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Effect of Dietary Protein on Nutrient Digestibility and Nitrogen Metabolism in Thai-Indigenous Heifers

[1]Anut Chantiratikul, [1]Songsak Chumpawadee, [2]Wanna Kanchanamayoon and [2]Piyanete Chantiratikul
[1]Faculty of Veterinary and Animal Sciences, Mahasarakham University, Maung, Maha Sarakham, 44000, Thailand
[2]Department of Chemistry, Center of Excellence for Innovation in Chemistry (PERCH-CIC), Faculty of Science, Mahasarakham University, Kantarawichai, Maha Sarakham, 44150, Thailand

Abstract: Four Thai-indigenous heifers, average 132±11.71 kg of body weight, were used to determine effect of dietary protein levels on nutrient digestibility and nitrogen utilization using a 4×4 Latin square design with 21d period. Heifers were fed diet containing 6, 9, 12 and 15% of CP. The findings revealed that dietary protein levels did not significantly alter ($p>0.05$) DM, NDF and ADF digestibilities in heifers. However, CP digestibility of heifers markedly increased ($p<0.05$) with increasing dietary protein levels. N intake, N fecal and urinary N excretion, N digestion and N retention significantly increased ($p<0.05$) when dietary protein levels increased. N retentions of heifers fed diet containing 6, 9, 12 and 15% CP were 11.53, 18.64, 24.20 and 31.08 g day^{-1}, respectively. Based on the results, it could be concluded that dietary protein influenced significantly ($p<0.05$) CP digestibility and N utilization in Thai-indigenous heifers. Furthermore, the results demonstrated that protein requirement for maintenance of Thai-indigenous heifers was lower than 6% of dietary CP.

Key words: Dietary protein, nutrient digestibility, nitrogen utilization, Thai-indigenous heifers

INTRODUCTION

Although, the nutrient requirement of beef cattle has been established in many temperate countries, it cannot be accurately applied for beef cattle in the tropics. There are variations between animals and also between samples of a food. In NRC (1996) Crude Protein (CP) recommendations for calves under body weight of 250 kg exceed 16%. On the other hand, Kearl (1982) suggested that CP requirement for maintenance of crossbred heifers in the tropics was 8%. Thai-indigenous cattle of the family of *Bos indicus* are smaller frame and lower growth rate than Brahman and temperate beef cattle. However, the indigenous cattle are generally kept as draught animals mainly in rice field and considered to be adapted well to extensive grazing and to have an ability to utilize low quality feed. They therefore, required the lower protein for maintenance than Brahman cattle (Kawashima *et al*., 2003). Currently, there is an insufficient information concerning effect of protein on nutrient digestibility and Nitrogen (N) metabolism in Thai-indigenous cattle. Most of the above data obtained from male Thai-indigenous cattle. Kawashima *et al*. (2003), Paengkoum and Tatsapong (2008) and Senarath *et al*. (2008) reported that CP digestibility, N intake, N retention and N excretion of male Thai-indigenous cattle increased dramatically ($p<0.05$) with increasing dietary CP. Thus, to establish the protein requirement for Thai-indigenous heifers, the optimal dietary protein level needs to be certified and studied. Therefore, the objective of present study was to determine effect of dietary protein on nutrient digestibility and N utilization in Thai-indigenous heifers.

MATERIALS AND METHODS

Four-Thai-indigenous yearling heifers an initial body weight of 132±11.71 kg were used. The animals were dewormed using Ivomectin and injected with AD_3E vitamin-complex prior to the beginning of the experiment. They were housed in individual stall and fed at 2.0% of body weight daily at 0800 and 1600 h. Drinking water was available at all time.

Animals were randomly allocated to one of 4 dietary treatments in a 4×4 Latin Square Design with 21 day periods. Each period consisted of 14 day adaptation and 7 day test. Dietary treatments contained 6, 9, 12 and 15%

Corresponding Author: Wanna Kanchanamayoon, Department of Chemistry, Center of Excellence for Innovation in Chemistry (PERCH-CIC), Faculty of Science, Mahasarakham University, Kantarawichai, Maha Sarakham, 44150, Thailand

Table 1: Ingredients and chemical composition of dietary treatments

	Dietary CP (%DM)			
Ingredients	6.00	9.00	12.00	15.00
Rice straw	30.00	30.00	30.00	30.00
Cassava chip	56.00	46.50	39.00	32.00
Rice bran	2.70	6.30	7.20	7.40
Soybean meal, 44% CP	3.10	9.00	15.60	22.40
Urea	0.50	0.50	0.50	0.50
Molasses	5.00	5.00	5.00	5.00
Sulfur	0.20	0.20	0.20	0.20
Dicalcium phosphate	1.50	1.50	1.50	1.50
Salt	0.50	0.50	0.50	0.50
Premix[1]	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00
Chemical composition (%DM)				
Dry matter	86.43	87.91	87.07	87.97
Organic matter	88.78	88.59	90.20	88.29
Crude protein	5.80	8.86	11.45	14.56
RUP[2],% of CP	29.49	30.02	30.27	30.43
Neutral detergent fiber	27.38	28.61	30.47	26.65
Acid detergent fiber	20.99	22.02	22.87	19.82
Ca	0.67	0.68	0.71	0.73
P	0.38	0.42	0.47	0.51
ME[3,4] (Mcal kg^{-1} DM)	1.99	1.98	1.97	1.96

[1]The premix provided per kilogram of diet: 10,000 IU vitamin A; 2,000 IU vitamin D_3; 20 IU vitamin E; 0.01 g Cu; 0.08 g Mn; 0.04 g Zn; 0.05 g Fe; 0.0008 g I; 0.0003 g Co; 0.0003 g Se; 0.005 g Ethoxiquin; and 0.05 g SiO_2. [2]RUP = Rumen Undegradable Protein. [3]Calculated values. [4]ME = Metabolisable Energy

of CP (Table 1). The diets were prepared in total mixed ration with rice straw as a roughage source and formulated to be isocaloric diets according to NRC (1996).

Feed intake was recorded daily throughout the experiment, however only the intake during the 7 day test period was analyzed. During 7 day collection period, feed were weighed, collected daily and composite by animal for chemical analysis. Simultaneously, feces of each animal was weighed daily, thoroughly mixed and a 5% subsample was taken, stored at 4°C and later pooled at the end of each period. Feed and fecal samples were dried at 60°C and ground through 1 mm screen for determination of DM, CP (AOAC, 1990), ADF and NDF (Van Soest *et al*., 1991). The urinary sample was collected via adapted urinary cap and kept into plastic container pre-added with 250 mL of 10% H_2SO_4 (v/v). Acidified urine was kept at -20°C for N determination by macro-Kjeldahl method (AOAC, 1990). The chemical composition of feed, feces and urine were used to estimate nutrient digestibility (Schneider and Flatt, 1975) and N utilization (McDonald *et al*., 1995), respectively.

Rumen fluid and blood samples were taken at the end of collection period. Rumen fluid was sampled using esophageal tube. The sample was immediately measured for pH, filtered through a muslin cloth and rumen fluid was then acidified 10% H_2SO_4 (v/v) and frozen at -20°C. Subsequently, the samples of rumen fluid were analyzed for NH_3-N by stem distillation and titration using the macro-Kjeldahl procedure. Blood was drawn from jugular vein. Plasma was harvested by centrifugation of the whole blood for 15 min at 3000 g and kept at -20°C for Blood Urea Nitrogen (BUN) analysis using Stanboi Urea Nitrogen Liqui-UV®, procedure no. 2020 (Stanbio Laboratory, Texas, USA).

Statistical analyzes: The data were analyzed using the general linear models procedure of Statistical Analysis System Institute SAS (1988) according to the following statistical model:

$$Y_{ijk} = \mu + A_i + P_j + D_k + e_{ijk}$$

Where, A, P and D are animal, period and diet effects, respectively. The differences among means were compared by Least Significant Different (Steel and Torries, 1980). Significance was declared at $p<0.05$.

RESULTS AND DISCUSSION

Crude protein concentrations in dietary treatments were 5.80, 8.86, 11.45 and 14.56% of DM. However, the rest chemical compositions and RUP (% of CP) remained constant among dietary treatments (Table 1).

Feed intake of heifers was not affected by increasing dietary protein content. Dietary protein levels did not dramatically alter ($p>0.05$) DM, NDF and ADF digestibilities in heifers (Table 2). The results are in agreement with previous studies in growing-finishing Brahman cattle (Yuangklang, 2008) and in Friesian crossbred heifers (Devant *et al*., 2000) that observed DM, ADF and NDF digestibilities were not markedly affected by dietary protein concentrations. However Veira *et al*. (1980) and Hoffman *et al*. (2001), found feeding male Holstein calves or heifers increasing amounts of dietary CP resulted in significant increases in DM, ADF and NDF digestibilities. Additionally, Kawashima *et al*. (2003) and Paengkoum and Tatsapong (2008) observed DM and OM digestibilities increased ($p<0.05$), but NDF and ADF digestibilities ($p>0.05$) in male Thai-indigenous cattle were not modified with increment of dietary CP. The different reported results regarding effects of dietary CP on nutrient digestibility were probably due to multiple factors such as animal condition, breed, sex, dietary CP level, Rumen Undegradable Protein (RUP): Rumen Degradable Protein (RDP) ratio and balance of RDP and nonstructural carbohydrate. Therefore, the further researches are needed to certify the above effect in Thai-indigenous heifers. Crude protein digestibility of heifers significantly increased ($p<0.05$) with increasing dietary protein levels (Table 2). Similarly, the previous studies in *Bos indicus* cattle (Kawashima *et al*., 2003; Paengkoum and Tatsapong, 2008; Yuangklang, 2008) found the CP digestibility differed ($p<0.05$) accordingly to the levels of

Table 2: Effect of dietary protein on feed intake and nutrient digestibility (% of intake) in Thai-indigenous heifers

	Dietary CP (%DM)				
Items	6.00	9.00	12.00	15.00	SEM
Total feed intake					
Kg d^{-1}	2.75	2.67	2.76	2.66	0.07
BW (%)	1.85	1.85	1.87	1.86	0.01
g $(BWkg^{0.75})^{-1}$	64.51	64.13	65.01	64.08	0.50
Digestibility					
Dry matter	75.54	75.18	75.83	75.76	0.23
Crude protein	62.04[d]	75.62[c]	76.35[b]	81.88[a]	2.03
NDF[1]	63.55	57.97	55.32	53.74	1.58
ADF[1]	61.05	54.25	51.59	49.72	1.68

[a-d]Means on the same row with different superscripts are significantly different ($p<0.05$). [1]NDF = Neutral Detergent Fiber, ADF = Acid Detergent Fiber

Table 3: Effect of dietary protein on nitrogen balance in Thai-indigenous heifers

	Dietary CP (%DM)				
Items	6.00	9.00	12.00	15.00	SEM
N intake (g d^{-1})	28.17[d]	40.42[c]	54.55[b]	66.27[a]	3.94
N feces (g d^{-1})	8.64[d]	9.88[c]	12.07[a]	13.82[a]	0.77
N urine (g d^{-1})	8.00[d]	11.91[c]	18.27[b]	21.37[a]	1.67
N digested (g d^{-1})	19.53[d]	30.54[c]	42.54[b]	52.45[a]	3.44
N retained (g d^{-1})	11.53[d]	18.64[c]	24.20[b]	31.08[a]	2.72
N retained (%)	39.55[b]	46.01[a]	43.35[b]	45.83[a]	3.16
N retained g $(Wkg^{0.75})^{-1}$	0.18[d]	0.29[c]	0.37[b]	0.49[a]	0.03

[a-d]Means on the same row with different superscripts are significantly different ($p<0.05$)

Table 4: Effect of dietary protein on rumen pH, Blood Urea Nitrogen (BUN) and ammonia-N in Thai-indigenous heifers

	Dietary CP (%DM)				
Item	6.00	9.00	12.00	15.00	SEM
pH	6.66	6.46	6.58	6.68	0.11
BUN (mg dL^{-1})	2.81[a]	8.67[b]	9.52[ab]	12.38[a]	1.19
Ammonia-N (mg dL^{-1})	6.55	6.68	6.81	6.78	0.57

[a-d]Means on the same row with different superscripts are significantly different ($p<0.05$)

dietary CP content. However, Hoffman *et al*. (2001) found the highest nutrient digestibility occurring when heifers at 398±9.4 kg of BW were fed diet containing 13% of CP. They concluded that feeding 400-kg heifers diets containing 13% CP optimized heifer nutrient utilization. On the other hand, the current obtained results of nutrient digestibility could not be used to identify the optimum dietary CP for Thai-indigenous heifers.

N intake, N fecal and urinary N excretion, N digestion and N retention markedly increased ($p<0.05$) when dietary protein levels increased (Table 2). The outcomes are in accordance with the previous reports in male Thai-indigenous cattle (Kawashima *et al*., 2003; Paengkoum and Tatsapong, 2008), in yearling Brahman steers (Yuangklang, 2008) and in Holstein heifers (Gabler and Heinrichs, 2003). The present findings revealed that heifers consumed diet containing 6-15% of CP retained 11.53-31.08 gN day^{-1}. Theoretically, the lowest dietary protein level that promotes a nitrogen balance close to zero is the maintenance level (McDonald *et al*., 1995). Thai-indigenous heifers consumed daily 28.17 gN or 176.06 g CP retained 11.53 gN day^{-1} (Table 3). The current results demonstrated that protein requirement for maintenance of Thai-indigenous heifer was lower than 176.06 gCP day^{-1} or 6% of dietary CP. This value of Thai-indigenous heifers was comparable to the recommended protein requirement for maintenance of tropical crossbred heifers (178 gCP day^{-1}) at 100 kg of BW proposed by Kearl (1982). Furthermore, protein requirements for maintenance of male Thai-indigenous cattle were previously found to be lower than 6.1% of dietary CP (Kawashima *et al*., 2003) or ranged from 5-7% of dietary CP (Paengkoum and Tatsapong, 2008). Based on the present and foregoing results, protein requirement for maintenance of both male and female Thai-indigenous cattle was not much different.

Ruminal pHs of heifers were similar ($p>0.05$) among dietary treatments (Table 4). The ruminal pH of heifers fed all diets was within the optimum pH range to maintain normal ruminal fiber digestion (Theodorou and France, 1993). The microbial activities would be seriously inhibited when ruminal pH declined below pH 6.2 (Ørskov, 1992; Russell *et al*., 1992). Blood urea nitrogen (2.81-12.38 mg dL^{-1}) significantly increased ($p<0.05$), but ammonia-N (6.55-6.78 mg dL^{-1}) of heifers was not affected by increasing dietary CP concentrations (Table 4). This incidence perhaps associated with an imbalance of fermentable energy and N available for microbial metabolism, as a result BUN increased. Additionally, the BUN is related closely to CP intake in beef heifers (Hall *et al*., 1995). However, the values of ammonia-N in all diets were above 5 mgN dL^{-1} (McDonald *et al*., 1995) which required to support maximum growth rate of rumen bacteria for the optimal rumen fermentation and the maximize OM digestion in the rumen (Satter and Slyter, 1974; NRC, 1988). The present results demonstrate that the optimal microbial fermentation was not significantly affected by dietary CP levels.

CONCLUSION

Increasing dietary protein significantly increased ($p<0.05$) CP digestibility and modified N utilization ($p<0.05$) in Thai-indigenous heifers. Protein requirement for maintenance of Thai-indigenous heifers was lower than 176.06 gCP day^{-1} or 6% of dietary CP.

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