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Effects of Dietary Protein on Ruminal Fermentation, Nitrogen Utilization and Crude Protein Maintenance in Growing Thai-indigenous Beef Cattle Fed Rice Straw as Roughage

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Abstract: Six male growing Thai-indigenous beef cattle with Body Weight (BW) of 154±13.18 kg were randomly assigned in replicated 3×3 Latin square design and fed with differences levels Crude Protein (CP) Total Mixed Ratio (TMR) diets. Crude Protein (CP) levels in diets were 4, 7% and 10% base on Dry Matter (DM), respectively. Dry Matter Intake (DMI) increased iinearly ($p<0.01$) with increasing crude protein concentration. There was not significantly different ($p>0.05$) digestibility of DM, Organic Matter (OM), Acid Detergent Fiber (ADF) and Neutral Detergent Fiber (NDF). Ruminal ammonia nitrogen (NH_3-N) concentration increased ($p<0.01$) with increasing CP level. Rumen pH, total VFA, acetate, propionate and butyrate were not affected by CP levels ($p>0.05$). Nitrogen (N) intake, urinary N excretion, N digestibility, N retention (g day^{-1}) and N retention (Percentage of N intake) increased linearly with increasing dietary CP levels ($p<0.05$) and was affected significantly ($p<0.01$) by diet of CP levels, respectively. However, the fecal N excretion was not affected ($p>0.05$) by dietary CP levels. The CP for maintenance requirement in growing Thai-indigenous beef cattle was 3.54 g kg^{-1} BW$^{0.75}$ which was estimated by using linear regression of N retention and N intake.

Key words: Thai-indigenous beef cattle, crude protein, maintenance, nitrogen retention, digestibility, ruminal ammonia

INTRODUCTION

Protein requirements of livestock are thought to be a function of many variables and not a specific figure for all conditions. There are varying protein requirements with different factors, such as breed, sex, body weight, daily gain, body condition, production functions, compensatory growth, environmental variation, forage availability and microbial yield effects (NRC, 1996). The Institute National de la Recherche Agronomique suggested that the maintenance protein requirement was 5.07 CP kg^{-1} BW$^{0.75}$ or 3.25 g MP kg^{-1} SBW$^{0.75}$. Wilkerson *et al.* (1993) and Susmel *et al.* (1994) recommended that the maintenance protein requirement was 5.94 g CP kg^{-1} BW$^{0.75}$ or 3.8 g MP kg^{-1} BW$^{0.75}$. Thai-indigenous beef cattle were small in frame and low in growth rate. Furthermore, Thailand is a tropical country with different climates and environment conditions from other countries. The nutrient requirements recommended by NRC and ARC are widely adopted to formulate diets around the world. Nevertheless, the nutrient requirement equations were based on Italic cattle but the nutrient requirements in growing Thai-indigenous bulls may not be the same as those recommended.

Rice straw was the most abundant and appropriate feed for cattle in tropical regions but it has poor protein, energy, minerals and vitamins contents. Protein supplementation with urea, cassava pulp and molasses can improve the utilization of low quality roughages through the supply of Nitrogen (N) to rumen microbes. The objective of this study was to determine the crude protein maintenance requirement and effects of protein supplementation of rice straw-based diets on the ruminal fermentation and nitrogen utilization for Thai-indigenous bull calve.

MATERIALS AND METHODS

Experimental animal, diet and experimental design: About Six growing Thai-indigenous beef cattle, averaging 154±13.68 kg of Body Weight (BW) and approximately 18 months of age were selected and housed in individual

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pens and then all of cattle were treated against anthelmintics and intestinal parasites with Ivermectin. The experimental cattle were assigned randomly in replicated 3×3 Latin square design. Two weeks before the experiment was the adjustment period. Each experimental period consisted of 21 days, of which 7 days was the sample collection period, 4 days was transition between each experimental periods.

The cattle were fed with 7.0% CP diet during adjustment and each transition period. After the 2-week adjustment period, the cattle were fed with Total Mixed Ratio (TMR) diets (T1-T3) which contained 4.3, 7.3 and 11.3% Crude Protein (CP), respectively with similar amounts of 20% above maintenance Metabolizable Energy (ME), the ingredients and chemical composition are shown in Table 1. TMR diet was fed twice per day at 0830 h and 1530 h, according to 2.5% of cattle BW. Refiusals were weighed and recorded daily prior to the morning feeding to determine daily Dry Matter Intake (DMI). Drinking water was freely available. The experimental cattle Body Weight (BW) was measured at both the beginning and the end of each period. In the last week of each experimental period in order to determinate digestibility and nitrogen balance, samples of orts, fecal and urine were collected before new feed was given each morning.

Sampling and sample analysis procedures: The samples of feed, ort and fecal were dried in a forced draught oven at 65°C for 48 h ground through 1 mm screen and analyzed for proximate principles (AOAC, 1990) and fiber analysis was determined by methods supported by Van Soest *et al.* (1991). Daily collection of urine of each animal was acidified with 20% H_2SO_4 to keep the final pH <3. It is essential to acidify the urine to prevent bacterial activity. About 5% of daily urine and fecal were collected according to daily urine volume and daily fecal weight and they were pooled respectively after sampling.

Table 1: Ingredients and chemical composition of experimental diets (Percentage, DM basis)

Ingredient (Percentage of base dry matter of diet)	Dietary crude protein levels		
	T1	T2	T3
Rice straw	79.70	79.20	79.20
Cassava pulp	14.90	13.50	7.90
Soy bean meal	0.00	3.40	9.60
Molasses	3.00	1.00	0.40
Urea	0.40	0.90	0.90
Dicalcium phosphate	1.00	1.00	1.00
Vitamin-mineral premix[1]	1.00	1.00	1.00
Total	100.00	100.00	100.00
Chemical composition			
DM	90.00	90.40	90.30
CP	4.30	7.30	10.30
Ash	15.90	15.90	15.60
NDF	59.50	59.10	58.00
ADF	40.30	40.10	39.30
Metabolizable energy (MJ kg^{-1})	7.94	7.93	7.93

[1] The premix contained per kilogram of DM: 4×10^6 IU Vit. A, 0.4×10^6 IU Vit D_3, 4000 IU Vitamin E, 24 g, Fe, 0.2 g Co, 2 g Cu, 10 g Zn, 0.5g I, 50 mg Se. T1 = 4.3% CP, T2 = 7.3% CP, T3 = 10.3% CP. DM = Dry Matter, NDF = Neutral Detergent Fiber, ADF = Acid Detergent Fiber

After that, a sample of 50 mL duplicate urine and a sample of 500 g duplicate fecal were taken and stored at -20°C for analysis. Rumen fluid was sampled at 0 and 4 h after feeding on the last day of each period. The rumen fluid sample was filtered by layers of cheesecloth. It was measured with a pH meter immediately and was acidified with 6NHCl and stored at -20°C for analysis of rumen ammonia N (NH_3-N) (Bremner and Keeney, 1965). Total Volatile Fatty Acids (VFA) was determined by GC (Hewlett-Packard GC system HP6890A; Hewlett-Packard Avondale, PA). Nitrogen requirement was estimated by using linear regression of nitrogen retained and nitrogen intake. Nitrogen balance equals nitrogen intake minus nitrogen lost. When nitrogen balance equals zero, nitrogen intake is the maintenance nitrogen requirement.

Statistics analysis: The GLM procedure (SAS, 1996) was used for statistical analysis of the data for the replicated 3×3 Latin square design. The model for Using Duncan's New Multiple Range Test and Orthogonal Analysis compares treatment means (SAS, 1996).

RESULTS AND DISCUSSION

Effects of CP levels on Dry Matter Intake (DMI) and digestibility: Effects of CP concentration on Dry Matter Intake (DMI) and apparent digestibility of Dry Matter (DM), Organic Matter (OM), ADF and NDF were shown in Table 2. DMI of Thai-indigenous bull calves in terms of kg per day (kg day^{-1}) and g per kilogram metabolic body weight (g kg^{-1} $w^{0.75}$) increased linearly with increasing crude protein concentration ($p<0.01$). DMI of 4% CP diet

Table 2: Effect of dietary level on DMI, digestibility of DM, OM, ADF and NDF

	Treatment				Contrast p-value	
Item	T1	T2	T3	SEM	Linear	Quadratic
DMI(kg day^{-1})	3.15^B	3.64^A	3.77^A	0.09	**	NS
DMI (g kg^{-1} $W^{0.75}$)	71.54^B	83.39^A	85.62^A	0.53	**	NS
Digestibility (%)						
DM	54.66	58.84	59.27	2.64	NS	NS
OM	60.87	64.39	64.95	3.42	NS	NS
ADF	46.25	50.05	50.90	4.29	NS	NS
NDF	55.76	59.22	59.48	3.12	NS	NS

$^{A-C}$Means in same row with different superscript letters differ ($p<0.05$); SEM = Standard Error of Means; NS = No Significantly different ($p>0.05$); *Means Significantly different ($p<0.05$); **Means Significantly different ($p<0.01$) DMI = Dry Matter Intake, DM = Dry Matter, OM = Organic Matter, NDF = Neutral Detergent Fiber, ADF = Acid Detergent Fiber, T1 = 4.3% CP, T2 = 7.3% CP, T3 = 10.3% CP

(3.15 kg day^{-1}) was <7% CP diet (3.64 kg day^{-1}) and 10% CP diet (3.77 kg day^{-1}) ($p<0.05$) but there was no significantly different between 7% CP diet and 10% CP diet ($p>0.05$). Similar, Paengkoum and Tatsapong (2009) used Thai native beef cattle to meet 5, 7, 9 and 11% CP diets demonstrated the DMI were not different ($p>0.05$) between 7, 9 and 11, 5% CP was lower ($p<0.05$) others protein levels. Also, this result agree with Perry *et al.* (1983) who indicated DMI were improved ($p<0.01$) with increasing protein level. But Devant *et al.* (2000), Basra *et al.* (2003), Yuangklang (2009); Chantiratikul *et al.* (2009) and Tatsapong *et al.* (2010) suggested that DMI for calves did not affect by different protein level diet.

There were not significantly different digestibility about DM, OM, ADF and NDF ($p>0.05$) but have a increased trendy with increasing CP level, similar result has been reported by Atkinson *et al.* (2007). Also, Devant *et al.* (2000) indicated digestibility about DM and OM were not affected by dietary protein concentration (14% CP vs. 17% CP) in growing crossbred heifers.

Effect of CP concentration on ruminal fermentation: Effects of CP concentration on rumen fermentation are shown in Table 3. Ruminal pH was not different ($p>0.05$) at 0, 4 h and mean pH between 4.3, 7.3 and 10.3% CP. The pH values at 0 h were 7.03, 7.02 and 6.93 and mean pH values were 7.07, 7.03 and 7.02, respectively. The values decreased with increasing CP level. Reed *et al.* (2007) demonstrated ruminal pH was not different between control and protein supplemented steers. However, numerous researchers (Heldt *et al.*, 1999; Mathis *et al.*, 2000) have indicated lower pH with increasing levels of DIP supplementation. Because changes in pH are a result of changes in ruminal fermentation, wide range level protein may affect ruminal fermentation.

Table 3: Effects of dietary CP levels on ruminal pH, ruminal NH_3-N and VFA

Item After-feeding (h)	Treatments T1 (4%)	T2 (7%)	T3 (10%)	SEM	Contrast p-value Linear	Quadratic
Ruminal pH						
0	7.03	7.02	6.93	0.49	NS	NS
4	7.10	7.05	7.11	0.61	NS	NS
Mean	7.07	7.03	7.02	0.58	NS	NS
Ruminal NH3-N (mg%)						
0	13.75^{B}	16.21^{B}	20.00^{A}	1.62	**	NS
4	12.45^{C}	15.65^{B}	19.54^{A}	1.47	**	NS
Mean	13.10^{B}	15.93^{B}	19.77^{A}	1.52	**	NS
Total VFA (mM L^{-1})						
0	63.27	70.88	74.42	1.45	NS	NS
4	63.25	64.03	65.85	0.47	NS	NS
Mean	63.26	68.45	69.85	9.16	NS	NS
Acetate(mol/100 mol)						
0	77.16	73.66	73.27	3.96	NS	NS
4	76.47	72.75	69.06	1.34	NS	NS
Mean	76.80	73.41	71.23	4.17	NS	NS
Propionate(mol/100 mol)					NS	NS
0	12.20	12.41	13.40	0.73	NS	NS
4	12.02	12.76	16.58	0.26	NS	NS
Mean	12.11	12.49	14.94	1.08	NS	NS
Butyrate(mol/100 mol)					NS	NS
0	10.64	13.94	13.33	0.32	NS	NS
4	11.52	14.49	14.36	0.41	NS	NS
Mean	11.09	14.10	13.83	0.41	NS	NS

$^{A-C}$Means in same row with different superscript letters differ ($p<0.05$); SEM = Standard Error of Means; NS = No Significantly different ($p>0.05$); *Means Significantly different ($p<0.05$); **Means Significantly different ($p<0.01$). T1 = 4.3% CP, T2 = 7.3% CP, T3 = 10.% CP

Ruminal ammonia N (NH_3-N) concentration (0, 4 h and mean value) increased with increasing levels of dietary protein from 4-10% CP ($p<0.01$) respectively. Similarly, a few researchers (Devant *et al.*, 2000; Arroquy *et al.*, 2004; Klevesahl *et al.*, 2003) suggested the concentration of ruminal NH3-N increased with increasing protein level.

Total Volatile Fatty Acid (VFA) concentration, molar proportions of acetate, propionate and butyrate were not affected by increased CP concentration at 0, 4 h and mean value ($p>0.05$). Similar result was reported by other studies (Reed *et al.*, 2007; Atkinson *et al.*, 2007). The dietary protein level from 4.3-7.3-10.3% CP, mean total VFA from 63.26-68.45-69.85, mean acetate from 61.52-65.72-63.95, mean propionate from 9.70-11.18-14.26 and butyrate from 8.88-12.62-13.20.

Paengkoum and Tatsapong (2009) used yearling Brahman x Thai native beef cattle to meet 6P, 9, 12 and 15% CP diets which indicated no difference in total VFA concentration between 9, 12 and 15% CP protein levels except that 6% CP was lower ($p<0.05$) than other protein levels. In addition, the present experiment confirmed the finding of Paengkoum and Tatsapong (2009) that molar proportions of acetate, propionate and butyrate are not affected by increased CP concentration.

Nitrogen utilization: Nitrogen utilization data are shown in Table 4. Effects of CP level on Nitrogen (N) intake, urinary N excretion, N digestibility, N retained (g day^{-1}) and N retained (Percentage of N intake) increased linearly with increasing dietary CP concentration ($p<0.01$)

Table 4: Effects of dietary CP levels on N utilization (%, DM basis)

Item	Treatment T1	T2	T3	SEM	Contrast P-value Linear	Quadratic
N-intake (g day^{-1})	22.21^{C}	43.86^{B}	63.06^{A}	1.96	**	NS
N-Fecal (g day^{-1})	17.59	19.02	19.18	1.62	NS	NS
N-Urinary (g day^{-1})	6.81^{C}	10.98^{B}	18.29^{A}	1.69	**	NS
N-Retaine d (g day^{-1})	-2.19^{C}	13.71^{B}	25.56^{A}	3.78	**	NS
N-digestibility (%)	21.31^{C}	56.18^{B}	69.67^{A}	4.38	**	NS
N-retained (Percentage of N-intake)	-9.65^{B}	31.04^{A}	34.60^{A}	9.59	**	NS

NS^{A-C} Means in same row with different superscript letters differ ($p<0.05$); SEM = Standard Error of Means; NS = No Significantly different ($p>0.05$); * Means Significantly different ($p<0.05$); ** Means Significantly different ($p<0.01$). T1 = 4.3% CP, T2 = 7.3% CP, T3 = 10.3% CP and were affected significantly by dietary CP levels, respectively ($p<0.01$). Similar results were reported by other studies (Devant *et al.*, 2000; Castillo *et al.*, 2001; Cole *et al.*, 2003; Reed *et al.*, 2007). The fecal N excretion was not affected by dietary CP concentration ($p>0.05$). This result agrees with Marini and van Amburgh (2003), Archibeque *et al.* (2001, 2002) and Reed *et al.* (2007) who observed no increase in fecal N excretion as N intake increased ($p>0.05$).

In this finding, the major effect of N excretion (g day^{-1}) was on urinary N output, the increase in dietary CP level increased urinary N excretion by 4.17 gN day^{-1} and 7.31 g N day^{-1}, respectively ($p<0.05$), In agreement with Sutton *et al.* (1998), Wright *et al.* (1998), Castillo *et al.* (2001), Cole *et al.* (2003) the most significant effect of protein conentration in the diet on N outputs was on urinary N excretion.

In contrast, Hunter and Siebert (1980) observed increases in fecal N excretion with increasing protein supplementation. There was no significantly different N retention (Percentage of N intake, $p>0.05$) between 7% CP diet and 10% CP diet but 4% CP diet was <7 and 10% CP. Castillo *et al.* (2001) indicated that N retention was not affected by level of CP concentration (210 g kg^{-1} DM and 290 g kg^{-1} DM). In this experiment, low N retention of 4% CP might be caused by inadequate RUP provision to help maintain the normal amount of ruminal microbial.

The crude protein requirement for Thai-indigenous beef cattle: Crude Protein for maintenance (CP_m) was determined by regression of crude protein retained on crude protein intake.

A significant linear relationship between crude protein retained and crude protein intake, the equation being $y = 0.6895x-2.4434$ ($r^2 = 0.88$, $p<0.0001$, Fig. 1). When crude protein retained was zero, the value of crude protein intake was crude protein maintenance, thus with the estimated equation, the value of maintenance of crude protein was computed to be 3.54 g CP kg^{-1} BW$^{0.75}$ which was lower than the crude protein requirement of *Bos Taurus* cattle suggested by NRC (1996) (5.94g CP kg^{-1} BW$^{0.75}$) and ARC (1980) for British breed (4.42 g CP kg^{-1} BW$^{0.75}$).

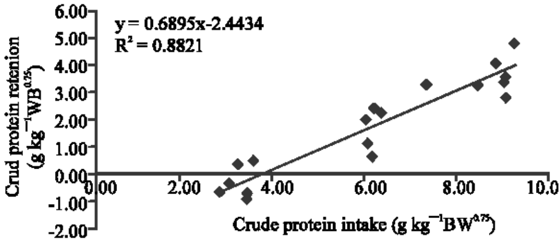

Fig. 1: The relationship between crude protein intake adjusted crude protein retenion

CONCLUSION

Digestibility of DM, OM, NDF and ADF was not affected ($p>0.05$) by dietary CP level. DMI increased linearly with increasing crude protein. Ruminal fermentation was not affected by dietary protein level except ruminal NH_3-N concentration increased strongly with increasing dietary CP level. N utilization was improved by increasing dietary concentration, the major effect of N excretion (g day^{-1}) was on urinary N output. The crude protein requirement for Thai-indigenous beef cattle is 3.54 g CP kg^{-1} BW$^{0.75}$ which is lower than recommended by NRC and ARC.

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