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Effect of Rumen Undegradable Protein Levels on Performance of Thai Native x Brahman Beef Cattle

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Abstract: This experiment aimed to study the effects of rumen undegradable protein levels on productive performance of Thai Native-Brahman beef cattle. Four yearling Thai Native x Brahman beef cattle with an average Body Weight (BW) of 175.5±18.6 kg were used in a 4 x 4 Latin square arrangement. The treatments were levels of Rumen Undegradable Protein (RUP) in concentrate at 30, 35, 40 and 45%. Concentrates were formulated to contain 14% CP and were fed at 2.0% BW. The results showed that the DM intake, OM digestibility and TVFA increased linearly ($p<0.05$) while the level of RUP increased. Moreover, the ruminal NH_3-N ($p<0.01$) and BUN linearly decreased, whereas at 45% RUP the ruminal NH_3-N concentration increased (quadratically, $p<0.01$). The bacteria and protozoa populations also increased as the level of RUP increased. However, the bacteria and protozoa populations decreased quadratically ($p<0.05$) when the level of RUP changed from 40 to 45%. The N retention (g/d) tended to increase with increasing the level of RUP. However, the N retention tended to decrease at the level of 45% RUP. It could be concluded that RUP level at 40% in concentrate had positive effects on productive performances.

Key words: Rumen undegradable protein, beef cattle

INTRODUCTION

Previous work with NPN supplementation showed that excessive Rumen Degradable Nitrogen (RDN) from NPN sources such as urea 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 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 work to determine the efficiency of N utilization from levels of RUP in concentrate.

MATERIALS AND METHODS

Four, Thai Native-Brahman beef cattle were used in the experiment. The animals were randomly assigned in a 4 x 4 Latin square designs with 21-d periods. The dietary treatments were formulated to contain various levels of Rumen Undegradable Protein (RUP) in concentrate at 30, 35, 40 and 45%. All animals were fed ad libitum of urea-treated rice straw with fed concentrate (14% CP) at 2.0% BW, twice daily at 08.00 am and 17.00 pm.

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 lower than 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 sample of faces was oven dried and ground (1 mm Screen) for determination of DM, ash, OM, NDF, ADF and N content. Rumen fluid and jugular blood were collected on the last day of each period. Ruminal pH was

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measured immediately after ruminal fluid sampling, 5 ml of 6 N HCL 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 x g for 15 min. Both rumen fluid and blood were stored at 5°C until analysis. Live weight of animals was measured at the beginning and at the end of each feeding period. Urea treated rice straw and concentrate were sampled every two weeks and the composted samples analyze for DM, NDF, ADF, ADL, CP and ash content. Neutral detergent fiber, acid detergent lignin of feeds and faeces were determined by the methods of Goering and Van Soet (1970) and DM, ash and crude protein were determined by the methods of AOAC (1985). Rumen fluid TVFA concentration was determined by titration technique of Briggs *et al.* (1957) and NH_3-N were determined by the methods of Bromner 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 (Steel and Torrie, 1980) and all data obtained from the experiment were subjected to the General Linear Models (GLM) procedure for orthogonal polynomial analysis of SAS (SAS, 1996).

Table 1: Feed formulation and chemical composition of dietary treatments

	Rumen undegradable protein (RUP) (%)			
Feed stuffs	30.0	35.0	40.0	45.0
Cassava pulp	55.5	41.7	37.7	15.1
Rice bran	3.0	15.0	22.0	36.0
Soybean meal	23.0	19.0	17.0	13.0
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	0.2
Lime stone	0.5	0.5	0.5	0.5
Salt	0.5	0.5	0.5	0.5
Mixed mineral	0.7	0.7	0.7	0.7
Total	100.0	100.0	100.0	100.0

Table 2: Chemical composition of dietary treatments

	RUP (%)				
	30	35	40	45	UTRS
DM	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	14.3	7.1

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. UTRS = Urea Treated Rice Straw

RESULTS AND DISCUSSION

The chemical composition of the diets was shown in Table 2. 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 3. These results indicated that total dry matter intake (5.9, 5.8, 6.0, 6.0 kgDM/d; $p<0.05$) increased linearly ($p<0.01$) as the levels of RUP 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 4. 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 RUP 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 given diet can be estimated from the amount and digestibility of organic matter digested by the animal (Orskov, 1976).

Body weight change (0.41, 0.49, 0.52, 0.42 kg/d) (Table 4) was highest in 40% RUP and lowest ($p<0.01$) in 30% RUP. However, in 35% and 40% RUP were not differ. 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. When the Ruminal Undegradable Protein (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.

Ruminal pH data are showed in Table 5. 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

Table 3: Effect of dietary treatments on feed intake

	RUP (%)					Contrast*	
Items	30	35	40	45	SEM	L	Q
Feed intake (kgDM/d)							
Concentrate	2.40	2.40	2.40	2.50	0.01	0.08	0.08
Roughage	3.40^{b}	3.40^{b}	3.60^{a}	3.60^{a}	0.03	0.01	0.72
Total intake							
kgDM	5.87^{b}	5.83^{c}	5.99^{ab}	6.04^{a}	0.04	0.01	0.34
%BW	3.18^{b}	3.17^{b}	3.24^{a}	3.24^{a}	0.02	0.00	0.46
(g/kgBW$^{0.75}$)	116.90^{b}	116.70^{b}	119.60^{a}	120.60^{a}	0.72	0.00	0.86

^{a,b}Means within a row with different superscripts differ (p<0.05). SEM = Standard Error of the Mean, NDF = Neutral Detergent Fiber, CP = Crude Protein, *Orthogonal polynomial contrast L = Linear, Q = Quadratic

Table 4: Effect of dietary treatments on digestibility and body weight change

	RUP (%)					Contrast*	
Items	30	35	40	45	SEM	L	Q
Digestibility (%)							
DM	67.80^{b}	68.90^{b}	71.90^{a}	69.90^{a}	0.65	0.02	0.06
NDF	56.20^{b}	57.50^{b}	61.90^{a}	57.50^{b}	0.75	0.05	0.01
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	0.94	0.04
Body weight change (kg/d)	0.41^{b}	0.49^{a}	0.52^{a}	0.42^{b}	0.02	0.40	0.00

^{a,b}Means within a row with different superscripts differ (p<0.05). SEM = Standard Error of the Mean, *Orthogonal polynomial contrast L = Linear, Q = Quadratic

Table 5: Effect of dietary treatments on rumen fermentation

	RUP (%)					Contrast*	
Items (hr-post-feeding)	30	35	40	45	SEM	L	Q
pH							
0	7.2^{a}	7.0^{b}	6.8^{c}	6.7^{c}	0.04	0.00	0.24
3	6.7^{a}	6.5^{b}	6.4^{b}	6.5^{b}	0.04	0.00	0.02
6	7.1^{a}	6.8^{b}	6.8^{b}	6.7^{c}	0.03	0.00	0.01
Mean	7.0^{a}	6.8^{b}	6.7^{b}	6.6^{b}	0.09	0.00	0.17
NH_3-N (mg%)							
0	11.2^{a}	9.7^{b}	8.4^{c}	9.1^{bc}	0.27	0.00	0.00
3	13.3^{a}	12.6^{b}	10.6^{c}	12.9^{ab}	0.20	0.00	0.00
6	12.4^{a}	10.6^{b}	9.5^{c}	11.1^{b}	0.18	0.00	0.00
Mean	12.3^{a}	11.0^{b}	9.5^{c}	11.0^{b}	0.65	0.00	0.00
TVFAs (mM/l)							
0	83.4^{d}	99.4^{c}	106.7^{a}	103.0^{b}	0.89	0.00	0.00
3	101.8^{c}	125.2^{ab}	125.9^{a}	122.6^{b}	0.98	0.00	0.00
6	94.8^{c}	114.9^{a}	117.4^{a}	110.6^{b}	0.85	0.00	0.00
Mean	94.3^{b}	113.2^{a}	116.7^{a}	112.1^{a}	4.57	0.00	0.00

^{a,b,c}Means within a row with different superscripts differ (p<0.05). *Orthogonal polynomial contrast L = linear, Q = quadratic

than by ammonia or lactate production (Stiles *et al*., 1970). Moreover, the correlation between rumen pH and VFA was 0.8. Ruminal ammonia-N concentration (12.3, 11.0, 9.5, 11.0 mg%) (Table 5) 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. In addition, decreases ammonia concentration were the results of a more efficient capture of N for microbial protein synthesis. Ruminal NH_3-N concentration at 0, 3 and 6 h post feeding are presented in Table 4. Ruminal NH_3-N concentration 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. 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 (Witt *et al*., 1999). Total VFA concentrations in the rumen (Table 5) (94.3,

Table 6: Effect of dietary treatments on microbial population and blood urea nitrogen

	RUP (%)					Contrast*	
Items	30	35	40	45	SEM	L	Q
Blood urea nitrogen (mg%) (hr-post-feeding)							
0	20.1[a]	19.5[a]	18.4[b]	18.3[b]	0.33	0.00	0.49
3	25.4[a]	23.5[b]	23.7[b]	24.7[ab]	0.77	0.40	0.02
6	21.8[a]	21.8[a]	19.1[c]	20.6[b]	0.31	0.00	0.03
Mean	22.4	21.6	20.4	21.2	1.22	0.13	0.25
bacteria (x 10^{10} cell/ml)	2.2	2.5	2.6	2.4	0.89	0.07	0.03
Protozoa (x 10^{5} cell/ml)	1.9[c]	2.1[ab]	2.2[a]	1.9[bc]	0.05	0.29	0.01

[a,b,c]Means within a row with different superscripts differ (p<0.05). *Orthogonal polynomial contrast L = Linear, Q = Quadratic

113.25, 116.7, 112.1 mM/l; p<0.01) 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. 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). Blood urea nitrogen concentrations are presented in Table 5. Blood urea nitrogen concentration at 3 h post feeding BUN increased quadratically (p<0.05) as the levels of RUP decreased and 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 (Hungate, 1966; Hobson, 1971).

Rumen microbe populations are presented in Table 6. Bacteria population [2.2, 2.5, 2.6, 2.4 (x 10^{10} cell/ml)] and protozoa population [1.9, 2.1, 2.2, 1.9 (x 10^{5} cell/ml)] were lowest (p<0.05) in 30% RUP. However, in 35% and 40% microbial populations were not differ. Moreover, these data showed that increase in bacteria and protozoa populations reflected increased in level of RUP from 30 to 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 (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*., 1989). 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, Jouaney and Ushida (1999) reported that ruminal protozoa growth depends on high rate of soluble sugars and starches in the ration.

Nitrogen balance study is shown in Table 7. 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/d; p>0.05) was not significantly different among dietary treatments. Nitrogen retention (20.9, 19.7, 31.0, 21.5 g/d) was highest (p<0.05) in 40% RUP. Nitrogen retention tended to increased as level of RUP increased. In addition, at 45% RUP N retention tended to decreased (quadratically, p = 0.1). The 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. Supplementing diets of relatively low digestible organic matter content with NPN with is useless, as the bacteria are unable to utilize all the

Table 7: Effect of dietary treatments on nitrogen balance

	RUP (%)					Contrast*	
Items	30	35	40	45	SEM	L	Q
Urine N (g/d)	51.0	50.7	41.9	48.1	2.23	0.13	0.20
Urine N (g/kgBW$^{0.75}$)	1.04	1.04	0.86	0.98	0.05	0.14	0.24
Feces N (g/d)	23.0^{b}	22.8^{b}	22.3^{b}	25.6^{a}	0.68	0.05	0.04
N absorption (g/d)	71.8	70.4	72.9	69.6	1.12	0.44	0.44
N retention (g/d)	20.9^{b}	19.7^{b}	31.0^{a}	21.5^{b}	2.23	0.23	0.11
N absorption (%N intake)	75.3^{ab}	75.4^{ab}	76.5^{a}	73.2^{b}	0.76	0.17	0.07
N retention (%N intake)	21.6^{b}	21.1^{b}	32.5^{a}	22.7^{b}	2.46	0.23	0.11

^{a,b}Means within a row with different superscripts differ ($p<0.05$). *Orthogonal polynomial contrast L = Linear, Q = Quadratic

ammonia released in the rumen, the excess being absorbed into the bloodstream, converted to urea and excreted in the urine.

Conclusion and recommendations: The results indicated that 40% RUP in concentrate can improve feed intake, body weight change, blood metabolites, rumen microbe populations, end-products of ruminal fermentation, digestibility and nitrogen balance. Therefore this study suggests that the diet contain 40% RUP has positive benefit on Thai Native x Brahman beef cattle productive performance.

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