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INFLUENCE OF CRUDE PROTEIN AND ENERGY LEVEL ON FEED INTAKE, RUMINAL AMMONIA NITROGEN, AND METHYLGLYOXAL PRODUCTION IN SWAMP BUFFALOES (*BUBALUS BUBALIS*)

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ABSTRACT

The present experiments were conducted to investigate the effect of protein (CP) and energy levels in concentrate supplementation on feed intake, ruminal ammonia nitrogen (NH_3-N) and methylglyoxal (MG) production in swamp buffaloes (*Bubalus bubalis*) fed rice straw based diet. Eight Thai - rumen fistulated male swamp buffaloes, about 4 years old with body weight (BW) of 381±10 kg, were randomly assigned to four dietary treatments in two consecutive 4 × 4 Latin square design experiments. Four buffaloes in first square received four different concentrate supplementations containing different CP levels at 92, 124, 181 and 219 g/kg while other four in the second square received different concentrate containing different total digestible nutrient (TDN) levels at 740, 761, 806 and 843 g/kg, respectively. All buffaloes were fed concentrate mixtures at 10 g/kg BW and rice straw was offered *ad libitum*. Under this investigation, the result revealed that total dry matter feed intake (DMI) increased with the increasing levels of CP ($P<0.05$) while increasing TDN up to 843 g/kg in concentrate mixture reduced DMI ($P<0.05$). Moreover, increasing CP and TDN levels in concentrate mixture reduced ruminal pH of buffaloes. However, ruminal temperature was not affected by either CP or TDN levels in concentrate mixture ($P>0.05$). In addition, ruminal NH_3-N concentration and blood urea nitrogen of buffaloes increased with the increasing levels of CP in the concentrate ($P<0.05$) while there was no effect of TND level in concentrate on NH_3-N and BUN of buffaloes in the second square ($P>0.05$). In addition, rumen MG productions were different among treatments by either CP or TDN levels in concentrate mixture, especially at 6 and 8 hour post morning feeding ($P<0.05$). The highest ruminal concentration of MG production was found in buffaloes received concentrate mixture containing CP and TDN levels at 124, 181 g/kg CP and 761, 806 g/kg TDN, respectively. The mean values of MG production concentration influenced by CP and TDN levels in concentrate mixtures were in the range of 24.5-28.1 and 3.8-4.5 mg/dl, respectively. Based on the present study, increasing CP and TDN levels in concentrate diet increased feed intake while ruminal pH was reduced. NH_3-N and BUN concentration were increased by CP levels. Levels of CP and TDN between 124-181 g/kg CP and 761-806 g/kg TDN, respectively, in the concentrate mixture showed the highest ruminal MG concentration in swamp buffaloes fed on rice straw.

Key words: Energy; methylglyoxal; protein; rumen; swamp buffaloes; rice straw.

INTRODUCTION

Ruminal ammonia nitrogen, blood urea nitrogen and milk urea nitrogen are the most common indicators of assessing ruminal N availability in ruminant model. However, ruminal ammonia nitrogen may not give an accurate picture of nitrogen availability for microbial protein synthesis by rumen microbes. Recent efforts to enhance productive performance of ruminants through synchronization of carbohydrate and nitrogen (N) to improve fermentation in the rumen did not result in detectable benefits for the animals (Richardson *et al*., 2003). Furthermore, the introduction of imbalance between N and energy supplies in the rumen did not negatively influence microbial protein synthesis or N use in growing bulls (Valkeners *et al*., 2004). Both the ruminal microbes and their host animal possess the means to compensate for variations in kinetics of feed degradation to ensure a relatively continuous supply of nutrients of biosynthesis to optimize microbial growth (Dawson, 1999). As reported, microbial growth is dependent on the supply of fermentable carbohydrate and the end-products of protein metabolism are influenced by the availability of carbohydrate (Russell *et al*., 2009).

Nocek and Russell (1988) explained that when ATP from rumen carbohydrate fermentation is available NH_3-N and/or amino acids from the diet can be incorporated into microbial cells and ultimately utilized as microbial protein by the ruminants. If ATP is not sufficient to drive protein synthesis, amino acids will be fermented as an energy source and NH3-N will accumulate, changes in any of these factors will alter NH_3-N concentration in the rumen (Ørskov, 1982).

Horvath *et al*. (2007) and Lodge-Ivey *et al*. (2002) brought out very interesting data on ruminal methylglyoxal (MG) production influenced by protein supplementation. Methylglyoxal is a dicarbonyl compound (Staniszewsha and Nagaraj, 2006) produced as a by-product of glycolysis in most living organisms (Chaplen *et al*., 1996). Methylglyoxal is produced via the MG shunt of glycolysis as described by Russell (1998). Methylglyoxal production is a form of energy spilling utilized by the ruminal bacteria as a survival mechanism. Energy spilling is based around the ATP pool. When ATP levels exceeds anabolic reaction needs i.e. protein synthesis or other microbial requirements additional ATP is spilled and not utilized by the bacteria. When N is limiting, anabolic reactions are greatly reduced, thus ATP within the pool is more abundant and bacteria are more apt to spill energy (Ferguson *et al*., 1998). Unfortunately, if glucose levels do not normalize, MG buildup will ultimately lead to bacterial lysis (Ferguson *et al*., 1998).

In addition to production of MG via glycolysis, this toxin may result from amino acid breakdown and/or metabolism of fatty acids in the tissues (Beisswenger *et al*., 2005). Therefore, the aim of this study was to determine effect of energy and protein levels in concentrate mixtures supplement on ruminal MG production as a predictor of nutrient imbalance in the rumen of swamp buffaloes.

MATERIALS AND METHODS

Animals, feeds and management: Eight rumen-fistulated swamp buffaloes (*Bubalus bubalis*), about 4 years old with live BW of 381±10 kg, were randomly assigned to four dietary treatments in two 4 × 4 Latin square design experiments with four animals per square. In first square, animals received four different concentrate supplementations containing different levels of protein at 92, 124, 181 and 219 g/kg DM while animals in second square received concentrates containing different TDN at 740, 761, 806, 843 g/kg DM, respectively. All buffaloes were fed concentrate diet at 10 g/kg of BW, while rice straw was offered *ad libitum* as a roughage source. All buffaloes were fed diets twice per day in the morning (6:00 am) and afternoon (15:00 pm). The feed ingredients and chemical compositions of the experimental diet are shown in Tables 1 and 2. Each Latin square design with four buffaloes per square consisted of four periods and each periods was run for 21 days. First 14 days were for feed adaptation and intake measurement, while the last 7 days were for sample collection using total collection method while animals were on metabolism crates. All animals were kept in individual pens and given an *ad-libitum* access to water and mineral block throughout the experimental period.

Data collection, sampling procedures and analysis: Feed offered and refusals were recorded daily throughout the experimental period. To determine the DM contents of feeds, samples were randomly collected twice weekly. Samples of concentrate mixture and rice straw including refusals were collected daily during the collection period. Samples of rice straw and concentrate mixtures were composited by period and stored at -20ºC for later chemical analyses. The samples were divided into two parts: first part for DM analyses and second part pooled at the end of each period to determine ash, crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF). Feeds and refusals samples were dried at 60ºC and ground (1mm screen using the Cyclotech Mill, Tecator, Sweden) and analyzed using standard methods of AOAC (1995) for DM (ID 967.03) and ash (ID 942.05). Acid detergent fiber contents were determined according to an AOAC method (1995; ID 973.18) and was expressed inclusive of residual ash. The NDF in samples was estimated according to Van Soest *et al*. (1991) with addition of α-amylase but without sodium sulphite and results are expressed with residual ash. Total nitrogen (N) was determined according to AOAC (1995; ID 984.13).

At the last day of each period at 0, 2, 4, 6, 8, and 12 hour post morning feeding, approximately 200 ml of rumen fluid was collected from the middle part of the rumen by using a 60 ml hand syringe at each time. Rumen fluid was immediately measured for pH and temperature using a portable pH temperature meter (HANNA Instruments HI 8424 microcomputer, Singapore). Thereafter, rumen fluid was strained through a four layer of cheesecloth and transferred to a plastic bottle containing 5 ml of 1 M H_2SO_4. Rumen samples were then centrifuged at 3,000 x *g* for 10 min. About 20-30 ml of supernatant was collected and analyzed for NH_3-N by Kjeltech Auto 1030 Analyzer (AOAC, 1995; ID 973.18).

A 10 ml blood sample was collected from the jugular vein kept in heparinized tube, placed on ice immediately, and transported to the laboratory. Samples were refrigerated for 1 h and centrifuged at 3,500 x *g* for 20 min. The plasma were harvested and stored at -20°C to determine blood urea nitrogen (BUN) according to method described by Crocker (1967).

Methylglyoxal sampling and analysis: Rumen fluid was collected at 0, 2, 4, 6, 8, and 12 hour post morningfeeding. The samples were acidified using 30 ml

of 1M HCl as the pH of the each acidified samples was adjusted to a pH of 4.0 spiked with known quantities of MG. The ruminal fluid was strained through a four layered cheesecloth into an insulated thermal container, returned to the laboratory, and centrifuged (10000*g*, 10 min, 10°C) to remove bacterial cells. The supernatant was removed and centrifuged (10000*g*, 10 min, 10°C) and frozen at (-20°C) until further analysis of MG using HPLC (Agilent 1100 Series, Agilent Technologies, Santa Clara, CA) following the procedures described by Lodge-Ivey *et al*. (2004).

Methylglyoxal concentration was determined on acidified ruminal samples by the method of Espinosa-Mansilla *et al*. (1998). Ruminal samples were purified using cation exchange columns (Alltech) and 200 µl was used for MG estimation. The samples were incubated at 60ºC for 45 min with 7.02 mM 6-hydroxy-2, 4, 5-triaminopyrimidine (TRI) in 1.6 ml of sodium acetate buffer (pH 4.05). The samples were passed through a 0.45 µm syringe filter and 50 µl of purified sample was injected into a C-18 reverse phase HPLC column (Waters Corp. Milford, MA). The mobile phase was 20 mM sodium acetate buffer (pH 4.05). The flow rate was 1.5 ml/min. The pteridin derivative from the reaction of MG and TRI was detected by fluorescence at 447 nm (excitation at 352 nm) and quantitated by comparison with known quantities of similarly processed MG and 6-methylpterin (injected directly) as documented by Lodge-Ivey *et al*. (2004).

Statistical analysis: Data were subjected to ANOVA for a 4×4 Latin square design using the PROC GLM and orthogonal contrast of SAS (SAS Institute Inc., 1996). Data were analyzed using the model of $Y_{ijk} = \mu + M_i + A_j + P_k + \varepsilon_{ijk}$; where Y_{ijk} observation from animal; j, receiving diet I in period k; µ, the overall mean; M_i, effect of different crude protein and TDN (i=1, 2, 3, 4); A_j, the effect of animal (j=1, 2, 3, 4); P_k, the effect of period (k=1, 2, 3, 4); and ε_{ijk} the residual effect. The results are presented as mean values and standard error of the means. Differences between treatment means were determined by Duncan's New Multiple Range Test (Steel and Torrie, 1980). Differences among means were declared statistically significant at P<0.05.

RESULTS

Effect of protein levels: Table 3 shows the result of feed intake, rumen metabolites and MG production affected by CP level in concentrate supplementation in swamp buffaloes. Total dry matter feed intake increased (P<0.05) with the increasing levels CP in concentrate diet. Highest intake was documented in the diet containing 219 g/kg CP (7.3 kg/d) and lowest in the diet with lower CP 92 g/kg CP (5.5 kg/day). All buffaloes consumed different intake of CP (P<0.05) and the highest was in buffalo consumed concentrate containing CP at 219 following by 181, 124, and 92 g/kg CP respectively, whereas TDN intake of each buffaloes was similar Ruminal pH was affected by CP levels supplementation (P<0.05) and buffaloes consumed concentrate containing higher level of CP had lower ruminal pH. However, ruminal temperature was not affected (P>0.05) by the CP levels supplemented. Ruminal ammonia concentration increased (P<0.05) linearly with the increasing levels of CP in the diet. BUN was found the highest in buffalo consumed concentrate containing CP at 219 g/kg CP. The result on ruminal MG concentrations are shown in Table 3. The means of MG production was significant increased (P< 0.01) and ranged at 24.5-28.1 mg/dl .Ruminal MG was not affected at 0 to 4 h post feeding; whereas, at 6 and 8 h MG concentrate concentration increased linearly (P<0.05) with increasing level of CP in the diet. The highest concentration of MG was in the groups of buffaloes consuming concentrate mixture containing CP at 124 and 181 g/kg CP supplements.

Effect of energy levels: The effects of increasing levels of TDN on feed intake and rumen parameters and MG production in buffalo fed on rice straw based diets are presented in Table 4. Total dry matter feed intake was higher (P<0.05) in the treatment 841g/kg TDN compared with at 761 and 806 g/kg TDN level. Crude protein intake was not affected by dietary treatments (P>0.05); however, TDN intake quadratically increased with the increasing levels of TDN in concentrate mixture. Buffaloes consumed concentrate contained TDN at 761 g/kg TDN showed the highest result of total intake at 9.5 kg/day and the lowest was in the treatment group containing TDN at 843 g/kg TDN (8.5 kg/day). Ruminal pH was dropped in buffalo consumed concentrate containing TDN at 843 g/kg TDN.. However, there was no effect of TDN level on ruminal temperature. Moreover, TDN level did not show any effect on ruminal NH_3-N and BUN (P>0.05). The mean values of NH_3-N and BUN were in the range of 11.5-12.9 and 8.0-9.5 mg/dl, respectively. The result showed that MG were affected by different levels of TDN in concentrate mixture (P< 0.01) at 2, 6 and 8 hour post morning feeding. Buffaloes received concentrate containing TDN at 761 and 806 g/kg had higher MG production. The mean value of MG concentration in buffaloes affected by different levels of TDN was in the range of 3.8-4.5.

Table 1. Ingredients and chemical composition of the concentrates and rice straw

Items	Treatments, g/kg CP				RS
	92	124	181	219	
Ingredients, g/kg of DM					
Cassava chip	600	600	600	700	
Rice bran	150	150	170	20	
Coconut meal	150	100	40	60	
Cassava hay	50	50	50	50	
Urea	00	20	40	60	
Molasses	10	30	40	50	
Tallow	10	20	30	30	
Salt	10	10	10	10	
Sulphur	10	10	10	10	
Mineral mixture[1]	10	10	10	10	
Chemical composition, g/kg of DM					
TDN[2]	770	767	756	753	402
DM	925	924	906	907	942
OM	900	894	889	885	896
CP	92	124	181	219	28
NDF	510	455	420	271	779
ADF	221	196	178	163	452

RS = rice straw,
[1] Chemical composition = Ca, 164g; P, 80g; Mn, 11g; Na, 132.6g; S, 19.2g; Zn, 3.582g; Fe, 2.0g; Mg, 2.0g; Cu, 1000mg; Co, 42mg; I, 35mg; Selenium, 27ppm.
[2]TDN = total digestible nutrient (calculated value), DM = dry matter, OM = organic matter, CP = crude protein, NDF = neutral detergent fiber, ADF = acid detergent fiber.

Table 2. Ingredients and chemical composition of the concentrates

Items	Treatments, g/kg TDN of concentrates				RS
	740	761	806	843	
Ingredients					
Cassava chip	450	500	550	600	
Rice bran	240	150	50	30	
Coconut meal	50	50	150	150	
Palm kernel meal	195	170	100	20	
Soybean meal	10	70	50	60	
Tallow	5	10	30	50	
Molasses	5	10	30	50	
Urea	15	10	10	10	
Mineral mixture[1]	10	10	10	10	
Sulfur	10	10	10	10	
Salt	10	10	10	10	
Chemical composition g/kg of DM					
TDN[2]	740	761	806	843	470
CP	122	121	124	120	27
NDF	410	355	320	271	727
ADF	151	146	158	123	432

[1] Chemical composition = Ca, 164g; P, 80g; Mn, 11g; Na, 132.6g; S, 19.2g; Zn, 3.582g; Fe, 2.0g; Mg, 2.0g; Cu, 1000mg; Co, 42mg; I, 35mg; Selenium, 27ppm.
[2]TDN=total digestible nutrient; estimated value, RS = rice straw, DM = dry matter, OM = organic matter, CP = crude protein, NDF = neutral detergent fiber, ADF = acid detergent fiber.

Table 3. Influence of level of crude protein in concentrates on feed intake, ruminal metabolites and methylglyoxal in swamp buffaloes.

Items	Treatments, g/kg CP of concentrate				SEM	Contrasts	
	92	124	181	219		L	Q
Total DM intake							
kg/d	5.5[a]	6.4[b]	6.7[b]	7.3[c]	0.10	**	**
%BW	1.4[a]	1.8[b]	1.9[b]	2.1[c]	0.02	**	**
CP intake, kg/d	0.30[a]	0.45[b]	0.62[c]	0.64[d]	0.01	**	**
TDN intake, kg//d	5.2	5.4	5.6	5.2	0.30	NS	NS
Ruminal metabolites							
pH	6.5[a]	6.5[a]	6.2[b]	6.3[b]	0.05	**	0.07
Temperature, °C	38.2	38.5	38.6	38.3	0.2	NS	NS
BUN, mg/dl	7.3[a]	8.4[a]	10.0[a]	18.1[b]	1.27	**	*
Ruminal NH_3-N, mg/dl							
0 h post-feeding	4.3[a]	5.5[b]	6.3[b]	7.4[c]	0.31	**	NS
2	4.5[a]	9.7[b]	13.3[b]	19.7[c]	1.41	**	NS
4	4.7[a]	8.8[a]	17.0[b]	18.3[b]	1.72	**	NS
6	5.0[a]	8.1[b]	11.3[c]	15.5[d]	0.66	**	NS
8	5.3[a]	6.0[b]	6.3[b]	6.9[c]	0.21	**	NS
12	4.3[a]	5.5[b]	7.9[c]	9.1[d]	0.15	**	NS
Mean	5.0[a]	7.3[b]	10.3[c]	12.8[d]	0.52	**	NS
Methylglyoxal, mg/dl							
0 h post-feeding	7.8	8.9	8.9	9.0	0.51	NS	NS
2	16.9	17.1	19.2	16.2	1.15	NS	NS
4	23.3	30.6	25.9	26.1	4.13	NS	NS
6	29.7[a]	33.9[b]	33.7[b]	30.5[ab]	1.10	NS	**
8	29.5[a]	36.5[b]	39.3[b]	29.5[a]	1.48	NS	**
12	40.8	41.6	40.6	35.7	2.36	NS	NS
Mean	24.6[a]	28.1[b]	27.9[b]	24.5[a]	0.87	NS	**

[a,b,c,d] Means in the same row with different superscripts differ (P < 0.05), * P<0.05, ** P<0.01, SEM = standard error of the mean, NS = non-significance, L = linear, Q = quadratic.

Table 4. Influence of level of TDN in concentrates on ruminal ammonia and methylglyoxal in swamp buffaloes

Items	Treatments, g/kg TDN of concentrates				SEM	Contrasts	
	740	761	806	843		L	Q
Total DM intake							
kg/d	8.9[a]	9.5[b]	9.4[ab]	8.5[c]	0.36	NS	*
%BW	2.1[a]	2.3[ab]	2.1[a]	1.9[c]	0.14	NS	*
CP intake, kg/d	0.60	0.58	0.59	0.54	0.04	NS	NS
TDN intake, kg//d	3.1[a]	3.5[b]	3.9[c]	3.7[c]	0.04	**	**
Ruminal metabolites							
pH	6.5[a]	6.3[a]	6.3[a]	6.2[b]	0.10	*	NS
Temperature, °C	39.4	39.6	39.7	39.6	0.14	NS	NS
BUN, mg/dl	9.0	9.5	8.5	8.0	0.84	NS	NS
Ruminal NH_3-N, mg/dl							
0 h post-feeding	10.3	10.2	9.3	9.8	0.92	NS	NS
2	16.8	19.1	18.1	15.7	3.09	NS	NS
4	9.2	10.5	10.8	8.7	1.02	NS	0.09
6	9.4	9.5	9.3	8.8	0.26	0.09	NS
8	9.4	8.5	9.5	9.0	0.46	NS	NS
12	13.6	14.9	20.5	18.6	3.68	NS	NS
Mean	11.5	12.1	12.9	11.8	0.94	NS	NS
Methylglyoxal, mg/dl							
0 h post-feeding	4.2	2.7	3.2	3.1	0.46	NS	NS

2	4.6[a]	5.4[a]	5.7[a]	3.2[b]	0.43	0.06	**
4	3.4	3.2	4.3	3.9	1.37	NS	NS
6	2.2[a]	3.6[ab]	4.4[b]	3.5[ab]	0.19	*	*
8	4.6[a]	5.7[ab]	5.8[b]	3.9[c]	0.30	NS	*
12	4.2	3.6	3.6	6.1	1.23	NS	NS
Mean	3.8[a]	4.0[ab]	4.5[b]	3.9[a]	0.16	NS	*

[a,b,c] Means in the same row with different superscripts differ (P < 0.05), * P<0.05, ** P<0.01, SEM = standard error of the mean, NS = non-significance, L = linear, Q = quadratic.

DISCUSSION

Effect of protein level in concentrate: The results of CP level in concentrate mixture fed with untreated rice straw in swamp buffalo was highly different among treatments on ruminal NH_3-N. Mean ruminal NH_3-N concentrations ranged from 4.3 to 19.7 mg/dl, while there was increased at 5.0, 7.3, 10.3 and 12.8 mg/dl by 92, 124, 181 and 219 g/kg CP supplements, respectively. as a result of low CP concentration of the untreated rice straw suggesting a possible deficiency in degradable intake CP and therefore, satisfying an assumption of the experimental model. Ruminal NH_3-N values ranged between 10 to 20 mg/dl are thought to be adequate for microbial growth and fiber digestion (Wanapat and Pimpa, 1999). According to NRC (1996) guidelines (Level 1), assuming 110 g/kg microbial protein yield from TDN, the cows were deficient in degradable intake protein (60, 80, or 120%, low concentrate, medium concentrate or high concentrate, respectively). Bodine *et al*. (2001) reported ruminal NH_3-N concentrations were slightly lower than those observed in the current study and suggested these levels were characteristic of a deficiency of degradable intake protein. On the contrary, according to Chanthakhoun *et al*. (2012), it was found that higher level of C in the concentrate mixtures improved DMI, nutrients digestibilities of DM, OM, CP, and NDF, rumen fermentation, and microbial CP synthesis in swamp buffaloes.

Methylglyoxal has been shown to be produced in this experiment responded to excessive nitrogen and carbohydrate limitation. The means of MG production increased from 28.1-27.9 mg/dl as influenced by 124-181 g/kg CP supplements respectively, however from 6 and 8 h sampling period, there was a difference in MG production across treatments, particularly higher at crude protein in concentrate mixture at 124 and 181 g/kg CP. These data combined with ruminal NH_3-N values collected in the current study indicated that there was an imbalance in the nitrogen to carbohydrate ratio in the rumen of these buffaloes. Methylglyoxal concentrations in the rumen appeared to be a more sensitive tool to assess nitrogen status of the rumen than ruminal NH_3-N as a large extent of NH_3-N excess in the rumen may be excreted as urea in the urine by growing ruminants (Dehareng and Ndibualonji, 1994). In addition, production of MG via glycolysis, this toxin may result from amino acid breakdown and/or metabolism of fatty acids (Beisswenger *et al*, 2005; Lodge-Ivey, 2004). There have been not reported values for rumen MG concentration in swamp buffalo.. The values obtained in this study are comparable to the values obtained by other authors studies in *in-vitro* with cultures of *Escherchia coli* (Tötemeyer *et al*., 1998), *P. ruminicola* B14 (Russell, 1993), and *Clostridium acetobutylicum* ATCC 824 (Huang *et al*., 1999). On the other hand, Horvath *et al*. (2007) who found that crude protein levels in year one were greater (8.2%) than in years 2 and 3 (6.7%). It appears that the nutrient composition of the forage in year 1 better fit the needs of the rumen bacteria than in years 2 or 3 and these were indicated by lower MG levels produced (0.65 ± 0.12mM) in year one. Meanwhile, the result of this study were due to MG concentration, which serves as an indicator of ruminal fermentation, showed no effect for treatment at 0, 2, 4 and 12 h post-feeding. However, at 6 and 8 h post-feeding was detected. Total of MG concentration with level of CP in concentrate mixtures supplements were increased approximately 28.1 and 27.9 mg/dl.

Effect of energy level in concentrate: Based on this experiment, it was found that using 761 to 806 g/kg TDN, in the concentrate mixtures as supplements could improve DMI, nutrient OM intake, estimated ME, OM, and CP, digestibility in swamp buffalo (Chanthakhoun *et al*., 2012). The addition of rapidly fermentable carbohydrate, such as cassava chip and molasses, , would exacerbate a deficiency of ruminal NH_3-N creating an imbalance of nitrogen to carbohydrate in the rumen. Methylglyoxal concentration was shown in this experiment as responded to excessive energy or TDN and nitrogen limitation. There was a difference in MG production across treatments, particularly TDN in concentrate mixture at 761 and 806 g/kg TDN. Result of ruminal NH_3-N indicated that there was an imbalance in nitrogen to carbohydrate ration and lower MG production in the rumen of these buffaloes. Lodge-Ivey *et al*. (2002) indicated that the ruminal bacteria produced MG when exposed to an imbalance of carbohydrate to nitrogen and found that MG appears to be a more sensitive than ruminal NH_3-N in predicting nitrogen deficiency in the rumen. Moreover, the formation of MG was observed during the heating of glucose, fructose, maltose, maltulose; whereas, amount of MG obtained from

monosaccharides was markedly higher than that from disaccharides and glucose (Hollnagel and Kroh, 1998). Methylglyoxal formation was performed by carbohydrate decomposition during caramelization of mono-, oligo- and polysaccharides (glucose, dextrin 15, and starch) (Homoki-Farkas *et al*., 1997). These results are comparable to those of Horvath *et al*. (2007), who showed that MG production differed depending on sample day, no noticeable change occurred amongst sample days 1 through 4, but on the sampling day 5, it was different from all other sample days. The increase in MGruminal concentrations resulted from excessive fermentable energy in the rumen and validated the hypothesis for this study. Methylglyoxal concentrations in the rumen appeared to be a more sensitive tool to assess nitrogen status of the rumen than ruminal NH_3-N production.

Conclusions and Recommendations: Based on this experiment, increasing CP and TDN levels in concentrate diet increased feed intake while ruminal pH was reduced. NH_3-N and BUN concentration were increased by CP levels, but not by TDN. Ruminal MG concentration increased in buffaloes received concentrate mixture containing CP and TDN at 124 and 181 g/kg and 761 and 806 g/kg, respectively. These data indicated that MG could be an effective tool to assess ruminal N status for measuring the relationship of dietary energy and CP in swamp buffaloes. Furthermore, the finding under this experiment showed lower dietary CP requirement level for swamp buffaloes.

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