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Effects of Dietary Crude Protein Levels on Nutrient Digestibility, Ruminal Fermentation and Growth Rate in Thai-Indigenous Yearling Heifers

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Abstract: The objective of this research was to determine the effect of dietary crude protein levels on nutrient digestibility, ruminal fermentation and growth rate in Thai-indigenous yearling heifers. Sixteen Thai-indigenous yearling heifers, with an average initial body weight of 118±15.39 kg were assigned to one of 4 treatments according to a completely randomized design. Dietary treatments contained 4 levels of crude protein (6, 8, 10 and 12%). Diets were fed at the rate of 3% BW. The findings revealed that dietary crude protein levels did not significantly alter ($p>0.05$) DM and OM digestibility in yearling heifers. However, CP, NDF and ADF digestibility of heifers increased ($p<0.01$) with increasing dietary crude protein levels. Ammonia nitrogen, blood urea nitrogen and average daily gain linearly increased ($p<0.01$) with increasing dietary protein. Base on the results, it can be concluded that increases dietary crude protein level did improve nutrient digestibility, ruminal fermentation and growth rate of Thai-indigenous yearling heifers. Additionally, it has been suggested that level of dietary crude protein should be higher than 12% during accelerated Thai-indigenous yearling heifer growth periods.

Key words: Crude protein, nutrient digestibility, growth, ruminal fermentation, Thai-Indigenous heifers

INTRODUCTION

Dietary protein supply is one of major factors that influence the productivity of animals. High levels of protein feeding may be effective in promoting rapid live weight gains, especially in growing cattle. Although, the nutrient requirement of beef cattle has been established in many temperate countries, it can not be accurately applied for beef cattle in the tropics. There are variations between animals such as growth rate, stage of production and animal species (Church and Pond, 1982) and also between samples of a food (Chantiratikul *et al*., 2009). In NRC (1996), Crude Protein (CP) recommendations for calves under body weight of 250 kg exceed 16%. Hoffman *et al*. (1996) suggested that protein requirement of Holstein heifer should be >12%. On the other hand, Kearl (1982) suggested that CP requirement for growth of heifers (150 kg, 0.25 kg day^{-1} body weigh gained) in developing countries was 10%. Thai-indigenous cattle are smaller frame and lower growth rate than Brahman and temperate beef cattle. They, therefore, probably require the different levels of protein for growth, comparing to bigger frame cattle. Therefore, this experiment was to determine the effect of increasing dietary protein levels on nutrient digestibility, ruminal fermentation, blood urea nitrogen and growth rate. An attempt was made to suggest protein requirement of Thai-indigenous yearling heifer.

MATERIALS AND METHODS

Animals and experimental design: Sixteen Thai-indigenous yearling heifers an initial body weight of 118±15.39 kg were used. The animals were housed in individual stall and fed at 3.0% BW in 2 equal meals at 7.00 and 17.00 h. Drinking water was available at all time. The experiment was carried out at Mahasarakham Research and Technology Transfer Center, during 18 July to 18 October, 2008. Animals were randomly allocated to one of 4 dietary treatments in CRD design (4 heads $treatment^{-1}$). The dietary treatment contained 6, 8, 10 and 12% of crude protein with similar amount of

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2.5 Mcal ME kg^{-1} of DM. The diets were prepared in total mixed ration with rice straw as a roughage source. The animals were weighed at the beginning of the trial and every 4 weeks.

Data collection and sampling procedures: Rumen fluid samples were collected in the last day of experiment at 4 h post feeding by stomach tube connected with a vacuum pump. Ruminal pH was measured immediately after sampling using pH meter (Handy Lab 1, CG842 Schott). Rumen fluid samples were then filtered through 4 layers of cheesecloth. Fifty milliliter of rumen fluid was acidified with 5 mL of 6 N HCl and centrifuged at 16000 g for 15 min and the clear supernatant was stored in plastic tubes at -20°C prior to NH_3-N analysis using the micro Kjeldahl methods (AOAC, 1990). A blood sample (about 10 mL) was drawn from the jugular vein at the same time as rumen fluid sampling, separated by centrifugation at 5000 g for 10 min and stored at -20°C until analysis of Blood Urea Nitrogen (BUN) using the Stanbio Urea Nitrogen (Liqui-UV® procedure No. 2020, Stanbio Laboratory, Texas, USA).

Feeds were randomly collected and composite prior to analyses. Composites samples were ground to pass through a 1 mm screen and the analyzed for DM, ash and CP (AOAC, 1990) NDF, ADF, ADL (Van Soest *et al*., 1991) and Acid Insoluble Ash (AIA) (Van Keulen and Young, 1977).

Fecal samples were collected by rectal sampling at 10.00 h for 3 consecutive days and composites. The feces were placed into an oven at 65°C for 72 h, weighed and ground to pass through a 1 mm screen and the analyzed for DM, ash CP, NDF, ADF and AIA. The chemical composition of feed and feces were estimated for nutrient digestibility using AIA as internal marker (Schneider and Flatt, 1975).

Statistical analyses: The experimental data was subjected to the General Linear Models (GLM) procedure for orthogonal polynomial contrast analysis of SAS (1996) according to a Completely Randomized Design (CRD). Significance was shown at $p<0.05$ unless otherwise noted.

RESULTS AND DISCUSSION

Chemical composition of dietary treatment: The chemical compositions of dietary treatments are presented in Table 1. All diets had similar chemical composition, but difference in crude protein levels. Additionally, the RUP (CP%) remained constant among dietary treatments.

Feed intake of yearling heifer was fixed rate at 3% BW. Thus, crude protein intake expressed as g day^{-1} and g/g/kg$BW^{0.75}$ linearly increased as increased protein levels (Table 2).

Table 1: Ingredients and chemical composition of dietary treatments

	Dietary crude protein levels (DM%)			
Ingredient	6.0	8.0	10.0	12.0
Rice straw	30.0	30.0	30.0	30.0
Cassava chip	45.6	35.9	32.2	29.2
Rice bran	14.2	20.9	19.6	16.7
Molasses	5.0	5.0	5.0	5.0
Soybean meal	2.0	5.0	10.0	15.9
Urea	0.5	0.5	0.5	0.5
Sulfur	0.2	0.2	0.2	0.2
Dicalcuim phosphate	1.5	1.5	1.5	1.5
Salt (NaCl)	0.5	0.5	0.5	0.5
Mineral premix	0.5	0.5	0.5	0.5
Total	100.0	100.0	100.0	100.0
Analyzed chemical composition				
Dry matter (%)	90.2	90.6	90.3	90.6
Ash (%)	10.3	10.8	10.5	10.7
Crude protein (%)	6.6	8.6	10.8	13.6
RUP* (CP%)	39.6	39.0	37.6	37.4
Neutral detergent fiber (%)	66.8	62.1	60.0	61.8
Acid detergent fiber (%)	24.9	23.9	26.7	28.8
Acid detergent lignin (%)	9.6	9.2	7.5	8.5
Total digestible nutrient* (%)	67.0	66.9	67.2	67.7
Metabolizable energy* (Mcal kg^{-1})	2.5	2.5	2.5	2.5

*Calculated value; RUP = Rumen Undegradable Protein

Nutrient digestibility: Nutrient digestibilities are shown in Table 2. Dry mater and organic matter digestibilities were not significant different ($p>0.05$) among treatments. The results are in agreement with previous studies in Thai-Indigenous heifer (Chantiratikul *et al*., 2009), growing-finishing Brahman cattle (Yuangklang, 2008), Thai native and Brahman crossbred (Paengkoum and Yanee, 2008) and Friesian crossbred heifers (Devant *et al*., 2000) weaned Holstein calves (Veira *et al*., 1980) that observed DM and OM digestibility were not affected by dietary crude protein concentrations. However, these observations did not agree with those of Kawashima *et al*. (2003) and Paengkoum and Tatsapong (2008), who reported that DM and OM digestibility increased with increasing dietary protein concentration. Digestibility of CP, NDF and ADF linearly increased ($p<0.01$) as increased protein levels. This result implies that the animal have high nutrient uptake when fed high dietary crude protein level. Hoffman *et al*. (2001) and Veira *et al*. (1980) also reported that increasing dietary protein levels resulted in significant increases in NDF and ADF digestibilities. However, this observation do not agree with those of Kawashima *et al*. (2003) and Chantiratikul *et al*. (2009), who reported that NDF and ADF digestibility were not markedly affected by dietary protein levels. The crude protein digestibility was similar to reports by Chantiratikul *et al*. (2009), Kawashima *et al*. (2003) and Yuangklang (2008). Many factors may influence nutrient digestibility such as protein levels in the ration (Kawashima *et al*., 2003), protein source and nature of protein source providing the rumen undegradable protein (Milis and Liamadis, 2007), protein fraction

Table 2: Effect of dietary crude protein levels on protein intake and nutrient digestibility

	Dietary crude protein levels (%)					Contrast		
Parameters	6.0	8.0	10.0	12.0	SEM	L	Q	C
Feed intake (kg day^{-1})	3.6	3.7	3.8	3.9	-	-	-	-
BW (%)	3.0	3.0	3.0	3.0	-	-	-	-
$BW^{0.75}$ (g kg^{-1})	97.8	99.0	100.0	101.0	-	-	-	-
Crude protein intake (g day^{-1})	237.2	314.0	406.3	531.0	30.5	0.003	ns	ns
$BW^{0.75}$ (g kg^{-1})	6.4	8.5	10.8	13.8	0.7	0.001	ns	ns
Nutrient digestibility (%)								
DMD	74.1	71.8	74.3	75.1	0.8	ns	ns	ns
OMD	78.7	77.7	79.2	79.6	0.8	ns	ns	ns
CPD	70.2	71.9	78.3	85.2	1.8	0.001	ns	ns
NDFD	71.4	72.1	74.2	79.6	1.7	0.01	ns	ns
ADFD	55.8	59.6	60.9	62.0	1.8	0.01	ns	ns

SEM = Standard Error of the Means; DMD = Dry Matter Digestibility; OMD = Organic Matter Digestibility; CPD = Crude Protein Digestibility; NDFD = Neutral Detergent Fiber Digestibility; ADFD = Acid Detergent Fiber Digestibility; ns = not significantly different ($p>0.05$); L, Q, C = Linear, Quadratic and Cubic effects of difference crude protein levels

Table 3: Effect of dietary crude protein levels on ruminal fermentation, blood urea nitrogen and ADG

	Dietary crude protein levels (%)					Contrast		
Parameters	6.0	8.0	10.0	12.0	SEM	L	Q	C
Ruminal fermentation (pH)	7.00	6.70	6.80	6.80	0.10	ns	ns	ns
NH_3-N	4.70	6.00	8.10	7.50	0.50	0.008	ns	ns
TVFAs (mM)	89.10	105.90	100.70	104.30	0.30	ns	ns	ns
Acetic (%)	70.00	55.90	62.70	48.80	2.76	0.01	ns	0.04
Propionic (%)	22.40	29.00	24.50	32.20	1.94	ns	ns	ns
Butyric (%)	7.60	15.10	12.80	19.00	1.84	0.05	ns	ns
A:P	3.47	1.94	3.02	1.58	0.30	ns	ns	ns
BUN	1.80	4.00	7.50	10.50	1.00	0.002	ns	ns
ADG	0.13	0.12	0.16	0.20	0.01	0.01	ns	ns

NH_3N = ammonia nitrogen; TVFAs = Total Volatile Fatty Acid; BUN = Blood Urea Nitrogen; ADG = Average Daily Gain; SEM = Standard Error of the Means; ns = not significantly different ($p>0.05$); L, Q, C = Linear, Quadratic and Cubic effects of difference crude protein levels

(Chumpawadee and Pimpa, 2008), RUP:RDP ratio, animal condition, breed, sex, non structural carbohydrate (Chantiratikul *et al*., 2009) and proportion of lignified cell walls (Chumpawadee and Pimpa, 2008).

Ruminal fermentation and blood urea nitrogen: Concentrations of NH_3-N, Volatile Fatty Acid (VFA) and pH in rumen fluid were used to monitor the ruminal fermentation pattern (Table 3). Ruminal pH did not differ significantly at any level of crude protein. This finding was similar to those reported by other researchers (Chantiratikul *et al*., 2009; Paengkoum and Tatsapong, 2008). The pH values were relatively stable at 6.7-7.0 and all treatment means were within the normal range, which has been reported as optimal for microbial digestion of protein (6.0-7.0) (Hoover, 1986) and fiber digestion (Theodorou and France, 1993). Generally, rate and extent of carbohydrates degradation are influenced ruminal pH (Nocek and Russell, 1988). The large amount of soluble carbohydrate may reduce the pH of rumen fluid and this can affect the rate of fermentation of structural carbohydrate (Sotton and Alderman, 2000). In addition, ruminal pH was partly regulated by the NH_3-N concentration and VFA concentration in the rumen (Stokes *et al*., 1991). In spite of the ammonia nitrogen in the rumen linearly, increased as increased protein levels (Table 3), but it did not alter ruminal pH. It was possibly, the buffering capacity can maintained the ruminal pH.

Ammonia nitrogen in the rumen fluid and blood urea nitrogen linearly ($p<0.01$) increased with increasing dietary protein concentrations. The results are in agreement with previous studies in Thai-indigenous steers (Paengkoum and Tatsapong, 2008), growing-finishing Brahman cattle (Yuangklang, 2008), Thai native and Brahman crossbred (Paengkoum and Yanee, 2008) weaned Holstein calves (Veira *et al*., 1980) that observed NH_3-N increased with increasing dietary crude protein concentrations. However, these observations do not agree with those of Chantiratikul *et al*. (2009), who reported that NH_3-N was not affected by dietary protein concentration. The difference in NH_3-N concentrations among treatments may have been related directly to dietary protein concentration and degradability of protein in the diet. There was highly correlated between BUN and NH_3-N concentration in the rumen (Church, 1972). Thus, animals fed high dietary protein was also high BUN (Table 3). It was possibly protein degradation is more rapidly than synthesis or imbalance of fermentable energy

and nitrogen available, thus, ammonia will be accumulated in the rumen liquor, absorbed in to the blood, carried to the liver converted to urea and excreted via urine (Kebreab *et al*., 2002). This finding, was similar to Veira *et al*. (1980), Bal and Yarar (2006), Cole *et al*. (2006) Bailay *et al*. (2008), Yuangklang (2008) and Chantiratikul *et al*. (2009).

Total volatile fatty acid concentrations were not significantly different among treatment ($p>0.05$). The results are in agreement with previous studies in weaned Holstein calves (Veira *et al*., 1980) that observed TVFAs were not affected by dietary crude protein concentrations. However, acetic concentrations was cubic response ($p = 0.05$) with increasing dietary protein concentrations. In addition, butyric acid was increased linearly ($p<0.01$) with increasing dietary protein concentration, but propionic and proportion of acetic and propionic were not significantly different. In contrast, Paengkoum and Yanee (2008), who reported that TVFAs, acetic and propionic concentration were increased linearly ($p<0.01$) with increasing dietary protein concentration, but butyric, Iso-butyric and valeric acids were not significantly different. Normally, rate and extent of VFAs production were influenced by carbohydrate fraction (Chumpawadee and Pimpa, 2009) and degradability of carbohydrate (Cheng *et al*., 1991). The incidence of this experiment might have been associated with high dietary protein promoted microbial activity and leading to improved fiber digestibility (Table 2).

Average daily gain: Average daily gain linearly increased ($p<0.01$) with increasing dietary protein concentration (Table 3). The results are in agreement with previous studies in Holstein heifers and steers (Bal and Yarar, 2006) finishing beef steers (Cole *et al*., 2006). In contrast, Bailay *et al*. (2008), who reported that average daily gain was quadratic increased with increasing dietary protein concentration. Additionally, Pasinato *et al*. (2008) reported that dietary protein concentration did not alter average daily gain in Holstein steers. The incidence of this experiment might have been associated with protein intake and nutrient digestibility (Table 3). When the animals were fed high dietary protein and improved nutrient digestibility, leading to high nutrient uptake and promoted average daily gain.

CONCLUSION

Increasing dietary protein significantly increased CP, NDF and ADF digestibility and improved ruminal fermentation and growth rate in Thai-indigenous yearling heifers. Base on this study, it has been suggested that level of crude protein should be >12% during accelerated Thai indigenous yearling heifer growth periods.

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