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Effects of Dietary Protein on Nitrogen Metabolism and Protein Requirements for Maintenance of Growing Thai Swamp Buffalo (*Bubalus bubalis*) Calves

[1]P. Tatsapong, [1]P. Peangkoum, [2]O. Pimpa and [3]M.D. Hare
[1]Institute of Agricultural Technology, Suranaree University of Technology, Nakhon Ratchasima, 30000, Thailand
[2]Faculty of Technology and Management, Price of Songkla University, Surat Thani Campus, Surat Thani, 84100, Thailand
[3]Faculty of Agriculture, Ubon Ratchathani University, Ubon Ratchathani, 34190, Thailand

Abstract: The experiment was conducted to investigate the effect of dietary crude protein on nitrogen utilization, nutrient digestibility and protein requirement for maintenance of swamp buffalo calves. Four growing swamp male buffaloes with 12-18 months old (average initial weight 209±17 kg) were used. The calves were assigned to use a 4×4 Latin square design to receive four diets. In terms of treatment, Crude Protein (CP) levels in the diets were 5, 7, 9 and 11% of Dry Matter (DM) and all diets were isocaloric (20% above maintenance of ME). CP intake and CP digestibility of calves clearly increased ($p<0.05$) with increasing CP content in diet. However, increasing the levels of dietary protein did not significantly alter ($p>0.05$) DM intake, digestibility of DM, organic matter, neutral detergent fiber and acid detergent fiber, body weight change and ruminal pH in calves. As the level of CP in diet increased, blood urea nitrogen, ruminal ammonia N, total volatile fatty acid, urinary N, N retention and N balance were observed to increase linearly ($p<0.05$). While N fecal was not significantly different ($p>0.05$), when dietary protein levels increased. The relationship between N Balance (NB) and N intake (NI, g kg^{-1} $W^{0.75}$) in swamp buffalo calves was NB = 0.883NI-0.653 (R^2 = 0.855). Present findings suggested that nitrogen requirements for maintenance of growing Thai swamp buffalo calves were 0.74 g N or 4.63 g CP kg^{-1} $BW^{0.75}$.

Key words: Swamp buffalo, buffalo calves, *Bubalus bubalis*, protein requirement, N balance, protein for maintenance

INTRODUCTION

In order to meet their production potential, ruminants have to consume the required amounts of nutrients from the diet. The nutrition of buffalo male calves is important as it plays a role in the onset of puberty in calves raised for breeding and it influences the quantity and quality of the meat produced by the calves. Subsequently, dietary protein supply is one of the factors that influence the productivity of animals. Feeding high levels of protein may be effective in promoting rapid live weight gains, especially growing buffalo (Basra *et al*., 2003b). Currently, there is an insufficient information concerning the effect of protein on nutrient digestibility and nitrogen metabolism in Thai swamp buffalo. Chantiratikul *et al*. (2009) and Chumpawadee *et al*. (2009) found that CP digestibility and N retention of Thai indigenous heifers increased with increasing dietary CP levels. Study of nutritional requirements of buffalo is necessary because of the NRC (1996, 2001) standard suggested for beef or dairy cattle. Although, the nutrition requirement of buffalo has been determined by Kearl (1982), it can not be accurately applied for swamp buffalo. Basra *et al*. (2003b) reported that lower protein requirements for *Nii ravi* buffalo male calves than cattle calves, while the CP requirements for growth may be the same as suggested for Holstein Friesian calves (Basra *et al*., 2003a).

On the other hand, the digestible CP requirement for maintenance of buffalo was 2.54 g kg^{-1} $BW^{0.75}$ day (Kearl, 1982), the Metabolisable Protein (MP) requirement for maintenance was 4.03-6.3 g kg^{-1} $BW^{0.75}$ day for growing *Nii ravi* buffalo at 125-400 kg (Paul and Patil, 2007). It has been indicated that the optimum fattening performance of 15 months old *Nii ravi* buffalo male calves may be obtained by providing 10.22% CP (Mahmoudzadeh *et al*., 2007) and 12% CP for 11-12 months old (Tipu *et al*., 2009). The nutrition needs of the buffalo probably differ from type of breeds found

Corresponding Author: P. Peangkoum, Institute of Agricultural Technology, Suranaree University of Technology, Nakhon Ratchasima, 30000, Thailand

in temperate countries because of difference in genetic make-up, mature body size, growth rate, composition of body tissue, quality of feed and climatic conditions (Kearl, 1982).

Therefore, research on the requirement of nutrients for Thai swamp buffalo does not exist and adequate information on the nutritional requirements of growing buffalo male calves is lacking. Hence, this experiment was conducted to determine the effect of increasing dietary protein levels on nitrogen utilization, nitrogen balance and protein requirements for maintenance of growing Thai swamp buffalo calves.

MATERIALS AND METHODS

Animals, diet and experimental design: Four growing male swamp buffalo (*Bubalus bubalis*) calves (12-18 months of age and 209±17 kg average body weight) were randomly assigned into a 4×4 Latin square design to receive four levels of crude protein (5, 7, 9 and 11% CP) in the diets.

The energy content of diets were formulated to contain a metabolisable energy intake of 20% above Maintenance (M) as determined by a review literature of the M = 1.62 Mcal kg^{-1} DM (Kearl, 1982). All animals were fed rice straw as roughage, cassava pulp and soybean meal as an energy and protein source according to the respective treatments. In order to increase the palatability of the diet, molasses was added. All concentrate diets were mixed daily and fed twice daily at 08:00 h in the morning and 16:00 h in the evening. During the experiment period, the animals were kept in well ventilated shed and concrete floor with individual feeding and watering arrangement. The animals were given 14 days for adaptation to diets at the start of each period, followed by 7 days collection period and 7 days for rest period. For the rest period, all animals were fed *ad libitum* of rice straw and 1.5% BW of concentrate (12% CP). Animals were fasted to measure N-retention on the last 4 days of the experiment.

Data collection and sampling procedures: Feed offered and orts were weighed and recorded daily and sampled every 3 days and the composites prior to chemical composition analysis. The feces and urine were collected daily from day 3-7 of collection period. The daily feces was weighed and mixed well and a 10% sub sample was taken and stored frozen. At the end of each collection period the daily fecal samples were bulked for each animal. Ten percent of each mixed well-bulked sample was taken for calculation of digestibility of DM, OM, CP, NDF and ADF. Daily collection of urine were acidified with 25% H_2SO_4 to kept the final pH of the urine below 3 and then weighed and it was sampled the same fecal samples for determination of urine nitrogen. Feed, orts and fecal sample were dried at 60°C and analyzed for proximate principles (AOAC, 1990) and fibre analysis was determined by methods supported by Van Soest *et al.* (1991).

At the end of each collection period, the rumen fluid was taken (200 mL) from the middle part of the rumen by a stomach tube connected with a vacuum pump at 0 and 4 h after morning feed. Ruminal pH was measured immediately after sampling using a potable pH meter and then, samples were filtered through nylon bag. Five milliliter of 1 M H_2SO_4 was added to 50 mL of rumen fluidto terminate fermentation. The mixture was centrifuged at 3,500 rpm for 15 min and the clear supernatant was stored in plastic bottle at -20°C until analyzed for ruminal ammonia nitrogen (NH_3-N) (Bremner and Keeney, 1965) and Volatile Fatty Acids (VFA) concentrations were determined by GC (Hewlett-Packard GC system HP6890 A; Hewlett-Packard Avondale, PA).

Blood samples were collected at the same time rumen fluid sampling for determine Blood Urea Nitrogen (BUN) concentration. The blood was drawn from the jugular vein into tube (10 mL for sample) and centrifuged at 3,500 rpm for 15-20 min and serum was separated and stored frozen at -20°C until analyzed for BUN (Crocker, 1967).

Statistical analysis: Nitrogen requirement for maintenance were estimated by determining the average Nitrogen Balance (NB) of buffaloes fed different levels of protein. In order to estimate dietary N requirement for maintenance, the NB and N intake were inserted into regression equation: Nitrogen balance = N balance index x (N intake) - N loss at zero N intake, where N requirement (Nm) for maintenance equals to N intake, when N balance is zero.

The data were analyzed as a 4×4 Latin Square Design using the GLM procedure of SAS (1996). To treatment means comparisons were conducted by Duncan's New Multiple Rang Test using Proc GLM (SAS, 1996).

RESULTS AND DISCUSSION

Chemical composition of dietary treatments: Chemical composition of dietary treatment and ingredients feed used in the experiment are shown in Table 1. All diet treatments had similar chemical composition but difference in crude protein levels. Crude protein concentrations in dietary treatments were 5.1, 7.09, 9.12 and 11.16% of DM and Metabolisable Energy (ME) concentrations in all diets were 2.14 Mcal kg^{-1} DM. However, CP and ME remained constant among dietary treatments.

Table 1: Ingredients and chemical composition of dietary treatments

	Dietary crude protein levels (%)			
Ingredients	5	7	9	11
Rice straw	66.22	65.48	65.39	65.78
Cassava pulp	26.04	22.50	18.18	13.38
Soybean meal	4.26	8.56	12.94	17.32
Molasses	3.37	3.36	3.38	3.40
Premix[1]	0.11	0.11	0.11	0.11
Total	100.00	100.00	100.00	100.00
Chemical composition (DM %)[2]				
Dry Matter (DM, kg)	3.90	3.91	3.88	3.85
Organic Matter (OM)	87.30	87.69	87.55	87.36
Crude Protein (CP)	5.10	7.09	9.12	11.16
Neutral Detergent Fibre (NDF)	56.78	56.16	55.91	55.92
Acids Detergent Fibre (ADF)	40.58	39.87	39.42	39.11
ME (Mcal kg^{-1} DM)	2.13	2.14	2.14	2.14

[1]The premix provided per kilogram of DM: 10,000 IU vitamin; 2,000 IU vitamin D_3; 20 IU vitamin E; 10 mg Cu; 80 mg Mn; 40 mg Zn; 50 mg Fe; 0.8 mg I; 0.3 mg Se; 0.3 mg Co; [2]Calculated

Live-weight change, intake and digestibility: Average body weight and body weight change of buffalo calves did not significantly different among treatment (Table 2). However, the animals lost weight, when they were fed low dietary protein during the experiment. In this way, they received 20% above maintenance of ME. The unbalance in nutrient intake to compare with minimum requirement of buffalo calves for growth (Kearl, 1982) may explain the weight loss.

Total DM intake of the calves, in terms of kg per day (kg day^{-1}) and g per kilograms metabolic weight (g kg^{-1} $W^{0.75}$) were not affected by increasing dietary protein content (Table 2). Similarly, Basra *et al.* (2003a), Yuangklang (2009) and Chantiratikul *et al.* (2009) demonstrated that daily DM intake for calves fed different CP diet did not significantly. Average of DM intake in calves was 3.86 kg $calf^{-1}$ day or 68 g kg^{-1} $W^{0.75}$day. Therefore, the daily CP intake in calves, in terms of g day^{-1} and (g kg^{-1} $W^{0.75}$) were increased ($p<0.05$) with increasing CP levels in diet. The CP intake (g kg^{-1} $W^{0.75}$) was 3.47, 4.85, 6.15 and 7.55 with ratios 5, 7, 9 and 11% CP, respectively, where as digestible CP intake was 0.97, 2.26, 3.40 and 5.02 (g kg^{-1} $W^{0.75}$), respectively (Table 2).

This result are in agreement with previous studies in growing *Nii ravi* buffalo (Basra *et al.*, 2003a, b), in growing indigenous heifers (Chantiratikul *et al.*, 2009; Chumpawadee *et al.*, 2009), in Korean black goats (Hwangbo *et al.*, 2009) and in yearling indigenous Thai native cattle (Paengkoum and Tatsapong, 2009) that observed CP or nitrogen intake was sharply affected by dietary protein concentration.

Digestibility of CP of calves shows in Table 2, it was higher >50% in comparison to the 5 and 11% CP in diet. CP digestibility was significantly higher in increasing CP content in diets (Paengkoum and Tatsapong, 2009; Chantiratikul *et al.*, 2009; Chumpawadee *et al.*, 2009). Dietary protein levels did not considerably alter ($p>0.05$) DM, OM, NDF, ADF and TDN digestibility in animals (Table 2). This result similarly, the previous research in Thai-indigenous heifers (Chantiratikul *et al.*, 2009) that observed DM, NDF and ADF digestibilities were not affected by dietary protein content. However, Chumpawadee *et al.* (2009) reported that increasing dietary CP indicated in significant increase in NDF and ADF digestibilities but DM and OM digestibilities did not different in yearling heifers. In additionally, Paengkoum and Tatsapong (2009) found that DM and OM digestibilities increased but NDF and ADF digestibilities did not differed following to the levels of dietary protein content. The different suggested that results regarding effects of dietary protein on nutrient digestibility were possibly due to multiple factors such as animal condition, breed, sex, dietary CP levels and balance of dietary protein and available carbohydrate (Mcdonald *et al.*, 1995). Javaid *et al.* (2008) demonstrated that increasing the levels of dietary Ruminally Degradable Protein (RDP) resulted in a linear decreased in crude protein and neutral detergent fiber digestibility but increase in dry matter digestibility in *Nii ravi* buffalo bulls.

Blood Urea Nitrogen (BUN) and ruminal fermentation: As shown in Table 3, BUN, concentration of NH_3-N, Volatile Fatty Acid (VFA) and pH in rumen fluid were presented to monitor the ruminal fermentation pattern and valves measured at 0 and 4 h post feeding. Ruminal pH was similar among diet and the values were quite stable at 7.0-7.1. This finding was similar previous reviewed (Chantiratikul *et al.*, 2009; Peangkoum and Tatsapong, 2009; Chumpawadee *et al.*, 2009) who explained that the ruminal pH was not affected by increasing dietary protein. Nisa *et al.* (2006) and Khan *et al.* (2006) reported that ruminal pH values at 12 h after feeding of buffaloes fed 12% CP in the diet were ranged 7.0-7.2. However, ruminal pHs of all calves were in the normal ranged (6.7-7.0) which has been found as optimal for microbial digestion of protein (Wanapat, 1999). Normally, rate and extent of carbohydrates digestion are influenced ruminal pH which in the large amount of soluble carbohydrates may reduce the ruminal pH. Based on this study the animals were received 20% above maintenance of energy. Moreover, increasing dietary RDP due to reduced ruminal pH and increased ruminal NH_3-N (Javaid *et al.*, 2008). In spite of the ammonia nitrogen in the rumen linearly, increased with increasing protein content (Table 3) but it did not alter ruminal pH. It was probably, the buffering capacity can maintained the ruminal pH.

Table 2: Effect of dietary protein on nutrients intake, digestibility and body weight change of swamp buffalo calves

	Dietary crude protein levels (%)					Contrast		
Items	5	7	9	11	SEM	L	Q	C
BW change (kg day^{-1})	0.04	-0.01	-0.13	0.23	0.12	NS	NS	NS
DMI (kg day^{-1})	3.84	3.89	3.85	3.84	0.04	NS	NS	NS
DMI (g kg^{-1} W $^{0.75}$)	67.85	68.34	67.65	67.54	0.31	NS	NS	NS
CPI (g kg^{-1} DM)	51.1^{d}	71.01^{c}	90.94^{b}	111.78^{a}	0.20	**	NS	NS
CPI (g kg^{-1} W $^{0.75}$)	3.47^{d}	4.85^{c}	6.15^{b}	7.55^{a}	0.04	**	NS	NS
DCPI (g kg^{-1} W $^{0.75}$)	0.97^{d}	2.26^{c}	3.40^{b}	5.02^{a}	0.13	**	NS	NS
Apparent digestibilities (%)								
DM	55.88	55.30	53.25	57.06	2.15	NS	NS	NS
OM	64.33	63.89	62.46	65.67	1.77	NS	NS	NS
CP	27.82^{d}	46.49^{c}	55.11^{b}	66.50^{a}	1.95	**	NS	NS
NDF	47.51	46.03	43.68	49.30	2.48	NS	NS	NS
ADF	42.89	40.89	37.24	41.88	2.90	NS	NS	NS
TDN	93.84	94.10	93.32	93.60	0.74	NS	NS	NS

$^{a-d}$Values on the same row under each main effect with different superscript differ significantly (p<0.05); BW = Body Weight; DMI = Dry Matter Intake; CPI = Crude Protein Intake; DM = Dry Matter; OM = Organic Matter; CP = Crude Protein; ADF = Acid Detergent Fiber; NDF = Neutral Detergent Fiber; TDN = Total Digestibility Nutrient; SEM = Standard Error of Means; NS = Not Significantly different (p>0.05); L, Q, C = Linear, Quadratic and Cubic effects of difference crude protein levels; **Significantly different (p<0.01)

Table 3: Effects of dietary protein on ruminal pH, ruminal ammonia nitrogen (NH_3-N, mg %), blood urea nitrogen (BUN, mg %) and Volatile Fatty Acid (VFA) of swamp buffalo calves

	Dietary crude protein levels (%)					Contrast		
Items	5	7	9	11	SEM	L	Q	C
h-post-feeding								
Ruminal pH								
0	7.03^{b}	7.33^{a}	7.18^{ab}	7.28^{a}	0.05	*	NS	*
4	7.05	6.88	7.12	7.10	0.10	NS	NS	NS
Mean	7.04	7.10	7.15	7.18	0.05	NS	NS	NS
Ruminal NH3-N (mg %)								
0	12.20^{c}	16.25^{b}	19.36^{a}	19.52^{a}	0.33	**	**	NS
4	11.77^{c}	14.56^{bc}	17.03^{ab}	18.42^{a}	1.07	**	NS	NS
Mean	11.98^{c}	15.41^{b}	18.20^{a}	18.97^{ab}	0.62	**	NS	NS
Blood urea nitrogen (mg %)								
0	12.63^{c}	18.56^{bc}	23.81^{ab}	27.31^{a}	2.42	**	NS	NS
4	12.39^{c}	21.13^{b}	22.67^{ab}	29.03^{a}	2.16	**	NS	NS
Mean	12.51^{b}	19.85^{ab}	23.24^{a}	28.17^{a}	2.25	**	NS	NS
Total volatile fatty acids (mM L^{-1})								
0	66.98	65.13	66.15	68.82	1.52	NS	NS	NS
4	64.12^{b}	67.11^{b}	70.50^{ab}	75.95^{a}	2.02	**	NS	NS
Mean	65.55^{b}	66.12^{b}	68.32^{b}	72.39^{a}	0.84	**	NS	NS
Acetate (mol/100 mol)								
0	69.28^{b}	71.23^{ab}	71.08^{ab}	73.30^{a}	0.89	*	NS	NS
4	70.74	69.99	67.69	68.46	0.93	NS	NS	NS
Mean	70.01	69.61	69.39	70.88	0.77	NS	NS	NS
Propionate (mol/100 mol)								
0	21.87	20.57	21.89	20.23	0.80	NS	NS	NS
4	21.43	22.34	22.52	22.49	0.68	NS	NS	NS
Mean	21.65	21.45	22.21	21.36	0.66	NS	NS	NS
Butyrate (mol/100 mol)								
0	8.85^{a}	8.20^{ab}	7.04^{bc}	6.47^{c}	0.48	**	NS	NS
4	7.83^{b}	9.68^{a}	9.79^{a}	9.05^{ab}	0.44	NS	*	NS
Mean	8.34	8.94	8.42	7.76	0.40	NS	NS	NS
C_2:C_3	3.24	3.26	3.13	3.33	0.13	NS	NS	NS

$^{a-d}$Values on the same row under each main effect with different superscript differ significantly (p<0.05); SEM = Standard Error of Means; NS = Not Significantly different (p>0.05); L, Q, C = Linear, Quadratic and Cubic effects of difference crude protein levels; *Significantly different (p<0.05) ; **Significantly different (p<0.01)

Ruminal NH_3-N concentration were also different (p<0.05) at each hour of sampling. It has been demonstrated that increased protein intake increased ammonia nitrogen concentration in rumen. In the study, values of ruminal NH_3-N ranged from 12-19 mg dL^{-1} and its normal range was considered optimum in swamp buffaloes (Wanapat and Pimpa, 1999). Furthermore, the increases in rumen NH_3-N levels also indicated in increasing levels of BUN (Javaid *et al*., 2008; Wanapat and Pimpa, 1999) and the values were linearly increased as levels of dietary protein increased in the diets (Table 3). Average BUN were 12.5, 19.8, 23.2

and 26.5% mg in calves fed diets 5, 7, 9 and 11% CP, respectively. The result are in agreement with previous research in Thai-indigenous yearling heifers (Chumpawadee *et al.*, 2009), Thai-indigenous steers (Paengkoum and Tatsapong, 2009), growing finishing Brahman cattle (Yuangklang, 2009) and Thai native and Brahman crossbred (Paengkoum and Yanee, 2009) found that increased BUN was associated with the elevation of NH_3-N in the rumen. Therefore, the ruminal NH_3-N increased, when protein intake increased may have been related directly to protein degradation is more rapid than synthesis, high dietary RDP (Javaid *et al.*, 2008; Sultan *et al.*, 2009) or imbalance of fermentable energy, so ammonia will accumulate in rumen fluid and the optimum concentration will be exceeded. Thus, ammonia is absorbed into the blood and also excreted via urine in to high levels of urine nitrogen (Javaid *et al.*, 2008; Yuangklang, 2009; Chantiratikul *et al.*, 2009).

Total Volatile Fatty Acids (TVFAs) concentrations in rumen fluid were significantly different among treatment ($p<0.05$), which ranged from 65-72 mM L^{-1}. The values increased linearly ($p<0.01$) as a consequence of additional CP in the diet. This results similarly reported by Paengkoum and Yanee (2009), who study in yearling Brahman x Thai native beef cattle. In addition, acetic acid was increased linearly with increasing protein levels in diet that observed before feeding, while butyric acid was decreased, when protein increased. However, propionic acid and proportion of acetic and propionic were not affected by dietary protein concentrations. The results are in agreement with previous observed in Thai-Indigenous heifers (Chumpawadee *et al.*, 2009). Generally, rate and extent of volatile fatty acids production were influenced by carbohydrate fraction and degradability of carbohydrate (Mcdonald *et al.*, 1995).

N metabolism: The N intake, N balance, N excretion in urine and N retention were increased significantly ($p<0.05$) when dietary protein levels increased (Table 4). Similarly results were suggested in male Thai native beef cattle (Paengkoum and Tatsapong, 2009; Sereethai *et al.*, 2009), in growing finishing Brahman cattle (Yuangklang, 2009) and in Thai-indigenous heifers (Chantiratikul *et al.*, 2009). The present study found that nitrogen balance ranged 0.03-0.3 g N kg^{-1} $W^{0.75}$ and N retained ranged 0.54-0.86 g N kg^{-1} $W^{0.75}$, when the calves consumed dietary protein from 5-11% CP of DM. There was linear increase ($p<0.05$) in urinary N (6.93, 11.70, 22.93 and 28.52 g day^{-1}) by increasing (5, 7, 9 and 11% CP) protein content in the diets (Table 4). However, there was no significant difference in fecal N among diets, it can be explained that endogenous loss from digestive tract may be not different in each animal (Kearl, 1982). It has been reported that N excretion through urine was increased due to CP diet and N intake (Pimpa *et al.*, 2009; Mehra *et al.*, 2006) and also directly to relate RDP ratio in dietary protein (Sultan *et al.*, 2009), high RDP ratio in dietary protein were increased urine N but decreased N balance (Javaid *et al.*, 2008). Normally, when protein degradation is more rapid than synthesis, so ammonia will accumulate in rumen liquor and is absorbed into the blood carried to the liver and converted to urea and it was excreted via to urine N (Mcdonald *et al.*, 1995). The values of N balance were regressed linearly for the determination of dietary nitrogen requirement for maintenance (Fig. 1). The regression equation between N balance and N intake of calves is N balance (g N kg^{-1} $W^{0.75}$) = 0.883N intake - 0.653 (R^2 = 0.855). This equation can be used to estimate N requirement for maintenance, the N intake at which N balance equal to zero were 0.74 g N kg^{-1} $W^{0.75}$ day. Consequently, nitrogen requirements for maintenance for growing swamp buffalo calves are 0.74 g N kg^{-1} $W^{0.75}$ or equivalent to 4.63 g CP kg^{-1} $W^{0.75}$ day or as approximately 6% of dietary crude protein. These finding were in an agreement with previous reported in yearling Thai native cattle (4.36 g CP kg^{-1} $W^{0.75}$ day) by Sereethai *et al.* (2009), in Thai southern

Table 4: Effect of dietary protein on N metabolites of swamp buffalo calves

	Dietary srude protein levels (%)					Contrast		
Items	5	7	9	11	SEM	L	Q	C
N intake (g day^{-1})	31.40^{d}	44.16^{c}	56.04^{b}	68.71^{a}	0.75	**	NS	NS
N intake (g kg^{-1} $W^{0.75}$)	0.55^{d}	0.78^{c}	0.98^{b}	1.21^{a}	0.006	**	NS	NS
Urine N (g day^{-1})	6.93^{b}	11.70^{b}	22.93^{a}	28.52^{a}	1.76	**	NS	NS
Urine N (% of N intake)	22.00^{b}	26.56^{b}	40.83^{a}	41.21^{a}	2.85	**	NS	NS
Fecal N (g day^{-1})	22.71	23.58	25.09	23.12	0.94	NS	NS	NS
Fecal N (% of N intake)	72.18^{a}	53.51^{b}	44.89^{c}	33.50^{d}	1.95	**	NS	NS
N balance (g day^{-1})	1.76^{c}	8.87^{b}	8.02^{b}	17.06^{a}	1.36	**	NS	*
N balance (g kg^{-1} $W^{0.75}$)	0.03^{d}	0.16^{b}	0.14^{b}	0.31^{a}	0.03	**	NS	*
N endogenous (g $kg^{-1}$$W^{0.75}$)	0.56	0.55	0.56	0.56	0.004	NS	NS	NS
N retention (g day^{-1})	30.62^{c}	40.28^{b}	39.43^{b}	48.47^{a}	1.57	**	NS	*
N retention (g kg^{-1} $W^{0.75}$)	0.54^{c}	0.71^{b}	0.70^{b}	0.86^{a}	0.03	**	NS	*

[a-d]Values on the same row under each main effect with different superscript differ significantly ($p<0.05$); SEM = Standard Error of Means; NS = Not Significantly different ($p>0.05$); L, Q, C = Linear, Quadratic and Cubic effects of difference crude protein levels; *Significantly different ($p<0.05$); **Significantly different ($p<0.01$)

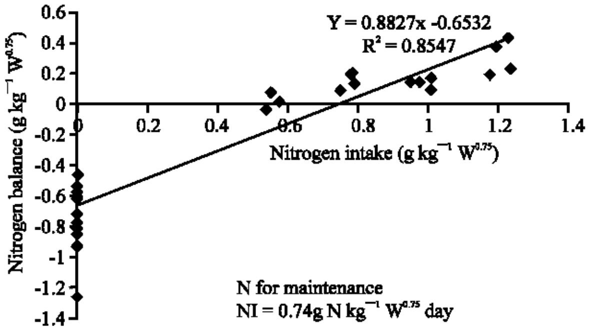

Fig. 1: Relationship between N balance and N intake ($g\ kg^{-1}\ W^{0.75}$) in swamp buffalo calves

native cattle (0.17-0.78 g N kg^{-1} $W^{0.75}$ day^{-1}) by Pimpa *et al.* (2009), in Thai-indigenous heifers (176 g CP day^{-1} or 4.5 g CP kg^{-1} W $^{0.75}$ day) by Chantiratikul *et al.* (2009). Based on the result indicated that protein requirement for maintenance of calves are approximately 14% lower than (Kearl, 1982) 5.4 g CP kg^{-1} $W^{0.75}$ and 12% <5.3 g CP kg^{-1} $W^{0.75}$ (NRC, 1996).

This results are in an agreement with Basra *et al.* (2003b), who indicated that in *Nii ravi* buffalo male calves (12-15 months of age) and Tauqir *et al.* (2009a), who reported that in *Nii ravi* buffalo calves (6-7 months of age) found that protein requirements of *Nii ravi* buffalo calves are lower than dairy cattle calves was recommended by NRC (2001). However, Paul and Patil (2007) demonstrated that the maintenance requirement of CP of *Nii ravi* buffalo heifers were 5.89-9.38 g CP kg^{-1} $W^{0.75}$ for 125-400 body weight ranges. Whereas protein requirement for growth of *Nii ravi* buffalo male calves (9-12 months of age) is same as NRC described for cattle (Basra *et al*., 2003a). In contrast, Tauqir *et al.* (2009b) suggested that CP requirements of *Nii ravi* buffalo calves (12-15 months of age) is higher than recommended by NRC (2001) for cattle.

CONCLUSION

From this study, it can be concluded that increasing dietary protein significantly increased ($p<0.05$) CP digestibility and modified N utilization ($p<0.05$) in growing Thai swamp buffalo calves. Protein requirements for maintenance for growing swamp buffalo calves are 4.63 g CP kg^{-1} $W^{0.75}$ day and the result was lower than Kearl and NRC recommendation.

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