

Effects of Energy Sources and Level of Supplementation on Ruminal Fermentation and Microbial Protein Synthesis in Dairy Steers

ผลของแหล่งอาหารพลังงานและระดับที่เสริมต่อ กระบวนการหมักในกระเพาะรูเมนและการสังเคราะห์ จุลินทรีย์โปรตีนในโคนมเพศผู้

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ABSTRACT

The experiment was aimed at studying the effect of different energy sources and level of supplementation on ruminal fermentation and microbial protein synthesis efficiency with urea-treated rice straw (UTS) as roughage in dairy steers. 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 at 1 and 2 %BW). Four dietary treatments were CM1, CM2, CC1 and CC2 respectively. UTS was offered ad libitum and water was available at all times. The results exhibited the differences between levels of CM and CC as energy sources especially supplementation of cassava chip at 2 %BW affect on ruminal pH was lowest than CM1, CM2 and CC1. Rumen total VFA concentrations were similar while molar proportions of acetate were lower at the higher level of supplementation but with higher propionate in treatment CM2 and CC2. However, microbial protein synthesis efficiency tended to be higher in dairy steers fed on groups CM1 and CC1 treatments than in those fed CM2 and CC2. Therefore, the results from this experiment suggest that CM and CC had different effect rumen ecology, with these effects being influenced by level of supplementation.

Key Words : cassava chip, corn meal, rumen fermentation, dairy steers

คำสำคัญ : มันเส้น ข้าวโพดบด กระบวนการหมักในกระเพาะหมักรูเมน โคนมเพศผู้

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บทคัดย่อ

การวิจัยครั้งนี้เพื่อศึกษาผลของแหล่งอาหารพลังงานและระดับที่เสริมต่อกระบวนการหมักของกระเพาะหมักหมูเมน ประสิทธิภาพการสังเคราะห์จุลินทรีย์โปรตีนในกระเพาะหมูเมนในโคนมเพศผู้ที่ได้รับฟางหมักยูเรียเป็นแหล่งอาหารหยาบ โดยใช้โคนมเพศผู้เจาะกระเพาะจำนวน 4 ตัว สุ่มให้ได้รับทริทเมนต์ตามการจัดทริทเมนต์แบบ 2×2 แฟคตอเรียลตามแผนการทดลองแบบ 4×4 ลาตินดิสแควร์ โดยมีปัจจัยแหล่งอาหารพลังงาน 2 แหล่งได้แก่ ข้าวโพดบดและมันเส้น และระดับที่เสริมได้แก่ 1 และ 2 เปอร์เซ็นต์ของน้ำหนักตัว ซึ่งมีทริทเมนต์ทั้งหมด 4 ทริทเมนต์ ได้แก่ ทริทเมนต์ที่ 1 เสริมข้าวโพดบด 1 เปอร์เซ็นต์ของน้ำหนักตัว (CM1) ทริทเมนต์ที่ 2 เสริมข้าวโพดบด 2 เปอร์เซ็นต์ของน้ำหนักตัว (CM2) ทริทเมนต์ที่ 3 เสริมมันเส้น 1 เปอร์เซ็นต์ของน้ำหนักตัว (CC1) และทริทเมนต์ที่ 4 เสริมมันเส้น 2 เปอร์เซ็นต์ของน้ำหนักตัว (CC2) ตามลำดับสัตว์ทุกตัวได้รับฟางหมักยูเรีย 5 เปอร์เซ็นต์แบบกินเต็มที่และมีน้ำสะอาดได้รับตลอดเวลา ผลจากการทดลองแสดงให้เห็นว่าแหล่งพลังงานและระดับที่เสริมมีผลต่อกระบวนการหมักในกระเพาะหมูเมนแตกต่างกัน โดยเฉพาะอย่างยิ่งโคที่ได้รับการเสริมมันเส้น 2 เปอร์เซ็นต์ของน้ำหนักตัว มีผลทำให้ความเป็นกรดในกระเพาะหมูเมนต่ำสุด ความเข้มข้นของระดับกรดไขมันที่ระเหยได้ง่ายทั้งหมดในกระเพาะหมักไม่แตกต่างกัน ขณะที่สัดส่วนความเข้มข้นของกรดอะซิเตตพบว่า มีระดับลดลงแต่สัดส่วนของกรดโพรพionate เพิ่มขึ้นเมื่อได้รับการเสริมแหล่งพลังงานในระดับที่สูงที่ระดับ 2 เปอร์เซ็นต์ของน้ำหนักตัว อย่างไรก็ตามประสิทธิภาพการสังเคราะห์จุลินทรีย์โปรตีนในกระเพาะหมักในโคที่ได้รับการเสริมแหล่งพลังงานที่ระดับ 1 เปอร์เซ็นต์ของน้ำหนักตัว (CM1, CC1) มีแนวโน้มสูงกว่ากลุ่มที่ได้รับการเสริมที่ระดับ 2 เปอร์เซ็นต์ของน้ำหนักตัว (CM2, CC2) ดังนั้นจากการศึกษาวิจัยครั้งนี้ชี้ให้เห็นว่าแหล่งพลังงานและระดับที่เสริมต่างกันมีผลต่อนิเวศวิทยาของจุลินทรีย์ในกระเพาะหมักหมูเมนแตกต่างกัน

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 climates 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-fibre carbohydrates (TNFC) per kg dry matter (DM) and has been used extensively as a feed for livestock (Kanjaputhipong, 2001). Cassava compared to cereal or 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). Cassava has a similar digestibility value to steam-flaked corn, but higher than sorghum grain. In addition, corn grain is a major source of dietary starch for lactating dairy cows. Ruminal starch digestibility of corn grain can be altered by fineness of grinding and by conservation method. Ying et al. (1998) reported that ruminal starch digestibility was reduced by more than 19 % when dry ground corn was substituted for ground high-moisture corn with no difference in total tract starch digestibility. However, the responses to cassava chip, which is highly degradable in the rumen when compared with corn meal as energy sources have not been extensively studied in dairy steers when fed on

urea-treated rice straw. Therefore, this study was conducted to evaluate the two energy sources at two levels of supplementation on ruminal fermentation, microbial protein synthesis and digestibility 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 2×2 Factorial arrangement in a 4×4 Latin square design to investigate the comparative study of two energy sources and two levels of supplementation to dairy steers on ruminal fermentation, nitrogen balance, feed intake, digestibility of nutrients and ruminal microbial protein synthesis. The dietary treatments were as follows: T1= Supplementation with corn at 1 % BW (CM1); T2=Supplementation with corn at 2 %BW (CM2).; T3=Supplementation with cassava chip at 1 %BW (CC1).; T4=Supplementation with cassava chip at 2 %BW (CC2).

All animals received cottonseed meal at 0.5 kg/head/day and urea-treated rice straw (5%) (UTS) was offered ad libitum as a roughage source. All animals were kept in individual pens and received free access to water. The experiment was conducted over four periods, each period lasted for 21 days. During the first 14 days, all animals were fed on their respective diets, while during the last 7 days, the animals were in metabolism crates for total collection during which they were restricted to 90% of the previous voluntary feed intake of straw.

Data collection, sampling procedures and analysis

Regular samples of feeds and faecal samples from the total collection of each individual cow on each treatment during the last 7 days of each period were collected. UTS, faeces and concentrates were sampled to be analyzed for chemical compositions (DM, Ash, CP, NDF, ADF) (AOAC, 1985; Georing and Van Soest, 1970).

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 during 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 instruments HI 8424 microcomputer) after withdrawal. Rumen fluid samples were then filtered through four layers of cheesecloth. Samples were divided into two portions; one portion was used for $\text{NH}_3\text{-N}$ analysis where 5 ml of H_2SO_4 solution (1M) was added to 50 ml of rumen fluid. The mixture was centrifuged at $16,000 \times g$ for 15 minute and supernatant stored at -20°C prior to $\text{NH}_3\text{-N}$ and VFA analyses using a HPLC according to Zinn and Owens (1986). Another 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 haemocytometer (Boeco) and cultured groups of bacteria using the roll-tube technique, described by Hungate (1969), for identification of bacteria groups (cellulolytic, proteolytic, amylolytic and total viable count bacteria).

Samples of blood (about 10 ml) were drawn from the jugular vein at the same time as rumen fluid sampling and was separated by centrifugation at 500x g for 10 minute and stored at -20 °C until analysis of blood urea nitrogen (BUN) according to the method of Crocker (1967). Urine samples were analyzed for urinary nitrogen (IAEA, 1997) and allantoin in urine was determined by high-performance liquid chromatography (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 the microbial purines absorbed (mmol/day).

The supply of microbial N in g/day was estimated as follows:

Microbial N (g/day)

$$= \frac{X \times 70}{0.116 \times 0.83 \times 1000}$$

$$= 0.727 \times X,$$

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
- * The ratio of purine-N : total N in mixed rumen microbes is 11.6:100

The Efficiency of Microbial Nitrogen Synthesis (EMNS) to denote the microbial N supplied to the animal per unit of DOMR was calculated using the following formula:

$$\text{EMNS (g N/kg of OMDR)} \\ = \frac{\text{MN (g/day)} \times 1000 \text{ (g)}}{\text{DOMR (g)}}$$

where DOMR = DOMI × 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, nutrient intake were analysed by the analysis of variance (ANOVA) techniques using the General Linear Model (GLM) procedures of the Statistical Analysis System Institute (Statistical Analysis Systems Institute, 2001). 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 AND DISCUSSION

Composition of the diets

Values of nutrient composition (DM basis) for corn meal (CM), cassava chip (CC), cottonseed meal (CSM) and urea-treated rice straw (UTS) are shown in Table 1. The UTS had 8.7% CP which was a slightly value than those reported by Wanapat (2000), this could be due to slight leakage of solution. Most importantly, farmers can grow their own cassava and prepare cassava chip by themselves.

Effect on feed intake and digestibility of nutrients

The daily dry matter intake and nutrient digestibility values are presented in Table 2. DMI of UTS was significantly affected ($p < 0.05$) by energy source and level, being higher with CM and on the low level of concentrate feeding. Concentrate intake did not differ between energy sources but were significantly higher ($p < 0.01$) on the high level of supplementation. Total intakes were significantly affected by energy source ($p < 0.05$) being highest on CM2 and lowest on CC1. Furthermore, apparent digestibility of DM, CP and ADF were significantly influenced by energy source and level of supplementation while OM and NDF were similar 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 Stockes, 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 the crystalline or physical nature of fibre. 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. Furthermore, 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 fibre. 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 silage and total feed intake, ruminal pH and total VFA concentration in rumen fluid. Digestibility of crude fibre was lower when barley or wheat was included in the diets of cows. In addition, they reported higher flows of starch to the 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 following rumen parameters were measured temperature, pH, ammonia - nitrogen ($\text{NH}_3\text{-N}$) and blood urea nitrogen (BUN) concentrations. As shown in Table 3, rumen temperatures were similar among treatments and the values were quite stable at around 40°C. Ruminal pH values measured at 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5 and 6 h-post feeding as shown in Figure.1, they were found in range of 5.2-6.7

and were significantly different. As shown, supplementation with CC2 resulted in the lowest pH, whilst supplementation with CM1, CM2 and CC1 were similar. 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., *Streptococcus 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 the three predominant cellulolytic species grow at pH < 6.0 (Weimer, 1996). Moreover, Weimer et al. (1999) also reported that populations of three prominent fibre-digesting bacteria (*Ruminococcus albus*, *Ruminococcus flavefacians* and *Fibrobacter succinogenes*) were not affected by a prolonged 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.

Ruminal $\text{NH}_3\text{-N}$ concentration was significantly different as affected by energy source and were lower than the optimal ruminal $\text{NH}_3\text{-N}$ (15–30 mg %, Perdok and Leng, 1990) for improving rumen ecology, microbial protein

synthesis, digestibility and voluntary feed intake (Table 3). However, $\text{NH}_3\text{-N}$ concentrations in all diets were above the minimum level of 50 mg/L to support maximum growth rates of rumen bacteria (Satter and Slyter, 1974). Furthermore, blood urea- nitrogen concentration was significantly different between treatments. The differences in $\text{NH}_3\text{-N}$ and BUN concentrations among treatments may have been related directly to CP levels of concentrate. Preston et al. (1965) reported that concentrations of BUN are highly correlated to protein intake and reflect the level of ammonia production in the rumen. This would indicate that available rumen $\text{NH}_3\text{-N}$ could be used and/or absorbed in the rumen for further synthesis. This study revealed that supplementation with concentrate has increased $\text{NH}_3\text{-N}$ concentration with ammonia being the main nitrogen source for growth and protein synthesis by ruminal bacteria which could result in maximum fermentation (Satter and Slyter, 1974).

Total VFA concentrations were not significantly affected by dietary treatments and were present at normal concentrations of 70 to 130 mM, (France and Siddons 1993) (Table 3). In addition, the molar percentage of acetate in dairy steers fed CM1 and CC1 were significantly higher than those fed CM2 and CC2. According to Murphy et al. (1994), 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, the molar percentages of propionate in dairy steers fed either CM2 or CC2 were relatively higher than those fed CM1 or CC1. These 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 dietary treatments. 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 favoured. 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 fibre 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

Table 4 presents rumen microorganism populations. Supplementation with energy sources from CM has resulted in a trend forwards an increase in cellulolytic, proteolytic and total viable bacteria populations whilst amylolytic and proteolytic bacteria tended to decrease. Protozoal populations 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). $\text{NH}_3\text{-N}$ is an essential source of nitrogen for microbial protein synthesis. Furthermore, Song and Kennelly (1990) found that total mixed bacteria tended to increase with increasing level of ammonia nitrogen in the rumen fluid of cattle. The ranges of $\text{NH}_3\text{-N}$ level for optimal rumen ecology has been reported to be 15 to 30 mg % (Leng, 1999). In addition, Jouaney and Ushida (1999) reported that the number of protozoa per ml rumen fluid depends on the rate of soluble sugars and starch 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. However, the decrease in protozoal count may be attributed 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 on all diets. There was no significant treatment difference for either allantoin excreted in urine or microbial-N 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 rumen ecology and characteristics. Overall cassava chip resulted in similar rumen parameters as compared to corn at high levels of supplementation. Therefore, further studies involving high levels of cassava chip in concentrate should be conducted particularly with non-protein nitrogen (NPN) sources.

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

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

DM = dry matter, CP = crude protein, OM = organic matter, NDF = neutral detergent fiber, ADF = acid detergent fiber.

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

Item	Treatment ^d				S.E.M.	Contrast ^e		
	CM1	CM2	CC1	CC2		ES	L	ES x L
DM intake, kg/hd/df								
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	4.4 ^{ab}	5.2 ^a	3.7 ^b	4.2 ^{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 on 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.

^f UTS = Urea-treated rice straw, Conc. = concentrate.

(* = $P < 0.05$, ** = $P < 0.01$, NS = $P > 0.05$).

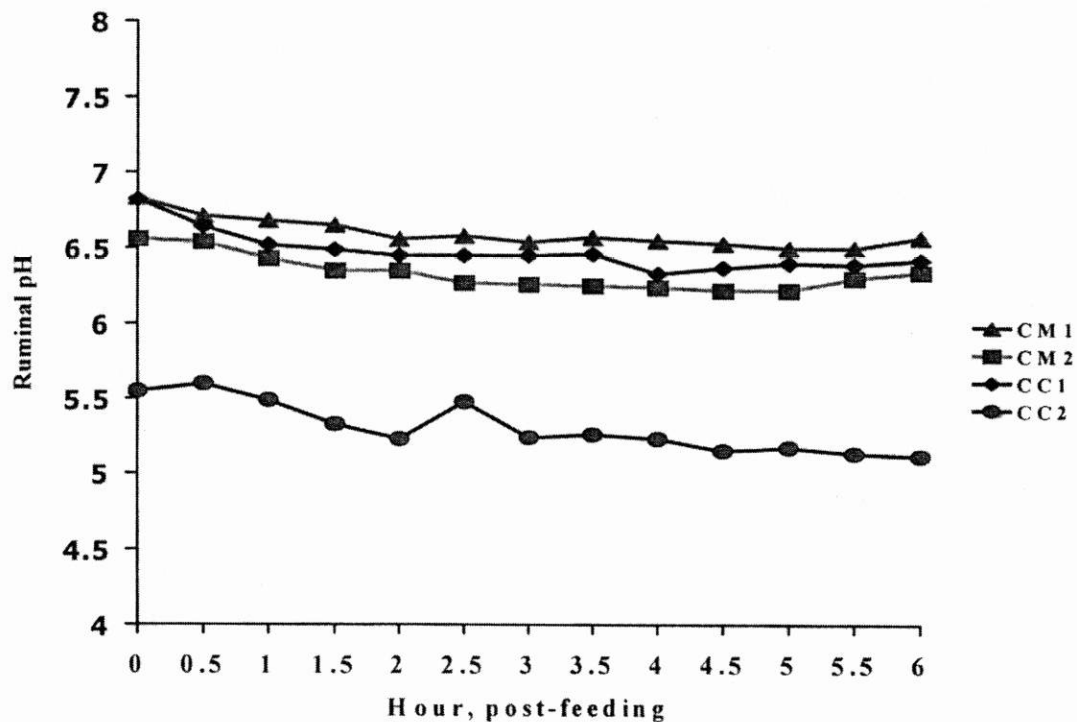


Figure 1 Effect of levels of energy feed supplementation (corn meal at 1 %BW, CM1; corn meal at 2%BW, CM2; cassava chip at 1 %BW, CC1; cassava chip at 2%BW, CC2) on ruminal pH in dairy steers.

Table 3 Influence of different energy sources and levels on rumen fermentation characteristics in dairy steers.

Item	Treatment ^d				S.E.M.	Contrast ^e		
	CM1	CM2	CC1	CC2		ES	L	ES x L
Ruminal Temperature, °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
NH ₃ -N, mg/100mL	7.3 ^a	7.3 ^a	5.1 ^b	5.8 ^b	0.55	*	NS	NS
BUN, mg/100mL	2.9 ^a	2.3 ^{ab}	1.4 ^b	1.3 ^b	0.38	*	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 (C2)	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 on 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 = $P > 0.05$).

Table 4 Influence of different energy sources and levels on microorganisms in dairy steers.

Item	Treatment ^d				S.E.M.	Contrast ^e		
	CM1	CM2	CC1	CC2		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	2.5 ^b	3.7 ^c	4.87	*	*	NS
Fungal zoospores (x 10 ⁶)	1.1	1.0	1.1	1.0	1.56	NS	NS	NS
Variable 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 on 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 = $P > 0.05$).

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				S.E.M.	Contrast ^d		
	CM1	CM2	CC1	CC2		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, 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
Calculated Efficiency of Microbial Nitrogen Synthesis, g N/kg of OMDR	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. (* = $P < 0.05$, ** = $P < 0.01$, NS = $P > 0.05$).