, Gadjah Mada University (2015) and ****Analysis results from the Laboratory Chemistry and Biochemistry, Study Center Food And Nutrition, Gadjah Mada University (2015)

Table 3: *In vitro* average pH, DMD, OMD, N-NH_3 yield, VFA yield and microbial protein synthesis (Mean±SD) of fattening rations with different protein-energy levels fed to Bali cattle

	Treatment					
Item	T_1	T_2	T_3	T_4	SEM	p-value
pH	6.80±0.00	6.78±0.08	6.80±0.00	6.80±0.00	0.007	0.829^{ns}
Dry matter digestibility (%)	71.23±2.31^{b}	73.54±2.26^{c}	66.68±0.45^{a}	74.67±1.55^{c}	0.728	0.000
Organic matter digestibility (%)	70.46±1.96^{b}	75.28±2.17^{c}	63.91±1.19^{a}	74.22±1.27^{c}	0.983	0.000
N-NH_3 (mg/100 mL)	22.73±2.35^{a}	27.40±2.51^{b}	33.21±2.40^{c}	32.72±1.63^{c}	0.993	0.000
VFA (mmol L^{-1})						
VFA total	21.30±1.22^{a}	26.11±2.55^{b}	28.45±2.42^{b}	25.82±3.58^{b}	0.730	0.001
Acetic (C_2)	15.76±0.96^{a}	19.38±1.91^{bc}	21.54±1.71^{c}	18.67±1.74^{b}	0.567	0.001
Propionic (C_3)	4.10±0.30^{a}	5.05±0.51^{b}	5.35±0.70^{b}	5.24±0.65^{b}	0.149	0.004
Butyric (C_4)	1.44±0.15^{a}	1.68±0.22^{ab}	1.55±0.14^{a}	1.91±0.24^{b}	0.052	0.003
C_2: C_3 ratio	3.86±0.16^{b}	3.85±0.14^{b}	4.05±0.26^{b}	3.55±0.10^{a}	0.050	0.001
MPS (mg mL^{-1})	0.26±0.06^{d}	0.17±0.02^{b}	0.12±0.04^{a}	0.19±0.02^{bc}	0.013	0.000

*Different superscripts in the same column indicates the effect of significantly different at p<0.05, Ns: Not-significant, MPS: Microbial protein synthesis and SEM: Standar error of mean

including more concentrated and degradable carbohydrate in feed rations[27]. Nevertheless, T_1 and T_3 showed inconsistent results in terms of propionic acid wherein T_1 (standard protein-standard energy) with 19% degradable carbohydrate had a lower propionic yield than did T_3 (high protein-standard energy) which included only 2% degradable carbohydrates.

The butyric acid yield increased linearly with the concentration of degradable carbohydrate in rations with different protein and energy levels (p<0.01). Treatment T_4 was significantly higher (p<0.05) than T_1 and T_3 but essentially equal to that of T_2 while, T_1 was equal to that of T_2 and T_3.

The acetic:propionic acid ratio (C_2:C_3 ratio) of T_4 was lower (p<0.01) than that of T_1, T_2 and T_3. An increase in the C_2:C_3 ratio can increase the rate of body tissue synthesis which in turn enhances cattle growth performance. Moreover, the acetic (C_2): propionic (C_3) acid synthesis ratio is generally used as an efficiency standard of energy allocation in ruminants wherein a high ratio translates to low energy efficiency, particularly in fattening rations[28].

Perry *et al.*[29] showed that increased starch concentrations in rations could diminish the acetic:propionic acid ratio meanwhile, structural carbohydrates such as hemicellulose produce higher acetic:propionic acid ratios. Thus, lower C_2:C_3 ratios are correlated with more efficient energy use because very little energy is devoted to CH_4 (methane) production[30].

Protein microbe synthesis: Protein microbe synthesis (mg mL^{-1}) by cattle fed T_1 was higher (p<0.01) than for cattle fed T_4, T_2 and T_3, respectively (Table 3). The relatively high amount of microbial protein synthesis was closely related to the low proportion of N-NH_3 that is caused by adequate and synchronized N-NH_3 and energy supply for protein microbe synthesis which was likely responsible for the higher values seen for the T_2 and T_4 rations. Ration T_1 had a better balance between protein and carbohydrate degradation that resulted in an optimum efficiency of microbial protein synthesis. Carbohydrates are the main energy source for bacteria although, carbohydrates also are viable carbon frames for protein synthesis combined with ammonia[16]. Protein synthesis

by rumen bacteria depends on the amount and type of carbohydrates that are used as energy sources for peptide bond synthesis. Highly fermentable carbohydrates such as starch or sugar are more effective for stimulating and inducing microbe growth than alternate sources (e.g., cellulose)[31].

Ration treatment T_2 (high energy-standard protein) consisted of 35% forage and 65% carbohydrate and was easily digestible while, T_4 (high protein-high energy) had 57% forage and 43% digestible carbohydrate. Both showed significant protein microbe synthesis was observed from the high N-NH_3 yields. This result indicated that the N-NH_3 yield was not optimized, even though these rations had adequate amounts of available energy in the form of digestible carbohydrates. The suboptimal N-NH_3 yield could be due to a lack of synchronization between protein and carbohydrate degradation rates that restricted microbial protein synthesis in rations T_2 and T_4. When the level of digestible protein was higher than that of carbohydrate fermentation occurs and most nitrogen is dissipated as ammonia. Conversely, when the carbohydrate fermentation rate was higher than that of protein degradation and microbial protein synthesis could decrease[32]. Although, the effect of an unsynchronized supply of carbohydrate and nitrogen for rumen microorganisms is unclear, the combined ecosystem of rumen microorganisms is sufficiently complex such that the nutrition supply could be synchronized to certain subpopulations but not to others[33]. Rumen fermentation products, VFAs expressed as molar concentrations of C_2 (acetic), C_3 (propionic) and C_4 (butyric) acids, multiple chain fatty acids and N-NH_3 significantly determined the energy efficiency and the production rate of microbial protein synthesis in the rumen. As such, consideration of rumen fermentation patterns and how they can be manipulated through changes in feed ration composition is essential to optimize cattle productivity[34].

The less than optimal production of protein by rumen microbes in cattle fed T_2 and T_4 correlated with the high amount of digestible carbohydrate in these rations. Several previous studies reported that the efficiency of protein microbe synthesis tended to increase if the fermentable carbohydrate supply represented less than 30% of the total ration[35] but decreased when the supply was above 70%. This decreased efficiency of microbial protein synthesis in the small intestines of cattle occurred when the feed contained more than 70% concentrate which could accelerate the degradation rate of non-structural carbohydrates and lead to unsynchronized fermentation[36]. Indeed, Devant *et al.*[20] reported that rations with high carbohydrate levels and low protein concentrations had adequate nitrogen to support microbe growth but restricted microbe protein synthesis and nutrition digestibility.

Ration treatment T_3 (high protein and standard energy) composed of 98% forage and 2% carbohydrate was easily digestible and had a higher N-NH_3 yield that indicated the lowest level of microbial protein synthesis relative to the other rations. This difference was due to the use of a carbohydrate source with lower digestibility as an energy source to convert N-NH_3 into microbial protein. This lowered digestibility resulted in an imbalance between protein and carbohydrate degradation and in turn the high yield of N-NH_3 was not optimized for microbial protein synthesis because of an imbalanced supply of carbon frames for use as an energy source.

Generally, the effects produced by T_3 were consistent with several previous findings showing that feed with high protein levels must also have a high availability of energy sources such that N-NH_3 derived from degradation of feed protein could be used to synthesize microbial protein. According to Huber and Herrera-Saldana[37], such synchronized energy-protein release in the rumen is one contributing factor that affects the efficiency of microbial protein synthesis. This synchronization makes energy and protein simultaneously available during cattle growth[3].

CONCLUSION

Conclusively, rations with different protein-energy levels fed to male Bali cattle being raised on smallholder farms did not severely affect rumen pH which remained within normal ranges. Dry matter and organic matter digestibility of these rations could be improved but N-NH_3 and VFA yields were not optimal to support microbial protein synthesis.

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