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# Influences of Energy Sources and Levels Supplementation on Ruminal Fermentation and Microbial Protein Synthesis in Dairy Steers

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**Abstract:** Four rumen fistulated dairy steers were randomly assigned according to a 4 x 4 Latin square design with a 2 x 2 factorial arrangement of dietary treatments (Factor A = source of energy; CM = corn meal, CC = cassava chip), (Factor B = level of supplementation; 1 and 2% of BW). Four dietary treatments were used: CM1, CM2, CC1 and CC2, respectively. Results revealed that supplementation of cassava chip at 2 %BW, was lower ruminal pH than that of CM1, CM2 and CC1, respectively. In addition, cellulolytic bacteria populations was decreased while protozoa increase with affected by energy source and level supplementation. The result from this experiment suggested that supplementation of energy sources at 2 %BW, especially from cassava chip was reduced ruminal pH lowest but increasing populations of protozoa in rumen of dairy steers.

**Key words:** Cassava chip, corn meal, rumen fermentation, microbial protein synthesis, dairy steers

## Introduction

Cassava (*Manihot esculenta*, Crantz) is an annual tuber crop grown widely in the tropical regions of Africa, Asia and Latin America. It thrives in sandy-loam soils with low organic matter, and climate characterized by low rainfall and high temperature (Wanapat, 2000). Cassava tubers contain high levels of energy and minimal levels of crude protein and have been used as readily fermentable energy in ruminant rations (Wanapat, 2003). Cassava contains 650-850 g of total non-fiber carbohydrates (TNFC) per kg dry matter (DM) and has been used extensively as a feed for livestock (Kanjanaputhipong *et al.*, 2001). Cassava compared to cereal such as maize grain has been reported to have a relatively low crude protein, accompanied by a high rate and extent of degradation in the rumen (Holzer *et al.*, 1997). However, the responses to cassava chip, which is highly degradable in the rumen compared with corn meal, as energy sources have not been extensively studied in dairy steers when fed with urea-treated rice straw. Therefore, this study was conducted to evaluate the comparative study of energy sources and supplementation level on ruminal fermentation and microbial protein synthesis in dairy steers.

## Materials and Methods

**Animals, diets and experimental design:** Four fistulated dairy steers (Holstein Friesian – based, 180 ± 10 kg initial BW) were randomly assigned according to a 2 x 2 factorial arrangement in a 4 x 4 Latin square design to investigate the comparative study of energy sources and supplementation levels with urea-treated rice straw (UTS) on feed intake, digestibility of nutrients, ruminal fermentation and ruminal microbial protein synthesis. The dietary treatments were as follows: T1=supplementation of corn at 1 % BW (CM1); T2=supplementation of corn at 2 %BW (CM2); T3=supplementation of cassava chip at 1 %BW (CC1); T4= supplementation of cassava chip at 2 %BW (CC2). All animals received cottonseed meal at 0.5 kg/head/day and urea-treated rice straw (5%) was offered *ad libitum* as a roughage source. All animals were kept in individual pens and received free choice of water. The experiment was conducted for four periods, each period lasted 21 days. During the first 14 days, all animals were fed on respective diets, while during the last 7 days, the animals were kept in metabolism crates for total feed collection during which they were restricted to 90 % of the previous voluntary feed intake.

**Data collection, sampling procedures and analysis:** Feeds were randomly collected and fecal samples were taken from total collection of individual cow during the last 7 days of each period. They were analyzed for chemical compositions (DM, CP, ash, NDF, ADF) (AOAC, 1985; Goering and Van Soest, 1970).

At the end of each period, rumen fluid and jugular blood samples were collected at 0, 1, 2, 4, 6 h post-feeding. Approximately 200 ml of rumen fluid was taken from the middle part of the rumen by using a 60-ml hand syringe at each time at the end of each period. Rumen fluid was immediately measured for pH and temperature using a portable pH and temperature meter (HANNA instrument HI 8424 microcomputer). Rumen fluid samples were then filtered through four layers of cheesecloth. The samples were divided into three portions. The first portion was used for volatile fatty acid (VFAs) analysis where 5 ml of $H_2SO_4$ solution (1M) was added to 50 ml

of rumen fluid. The mixture was centrifuged at 16,000 x *g* for 15 minutes and supernatant was stored at –20°C prior to VFA analyses using a high-performance liquid chromatography HPLC (Instruments by controller water model 600E; water model 484 UV detector; column novapak $C_{18}$; column size 4 mm x 150 mm; mobile phase 10 mM $H_2PO_4$ (pH2.5)) according to (Samuel *et al.,* 1997). Second portion was fixed with 10% formalin solution in normal saline (0.9% NaCl, Galyean, 1989). The total direct count of bacteria, protozoa and fungal zoospores were made using the methods of Galyean (1989) based on the use of a haemacytometer (Boeco). Third portion was taken to study cultured groups of viable bacteria using roll-tube technique (Hungate, 1969), for identifying bacteria groups (cellulolytic, proteolytic, amylolytic and total viable count bacteria). Urine samples were analyzed for urinary nitrogen (IAEA, 1997) and allantoin in urine was determined by HPLC as described by (Chen *et al.,* 1993).

The amount of microbial purines absorbed (X mmol/day) corresponding to the purine derivatives excreted (Y mmol/day) was calculated based on the relationship derived by Chen and Gomes (1995).

$$Y = 0.85X+(0.385W^{0.75})$$

where Y is the excretion of purine derivatives (mmol/day); X is the microbial purines absorbed (mmol/day).

The supply of microbial N in gram per day was estimated as follows:

$$\text{Microbial N (gram/day)} = \frac{X \times 70}{0.116 \times 0.83 \times 1000} = 0.727x\ X,$$

with X being the absorption of purine derivatives in mmol/day, following the
assumptions made by Chen and Gomes (1995).

Digestibility of microbial purine is 0.83, the N content of purines is 70 mg N/mmol and the ratio of purine-N: total N in mixed rumen microbes is 11.6:100.

The EMNS which denote the microbial N supplied to the animal per unit of DOMR was calculated using the following formula:

$$\text{EMNS} = \frac{\text{MN (g/ay) x1000 (g)}}{\text{DOMR (g)}}$$

Where DOMR = DOMI x 0.65 (ARC, 1990),
DOMR = digestible organic matter apparently fermented in the rumen, DOMI = digestible organic matter intake, EMNS = efficiency microbial nitrogen synthesis, OMDR = organic matter truly digested in the rumen.

**Statistical analysis:** The means of each parameter measured in the digestibility studies and nutrient intake were analyzed by the analysis of variance (ANOVA) techniques using the General Linear Model (GLM) procedures of the (SAS, 1998). Treatment means were compared by the least significant difference method (LSD). The 2x2 factorial analysis of variance was used to examine the effects of energy source and the level of supplementation as well as their interactions. Rumen fluid data (pH, ammonia-N and VFA) were analyzed by split-plot analysis of variance (Snedecor and Cochran, 1967) using the following model: $Y_{ijklm} = \mu + A_i + P_j + T_k + e_{ijk} + H_i + (AH)_{il} + (PH)_{jl} + (TH)_{kl} + e_{ijklm}$ while where μ is the mean of A, P, T and H which stand for animal, period, treatment and time effects, respectively $e_{ijk}$ is the main plot error and $e_{ijklm}$ is the sub-plot error.

Mean separations with a significant F ($P<0.05$) for treatment were statistically compared using the Duncan's New Multiple Rang Test (DMRT) (Steel and Torrie, 1980).

## Results

**Composition of the diets:** Values of nutrient compositions on DM basis to corn meal, cassava chip, cottonseed meal and urea-treated rice straw are shown in Table 1.

Table 1: Chemical compositions (% DM) of cassava chip (CC), corn meal (CM), cottonseed meal (CSM), and urea-treated rice straw 5% (UTS) in the experiment

| Item | CC | CM | CSM | UTS |
|---|---|---|---|---|
| DM | 90.1 | 89.2 | 93.7 | 53.6 |
| OM | 96.2 | 98.3 | 93.7 | 87.0 |
| Ash | 3.7 | 1.7 | 6.2 | 13.0 |
| CP | 2.9 | 10.1 | 41.9 | 8.7 |
| NDF | 7.0 | 15.8 | 28.0 | 72.0 |
| ADF | 6.2 | 10.6 | 21.4 | 55.0 |

**Effect on rumen fermentation and feed intake:** The daily DM intake and nutrient digestibility patterns are presented in Table 2. DM intake of UTS and concentrate were affected ($P<0.05$) by energy sources and levels of supplementation. Furthermore, apparent digestibility of DM, CP and ADF were significantly influenced by energy sources and levels of supplementation while OM and NDF were similar in all groups.

As shown in Table 3, rumen temperatures were similar among treatments and the values were quite stable at 39°C. Ruminal pH values measured immediately at half an hour from 1-6 h-post feeding were found in a range of 5.2-6.7 which were significantly different among group. As shown in Fig. 1, the supplementation of CC2 resulted in lowest pH, while the supplementation at CM1 and CC1 were similarly.

The effect of both energy sources and levels of supplementation were apparent in both acetate and propionate molar proportions. Molar proportion of acetate was significantly higher ($P<0.05$) in dairy steers fed CM1 (73.0%) than in CC2 (69.4%). In addition, the propionate was also affected ($P<0.01$) by the level of

Table 2: Influence of different energy levels on feed intake and digestibility of nutrients in dairy steers

| Item | Treatment[d] | | | | | Contrast[e] | | |
|---|---|---|---|---|---|---|---|---|
| | CM1 | CM2 | CC1 | CC2 | SEM | ES | L | ES x L |
| DM intake (kg/hd/d[f]) | | | | | | | | |
| UTS | 2.7[a] | 2.0[ab] | 2.0[ab] | 1.5[b] | 0.21 | * | * | NS |
| Conc. | 1.6[a] | 3.1[b] | 1.7[a] | 2.6[b] | 0.20 | NS | ** | NS |
| Total | 4.3[ab] | 5.2[a] | 3.7[b] | 4.1[ab] | 0.33 | * | NS | NS |
| Apparent total-tract digestibility (%) | | | | | | | | |
| DM | 69.6[a] | 78.5[b] | 77.1[b] | 82.2[b] | 2.37 | * | * | NS |
| OM | 70.4 | 79.0 | 79.9 | 79.8 | 2.88 | NS | NS | NS |
| CP | 75.1[a] | 74.6[ab] | 72.2[c] | 72.9[bc] | 0.93 | * | NS | NS |
| NDF | 62.5 | 64.7 | 64.0 | 61.5 | 1.28 | NS | NS | NS |
| ADF | 59.5[a] | 56.3[b] | 59.2[a] | 55.6[b] | 0.82 | NS | * | NS |

[abc]Values in the same row with different superscripts differ (p<0.05). [d]CM1 = corn meal at 1% BW, CM2 = corn meal at 2% BW, CC1 = cassava chip at 1% BW, and CC2 = cassava chip at 2% BW. [e]Probability of main effects of energy sources (corn meal vs cassava chip), levels (1 vs 2% BW), or the ES x L interaction. * = P<0.05, ** = P<0.01, NS = P>0.05. [f]UTS = urea-treated rice straw, Conc. = treatment

Table 3: Influence of different energy levels on rumen fermentation characteristic in dairy steers

| Item | Treatment[d] | | | | | Contrast[e] | | |
|---|---|---|---|---|---|---|---|---|
| | CM1 | CM2 | CC1 | CC2 | SEM | ES | L | ES x L |
| Ruminal Temperature ($^0$C) | 39.8 | 39.8 | 39.7 | 39.8 | 0.11 | NS | NS | NS |
| Ruminal pH | 6.5[a] | 6.3[b] | 6.5[a] | 5.3[c] | 0.65 | NS | ** | NS |
| Total VFA (mmol/L) | 81.1 | 85.9 | 85.9 | 89.4 | 3.55 | NS | NS | NS |
| Molar proportion of VFA (mol/100mol) | | | | | | | | |
| Acetate (C2) | 73.0[a] | 70.3[b] | 72.6[a] | 69.4[c] | 0.71 | * | ** | NS |
| Propionate (C3) | 16.8[a] | 19.8[b] | 17.0[a] | 19.9[b] | 0.24 | NS | ** | NS |
| Butyrate (C4) | 10.1 | 9.9 | 10.4 | 10.4 | 0.37 | NS | NS | NS |
| C2:C3 ratio | 4.3[a] | 3.5[b] | 4.2[a] | 3.4[b] | 0.68 | NS | ** | NS |
| C2+C4 : C3 ratio | 4.9[a] | 4.0[b] | 4.8[a] | 4.0[b] | 0.75 | NS | ** | NS |

[abc]Values in the same row with different superscripts differ (p<0.05). [d]CM1 = corn meal at 1% BW, CM2 = corn meal at 2% BW, CC1 = cassava chip at 1% BW, and CC2 = cassava chip at 2% BW. [e]Probability of main effects of energy sources (corn meal vs cassava chip), levels (1 vs 2% BW), or the ES x L interaction. * = P<0.05, ** = P<0.01, NS = P>0.05

supplementation when fed by CC being higher when supplemented with at 2%BW (19.9%) than with at 1%BW (17.0%). As a result, the acetate to propionate (C2:C3) ratio differed between supplementation level. In term of CM diets, CM2 was significantly lower (C2:C3) ratio (3.5) than those fed CM1 (4.3) and in CC diets, while in CC2 there was significantly lower ratio (3.4) than those fed CC1 (4.2). Molar proportion of butyrate tended (P>0.05) to be higher in CC diets (average of 10.4%) than in CM diets.

**Effect on rumen microbes:** Table 4 presents rumen microorganism population. As for groups of bacteria, there were not significantly differences among treatments; however, cellulolytic bacteria in CM group were higher than in CC group (5.6-6.6 vs 2.3-5.4 X $10^7$ CFU/g rumen content). Furthermore, total bacteria counts were similar in all treatments, meanwhile, protozoal populations were found dramatically increased as the level of cassava chip increased.

**Effect on nitrogen balance and microbial nitrogen supply:** As shown in Table 5, N balance in terms of N absorption and retention were similar among treatments (P>0.05). Nitrogen intakes were similar (in the range of 68.7 to 74.9 g/day) for all animals fed different diets. Furthermore, N absorbed, excreted in faces and urine in all groups were not significantly (P>0.05) affected by either energy sources or level of supplementation. Fecal-N output, urine-N excretion, N-balance of dairy steers and allantoin excretion in urine were not significantly (P<0.05) different among dietary treatments. The dairy steers were in positive N-balance. Urinary allantoin was in the range of 28.1 to 45.9 mmol/day/kg $BW^{0.75}$ for the four dietary treatments. The results on EMNS based on OMDR was significantly (P<0.05) different among energy sources and levels of supplementation. Dairy steer fed CC1 had higher EMNS (10.7 g N/kg of OMDR) than those fed CM1, CC2 and CM2 (7.7, 5.2 and 4.3 g N/kg of OMDR), respectively.

## Discussion

**Composition of the diet:** The chemical analysis of CC, CM, CSM and UTS are presented in table 1. The UTS had 8.7 % CP which was slightly lower than those reported by Wanapat (1999).

**Effect on feed intake:** Intake of UTS by dairy steers in treatments with supplementation of CM and CC were not significantly different. Moreover, apparent total-tract

Table 4: Influence of different energy levels on ruminal bacteria, protozoa, fungi population, total viable, amylolytic, proteolytic and cellulolytic bacteria in dairy steers

| Item | Treatment[d] | | | | | Contrast[e] | | |
|---|---|---|---|---|---|---|---|---|
| | CM1 | CM2 | CC1 | CC2 | SEM | ES | L | ES x L |
| Rumen microbes (cells/g) | | | | | | | | |
| Bacteria (x $10^{10}$) | 7.9[a] | 8.8[ab] | 7.3[a] | 7.5[b] | 0.53 | * | NS | NS |
| Protozoa (x $10^{6}$) | 1.0[a] | 1.3[a] | 1.5[b] | 1.7[c] | 4.87 | NS | * | NS |
| Fungal zoospores (x $10^{6}$) | 1.1 | 1.0 | 1.1 | 1.0 | 1.56 | NS | NS | NS |
| Viable bacteria (CFU/g) | | | | | | | | |
| Total (x $10^{7}$) | 17.4 | 15.2 | 17.3 | 13.7 | 3.25 | NS | NS | NS |
| Amylolytic (x $10^{7}$) | 10.1 | 8.9 | 11.4 | 10.6 | 2.26 | NS | NS | NS |
| Proteolytic (x $10^{6}$) | 4.5 | 4.2 | 4.3 | 7.1 | 0.82 | NS | NS | NS |
| Cellulolytic (x $10^{7}$) | 6.6 | 5.9 | 5.4 | 2.3 | 1.74 | NS | NS | NS |

[abc]Values in the same row with different superscripts differ ($p<0.05$). [d]CM1 = corn meal at 1% BW, CM2 = corn meal at 2% BW, CC1 = cassava chip at 1% BW, and CC2 = cassava chip at 2% BW. [e]Probability of main effects of energy sources (corn meal vs cassava chip), levels (1 vs 2% BW), or the ES x L interaction. * = $P<0.05$, NS = $P>0.05$

digestibility (%) of OM and NDF were similar in all groups while digestibility of DM, CP and ADF were significantly in all groups. In general, rate of digestion of carbohydrates is the major factor controlling the energy available for growth of rumen microbes (Hoover and Stokes, 1991). Mertens (1977) concluded that changes in the composition of cell wall involving lignin and possibly silica limited the potential extent of digestion whereas the rate of digestion is limited by the chemical entities other than by crystalline or physical nature of fiber. It is possible that the high fibrous fraction (ADL) could have attributed to lower digestibility (Hart and Wanapat, 1992), especially a large proportion lignified cell walls with low fermentation rate and digestibility, leading to low rate of disappearance through digestion or passage and limited feed intake. In this case, cassava chip, oats, wheat and barley contain high soluble fractions of starch and sugar and can be added to diets to increase utilization of ruminal ammonia-N for microbial protein synthesis. Although, previous reports (Hoover, 1986) have suggested that the reduced pH decrease digestion of fibers. Higher degradation rates can result in a substantial decrease in ruminal pH and fiber digestibility thus reducing feed intake. Moreover, when ruminal pH was reduced below 6.3 in dairy cows, ADF digestion could be reduced at 3.6 % unit per 0.1 pH and may result in depressed feed intake (Erdman, 1998). Grant (1994) has reported that source of starch influenced the rate of NDF digestion differently at pH 6.8 from 5.5 and led to dramatic differences in the apparent extent of ruminal NDF digestion using corn and sorghum starch. On the other hand, Lebzien and Engling (1995) have undertaken a comparison of cassava, corn, barley and wheat as sources of starch in non-lactating dairy cow diets. They found that the source of starch had no effect on silages and total feed intake, ruminal pH and total VFA concentration in rumen fluid. Digestibility of crude fiber was lower when barley or wheat was included in the diets of cows. In addition, they reported higher flows of starch to duodenum in animals fed corn-starch than when fed cassava. Total tract digestibility of cassava has been reported as ranging from 98.9 to 100 percent of intake (Lebzien and Engling, 1995).

**Effect on rumen fermentation:** The temperature of rumen was not significantly affected by treatments and all were within a normal range. Ruminal pH between CM and CC group were significantly different at 0, 1, 2, 4 and 6 h-post feeding. Especially, high level of supplementation of CC2 was the lowest in ruminal pH, while supplementation of CM1, CM2 and CC1 were similar among treatments. Normally, ruminant animals depend on cellulolytic bacteria to digest cellulose, but these bacteria cannot resist the low ruminal pH and an increase in pH gradient leads to anion toxicity (Russell and Wilson, 1996). In addition, most ruminal bacteria prefer pH near neutrality for growth although some species (e.g., *Steptococcus bovis* and *Prevotella ruminicola*) can grow in pH 5 to 6 ranges. The predominant ruminal cellulolytic bacteria are particularly sensitive to low pH. None of three predominant cellulolytic species grow at pH < 6.0 (Shi and Weimer, 1992; Weimer, 1993). Moreover, Weimer *et al.* (1999) also reported that populations of three prominent fiber-digesting bacteria (*Ruminococcus albus, Ruminococcus flavefacians* and *Fibrobacter succinogenes*) were not affected by prolong period of ruminal pH below 6.0. Chronic decreases in ruminal pH are most easily explained by VFA, ruminal motility, and fluid dilution rate. Ruminal movements are triggered by the presence of articulate materials in the rumen, and concentrate-fed cattle do not ruminate as often as forage-fed cattle (Church, 1969). Because VFA absorption is a passive process (Ash and Dobson, 1963), the transfer of VFA from the lumen to the surface of the epithelium via rumen movements would increase the removal of VFA from the rumen.

Total VFA concentration was not significant by dietary treatments. However, total VFA concentrations in all diets were in normal concentrations of 70 to 130 m*M*/L,

Table 5: Nitrogen balance (g/d), excretion of purine derivatives (mmol/d) and microbial protein supply in dairy steer given different energy levels

| Item | Treatment[c] | | | | | Contrast[d] | | |
|---|---|---|---|---|---|---|---|---|
| | CM1 | CM2 | CC1 | CC2 | SEM | ES | L | ES x L |
| Nitrogen balance (g/d) | | | | | | | | |
| N intake | 70.7 | 74.9 | 68.7 | 72.6 | 7.27 | NS | NS | NS |
| Faecal N | 2.1 | 3.0 | 2.3 | 3.0 | 0.33 | NS | NS | NS |
| Urinary N | 7.1 | 6.9 | 4.9 | 5.7 | 1.18 | NS | NS | NS |
| N absorption | 68.6 | 71.9 | 73.7 | 69.6 | 8.60 | NS | NS | NS |
| N retention | 61.5 | 65.0 | 68.9 | 63.9 | 8.13 | NS | NS | NS |
| Purine derivative (PD) (mmol/d) | | | | | | | | |
| Allantoin excretion | 37.7 | 29.1 | 45.9 | 28.1 | 5.61 | NS | NS | NS |
| Allantoin absorption | 27.9 | 17.6 | 37.6 | 16.5 | 6.61 | NS | NS | NS |
| Microbial protein supply (g N/d) | 20.3 | 12.8 | 27.3 | 12.0 | 4.81 | NS | NS | NS |
| EMNS (g N/kg of OMDR)[1] | 7.7[ab] | 4.3[a] | 10.7[b] | 5.2[ab] | 1.82 | NS | * | NS |

[ab]Values on the same row with different superscripts differ ($p<0.05$).[c]CM1 = corn meal at 1% BW, CM2 = corn meal at 2% BW, CC1 = cassava chip at 1% BW, and CC2 = cassava chip at 2% BW. [d]Probability of main effects of energy sources (corn meal vs cassava chip), levels (1 vs 2% BW), or the ES x L interaction. * = $P<0.05$, NS = $P>0.05$. [1]EMNS = efficiency microbial nitrogen synthesis, OMDR = organic matter truly digested in the rumen (Chen and Gomes, 1995)

(France and Siddons, 1993). In addition, the molar percentage acetate in dairy steers fed CM1 and CC1 were significantly higher than those fed CM2 and CC2. According to Murphy *et al*. (1982), ruminal fermentation of structural carbohydrates such as cellulose and hemicellulose in diets exceeding 60% roughage would yield high proportions of acetate and butyrate, respectively. The relatively high proportion of acetate and low proportion of propionate in CM1 and CC1 as compared to CM2 and CC2 could be due to the high content of ADF and low content of hemicellulose. Furthermore, molar percentage of propionate in dairy steers fed either CM2 or CC2 were relatively higher than those fed CM1 or CC1. The changes in acetate and propionate concentrations resulted in a decrease in acetate: propionate ratio when fed CM2 or CC2 and increased C2/C3 ratio in CM1 or CC1 groups. Satter and Esdale (1968) proposed that, although acetate and propionate are the important metabolites of lactate in the rumen, acetate is usually only an intermediate and is used in the synthesis of butyrate. The oxidation of lactate to pyruvate generates two hydrogen atoms, and formation of butyrate from acetate may serve as an electron sink. Formation of propionate from lactate is an alternative way of maintaining the oxidation reduction balance but seems less favored. Satter and Esdale (1968) reported that acetate and butyrate production from lactate was pH-dependent, with acetate production maximal at higher pH and butyrate production at lower pH. The inverse relationship between acetate:propionate ratio and the amount of concentrate in the diet has often been explained by the tendency of fiber fermenting bacteria to produce acetate and starch fermenting bacteria to produce propionate. This generalization is, however, not supported by the characteristics of pure cultures. *Ruminococcus albus* produces large amounts of acetate, but *Fibrobacter succinogenes* and *Ruminococcus flavefaciens* produce mostly succinate, an intermediate that is ultimately converted to propionate. Some starch fermenting bacteria can produce succinate or propionate but most are also able to produce large amounts of acetate (Hungate, 1966).

**Effect on rumen microbes:** Supplementation of energy sources from CM has resulted in increase in cellulolytic, proteolytic and total viable bacteria populations whilst amylolytic and proteolytic bacteria populations were dramatically decreased. However, it should be noted that protozoal population in all CC groups were higher than those in the CM. The high level of cassava-based diets had a higher number of protozoa than corn-based diets. The presence of protozoa in the rumen can also affect rumen fermentation of starch. As cellulolytic bacteria are sensitive to pH, when a high level of starch is fed, the pH may be decreased below 6.0. At this pH the cellulolytic bacteria are inhibited and feed intake depressed (Russell and Wilson, 1996). Optimum pH for maximum microbial growth is between 6.5 to 7.0 (Hungate, 1966). In addition, Jouaney and Ushida (1999) reported that the number of protozoa per ml rumen fluid depends on the rate of soluble sugars and starches in the ration and also pH. Moreover, if the ration is based on grain, protozoa engulfment of starch grains can modulate pH and protect the animals from acidosis (Russell and Hespell, 1981). However, the decrease in protozoal count may attribute to the increase in fungal zoospores per ml rumen fluid, as removal of protozoa has been associated with an increase in the concentration of fungi (Demeyer, 1981).

**Urinary excretion of purine derivatives and microbial nitrogen supply:** Excretion of allantoin in the urine was low in diets. There was no significant difference between the amount of allantoin excreted in urine and microbial

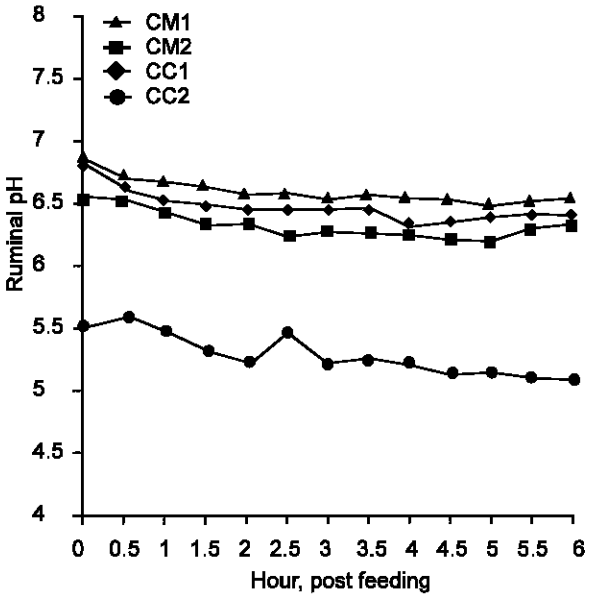


Fig. 1: Effect of levels of energy feed supplementation (corn meal at 1% BW, CM1; corn meal at 2% BW, CM2; cassava chip at 1% BW, CC1; cassava chip at 2% BW, CC2) on ruminal pH in dairy steers

nitrogen synthesis in the rumen. The higher microbial nitrogen supply in dairy steers fed CC1 may be due to synchronization of the available fermentable energy and degradable nitrogen in the rumen. Moreover, the rate of digestion of carbohydrates is a major factor controlling the energy available for microbial growth (Hoover and Stokes, 1991). Furthermore, the efficiency of rumen microbial protein synthesis was significantly higher in dairy steers fed CC1 supplement. Although, variability in efficiency of microbial protein synthesis exists as a result of various factors like concentration and sources of nitrogen and carbohydrates.

**Conclusions:** Based on this experiment it could be concluded that differences in energy sources between CM and CC and supplementation level could affect on rumen ecology. Overall cassava chip resulted in similar rumen parameters as compared to corn at high supplementation level would lower ruminal pH. Therefore, further studies of high level of cassava chip in concentrate should be conducted particularly with non-protein nitrogen (NPN).

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