

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 linearly ($p < 0.01$) 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 ($\text{NH}_3\text{-N}$) 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 $3.54 \text{ g kg}^{-1} \text{ BW}^{0.75}$ 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 $5.07 \text{ CP kg}^{-1} \text{ BW}^{0.75}$ or $3.25 \text{ g MP kg}^{-1} \text{ SBW}^{0.75}$. Wilkerson *et al.* (1993) and Susmel *et al.* (1994) recommended that the maintenance protein requirement was $5.94 \text{ g CP kg}^{-1} \text{ BW}^{0.75}$ or $3.8 \text{ g MP kg}^{-1} \text{ BW}^{0.75}$. 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 calves.

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

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. Refusals 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% H₂SO₄ 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.

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 (NH₃-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 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

¹The premix contained per kilogram of DM: 4×10⁶ IU Vit. A, 0.4×10⁶ IU Vit D₃, 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

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

Item	Treatment				Contrast p-value	
	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

Table 3: Effects of dietary CP levels on ruminal pH, ruminal NH₃-N and VFA

Item	Treatments			SEM	Contrast p-value	
	T1 (4%)	T2 (7%)	T3 (10%)		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)						
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)						
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 = 10.3% CP

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.

Ruminal ammonia N (NH₃-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			SEM	Contrast P-value	
	T1	T2	T3		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-Retained 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

^{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 concentration 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 $\text{g CP kg}^{-1} \text{BW}^{0.75}$ which was lower than the crude protein requirement of *Bos Taurus* cattle suggested by NRC (1996) (5.94 $\text{g CP kg}^{-1} \text{BW}^{0.75}$) and ARC (1980) for British breed (4.42 $\text{g CP kg}^{-1} \text{BW}^{0.75}$).

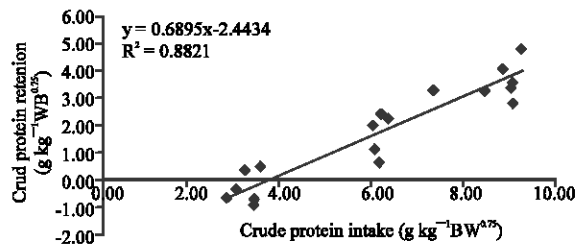


Fig. 1: The relationship between crude protein intake adjusted crude protein retention

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 $\text{NH}_3\text{-N}$ 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 $\text{g CP kg}^{-1} \text{BW}^{0.75}$ which is lower than recommended by NRC and ARC.

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REFERENCES

- AOAC., 1990. Official Methods of Analysis of Association of Analytical Chemistry. 15th Edn., AOAC., Arlington, Virginia, pp: 1298.
- ARC, 1980. The Nutrition Requirement of Rument Livestock. CAB International, Farnham Royal, UK.
- Archibeque, S.L., J.C. Burns and G.B. Huntington, 2001. Urea flux in beef steers: Effects of forage species and nitrogen fertilization. *J. Anim. Sci.*, 79: 1937-1943.
- Archibeque, S.L., J.C. Burns and G.B. Huntington, 2002. Nitrogen metabolism of beef steers fed endophyte-free tall fescue hay: Effects of ruminally protected methionine supplementation. *J. Anim. Sci.*, 80: 1344-1351.
- Arroquy, J.I., R.C. Cochran, M. Villarreal, T.A. Wickersham and D.A. Llewellyn *et al.*, 2004. Effect of level of rumen degradable protein and type of supplemental non-fiber carbohydrate on intake and digestion of low-quality grass hay by beef cattle. *Anim. Feed Sci. Technol.*, 115: 83-99.
- Atkinson, R.L., C.D. Toone, T.J. Robinson, D.L. Harmon and P.A. Ludden, 2007. Effects of supplemental ruminally degradable protein versus increasing amounts of supplemental ruminally undegradable protein on nitrogen retention, apparent digestibility and nutrient flux across visceral tissues in lambs fed low-quality forage. *J. Anim. Sci.*, 85: 3331-3339.
- Basra, M.J., M. Nisa, M.A. Khan, M. Riaz, N.A. Tuqeer and M.N. Saeed, 2003. *Nili-Ravi* buffalo II. Energy and protein requirements of 9-12 months old calves. *Int. J. Agric. Biol.*, 5: 380-381.

- Bremner, J.M. and D.R. Keeney, 1965. Steam distillation methods of determination of ammonia, nitrate and nitrite. *Anal. Chem. Acta*, 32: 485-495.
- Castillo, A.R., E. Kebreab, D.E. Beever, J.H. Barbi, J.D. Sutton, H.C. Kirby and J. France, 2001. The effect of protein supplementation on nitrogen utilization in lactating dairy cows fed grass silage diets. *J. Anim. Sci.*, 79: 247-253.
- Chantiratikul, A., S. Chumpawadee, W. Kanchanamayoon and P. Chantiratikul, 2009. Effect of dietary protein on nutrient digestibility and nitrogen Metabolism in Thai-indigenous Heifers. *J. Anim. Vet. Adv.*, 8: 297-300.
- Cole, N.A., L.W. Greene, F.T. McCollum, T. Montgomery and K. McBride, 2003. Influence of oscillating dietary crude protein concentration on performance, acid-base balance and nitrogen excretion of steers. *J. Anim. Sci.*, 81: 2660-2668.
- Devant, M., A. Ferret J. Gasa, S. Calsaminglia and R. Casals, 2000. Effects of protein concentration and degradability on performance, ruminal fermentation and degradability on performance, ruminal fermentation and nitrogen metabolism in rapidly growing heifers fed high-concentrate diets from 100-230 kg body weight. *J. Anim. Sci.*, 78: 1667-1676.
- Heldt, J.S., R.C. Cochran, C.P. Mathis, B.C. Woods and K.C. Olson *et al.*, 1999. Effects of level and source of carbohydrate and level of degradable protein on intake and digestion of low-quality tallgrass-prairie hay by beef steers. *J. Anim. Sci.*, 77: 2846-2854.
- Hunter, R.A. and B.D. Siebert, 1980. The utilization of spear grass (*Heteropogon contortus*). IV. The nature and flow of digesta in cattle fed on spear grass alone and with protein or nitrogen or sulfur. *Aust. J. Agric. Res.*, 31: 1037-1047.
- Klevesahl, E.A., R.C. Cochran, E.C. Titgemeyer, T.A. Wickersham, C.G. Farmer, J.I. Arroquy and D.E. Johnson, 2003. Effect of a wide range in the ratio of supplemental rumen degradable protein to starch on utilization of low-quality, grass hay by beef steers. *Anim. Feed Sci. Technol.*, 105: 5-20.
- Marini, J.C. and M.E. van Amburgh, 2003. Nitrogen metabolism and recycling in Holstein heifers. *J. Anim. Sci.*, 81: 545-552.
- Mathis, C.P., R.C. Cochran, J.S. Heldt, B.C. Woods and I.E. Abdelgadir *et al.*, 2000. Effects of supplemental degradable intake protein on utilization of medium- to low-quality forages. *J. Anim. Sci.*, 78: 224-232.
- NRC. (National Research Council), 1996. Nutrient Requirements of Beef Cattle. 7th Edn., National Academy Press, Washington, DC., USA.
- Paengkoum, P. and P. Tatsapong, 2009. Effect of Different Levels of Protein on Feed Intake, Digestibility and Growth Rate of Thai Native Beef Cattle Fed Pangola Grass as Roughages. In: Establishment of a Feeding Standard of Beef Cattle and a Feed Database for the Indochinese Peninsula, Oshio, S., M. Otsuka and K. Sommart (Eds.). JIRCAS., Tsukuba, Ibaraki, Japan, pp: 76-78.
- Perry, T.W., D.R. Shields, W.J. Dunn and M.T. Mohler, 1983. Protein levels and monensin for growing and finishing steeres. *J. Anim. Sci.*, 57: 1067-1075.
- Reed, J.J., G.P. Lardy, M.L. Bauer, T.C. Gilbery and J.S. Caton, 2007. Effect of undegradable intake protein supplementation on intake, digestion, microbial efficiency, *in situ* disappearance and plasma hormones and metabolites in steers fed low-quality grass hay. *J. Anim. Sci.*, 85: 1092-1101.
- SAS, 1996. SAS User's Guide: Statistics. 6th Edn., SAS Institute, Cary, NC.
- Susmel, P., M. Spanghero, B. Stefano, C.R. Mills and E. Plazzota, 1994. Digestibility and allantion excretion in cows fed diets differing in nitrogen content. *Livestock Prod. Sci.*, 39: 97-99.
- Sutton, J.D., S.B. Cammell, D.E. Beever, D.J. Humphries and R.H. Phipps, 1998. Energy and nitrogen balance of lactating dairy cows given mixtures of urea-treated whole-crop wheat and grass silage. *Anim. Sci.*, 67: 203-212.
- Tatsapong, P., P. Paengkoum, O. Pimpa and M.D. Hare, 2010. Effects of dietary protein on nitrogen metabolism and protein requirements for maintenance of growing Thai swamp buffalo (*Bubalus bubalis*) calves. *J. Anim. Vet. Adv.*, 9: 1216-1222.
- Van Soest, P.J., J.B. Robertson and B.A. Lewis, 1991. Methods for dietary fiber, neutral detergent fiber and non starch polysaccharides in relation to animal nutrition. *J. Dairy Sci.*, 74: 3583-3597.
- Wilkerson, V.A., T.J. Klopfenstein, R.A. Britton, R.A. Stock and P.S. Miller, 1993. Metabolizable protein and amino acid requirements of growing beef cattle. *J. Anim. Sci.*, 71: 2777-2784.
- Wright, T.C., S. Moscardini, P.H. Luimes and B.W. McBride, 1998. Effects of rumen-undegradable protein and feed intake on nitrogen balance and milk protein production in dairy cows. *J. Dairy Sci.*, 81: 784-793.
- Yuangklang, C., 2009. Protein Requirement for Growing Finishing Brahman Cattle. In: Establishment of a Feeding Standard of Beef Cattle and a Feed Database for the Indochinese Peninsula, Oshio, S., M. Otsuka and K. Sommart (Eds.). JIRCAS, Tsukba, Ibaraki, Japan, pp: 79-82.