Research Journal of Applied Sciences 5 (4): 267-273, 2010
ISSN: 1815-932X

Effect of Concentrate Mixed with Extrusion-processed Mixture of Cassava Pulp and Urea (EMCPU) and Varying Levels of Ruminal Undegradable Protein on Digestibility, Rumen Fermentation and Nitrogen Balance of Beef Cattle

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Abstract: This experiment aimed to study the effects concentrate mixed with Extrusion-processed Mixture of Cassava Pulp and Urea (EMCPU) and varying levels of Ruminal Undegradable Protein (RUP) on productive performance of crossbred (Thai Native x Brahman) beef cattle. Four cattle with an average bodyweight of 175.5±18.6 kg) were used in a 4×4 Latin square arrangement of treatments with 21 days periods. The treatments were the concentrate mixed with EMCPU and varying levels of RUP at 30, 35, 40 and 45% (T1, T2, T3 and T4). Animal were fed at 2.0% BW of concentrate mixed and all animals were fed *ad libitum* with urea treated rice straw as roughage. The results showed that dry matter intake increased linearly ($p<0.01$) as the level of RUP increased. Moreover, organic matter digestibility increased linearly ($p<0.05$) with increasing the level of RUP. Ruminal ammonia-N concentration was highest in 30% RUP. In addition, linearly decrease in ruminal ammonia-N ($p<0.01$) reflected increases in level of RUP however, at 45% RUP, NH_3-N was increased quadratically ($p<0.01$). Total volatile fatty acid (94.3, 113.25, 116.7, 112.1 mM L^{-1}; $p<0.01$) increased linearly ($p<0.01$) as the level of RUP increased. However, at 45% RUP, TVFAs decreased quadratically ($p<0.01$). Increase in bacteria and protozoa populations reflected increased in level of RUP from 30-40%. However, bacteria and protozoa populations were decreased (quadratically, $p<0.05$) as the level of RUP increased from 40-45%. Nitrogen absorption was not significantly different among dietary treatments. Nitrogen retention (20.9, 19.7, 31.0, 21.5 g day^{-1}; $p<0.05$) was highest in 40% RUP and tended to increase as level of RUP increased however, at N retention at 45% RUP tended to decrease (quadratically, $p = 0.1$). These results indicated that 35% CP from EMCPU replacement for RUP levels at 40% in concentrate have positive effects on crossbred Native x Brahman beef cattle production.

Key words: EMCPU, ruminal undegradable protein, beef cattle, nitrogen balance, nitrogen retention, quadratically

INTRODUCTION

Studies with an extrusion-processed mixture of grain starch and urea (Starea) indicated that ammonia was released slower from this product both *in vivo* and *in vitro* than when a non-heated control mixture was used and additionally, this product was superior to urea as a nitrogen supplement for lactating cows and for growing-fattening cattle (Helmer *et al*., 1970). Utilization of urea rations for milk production has been improved by use of a mixture of gelatinized starch and urea (Starea), processed through an extruder cooker (Jittakot, 1999). Rumen ammonia concentrations were reduced when this mixture was incubated with rumen microorganisms (Helmer *et al*., 1970). However, previous research with NPN supplementation showed that excessive Rumen Degradable Nitrogen (RDN) from NPN sources such as Starea can result in increased rumen ammonia concentration and low efficient incorporation of N into microbial protein (Helmer *et al*., 1970). Moreover, if Ruminally Undegradable Protein (RUP) is included in the diet in sufficient amounts, there is the potential to increase the amino acid flow from the abomasum and eventually modify the amino acid profile of protein reaching the duodenum, so that the efficiency of microbial growth and microbial protein production may be improved by balancing the overall daily ratio of RUP and RDP in the diet (Bach and Stern, 2000). The discovery by McDonald (1948) that soluble dietary proteins are extensively degraded to ammonia in the rumen and the subsequent observations that proteins or amino acids administered directly into the rumen (Schelling and Hatfield, 1968) has

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led to recent attempts to find ways of protecting soluble, high quality, dietary proteins from microbial degradation within the rumen. Decreasing the rumen solubility of casein (Faichney and Weston, 1971) and soybean meal protein (Peter *et al*., 1971) by treatment with formaldehyde would appear to be a potential method of decreasing rumen degradation of high quality proteins and allowing more dietary protein by bypass the rumen to the abomasum and lower digestive tract. Reported here is research to determine the efficiency of N utilization from EMCPU by balancing daily levels of RUP in concentrate. The objectives of this research were to compare productive performance of Thai native x Brahman beef cattle fed with concentrate mixed of extrusion-processed mixture of cassava pulp and urea and varying levels of RUP.

MATERIALS AND METHODS

Four crossbred (Thai native x Brahman) beef cattle were used in the experiment. The animals were randomly assigned in a 4×4 Latin square designs with 21 days periods. The dietary treatments were mixed concentrate with EMCPU and varying levels of RUP as follows:

T1 = Concentrate mixed with RUP at 30%
T2 = Concentrate mixed with RUP at 35%
T3 = Concentrate mixed with RUP at 40%
T4 = Concentrate mixed with RUP at 45%

All animals were fed *ad libitum* with urea-treated rice straw and concentrate mixed at 2.0% BW, twice daily at 08.00 and 17.00. Each cow was housed in an individual pen and free access to clean water all times. Daily collection of urine and faeces were made in the last 7 days of each period. Urine of individual animals was collected in 200 mL of 20% H_2SO_4 to keep the final pH of the urine <3 all times in a container. It is essential to acidify the urine to prevent bacterial activity. After recording the weight, urine was diluted 4 times to prevent precipitation of uric acid during storage. Duplicate urine samples of 50 mL were taken and stored at -20°C until analysis. Daily faeces collects in each period were bulked, mixed and a 5% sub sample taken. The samples of faeces were oven dried and ground (1 mm. Screen) for determination of chemical compositions.

Rumen fluid and jugular blood were collected on the last day of each period. Ruminal pH was measured immediately after ruminal fluid sampling, 5 mL of 6 NHCL was added to 50 mL. Rumen fluid was collected 0, 3 and 6 h post feeding and jugular blood was collected at 0, 3 and 6 h post feeding and placed into heparinized vacationer tubes and centrifuged at 2,500×g for 15 min. Both rumen fluid and blood were stored at 5°C until analysis. Live weights of animals were measured at the beginning and at the end of each feeding period. Urea treated rice straw and concentrate were sampled every 2 weeks and the composited samples analyze for chemical compositions. Neutral detergent fiber, acid detergent lignin of feeds and faeces were determined by the methods of Goering and van Soest (1970) and Dry Matter (DM), ash and Crude Protein (CP) were determined by the methods of AOAC (1985). Rumen fluid Total Volatile Fatty Acid (TVFA) concentration was determined by titration technique of Briggs *et al.* (1957) and NH_3-N were determined by the methods of Bremner and Keeney (1965).

All data obtained from the experiment were subjected to analysis of variance using Proc. GLM (SAS, 1996), treatment means were statistically compared by Duncan's New Multiple Range Test and all data obtained from the experiment were subjected to the General Linear Models (GLM) procedure for orthogonal polynomial analysis of SAS (1996).

RESULTS AND DISCUSSION

The composition of the diets was shown in Table 1. Variation in CP within concentrates was small (approximately 14% CP) and was slightly higher than formulated at 14% CP. The high RUP (45% RUP) rations contained slightly higher NDF and ADF than other treatments. The effect of diet on feed intake is shown in Table 2. These results indicated that total DM intake (5.9, 5.8, 6.0, 6.0 kgDM day^{-1}; $p<0.05$) increased linearly ($p<0.01$) as the levels of EMCPU increased. Increase in DM intake is probably due to amino acid balance arising from increased RUP. Balance of essential amino acids enhanced feed intake.

The effect of diet on feed digestibility is shown in Table 3. These results indicated that DM digestibility (67.8, 68.9, 71.9, 69.9%; $p<0.05$) were highest in 40% RUP. DM digestibility increased linearly ($p<0.01$) as the level of RUP increased. Moreover, OM digestibility increased linearly ($p<0.05$) with increasing the level of RUP. Crude protein digestibility of the diet containing RUP at 40% was higher ($p<0.05$) than other treatments. However, DM and NDF digestibility decreased (quadratically, $p<0.05$) as the levels of CP from EMCPU increased from 40-45%. These data indicated that matching supply of energy and N supply and balancing the overall daily ratio of RUP and RDP in the rumen may improve microbial growth and activity. The nitrogen requirement of rumen bacteria on a

Table 1: Feed formulation and chemical composition of dietary treatments

	Dietary treatments				
Feed stuffs	T1	T2	T3	T4	
EMCPU	10.9	10.9	10.9	10.9	
Cassava pulp	55.6	41.8	37.8	15.4	
Rice bran	3.0	15.0	22.0	36.0	
Soybean meal	12.0	8.0	6.0	2.0	
Palm meal	3.0	13.0	20.0	34.0	
Molasses	13.0	9.0	1.2	0.0	
Urea	0.8	0.6	0.4	0.0	
Sulfur	0.2	0.2	0.2	0.2	
Lime stone	0.5	0.5	0.5	0.5	
Salt	0.5	0.5	0.5	0.5	
Mixed mineral	0.5	0.5	0.5	0.5	
Total	100.0	100.0	100.0	100.0	Urea treated rice straw
Chemical composition (%)	89.9	90.2	90.4	91.0	49.8
	%DM				
OM	91.9	91.7	91.6	89.7	89.1
NDF	18.8	19.4	19.9	20.2	68.0
ADF	9.3	9.7	10.1	11.4	47.1
ADL	4.2	4.3	4.3	4.5	8.7
Ash	8.1	8.3	8.4	10.3	10.9
AIA	1.4	1.5	1.5	1.6	1.5
CP	14.2	14.1	14.1	14.0	7.1

EMCPU = Extrusion-processed Mixture of Cassava Pulp and Urea, DM = Dry Matter, OM = Organic Matter, NDF = Neutral Detergent Fiber, ADF = Acid Detergent Fiber, ADL = Acid Detergent Lignin, AIA = Acid Insoluble Ash, CP = Crude Protein, T1 = Concentrate mixed with RUP at 30%, T2 = Concentrate mixed with RUP at 35%, T3 = Concentrate mixed with RUP at 40% and T4 = Concentrate mixed with RUP at 45%

Table 2: Effects concentrate mixed with Extrusion-processed Mixture of Cassava Pulp and Urea (EMCPU) and varying levels of Ruminal Undegradable Protein (RUP) on feed intake and nutrient intake

	Concentrate mixed					Contrast*	
Parameters	T1	T2	T3	T4	SEM	L	Q
Feed intake (kgDM day^{-1})							
Concentrate	2.40	2.40	2.40	2.50	0.01	0.078	0.082
Roughage	3.40^{b}	3.40^{b}	3.60^{a}	3.60^{a}	0.03	0.010	0.716
Total intake							
kgDM	5.87^{b}	5.83^{c}	5.99^{ab}	6.04^{a}	0.04	0.009	0.339
BW(%)	3.18^{b}	3.17^{b}	3.24^{a}	3.24^{a}	0.02	0.004	0.455
(g kg^{-1} BW$^{0.75}$)	116.90^{b}	116.70^{b}	119.60^{a}	120.60^{a}	0.72	0.004	0.862
Nutrient intake (kg day^{-1})							
NDF	2.80^{b}	2.80^{b}	2.90^{a}	2.90^{a}	0.02	0.003	0.611
CP	0.59	0.58	0.60	0.59	0.01	0.253	0.506

^{a,b}Means within a row with different superscripts differ ($p<0.05$), SE = Standard Error of the mean, NDF = Neutral Detergent Fiber, CP = Crude Protein, *Orthogonal polynomial contrast L = Linear, Q = Quadratic, T1 = Concentrate mixed with RUP at 30%, T2 = Concentrate mixed with RUP at 35%, T3 = Concentrate mixed with RUP at 40% and T4 = Concentrate mixed with RUP at 45%

given diet can be estimated from the amount and digestibility of organic matter digested by the animal, bearing in mind that there should be at least 30 g nitrogen to be hydrolyzed to ammonia in the rumen, per kg of digestible organic matter in the diet (Orskov, 1976). If the basal diet offered to the animals is deficient in nitrogen but contains large amounts of readily fermentable carbohydrates, it is advisable to supplement the diet with NPN so as to ensure the correct proportions of nitrogen and energy for microbial protein synthesis. The magnitude of the latter is rarely limited by nitrogen deficiency as nitrogen can readily be added in form of NPN or in the first instance by the intake of sufficiently large amounts of rumen-digestible organic matter, mainly carbohydrates.

Table 3: Effects concentrate mixed with Extrusion-processed Mixture of Cassava Pulp and Urea (EMCPU) and varying levels of Ruminal Undegradable Protein (RUP) on digestibility and body weight change

	Concentrate mixed					Contrast*	
Parameters	T1	T2	T3	T4	SEM	L	Q
Digestibility (%)							
DM	67.80^{b}	68.90^{b}	71.90^{a}	69.90^{a}	0.65	0.020	0.059
NDF	56.20^{b}	57.50^{b}	61.90^{a}	57.50^{b}	0.75	0.048	0.009
CP	75.30^{ab}	75.40^{ab}	76.50^{a}	73.20^{b}	0.76	0.168	0.068
Body weight (kg)							
Initial weight	180.80	179.70	179.80	180.50	-	-	-
Final weight	189.40	190.10	190.70	189.30	0.40	0.936	0.036
Body weight change (kg day^{-1})	0.41^{b}	0.49^{a}	0.52^{a}	0.42^{b}	0.02	0.403	0.001

^{a,b}Means within a row with different superscripts differ ($p<0.05$) SE = Standard Error of the mean, *Orthogonal polynomial contrast L = Linear, Q = Quadratic, T1 = Concentrate mixed with RUP at 30%, T2 = Concentrate mixed with RUP at 35%, T3 = Concentrate mixed with RUP at 40% and T4 = Concentrate mixed with RUP at 45%

Body weight change (0.41, 0.49, 0.52, 0.42 kg day^{-1}) (Table 3) was highest in 40% RUP and lowest ($p<0.01$) in 30% RUP. However, in 35 and 40% RUP were not differing. Increase in body weight change reflected increases in level of RUP from 30-40%. Moreover, increases in level of RUP from 40-45% caused decreased in body weight change (quadratically, $p<0.01$). The animals fed the diet containing RUP at 40% had the highest body weight gains. The results indicated that the efficiency of microbial growth and microbial protein production may be improved by balancing the overall daily ratio of RUP and RDP in the diet.

Bach and Stern (2000) reported that when the RUP is included in the diet in sufficient amounts, there is the potential to increase the AA flow from the abomasum and eventually modify the AA profile of protein reaching the duodenum which enhanced body weight gains. The discovery by McDonald (1948) that soluble dietary proteins are extensively degraded to ammonia in the rumen and the subsequent observations that proteins or amino acids administered post-ruminally resulted in greater nitrogen retention than when these were administered directly into the rumen (Schelling and Hatfield, 1968) has led to recent attempts to find ways of protecting soluble, high quality, dietary proteins from microbial degradation within the rumen. Decreasing the

Table 4: Effects concentrate mixed with Extrusion-processed Mixture of Cassava Pulp and Urea (EMCPU) and varying levels of Ruminal Undegradable Protein (RUP) on rumen fermentation

	Concentrate mixed					Contrast*	
Parameters	T1	T2	T3	T4	SEM	L	Q
pH (hr-post-feeding)							
0	7.2[a]	7.0[b]	6.8[c]	6.7[c]	0.04	0.001	0.236
3	6.7[a]	6.5[b]	6.4[b]	6.5[b]	0.04	0.001	0.015
6	7.1[a]	6.8[b]	6.8[b]	6.7[c]	0.03	0.001	0.008
Mean	7.0[a]	6.8[b]	6.7[b]	6.6[b]	0.09	0.001	0.170
NH_3-N (mg%)							
0	11.2[a]	9.7[b]	8.4[c]	9.1[bc]	0.27	0.001	0.002
3	13.3[a]	12.6[b]	10.6[c]	12.9[ab]	0.20	0.003	0.001
6	12.4[a]	10.6[b]	9.5[c]	11.1[b]	0.18	0.001	0.001
Mean	12.3[a]	11.0[b]	9.5[c]	11.0[b]	0.65	0.003	0.001
TVFAs (mM L^{-1})							
0	83.4[d]	99.4[c]	106.7[a]	103.0[b]	0.89	0.001	0.001
3	101.8[c]	125.2[ab]	125.9[a]	122.6[b]	0.98	0.001	0.001
6	94.8[c]	114.9[a]	117.4[a]	110.6[b]	0.85	0.001	0.001
Mean	94.3[b]	113.2[a]	116.7[a]	112.1[a]	4.57	0.001	0.001

[a-d]Means within a row with different superscripts differ (p<0.05), *Orthogonal polynomial contrast L = Linear, Q = Quadratic, T1 = Concentrate mixed with RUP at 30%, T2 = Concentrate mixed with RUP at 35%, T3 = Concentrate mixed with RUP at 40% and T4 = Concentrate mixed with RUP at 45%

rumen solubility of casein (Faichney and Weston, 1971) and soybean meal protein (Peter *et al.*, 1971) by treatment with formaldehyde would appear to be a potential method of decreasing rumen degradation of high quality proteins and allowing more dietary protein by bypass the rumen to the abomasum and lower digestive tract.

Ruminal pH data are showed in Table 4. Ruminal pH decreased linearly ($p<0.05$) as the levels of RUP increased and was lower ($p<0.05$) than other treatments when RUP level in concentrate at 45% was fed. Ruminal pH at 3 h post feeding was increased similar in all dietary treatments. The data in this experiment indicated that ruminal fermentation was greatest at the 3 h post feeding. Low pH values appeared to be related to high VFA and high bacterial nitrogen production. H-ion concentration appeared to be influenced more by VFA than by ammonia or lactate production (Stiles *et al.*, 1970). Moreover, the correlation between rumen pH and VFA was 0.8 (Roman-Ponce *et al.*, 1974). Coombe *et al.* (1960) pointed out that there was a relationship between rumen pH and ammonia concentration which depended on quantities of VFA. These findings agree with many workers because rumen pH and VFA followed the above patterns.

Ruminal ammonia-N concentration (12.3, 11.0, 9.5, 11.0 mg%) (Table 4) was highest ($p<0.01$) in 30% RUP. Moreover, the data showed that decrease in ruminal ammonia-N linearly ($p<0.01$) reflected increases in level of RUP. However, at 45% RUP rumen NH_3-N increased (quadratically, $p<0.01$). These results showed that the rumen NH_3-N concentration in animal fed the diet containing RUP at 40% was lower ($p<0.01$) than other treatments. It is possible that more efficient capture of N for microbial protein synthesis. Kolver *et al.* (1998) reported that decrease ammonia concentration were the results of a more efficient capture of N for microbial protein synthesis.

Moreover, Helmer and Bartley (1971) have shown that slower hydrolysis of N results in more efficient incorporation of urea N into microbial protein. Ammonia liberated in the rumen is utilized by the microorganisms for growth and thus for increasing the amount of microbial protein. In this way a part of dietary protein is converted into microbial protein of high nutritive value. Not all ammonia of the ruminal pool is utilized by the microorganisms. A part, depending in the first instance on rumen ammonia concentration and acidity is absorbed from the rumen into the blood and is converted in the liver to urea of which a part is recirculated to the rumen with saliva or across the rumen wall while the remainder is excreted in urine as the end product of nitrogen metabolism in the ruminant. There is a close relationship between the quantity of energy obtained from the feed by anaerobic fermentation in the rumen and available to the microorganisms and the yield of microbial matter (Hungate, 1966; Hobson, 1971).

The yield of ATP from the fermentation of feeds is proportional to the quantity of organic matter fermented. The types of rumen fermentation and the kind of end-products, i.e., the molar proportion of volatile fatty acids have but little effect on the yield of microbial matter (Orskov *et al.*, 1974). If, however, a certain microorganism causing a different type of fermentation predominated in the rumen and if that microorganism were particularly efficient in utilizing ATP, then a more pronounced relationship between type of fermentation and bacterial cell yield would exist.

Ruminal NH_3-N concentration at 0, 3 and 6 h post feeding are shown in Table 4. Ruminal NH_3-N concentrations at 3 h post feeding were increased similar in every dietary treatment. The data in this experiment indicated that ruminal fermentation was greatest at the 3 h post feeding. Similar reported by Davis and Stallcup (1967) that peaks of ammonia concentration in rumen contents 2 or 3 h after feeding. In contrast, Thompson *et al.* (1972) found that peak rumen ammonia for Starea rations 90 min after feeding. Higher NH_3-N concentration at 3 h post feeding might have been related to the microbial population in the rumen that increased at the same time however, at the 6 h post feedings decreased indicating a more capture of N for increased microbial protein synthesis (Sinclair *et al.*, 1993; Witt *et al.*, 1999).

Total VFA concentrations in the rumen (Table 4) increased (linearly, $p<0.01$) as the level of RUP increased. However, at 45% RUP, TVFAs decreased (quadratically, $p<0.01$). These result showed that Total VFA concentration in animal fed the diet containing RUP at 40% was higher ($p<0.01$) than other treatments. The lowest ($p<0.01$) concentration recorded was on animals fed 30% RUP. The lowest concentration of VFA probably reflecting imbalances between RUP and RDP in the diet, resulting in decreased ruminal end product (Kim, 2001). Moreover, Witt *et al.* (1999) reported that higher VFA concentration might have been related to the microbial population in the same time as optimum pH. Moreover, Davis and Stallcup (1967) observed more alkaline rumen pH when urea was fed due to the high concentration of ammonia and the low VFA production. Rumen microbe populations are shown in Table 4. The levels of RUP in concentrate had a significant influence upon both total bacteria and protozoa population was lowest ($p<0.05$) in 30% RUP. It is possible that lower synchronizing rate of degradation of dietary energy and N release in the rumen beneficially increased rumen microbe population and microbial protein synthesis (Herrera-Saldana *et al.*, 1990).

Total VFAs concentrations at 0, 3 and 6 h post feeding are shown in Table 4. Ruminal TVFAs concentration at 3 h post feeding was increased similar in all dietary treatments. The data in this experiment indicated that ruminal fermentation was greatest at the 3 h post feeding. Higher VFA concentration at 3 h post feeding might have been related to the microbial population in the rumen that increased at the same time however, at the 6 h post feedings decreased indicating a more capture of C-skeleton for increased microbial protein synthesis (Sinclair *et al.*, 1993; Witt *et al.*, 1999). The result agrees with Stiles *et al.* (1970) who reported that the rumen VFA concentration usually peaked 4 h post-feeding. There is a close relationship between the quantity of energy obtained from the feed by anaerobic fermentation in the rumen and available to the microorganisms and the yield of microbial matter (Hungate, 1966; Hobson, 1971). The yield of ATP from the fermentation of feeds is proportional to the quantity of organic matter fermented. The types of rumen fermentation and the kind of end-products, i.e., the molar proportion of volatile fatty acids have but little effect on the yield of microbial matter (Orskov *et al.*, 1974). If, however, a certain microorganisms causing a different type of fermentation predominated in the rumen and if that microorganisms were particularly efficient in utilizing ATP, then a more pronounced relationship between type of fermentation and bacterial cell yield would exist. The result agrees with Stiles *et al.* (1970) who reported that the rumen VFA concentration usually peaked 4 h post-feeding.

Table 5: Effects concentrate mixed with Extrusion-Processed Mixture of Cassava Pulp and Urea (EMCPU) and varying levels of Ruminal Undegradable Protein (RUP) on microbial population and blood urea nitrogen

	Concentrate mixed					Contrast*	
Parameters	T1	T2	T3	T4	SEM	L	Q
Blood urea nitrogen (mg%)							
0	20.1^{a}	19.5^{a}	18.4^{b}	18.3^{b}	0.33	0.001	0.488
3	25.4^{a}	23.5^{b}	23.7^{b}	24.7^{ab}	0.77	0.397	0.015
6	21.8^{a}	21.8^{a}	19.1^{c}	20.6^{b}	0.31	0.001	0.028
Mean	22.4	21.6	20.4	21.2	1.22	0.125	0.245
Ruminal bacteria ($\times 10^{10}$ cell mL^{-1})	2.2	2.5	2.6	2.4	0.89	0.066	0.025
Ruminal protozoa ($\times 10^{5}$ cell mL^{-1})	1.9^{c}	2.1^{ab}	2.2^{a}	1.9^{bc}	0.05	0.288	0.005

$^{a-d}$Means within a row with different superscripts differ ($p<0.05$), *Orthogonal polynomial contrast L = Linear, Q = Quadratic, T1 = Concentrate mixed with RUP at 30%, T2 = Concentrate mixed with RUP at 35%, T3 = Concentrate mixed with RUP at 40% and T4 = Concentrate mixed with RUP at 45%

Blood urea nitrogen concentrations are shown in Table 5. Blood urea nitrogen concentration at 0 h after increased linearly ($p<0.05$) as the levels of RUP decreased. However, at 3 h post feeding BUN increased quadratically ($p<0.05$) as the levels of RUP decreased. Blood urea nitrogen concentration at 3 h post feeding was increased similar in all dietary treatments. The data in this experiment indicated that ruminal fermentation was greatest at the 3 h post feeding; rumen ammonia may have been absorbed into portal blood and incorporated in to urea in the liver. However, at the 6 h post feedings decreased indicating a more excreted in the urine (Sinclair *et al.*, 1993; Witt *et al.*, 1999).

Ammonia liberated in the rumen is utilized by the microorganisms for growth and thus for increasing the amount of microbial protein. In this way a part of dietary protein is converted into microbial protein of high nutritive value. Not all ammonia of the ruminal pool is utilized by the microorganisms. A part, depending in the first instance on rumen ammonia concentration and acidity is absorbed from the rumen into the blood and is converted in the liver to urea of which a part is recirculated to the rumen with saliva or across the rumen wall while the remainder is excreted in urine as the end product of nitrogen metabolism in the ruminant. There is a close relationship between the quantity of energy obtained from the feed by anaerobic fermentation in the rumen and available to the microorganisms and the yield of microbial matter (Hungate, 1966; Hobson, 1971). Rumen microbe populations are shown in Table 5. Bacteria

Table 6: Effects concentrate mixed with Extrusion-processed Mixture of Cassava Pulp and Urea (EMCPU) and varying levels of Ruminal Undegradable Protein (RUP) on nitrogen balance

	Concentrate mixed					Contrast*	
Parameters	T1	T2	T3	T4	SEM	L	Q
Urine N (gday^{-1})	51.00	50.70	41.90	48.10	2.23	0.132	0.197
Urine N (g kg^{-1}BW$^{0.75}$)	1.04	1.04	0.86	0.98	0.05	0.143	0.244
Feces N (g day^{-1})	23.00^{b}	22.80^{b}	22.30^{b}	25.60^{a}	0.68	0.052	0.044
Nabsorption (g day^{-1})	71.80	70.40	72.90	69.60	1.12	0.438	0.437
Nretention (g day^{-1})	20.90^{b}	19.70^{b}	31.00^{a}	21.50^{b}	2.23	0.231	0.110
N absorption (%N intake)	75.30^{ab}	75.40^{ab}	76.50^{a}	73.20^{b}	0.76	0.168	0.068
N retention (%N intake)	21.60^{b}	21.10^{b}	32.50^{a}	22.70^{b}	2.46	0.230	0.107

^{a,b}Means within a row with different superscripts differ (p<0.05), *Orthogonal polynomial contrast L = linear, Q = quadratic, T1 = Concentrate mixed with RUP at 30%, T2 = Concentrate mixed with RUP at 35%, T3 = Concentrate mixed with RUP at 40% and T4 = Concentrate mixed with RUP at 45%

population and protozoa population were lowest ($p<0.05$) in 30% RUP. However, in 35 and 40% microbial populations were not differing. Moreover, these data showed that increase in bacteria and protozoa populations reflected increased in level of RUP from 30-40%. However, bacteria and protozoa populations were decreased quadratically ($p<0.05$) as the level of RUP increased from 40-45%. These result showed that microbe populations in animal fed the diet containing RUP at 40% was higher treatments ($p<0.05$) than other treatments.

The lowest treatments ($p<0.05$) bacteria populations recorded was on animals fed 30% RUP. It is possible that lower synchronizing rate of degradation of dietary energy and N release in the rumen beneficially increased rumen microbe population and microbial protein synthesis (Herrera-Saldana *et al*., 1990). Sinclair *et al*. (1993) and Kim (2001) reported that higher synchronizing rate of degradation of dietary energy and N release in the rumen beneficially increased microbial protein synthesis. Moreover, Jouany *et al*. (1998) reported that ruminal protozoa growth depends on high rate of soluble sugars and starches in the ration. Therefore, high soluble N in diets and cassava starch possibly affected the rumen microbe population.

Nitrogen balance study is shown in Table 6. Urinary N was similar across treatment. However, fecal N excretion increased quadratically ($p<0.05$) as the levels of RUP increased. Fecal N excretion was higher ($p<0.05$) than other treatments when RUP level in concentrate at 45% was fed, Fecal N excretion increase indicating that excessive RUP can result in decrease protein digestibility. Nitrogen absorption (71.8, 70.4, 72.9, 69.6 g day^{-1}; $p>0.05$) was not significantly different among dietary treatments. Nitrogen retention (20.9, 19.7, 31.0, 21.5 g day^{-1}) was highest ($p<0.05$) in 40% RUP. Nitrogen retention tended to increase as level of RUP increased. In addition, at 45% RUP N retention tended to decreased (quadratically, p = 0.1). This result agrees with Cecava who reported that excessive RUP can result in decreased microbial yield and reduced ruminal OM and fiber digestion.

Therefore when protein digestibility decreased can result in increase fecal N excretion. Ammonia liberated in the rumen is utilized by the microorganisms for growth and thus for increasing the amount of microbial protein. In this way a part of dietary protein is converted into microbial protein of high nutritive value. Not all ammonia of the ruminal pool is utilized by the microorganisms.

A part, depending in the first instance on rumen ammonia concentration and acidity is absorbed from the rumen into the blood and is converted in the liver to urea of which a part is recirculated to the rumen with saliva or across the rumen wall while the remainder is excreted in urine as the end product of nitrogen metabolism in the ruminant.

There is a close relationship between the quantity of energy obtained from the feed by anaerobic fermentation in the rumen and available to the microorganisms and the yield of microbial matter (Hungate, 1966; Hobson, 1971). Supplementing diets of relatively low digestible organic matter content with NPN with is useless, as the bacteria are unable to utilize all the ammonia released in the rumen, the excess being absorbed into the bloodstream, converted to urea and excreted in the urine.

CONCLUSION

The results indicated that concentrate mixed of EMCPU (35% CP) in ration with 40% RUP can improve feed intake, body weight change, blood metabolites, rumen microbe populations end-products of ruminal fermentation, digestibility and nitrogen balance. Therefore, this study suggested that the diet containing of 35% EMCPU used as a protein in ration with 40% RUP has positive benefit on Thai native x Brahman beef cattle productive performances.

ACKNOWLEDGEMENTS

The researchers acknowledge Suranaree University of Technology and National Research Council of Thailand (NRCT) for their facilities and financially supporting this research.

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