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Article

Empirical evaluation and prediction of protein requirements for maintenance and growth of 18-24 months old Thai swamp buffaloes

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Abstract: Interpretation of increased gain in the bovine animals is difficult to be validated due to inherent genetic variation to meet their requirement for energy and protein, and those may relate to the bull species, e.g., Thai swamp buffalo. Therein, the study aimed at investigating and predicting protein requirement systems, with providing abundant energy intake 2.20 Mcal/kg DM for maintenance and growth of Thai swamp buffaloes using the comparative prolonged feeding trial for 90 days. Sixteen bull Thai swamp buffaloes at the initial (Age: 18-24 months; BW: 233 ± 25.0 kg) were assigned into four treatment groups, four buffaloes each, fed 5.42, 6.96, 8.94, and 10.71% DM crude protein (CP). CP intake, BW, and physiological fluid were determined. The net CP requirements for maintenance and growth of Thai swamp buffaloes were 5.41 g CP/kg $W^{0.75}$ and 0.46 g CP/g average daily gain (ADG), respectively. Our results indicated that CP requirement increases, when BW increases. An increased dietary CP resulted in an increased number at blood urine nitrogen (N), N absorption, total volatile fatty acid, urinary purine derivative, and the microbial N. Notably, the net CP requirement for growth of Thai swamp buffalo was higher than it reported in NRC, but the maintenance was lower.

Keywords: feeding trial; growth; maintenance; nutrient evaluation; protein utilization; Thai swamp buffalo

1. Introduction

A domesticated swamp buffalo (*Bubalus bubalis*) is one of economic-based strategies in tropical livestock production to supply important animal resources [1]. The domestic swamp buffalo plays a crucial role, which extends beyond its supply of primarily draught power, hides, social value, credit, and its contribution of stabilized population numbers as meat supply a secondary consideration in recent years in Thailand [2]. Molecular and morphological evidence suggests that swamp buffalo populations have strong phenotypic uniformity and geographic genetic distinction and it is recorded to disperse through south-east Asia [2]. Having a high meat production potential, multiple purpose as agricultural tool, and a well-adapted to the hot-humid tropical climate condition, therefore, Thai swamp buffalo are large domesticated in several areas in Thailand. Since the nutrient requirements of livestock is able to be determined by inherent genetic variation and environmental factors, mounting interest has been paid to closely regulate the nutrient requirements of Thai swamp buffalo to optimize their production in Thai-

land. Although, most of study had been done on energy and protein requirements for maintenance and growth of Thai-indigenous beef cattle [3], Nili-Ravi buffalo [4], and Murrah swamp buffalo [5]; however, it remains scanty to pay attention for the nutrient requirement, particularly protein in Thai swamp buffaloes; the future meat producing buffalo either in Thailand or other Southeast Asian countries.

The functional significance of protein at maintaining the health and performance farmed livestock is well-represent [6-8]. Amount absorbed protein represents animals be able to use the extent of protein fermentation. As pointed out by NRC [9], the true absorbed protein can be attributed to the metabolizable protein (MP) that is absorbed by the rumen associated with the intestine, and dispensed by undegradable intake protein (UIP) and bacterial crude protein (BCP). Thus, animals compensate by eating crude protein (UIP and degradable intake protein (DIP)) in the diet to get a higher efficiency of BCP to synthesize for amino acids, peptides, or branched-chain amino acids. Of note, there are animal-based protein commonly be used in animal ration and even plant-based protein in grazing system. However, either protein deficiency or imbalance can occur in each animal and result in a negative impact on response to stressors, and animal productivity, including body maintenance, reproduction, and growth. The latter form of excessively dietary crude protein with insufficient fermentable energy availability could lead to the poor metabolism of protein which gives a negative effect on the environment as nitrous oxide, nitric oxide, nitrate, and ammonia formed in feces and urine [10]. As a consequence, a precise prediction of protein requirement accompanied by their balanced energy supply could diminish their excretion and minimize harmful pollution to the environment [4,11-13].

Validation of protein requirement is more difficult with swamp buffalo on high-quality forage and finished on high-energy. The logically knowledge for expressing the optimum exert of rumen degraded protein for buffaloes and the extent to which a feed containing adequate protein and energy sources in which are simultaneously degraded and absorbed from the lumen of the gut into the central blood vessel supplying the liver needs more detailed. Those metabolized protein and net energy requirements set maintenance and potential growth. However, most difficult to interpret are data where energy intake increases with protein supplementation because one does not know whether the increased gain is the result of increased metabolized protein or net energy [9]. An excellent example of this is how Thai swamp buffalo performance is similar with the requirement for rumen degradable protein (including non-protein nitrogen, NPN) or protein supplementation either 0.75 g N or 4.69 g CP/ kg $BW^{0.75}$/d [14] for maintenance and net energy to convert feed N at energy intake roughly 2.20 Mcal/kg DM [15,16]. Buffaloes do not storage protein in their body, so these suggesting that its daily supply and observation are crucial. Therefore, the study aimed at investigating and predicting protein requirement systems, with providing abundant energy intake 2.20 Mcal/kg DM for maintenance and growth of Thai swamp buffaloes using the comparative prolonged feeding trial for 90 days.

2. Materials and Methods

2.1. Animal, diet, and experimental design

All procedures involving animals in present study were approved by Suranaree University of Technology Institutional Animal Care and Use Committee (SUT 4/2558; U1-02632-2559). The experiment was conducted at the Cattle Unit, Suranaree University of Technology farm, Thailand (14°53′21′′N, 102°00′08′′E). Sixteen growing male (bulls) Thai swamp buffaloes, 18-24 months of age and 233 ± 25.0 kg initial weight, were allocated to four experimental groups (n=4) in a randomized complete block design in 2 repeated periods. A period was 45 days and 7 days on the last week which were set as time of sample collection. Thai swamp buffaloes were housed individually in metabolic cage

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(length 3.5 m × width 1.6 m × height 1.9 m) with the ambient temperature ranging from 19.4 ± 2.52 to 29.2 ± 1.39 °C and the average relative humidity was 69.75 ± 6.24%, where each cage had a manual feeder and waterer. Those circumstances did not tend to buffalo stress. Each buffalo had a properly deworming using ivermectin at 1 mL/30 kg (Vermax, Bangkok, Thailand) on the day before the first adaptation period. Four buffalo each, fed rice straw and concentrate for a 90-day feeding trial (Table 1). Those materials were made freshly per a week as a total mixed ration (TMR) and prepared to avoid protein and energy deficiencies for maintenance and growth following to prior documented requirements [9,14-16]. Determination of protein level was based on our previous group result, thereby were 5.42, 6.96, 8.94, and 10.71% crude protein (CP) DM [14]. Provision of net energy was set to each buffalo based on the other buffalo studies [15,16] that were ranged of 2.16-2.25 Mcal/kg DM (1 kg TDN = 3.62 Mcal, Kearl [17]). Buffaloes had free access for clean drinking water throughout the experimental period. Buffaloes were trained-in with a metabolic cage for the 14-day-adaptation period, therefore, buffaloes could adapt the housing set before starting the experiment. All buffaloes were healthy throughout the study.

2.2. Sampling

Following the 14-day adaptation period, buffaloes were offered a TMR twice daily at 07:00 and 16:00 h and the daily feed mass was adjusted counted on the dry matter intake (DMI) of the previous day at considering the 90% voluntary feed intake. The daily feed intake and refusal diet were recorded daily, and 10% of remaining refusal diets were sampled daily (morning and afternoon before next fresh diets offered) and remaining refusal diets were stored at -20 °C for chemical composition.

Buffaloes were weighted monthly, which the first measurement was dedicated as a covariate. On Days 38-44 and 83-89, feces and urine were collected daily by preparing plastic container as given by Paengkoum, *et al.* [18]. Approximately, 10% collected feces and urine each, were collected and acidified to maintain the pH < 3. Feces and urine were composited by each sampling period and frozen at -20 °C for further observation. On Days 45 and 90, 200 mL of rumen fluids were by using a stomach tube connected to a manual pump [12,13,19] 0 and 4 h post-feedings. Monitoring an error by saliva, pump set, and other expectedly barriers during rumen-collecting management were carefully performed. Determination of strict pH, strained through 4 layers of medicinal gauze, and acidified with sulfuric acid had been done before those rumen fluids that were transferred to the laboratory. Each sample was partitioned and stored into three Falcon tubes at -80 °C until analysis of volatile fatty acid (10 mL), N ammonia (5 mL), and rumen microorganism population (20 mL). Furthermore, 10 mL of blood was collected subsequently after rumen fluids collected from the jugular vein into Vacuette tubes (Greiner Bio-One GmbH, Frickenhausen, Germany) containing K3-EDTA (0.47 mol/L). Collected blood samples were centrifuged at 3500 rpm for 20 min at 4 °C (Sorvall Legend XT/XF Centrifuge Series, Thermo Fisher Scientific, Waltham, MA) and plasma was stored at -20 °C to measure blood urea nitrogen (BUN).

2.3. Chemical analysis and calculation

Samples of diet (TMR), refusal diet, and feces were ground using a mesh size of 1 mm (Retsch SM 100 mill; Retsch Gmbh, Haan, Germany) after dried in an oven at 60 °C for 2 days. Those samples were analyzed for DM, ash, total crude protein (total N × 6.25) following to AOAC [20]. Organic matter (OM) content was calculated as a hundred percentage minus ash percentage, which was achieved after incineration in a muffle furnace at 600 °C for 4 h (AOAC#942.05). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were detected by the method of Van Soest, *et al.* [21]. Hemicellulose was calculated as NDF minus ADF. Total digestible nutrient (TDN) and metabolized energy (ME) were estimated using the respective equations: total nutrient digestible = TDN = tdNFC + tdCP

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+ (tdFA × 2.25) + tdNDF – 7 [9]; MP = 1 kg TDN = 3.62 Mcal [17]. In the present study, ingredients of diet, refusal diet, and feces were run (triplicate), and used for chemical analysis and calculation of apparent total tract digestibility.

Table 1. Ingredient and chemical composition of experimental diets (%DM).

Item	Crude protein level			
	5.42%	6.92%	8.92%	10.71%
Ingredient, %DM				
Rice straw	61.90	63.10	66.79	65.50
Cassava pulp	14.88	15.36	14.02	12.38
Ground corn	22.76	17.81	11.02	10.81
Soybean meal	0	3.10	7.38	10.44
Urea	0.37	0.55	0.71	0.80
Premix[1]	0.09	0.08	0.08	0.08
Total	100	100	100	100
Chemical composition				
Dry matter, %	89.35	89.24	88.46	88.23
Organic matter, %DM	5.42	6.96	8.94	10.71
Crude protein, %DM	56.94	55.16	57.70	57.02
Neutral detergent fiber, %DM	36.73	36.15	38.43	38.56
Acid detergent fiber, %DM	20.21	19.01	19.26	18.46
Hemicellulose, %DM	89.35	89.24	88.46	88.23
Total digestible nutrient, %DM[2]	62.40	62.20	60.15	59.82
Gross energy, Mcal/kg DM[3]	2.25	2.25	2.18	2.16

[1] The premix contained (per 5 kg): vitamin A, 20 mIU; vitamin D3, 2 mIU; vitamin E, 20 IU; Mn, 80 g; Zn, 50 g; Fe, 120 g; Cu, 10 g; Se, 0.25 g; Co, 1 g; I, 2.5 g. [2] Calculated from NRC [9]; TDN = tdNFC + tdCP + (tdFA ×2.25) + tdNDF – 7. [3]Gross energy = Metabolizable energy; calculated from Kearl [17]: 1 kg total digestive nutrient (TDN) = 3.62 Mcal.

Determination of total N in feces and urine was measured in triplicate based on prior study [22]. Separated samples of urine were equipped into the high-performance liquid chromatography (HPLC, Hewlett-Packard HPLC system HP series 1100, Agilent technologies, USA), injected triplicate in C18 reversed phase column with a UV detector wavelength 205 nm, a quat pump, and DAD detector (G1315A) to determine urinary urine derivatives (PD), creatinine excretion, microbial related to either purine or nitrogen efficiency [23]. The microbial purine based (MPB) was estimated according to Chen, *et al.* [24] equation, which following factors of digestibility of microbial purines is 0.83, the N content of purine is 70 mg N/mmol, and purine N: total N in mixed rumen microbes is 11.6:100. Thus, microbial N (g N/d) = 70 PB/ (0.116 × 0.83 × 1,000) = 0.727PB, where, PB = the corresponding number of microbial purines absorbed (mmol/d). In addition, calculation of daily purine absorption (mmol/d) was done by using equation according to Pimpa, *et al.* [25] equation. PDC index was calculated as PDC = PD/creatinine × $BW^{0.75}$.

Collected plasma gathered at 0 and 4 post-feedings were analyzed for blood urea nitrogen [26]. Similarly, portioned rumen fluids at 0 and 4 post-feedings were thawing gently. First portion of rumen fluids was prepared, fixed, and analyzed for volatile fatty acid (VFA) using gas chromatography (Agilent 6890 GC, Agilent Technologies, USA) with a 30 m x 0.25 mm x 0.25 µm column (DB-FFAP) and peak detection was compared and calculated as given by Purba, *et al.* [27]. Second portion of rumen fluids was centrifuged at 6,000 × g for 15 min at 4 °C, and the supernatant was subsequently measured for ruminal ammonia nitrogen (NH_3-N) using the micro-Kjeldahl methods (Kjeltec 8100, Hilleroed, Denmark, AOAC [20]). The last portion of rumen fluids were separated into two aliquots: direct counting cells for bacteria, protozoa, and fungal zoospore using

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Galyean [28] procedure; roll-tube technique in culture bacteria for groups of cellulolytic, proteolytic, and amylolytic using Hungate [29] method.

2.4. Statistical analysis

All data in this experiment including replication were statistically analyzed as a randomized complete block design (RCBD) following to the general linear model (GLM) procedure of the SAS 9.4 [30]. The model was: $Y_{ij} = \mu + B_i + T_j + \varepsilon_{ij}$; where; Y_{ij} = the observation, μ = overall mean, B_i = effect of block (i: buffaloes), T_j = effect of treatment (j: dietary crude protein), and ε_{ij} = random error. The model of covariance of RCBD was: $Y_{ij} = \mu + B_i + T_j + \beta(X_{ij} + x) + \varepsilon_{ij}$; where Y_{ij} = the observation, X_{ij} = the covariates, response of buffalo in dietary crude protein, μ = overall mean, x = mean of covariates B_i = effect of block (i: buffaloes), T_j = effect of treatment (j: dietary crude protein), β = the regression or slope, adjusted Y by X, and ε_{ij} = random error. Despite other parameters, initial weight of buffaloes and number of protozoa at 0 post feeding were dedicated as covariate. Comparison of treatment means used Duncan's new multiple range test and orthogonal contrast analysis which set the method of least significant difference the maximal significant at $p < 0.05$.

3. Results and discussion

Most difficulty in either validating or interpreting protein requirement systems for maintenance and growth among bovine animals is evidence that those requirements are influenced by inherent genetic variation and capability of animals to obtain greater protein synthesis as a considerably energy intake. Therefore, the discussion will be based on providing different protein supplies with abundance of energy supply for maintenance and growth of Thai swamp buffalo. Protein requirement, nutrient utilization, and microorganism profile are included to elaborate the influences mentioned.

3.1. Body weight and daily gain performance

The different protein supplies with abundance of energy supply fed to Thai swamp buffaloes resulted in improving numbers of final weight and average of daily gain ($p < 0.05$; Table 2). Average of daily gain (ADG) throughout the 90 days of feeding trial was a gradually increased ($p < 0.05$) in buffaloes receiving a greater protein level in diets. Despite buffaloes receiving a level of 5.42% protein in diet, the present study indicating that those buffaloes had a higher protein efficiency, where buffaloes received a range of 6.92-10.71% crude protein (CP) able to get impressive ADG. Our results were in agreement with Chumpawadee, *et al.* [31] who reported increasing ADG of Thai-indigenous yearling heifers is successfully obtained after those heifers fed diets containing high CP (6-12%). Assumption of more protein supply about more gaining weight receive that might be true to preliminary talk. Other bull observations, nevertheless, concluded that excessively dietary CP was no effect on ADG in bull animal performance. For instance, Devant, *et al.* [32] increased dietary CP level in diets ranged of 14-17% and Promkot and Wanapat [33] was attempted to decrease level of protein in diets ranged of 10.5-14.4%, those observations reported that no gaining weight of crossed heifers was occurred. In similar, Basra, *et al.* [34] suggested no shift of ADG, when CP level in diets is adjusted from 12 to 18% of DM in Nili-Ravi buffaloes. It, thus, suggesting that dietary CP over 10% fed to bull animals did not give a positive impact on gaining weight. Dietary CP ranged of 6.92-8.92% constituted an alternative strategy to improve body weight and ADG, without negatively affecting animal performance and farm budgeting. Other discussion had previously reviewed regarding efficiency of protein-rich diets to contribute to food security, employment, and rural economies [35].

Table 2. Effect of dietary crude protein on body weight and daily gain performance in Thai swamp buffaloes.

Item	Crude protein level				SEM	Significance		
	5.42%	6.92%	8.92%	10.71%		L	Q	C
INW, kg	233.8	234.0	234.0	232.8	6.74	ns	ns	ns
FW, kg	229.3^{c}	248.3^{bc}	260.5^{ab}	279.0^{a}	8.50	**	ns	ns
AW, kg	231.5^{b}	241.1^{ab}	247.3^{ab}	255.9^{a}	7.34	*	ns	ns
ADG, kg/d	-0.05^{c}	0.16^{b}	0.29^{b}	0.51^{a}	0.05	**	ns	ns
ADG/DMI, g/kg	-14.20^{c}	31.89^{b}	54.49^{b}	92.07^{a}	9.91	**	ns	ns
ADG/CPI, g/g	-0.28^{b}	0.48^{a}	0.62^{a}	0.86^{a}	0.17	**	ns	ns
ADG/TDNI, g/kg	-22.49^{c}	51.14^{b}	90.65^{b}	153.73^{a}	16.36	**	ns	ns

INW: initial weight. FW: final weight. AW: average weight. ADG: average daily gain. DMI: dry matter intake. CPI: crude protein intake. TDNI: total digestible nutrient intake. SEM: standard error of mean. $^{a, b, c}$ Values on the same row under each main effect with different superscript differ significantly ($p < 0.05$). Significance (*, $p < 0.05$; **, $p < 0.01$; ns, not significantly different). L, Q, C: linear, quadratic and, cubic effects of difference crude protein levels.

As expected, the present results aforementioned gained weights were derived from a greater feed efficiency (ADG/DMI, CPI or TDNI, $p < 0.01$, Table 2) as result of receiving more protein supplies in diets. These results were similar with a prior study [36], where Nili-Ravi buffaloes fed to diets containing CP (9.13%) and had relatively abundant energy intake. These achievements, in turn, lead to lower the feed efficiency and ADG pattern. If the reduction in a feed intake represents a shift of caloric restriction allowing hyperthermic animals to reduce heat generation [37], a reduction of in feed efficiency or ADG pattern was obtained from the limitation of nutrient digestion and metabolism in rumen host permitting to alleviate the protein synthesis rate, as indicated from insufficient energy intake [9].

3.2. Nutrient intake and nutrient digestibility

Nutrient intakes beneath requirements by bull animals results in deferred maturity of reproduction system and slowed down growth rates [3,5,9]. Generally, all of nutrient intakes on dry-matter (DM) basis increased, when dietary crude protein increased ($p < 0.01$, Table 3). CP intake (g/kg $W^{0.75}$) was a gradually increased at ranged of 3.55-9.44 g/kg $W^{0.75}$. It could be noted that no buffalo had restricted the nutrient access per group per experimental design (n=4) in the present study. Thus, latter outcomes could determine more validation for nutrient or protein requirement of maintenance and growth in Thai swamp buffaloes. Besides, the rate of nutrient intakes could be influenced by several factors such as rumen capacity, ruminal metabolic level (factual VFAs), digestion rate, physiological animal, and nutrient requirement setting [38]. We know of few serial observations regarding dietary DM intake influenced by dietary CP as similar as the present study. Comparative studies observed in Nili-Ravi buffaloes [34,36], Thai-indigenous heifers [31,39], and Murrah buffaloes [5,15] that either protein or nitrogen intake was varied when those bull animals fed to the extent of dietary CP. In addition, rumen capacity including limited passage rate suggested to microbial turnover growing up and leading to reduce the efficiency of microbial protein [9]. In this case, low feed intake might be occurred and tend to animals' severe nutrient deficiency.

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Table 3. Effect of dietary crude protein on nutrient intake and apparent digestibility in Thai swamp buffaloes.

Item	Crude protein level				SEM	Significance		
	5.42%	6.92%	8.92%	10.71%		L	Q	C
Dry matter intake								
kg/d	4.17^{c}	4.97^{b}	5.25^{ab}	5.60^{a}	0.16	**	ns	ns
g/kg W$^{0.75}$	70.43^{c}	81.25^{b}	84.13^{ab}	87.67^{a}	1.94	**	ns	ns
% BW	1.82^{b}	2.06^{a}	2.12^{a}	2.20^{a}	0.05	**	ns	ns
Crude protein intake								
kg/d	0.21^{d}	0.33^{c}	0.46^{b}	0.60^{a}	0.01	**	ns	ns
g/kg W$^{0.75}$	3.55^{d}	5.42^{c}	7.34^{b}	9.44^{a}	0.21	**	ns	ns
TDN								
kg/d	2.57^{b}	3.12^{a}	3.15^{a}	3.35^{a}	0.09	**	ns	ns
g/kg W$^{0.75}$	43.57^{b}	50.93^{a}	50.49^{a}	52.51^{a}	1.31	**	ns	ns
Other nutrient intakes								
OM, kg/d	3.70^{c}	4.44^{b}	4.65^{ab}	4.94^{a}	0.14	**	ns	ns
NDF, kg/d	2.45^{c}	2.77^{bc}	3.06^{ab}	3.21^{a}	0.11	**	ns	ns
ADF, kg/d	1.65^{c}	1.82^{bc}	2.07^{ab}	2.17^{a}	0.08	**	ns	ns
Hemicellulose, kg/d	0.80^{b}	0.96^{a}	1.00^{a}	1.03^{a}	0.04	**	ns	ns
Apparent digestibility								
Dry matter, %	60.81^{ab}	63.22^{a}	57.45^{ab}	56.40^{b}	1.77	*	ns	ns
Organic matter, %	66.64^{ab}	69.73^{a}	65.14^{b}	63.02^{b}	1.26	*	ns	ns
Crude protein, %	36.44^{c}	52.53^{b}	58.20^{ab}	63.30^{a}	2.76	**	ns	ns
TDN, %	91.08^{a}	91.92^{a}	89.36^{b}	88.59^{b}	0.47	**	ns	*
NDF, %	53.36^{ab}	59.95^{a}	47.85^{b}	44.82^{b}	3.19	*	ns	ns
ADF, %	51.41	50.36	45.92	43.97	2.67	ns	ns	ns
Hemicellulose, %	57.52^{ab}	67.82^{a}	51.65^{b}	47.26^{b}	4.60	*	ns	ns

BW: body weight. TDN: total digestible nutrient. OM: organic matter. NDF: neutral detergent fiber. ADF: acid detergent fiber. SEM: standard error of mean.

$^{a-d}$ Values on the same row under each main effect with different superscript differ significantly ($p < 0.05$). Significance (*, $p < 0.05$; **, $p < 0.01$; ns, not significantly different). L, Q, C: linear, quadratic and, cubic effects of difference crude protein levels.

Nutrient digestibility, especially digestibility of protein is essential—for both BCP and UIP during absorbing the metabolizable protein [9]. In the present study, digestibility rates of DM, OM, total digestible nutrient (TDN), neutral detergent fiber (NDF) were lower, whereas digestibility of protein was higher when dietary crude protein increased ($p < 0.05$, Table 3). Although, the present results were in contrast with previous study [5] that Murrah buffaloes showed the greater digestibility rates of DM, OM, and protein. Diets were consisted of berseem, wheat straw, and concentrate mixture without inclusion of urea. More previously, inclusion of urea from 10 to 30 g/kg in diet composition led to increase the digestibility rates both of OM and protein. To note, the present study provided dietary CP increased with simultaneously increasing urea level ranged of 3.7-8.0 g/kg DM in the buffalo diets (Table 1). It, thus, suggesting that presence of urea in bull animal diets might enhance deamination and modulate the apparent of protein; however, exceedingly urea supplementation of offered diets might considerably impact on ureagenesis. A generous hepatic ureagenesis becomes indispensable for ruminants to refrain poisoning from absorbed ammonia [40]. Furthermore, considering to carbohydrate digestion (TDN) in the rumen could be the most accurate predictor of BCP synthesis [9], present study expected to provide the protein and carbohydrate digestions, so that provision of 6.96% protein as inclusion of 5.5 g/kg urea and availability of energy intake at

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2.20 Mcal/kg DM in diet (Table 1) suggested the optimum level for buffaloes to obtain a greater digestibility rates of protein and TDN.

3.3. Ruminal fermentation and blood urea nitrogen

Ruminal fermentation end-product (pH, NH_3-N, VFAs) and blood metabolite such as blood urea nitrogen (BUN) are crucial parameters to assess whether animals meeting their nutrient requirement without reducing animal responses [5,15,34,36,41]. Increasing protein content in Thai swamp buffalo diets enriched the concentration of NH_3-N in the rumen ($p < 0.05$), but those were surprisingly not affecting to ruminal pH (Table 4). Ruminal pH of Thai swamp buffaloes fed to all diets in present study was unvaried as provision of different protein content and the values were expected pH rate (6.7-7.1). Current result was similar to prior observations [31,39]. To note, inclusion of urea in diets of present study suggested a similar finding by Wanapat, *et al.* [42] who added urea in buffalo diet ranged of 15-30 g/kg concentrate. Ruminal pH was in the normal range from 6.8 to 6.9, which provided optimal rumen circumstance to rumen host to grow and ferment nutrient digestion, especially protein. Hence, it might be the rumen-buffering capacity no occurred, but even ruminal NH_3-N changed. Further studies are needed to better explain the regression between rumen-buffering capacity, pH, and ruminal NH_3-N on manipulating protein supply in buffalo diets.

Table 4. Effect of dietary crude protein on ruminal fermentation end-product and blood metabolite in Thai swamp buffaloes.

Item	Crude protein level				SEM	Significance		
	5.42%	6.92%	8.92%	10.71%		L	Q	C
Ruminal Ph								
0 h post-feeding	7.08	7.05	7.08	6.98	0.04	ns	ns	ns
4 h post feeding	6.88	6.75	6.70	6.75	0.07	ns	ns	ns
NH_3-N concentration, mg/dL								
0 h post-feeding	10.39^{d}	12.44^{c}	16.14^{b}	18.44^{a}	0.26	**	ns	*
4 h post feeding	10.80^{d}	16.37^{c}	18.88^{b}	22.12^{a}	0.27	**	**	*
BUN concentration, mg/dL								
0 h post-feeding	11.94^{d}	16.02^{c}	19.34^{b}	24.34^{a}	0.30	**	ns	ns
4 h post feeding	13.69^{d}	19.02^{c}	21.34^{b}	26.83^{a}	0.21	**	ns	**
Total VFA, M*m*								
0 h post-feeding	82.55	88.34	86.84	85.43	3.95	ns	ns	ns
4 h post feeding	79.01^{b}	81.56^{b}	81.56^{b}	85.00^{a}	0.86	**	ns	ns
VFA profile, mol/100 mol								
Acetic acid								
0 h post-feeding	68.80	69.09	69.63	68.75	0.56	ns	ns	ns
4 h post feeding	69.14^{a}	68.43^{a}	68.69^{a}	65.96^{b}	0.69	*	ns	ns
Propionic acid								
0 h post-feeding	19.62	19.35	19.30	20.36	0.55	ns	ns	ns
4 h post feeding	18.78^{b}	20.04^{b}	19.95^{b}	22.46^{a}	0.61	**	ns	ns
Butyric acid								
0 h post-feeding	11.57	11.56	11.07	10.89	0.39	ns	ns	ns
4 h post feeding	12.07	11.53	11.36	11.57	0.34	ns	ns	ns

NH_3-N: ammonia. BUN: blood urea nitrogen. VFA: volatile fatty acid. SEM: standard error of mean. $^{a-d}$ Values on the same row under each main effect with different superscript differ significantly ($p < 0.05$). Significance (*, $p < 0.05$; **, $p < 0.01$; ns, not significantly different). L, Q, C: linear, quadratic and, cubic effects of difference crude protein levels.

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Furthermore, BUN concentration was shifted by dietary CP ($p < 0.01$; Table 4). As aforementioned a shift of ruminal NH_3-N, the mean of ruminal NH_3-N was at normal level (11.3-22.2 mg/dL) and considered safe for rumen host to avoid microbial turnover increasing [43]. However, other study suggested that a shift of ruminal NH_3-N related to altered BUN concentration as modulating dietary CP for bull animals [39,44,45]. Here, the mean of BUN in the present study was varied ($p < 0.01$, 13.7-26.8 mg/dL) and those numbers were still considerably safe for ruminal bull animals [39,45]. Moreover, there was high relationship between nitrogen intake as varied protein intake, ruminal NH_3-N, and BUN (Figure 1). Ruminal NH_3-N concentration increases when protein intake increases that relates to faster protein degradation than synthesis, higher dietary RDP [45], or an imbalance of fermentable energy [45,46]. This occur suggesting that ammonia would be accumulated to rumen fluid and result in exceedingly concentration. As aforementioned, all of the ammonia including urea retention absorbed in lumen of the gut was removed by the liver with, as a result, a net splanchnic flux of zero to perform detoxification of ammonia by the liver [40], and finally excreted via urine in to high levels of urine nitrogen [39,46].

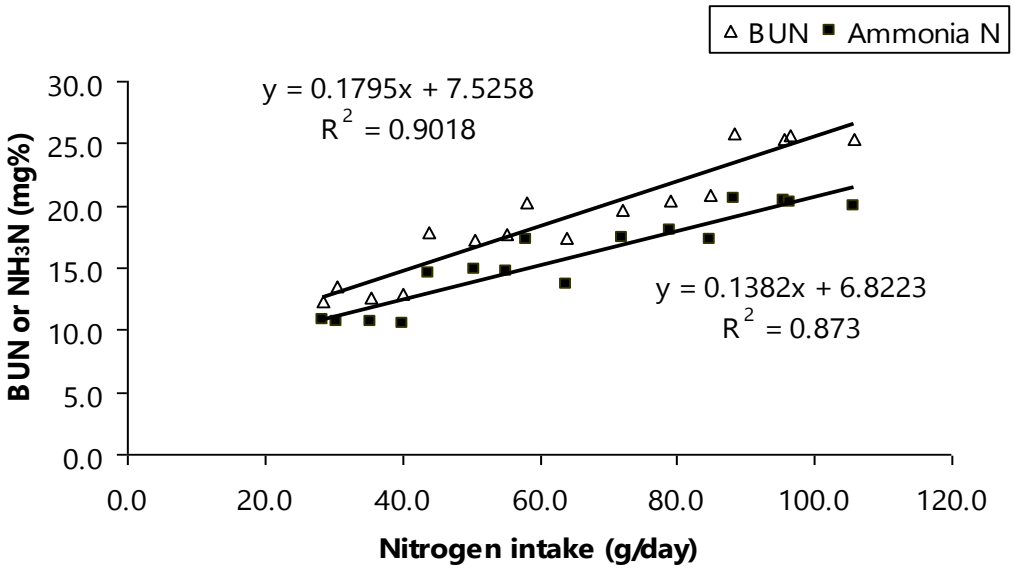

Figure 1. Relationship between nitrogen intake and either blood urea nitrogen (BUN) or ruminal ammonia nitrogen (NH_3-N).

Total VFA in the present study was varied ranged of 79.0-85.0 m*M*, and VFA fraction including acetate and propionate was changed but butyrate remained unchanged, where samples of rumen fluids observed at 0 and 4 h post-feedings by dietary CP (Table 4). In general, influences of dietary CP were occurred in buffaloes fed to diet consisted of 10.71% protein at 4 h post-feeding ($p < 0.05$). A shift of VFAs relates to the ruminal host on digesting and metabolizing the nutrient source in diet, here, buffaloes could manifest their efficiency of feed utilization that is represented by increasing a number of VFAs. A higher VFAs might be due to a greater digestibility by VFA-producing bacteria. More CP intake at 10.71% showed the highest protein digestibility compared with other protein supplementations (Table 3). However, those effects are time-dependent. Since fermentable energy availability (TDN, g/kg $W^{0.75}$) obtained in similar pattern, the present results reflecting that dietary CP allowed the VFA-producing bacteria to rapidly accelerate the protein breakdown on nutrient digestion and the latter fermentable products were forwarded to hydrolysis and stored at a shift of VFAs as a main source of buffalo energy [42,45]. Additionally, there was a shift in VFAs produced in the rumen toward more

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propionate with corresponding to reduce in acetate and remain in butyrate (Table 4). Acetate portion decreased when propionate increased indicating dietary CP in the present study altered fermentable carbohydrate to be major substrate for acetate fraction; however, a higher propionate suggested that propionate-producing bacteria was dominant to synthesize fermentable carbohydrate more propionate in gluconeogenesis by the pentose phosphate pathway produces NADPH [11,12,47]. These occurs might relate to the reduction of NDF digestion (Table 3). This declining availability of fermentable carbohydrate might relate to decrease acetate portion as the first major VFA absorbed from the rumen to have somewhat distinctive metabolic shift [11,27,47]. This achievement was similar with previous study [31] and was expected to the present study because of altered carbohydrate digestion.

3.4. Nitrogen balance, purine derivative excretion, microbial-related N characteristic, and rumen microorganim population

Provision of increased dietary CP in Thai swamp buffaloes increased the size of feces ($p < 0.05$, Table 5). This occur was expected to the efficiency of dietary CP which was suggested below 10% DM CP in diet as aforementioned [31-34]. Hence, the present results could be corroborating previous reports. This possibly occur appointed to a higher dietary CP increasing a greater fecal size might be also due to likely enlarge on DM intake and slightly dwindle number of DM digestibility (Table 3), so as to excrete lowered undigested dietary CP formed in a higher volume of feces. Therefore, a significant difference in fecal N among diets in the present study could be attributed to the endogenous losses from digestive tracts; however, it might not be varied among bull animals [17].

Concurrently, the current scenario resulted in N contents determined in N intake, N excretion, N absorption, and N retention as indicated a higher N amount of urinary and fecal ($p < 0.01$). It has been a well-documented that increasing protein content in diet inevitably increases N intake and this surpasses the numbers of N retention and N excretion in Thai-indigenous heifers [3,39], Murrah buffaloes [5], and *Nili-Ravi* buffaloes [44]. Notably, Thai swamp buffaloes fed to increased dietary CP as a depiction in the present study showing that there is relationship between N intake, N retention, and both of N absorption and excretion (g/kg $W^{0.75}$; Figure 2). These recent findings interpreting that N retention was ranged from 0.07 to 0.45 g N/kg $W^{0.75}$ and N absorption ranged from 0.21 to 0.96 g N/kg $W^{0.75}$, when the Thai swamp buffaloes consumed the increased dietary CP levels. This relationship gave an evidence that provision of increased dietary CP among bull animals resulted in increased the N numbers of feces, nitrogen fecal, and urine, if fermentable energy availability in sufficient supply.

Table 5. Effect of dietary crude protein on excreta volume and nitrogen balance in Thai swamp buffaloes.

Item	Crude protein level				SEM	Significance		
	5.42%	6.92%	8.92%	10.71%		L	Q	C
Excreta volume								
Urine, L/d	3.55	3.62	4.48	4.80	0.57	ns	ns	ns
Feces, kg DM/d	1.67^{b}	1.83^{b}	2.23^{a}	2.44^{a}	0.12	**	ns	ns
Urine nitrogen								
g/d	8.08^{c}	10.06^{c}	20.93^{b}	32.55^{a}	2.17	**	ns	ns
g/kg$W^{0.75}$	0.14^{c}	0.16^{c}	0.34^{b}	0.51^{a}	0.36	**	ns	ns
Feces nitrogen								
g/d	21.79^{c}	25.19^{bc}	30.77^{ab}	35.17^{a}	2.12	**	ns	ns
g/kg$W^{0.75}$	0.36^{c}	0.41^{bc}	0.49^{ab}	0.55^{a}	0.03	**	ns	ns
Nitrogen intake								
g/d	33.65^{d}	53.28^{c}	73.41^{b}	96.50^{a}	2.36	**	ns	ns
g/kg$W^{0.75}$	0.57^{d}	0.87^{c}	1.17^{b}	1.51^{a}	0.03	**	ns	ns

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Nitrogen excretion								
g/d	29.87[c]	35.24[c]	51.70[b]	67.73[a]	2.85	**	ns	ns
g/kgW$^{0.75}$	0.50[c]	0.57[c]	0.83[b]	1.06[a]	0.04	**	ns	ns
Nitrogen absorption								
g/d	11.86[d]	28.10[c]	42.64[b]	61.33[a]	2.33	**	ns	ns
g/kgW$^{0.75}$	0.21[d]	0.46[c]	0.68[b]	0.96[a]	0.04	**	ns	ns
Nitrogen retention								
g/d	3.78[b]	18.03[a]	21.70[a]	28.78[a]	3.56	**	ns	*
g/kgW$^{0.75}$	0.07[b]	0.29[a]	0.35[a]	0.45[a]	0.05	**	ns	*

SEM: standard error of mean.

[a-d] Values on the same row under each main effect with different superscript differ significantly ($p < 0.05$). Significance (*, $p < 0.05$; **, $p < 0.01$; ns, not significantly different). L, Q, C: linear, quadratic and, cubic effects of difference crude protein levels.

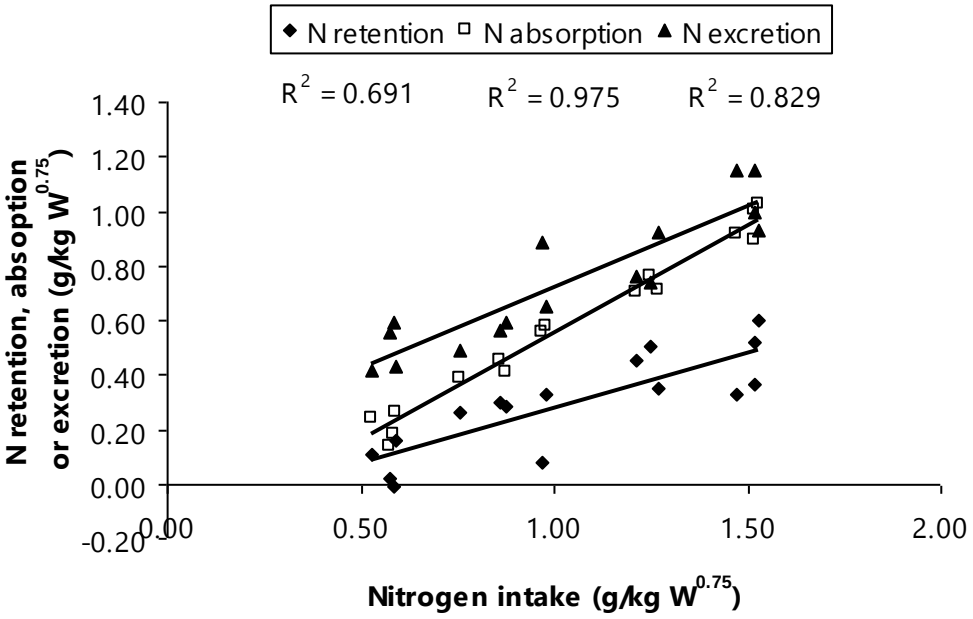

Figure 2. Relationship between nitrogen intake and nitrogen utilization.

It has been discussed that N excretion via urine increased with increasing protein level in diet (N intake), which may relate to RDP ration in dietary CP [46,48]. To note, high RDP ratio in dietary CP have been showing to increase N urine, but this occur led to decrease N balance [44]. Utilized nitrogen parameter such nitrogen or protein degradability has a main effect on urinary N output because of excess soluble N in the rumen from diets with high RDP [46]. Several factors, namely N origin, degree of N intake, degree of energy intake, age, sex, and physiological animal related to metabolic perspective could be considered as profoundly influencer on shifting nitrogen balance [6]. Nitrogen balance defines animal status to meet their protein requirement, as metabolized protein excess (supply minus requirements; NRC [9]). However, excessively N retaining no longer gives a metabolic benefit due to those N absorbed from the lumen of the gut into the portal vein, which must be partially removed by the liver to avoid a large hepatic ureagenesis as discussed above [40].

The urinary purine derivative (PD) excretion (mmol/d or μmol/kg W$^{0.75}$) increased, when dietary CP in Thai swamp buffaloes increased ($p < 0.05$; Table 6). Concentration of PD parameters (allantoin, uric acid, xanthine, and hypoxanthine) in the present study was ranged of 16.3-31.3 mmol/d and it was expected to similar range as reported in other buffalo investigations [25,49]. In addition, there was a significant increase ($p < 0.05$) in

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allantoin and total PD, and non-significant responses or remaining unchanged in relatively uric acid, xanthine and hypoxanthine excretions that observed in urine as influence of increased dietary CP. These achievements in the present study were similar to previous studies [25,46], which sizes of allantoin and uric acid excretions were relatively normal for bull animals as influenced protein content in diets. Moreover, the present study by adjusting dietary CP seemed to no have influence on urinary excretion of creatinine (μmol/kg $W^{0.75}$). However, dietary CP shifted ratio of A:C, PD:C and PDC index ($p < 0.01$; Table 6). Creatinine excretion might be influenced by the amount of protein intake by animals and the present study showed increased pattern of DM intake as well as concomitantly protein content in diets increased (Table 1 and 3). However, the recent findings were not in linear with previous studies that were observed in Zebu cattle and swamp buffaloes [50], and Murrah buffaloes [51]. To compare, those bull animals had been to show the difference of creatinine excretions due to the marginal variations among studies. Variation of animals, diets, experimental managements were expected to differ the characteristic and profile in creatinine shift [50]. We speculated also that breed or species specific and more closely correlated with muscle mass than body weight might differ the creatinine size, and not depend on dietary intake. Thus, the present study could be assumed to reconfirm that dietary intake of animals did not attribute to vary the creatinine excretion, if those fed to similar in diet concern and group of selected breeds.

Table 6. Effect of dietary crude protein on purine derivative and microbial-related N characteristic in Thai swamp buffaloes.

Item	Crude protein level				SEM	Significance		
	5.42%	6.92%	8.92%	10.71%		L	Q	C
Purine derivative (PD)								
Allantoin (A)								
mmol/d	10.52^{b}	14.83^{b}	13.77^{b}	25.26^{a}	1.90	**	ns	ns
μmol/kg $W^{0.75}$	180^{b}	240^{b}	225^{b}	405^{a}	39.8	**	ns	ns
%	65.33^{b}	77.13^{a}	78.28^{a}	80.61^{a}	2.54	**	ns	ns
Uric acid								
mmol/d	3.00	2.09	1.50	3.82	0.75	ns	ns	ns
μmol/kg $W^{0.75}$	56.25	34.00	24.50	61.50	15.8	ns	ns	ns
%	11.34	10.81	8.53	12.06	2.81	ns	ns	ns
Hypoxanthine								
mmol/d	0.97	0.83	0.65	0.50	0.16	ns	ns	ns
μmol/kg $W^{0.75}$	18.00	10.75	8.25	13.00	3.54	ns	ns	ns
%	5.67^{a}	3.46^{b}	2.92^{b}	2.71^{b}	0.62	*	ns	ns
Xanthine								
mmol/d	1.80	1.61	1.74	1.40	0.22	ns	ns	ns
μmol/kg $W^{0.75}$	3.00	27.50	27.50	25.00	2.88	ns	ns	ns
%	11.66^{a}	8.59^{ab}	10.28^{a}	4.62^{b}	1.58	*	ns	ns
Total PD								
mmol/d	16.28^{b}	19.18^{b}	17.51^{b}	31.32^{a}	2.59	**	ns	ns
μmol/kg $W^{0.75}$	287^{c}	312^{b}	287^{b}	503^{a}	55.4	*	ns	ns
Creatinine (C)								
mmol/d	30.08	25.63	19.68	32.56	4.23	ns	ns	ns
μmol/kg $W^{0.75}$	552	416	322	519	86.7	ns	ns	ns
Ratio A:C	0.39^{b}	0.59^{ab}	0.71^{a}	0.78^{a}	0.06	**	ns	ns
Ratio PD:C	0.58^{b}	0.76^{ab}	0.90^{a}	0.97^{a}	0.07	*	ns	ns
PDC index	34.86^{b}	47.34^{ab}	55.97^{a}	61.42^{a}	4.98	*	ns	ns
Microbial related N								
MPB flow, mmol/d	37.99^{c}	56.44^{b}	42.99^{b}	155.26^{a}	24.6	*	ns	ns

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Microbial N supply, g N/d	27.60[b]	41.03[b]	31.25[b]	112.87[a]	17.9	*	ns	ns
Microbial N efficiency								
g N/kg DOMR	17.70[b]	20.38[b]	15.90[b]	54.23[a]	8.34	*	ns	ns
g N/kg OMI	8.07[b]	9.23[b]	6.57[b]	22.39[a]	3.72	*	ns	ns
g N/kg DMI	7.19[b]	8.26[b]	5.81[b]	19.76[a]	3.29	*	ns	ns
g N/kg CPI	0.14	0.12	0.06	0.19	0.04	ns	ns	ns
g N/kg TDNI	36.04[c]	48.92[bc]	60.50[b]	85.26[a]	5.24	*	ns	ns

PD: purine derivative. MPB: microbial purine base. DOMR: digestibility of organic matter fermented in rumen. OMI: organic matter intake. DMI: dry matter intake. CPI: crude protein intake. TDNI: total digestive nutrient intake. SEM: standard error of mean.

[a-d] Values on the same row under each main effect with different superscript differ significantly ($p < 0.05$). Significance (*, $p < 0.05$; **, $p < 0.01$; ns, not significantly different). L, Q, C: linear, quadratic and, cubic effects of difference crude protein levels.

To determine and predict protein requirement for maintenance and growth of Thai swamp buffaloes on any given feeding program, it is necessary to the characteristic of microbial nitrogen supply and microbial nitrogen efficiency. Adjusting dietary CP was expected to increase linearly microbial purine base (MPB) flow (mmol/d), microbial N supply (g N/d), and microbial N efficiency (g N/kg nutrient utilization) in urinary Thai swamp buffaloes ($p < 0.01$; Table 6). However, a solely microbial N efficiency in term of CP intake remained unchanged. Our results were in agreement with previous study of Paengkoum *et al.* [47], their group assessed that microbial N synthesis grew up by adding urea from 10 to 30 g/kg steam-treated oil palm fronds. As mentioned above, urea stimulated to increase protein availability in rumen. Kim *et al.* [46] reported rumen richer in fermentable protein derived from dietary CP (9 vs. 11%DM) and solely RDP fraction (52 vs. 80%CP) resulting in plentiful microbial N supply to omasum. It may indicate to microbial N turnover decreasing as a greater N source. Exceedingly CP supplementation at 13.5 to 19.4% DM had been reported to show an inclination of omasal flow of total non-NH_3-N bacteria from 425 to 480 g/d [52]. More efficiency gains had been achieved by only increasing RDP level (10.6-13.2%) in the diets tending to enrich total non-NH_3-N bacteria [53]. It could be assessed that impressive numbers of total non-NH_3-N bacteria flowing from rumen to omasum due to dietary CP increased as protein intake and its degradability increased, but this efficiency of microbial protein synthesis had seemed to depend on the age of rumen itself. The age-related succession of rumen microbial communities, especially *Bacteroidetes* and *Proteobacteria* populations, defined the shift of ruminant animal productivity as growing higher in presence of older age rumen [54]. In other words, animal age might have influence on differing microbial efficiency. Moreover, the present study had provided the experimental design in numerous levels of CP and considerably energy supply. This was prepared because we still assumed that any protein catabolism converting to amino acid seeming to have sufficient fermentable energy availability. The traditional meta-analysis regarding microbial efficiency in rumen had been previously discussed to relate protein synthesis is based on ruminal carbohydrate as a main energy source in rumen [55]. The high-energy and low-protein diets shifted the available N for microbial growth in limited microbial protein synthesis [56]. Of note, therefore, several factors such as the availability of carbohydrates and N in rumen, ruminal pH, physiological effects, sources and levels of N components, and other stabilizing ruminal fermentations had been discussed to have a substantial role on modifying the efficiency of microbial protein in rumen [55-57].

Despite similar pH in rumen, dietary CP increased relatively fungal zoospore and total bacteria in rumen of Thai swamp buffaloes ($p < 0.05$; Table 7). Cellulolytic bacterial population had quadratic and cubic effects ($p < 0.05$). Amylolytic bacterial population had a linear effect ($p < 0.05$). However, increasing CP content in diets did not affect proteolytic bacterial population in the rumen of Thai swamp buffaloes neither observed in 0

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and 4 h post-feedings. The inclination of increased numbers in those recently mentioned findings were similar patterns as a well-documented in those prior reports [58,59]. There may be a big question mark in which the proteolytic bacterial population did not get affection by adjusting dietary CP, while the digestibility of CP (Table 3) was a greater appearance. The possibly reason might relate to the protein metabolism itself in rumen as present objective delivered. Protein metabolism in the rumen is metabolically manifestation by activity of ruminal microorganisms to have a corresponding effect towards the nutrient utilization. Here, protein utilization is depending on the structure of nutrient and it is defining whether having a susceptibility to microbial proteases and, thus, its degradability. Bach, *et al.* [60] had pointed out several findings by reviewing the nitrogen metabolism in the rumen. Ruminal protein degradation is shifted by pH and the major microbial population. Proteolytic population represents the protein degrader in rumen and its activity changes as pH changes with high-forage dairy cattle-type rations, but not in high-concentrate beef-type rations. It, thus, indicating that proteolytic population degrades the protein substrates in post feeding and finally shifts to the amino acid in rumen. However, some amino acid, such as Ile, Leu, and Phe which are synthesized by rumen microorganisms tend to limit the protein degradation in rumen [3,6,60]. Second reason pointed to lack of determining proteolytic population was in recently alternative and complementary technique of the present study, where measurement of proteolytic counting used the roll-tube technique observed in rumen culture no longer accurate. It is recently suggested that bacterial cells including N distribution therein, could be affected by indigenously factor in rumen such as rate of fermentation [60]. As consequence, further studies are needed to greater explain the protein utilization by proteolytic population in rumen determined by using sequence technique to define extracellular prokaryotic diversity in bovine rumen [61].

Table 7. Effect of dietary crude protein on microbial group in rumen of Thai swamp buffaloes.

Item	Crude protein level				SEM	Significance		
	5.42%	6.92%	8.92%	10.71%		L	Q	C
Direct count								
Protozoa, $\times 10^5$ cell/mL								
0 h post feeding	7.81	4.63	6.56	4.81	2.10	ns	ns	ns
4 h post feeding	9.25	4.75	8.63	7.38	3.17	ns	ns	ns
Fungal zoospores, $\times 10^7$ cell/mL								
0 h post feeding	1.72^{b}	3.35^{ab}	4.14^{ab}	6.54^{a}	0.97	**	ns	ns
4 h post feeding	3.36	3.63	4.51	3.35	0.56	ns	ns	ns
Total bacteria, $\times 10^9$ cell/mL								
0 h post feeding	1.33	1.40	1.39	1.80	0.18	ns	ns	ns
4 h post feeding	1.01^{b}	1.09^{b}	1.23^{b}	1.69^{a}	0.13	*	ns	ns
Roll-tube technique								
Amylolytic bacteria, $\times 10^6$ CFU/mL								
0 h post feeding	2.06^{b}	12.81^{ab}	6.94^{b}	39.25^{a}	9.61	*	ns	ns
4 h post feeding	6.19	4.19	19.88	18.44	6.91	ns	ns	ns
Cellulolytic bacteria, $\times 10^8$ CFU/mL								
0 h post feeding	0.98^{b}	1.20^{b}	3.15^{a}	1.27^{b}	0.53	ns	ns	*
4 h post feeding	0.68^{b}	0.90^{ab}	1.75^{a}	0.73^{b}	0.27	ns	*	ns
Proteolytic bacteria, $\times 10^6$ CFU/mL								
0 h post feeding	3.81	3.19	2.69	5.92	1.83	ns	ns	ns
4 h post feeding	3.38	3.19	4.13	5.00	1.20	ns	ns	ns

SEM: standard error of mean.

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[a-d] Values on the same row under each main effect with different superscript differ significantly ($p < 0.05$). Significance (*, $p < 0.05$; **, $p < 0.01$; ns, not significantly different). L, Q, C: linear, quadratic and, cubic effects of difference crude protein levels.

3.5. Nitrogen or crude protein requirement

The net nitrogen or crude protein requirements for maintenance and growth of Thai swamp buffaloes were determined based on ADG (g ADG/kg $W^{0.75}$) and N intake (g N/kg $W^{0.75}$; Figure 3). It depicted the linear regression between dietary nitrogen or crude protein and the net requirements for maintenance and growth of Thai swamp buffaloes, as equation = 0.0725ADG + 0.8663 ($R^2 = 0.577$, $p < 0.001$, n = 16). Nitrogen requirement could be estimated, the nitrogen intake in which ADG (equally at 0) was nitrogen requirement for maintenance, ADG index (at slope) was nitrogen requirement for growth of Thai swamp buffaloes. Of note, it could be mathematically calculated following to 0.866 g N/kg $W^{0.75}$ or equivalent to 5.41 g CP/kg $W^{0.75}$/d (maintenance requirement), and 0.073 g N/g ADG or equivalent to 0.46 g CP/g ADG/d (growth). The recently findings regard of the net CP requirement for growth of Thai swamp buffaloes was higher than it reported in NRC [9], but the maintenance was lower. Besides, the present results were slightly higher for maintenance of buffaloes and lower to roughly at 24% for growth of buffaloes compared to those reported in Kearl's report (maintenance, (5.24 g CP/kg $W^{0.75}$; growth, 0.65 g CP/g ADG), as recommendation CP requirements of ruminants in developing countries [17]. The possibly these differences were at UIP presence of the applied diet as a deciding factor to influence on unaffected nutrient apparent tract digestibility and the population of ammonia-producer bacteria. Rumen fermentation alleviated with the greater number of UIP in diet, but UIP intensified ammonia retention as higher efficiency of nitrogen utilization in rumen. For instance, Paengkoum *et al.* [3] had estimated the metabolized protein (MP) requirement to increase one g/kg $BW^{0.75}$ that was 0.34 g MP/kg $BW^{0.75}$ of Thai-indigenous growing cattle and MP requirement for maintenance was 2.77 g/kg $BW^{0.75}$. It was suggested to supplement not over 10% CP DM in diet with proportional ratio of UIP and DIP (35:65), which resulted in the optimum growth performance for growing Thai-indigenous beef cattle. Several previous studies also had been testing to prove the net nitrogen requirement for maintenance and growth among bull animals having a age-related succession of rumen microbial communities [54], breed and sex [34,36], and balanced content of protein and energy [15,51]. Collectively, the present findings could corroborate other possibly factors, protein requirement of bull animals might relate to specific domestication and climatic condition as Thai swamp buffaloes investigated in the present study.

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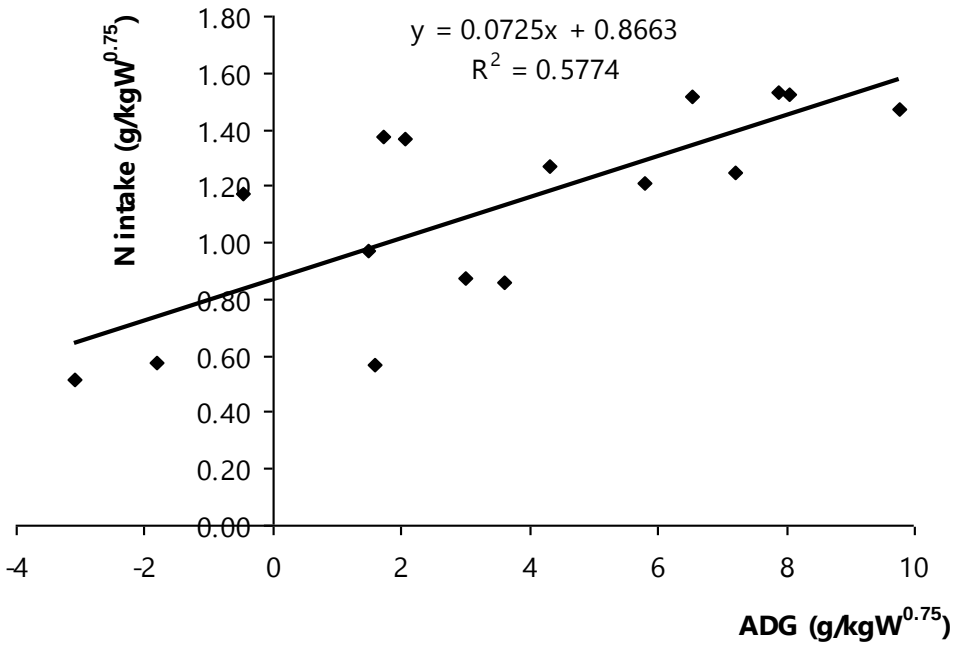

Figure 3. Relationship between average daily gain (ADG) and N intake in Thai swamp buffaloes.

4. Conclusions

In conclusion, the net crude protein requirement for maintenance of Thai swamp buffaloes was lower compared to NRC 2001. The net crude protein requirement for growth of Thai swamp buffaloes was higher compared to NRC 2001. The present results suggested that crude protein requirement increases, when body weight increases. An increased dietary crude protein resulted in an increased number at nutrient intake, nutrient digestibility, total volatile fatty acid, blood urine nitrogen, ammonia absorption, urinary purine derivative, and microbial related ammonia. Adjusting dietary crude protein ranged of 6.92-8.92% crude protein on dry-matter basis in diet is suggested an effective strategy to develop body weight and average daily gain in Thai swam buffaloes. More studies that use miscellaneous ages, inherent genetic variation, and varied production systems should be further carried out, so as to shed lighter on the changes in those dietary requirements of crude protein either in maintenance or growth stages.

Author Contributions: Conceptualization, S.P., P.T., N.T., T.S., R.A.P.P. and P.P.; methodology, S.P., P.T., N.T., T.S., R.A.P.P. and P.P.; investigation, P.T.; writing—original draft preparation, S.P., P.T., R.A.P.P. and P.P.; writing—review and editing, S.P.; supervision, S.P., R.A.P.P. and P.P.; funding acquisition, S.P. and P.P. All authors have read and agreed to the published version of the manuscript.

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