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EFFECT OF SYNCHRONIZATION OF CARBOHYDRATE AND PROTEIN SUPPLY IN THE SUGARCANE BAGASSE BASED DIET ON MICROBIAL PROTEIN SYNTHESIS IN SHEEP

A. T. Suhada, L. K. Nuswantara, E. Pangestu, F. Wahyono and J. Achmadi
Faculty of Animal and Agricultural Sciences, Diponegoro University,
Tembalang Campus, Semarang 50275 - Indonesia
Corresponding E-mail: jachmadi@gmail.com

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ABSTRAK

Penelitian bertujuan untuk mengkaji pengaruh penyelarasan pelepasan protein dan karbohidrat ruminal dalam pakan berbasis ampas tebu (PBAT) terhadap sintesis protein mikroba pada domba. Eksperimen pertama merupakan formulasi tiga PBAT dengan kandungan nutrien yang sama tetapi dengan indeks sinkronisasi yang berbeda (yaitu 0,36; 0,50 and 0,63). Penghitungan indeks sinkronisasi didasarkan atas koefisien degradabilitas nutrien secara *in sacco* dari masing-masing bahan pakan penyusun. Eksperimen kedua adalah penetapan pH rumen, konsentrasi total asam lemak volatil (TVFA) dan nitrogen amonia (NH_3-N) rumen, serta level nitrogen urea darah (BUN) pasca konsumsi pakan pada domba yang mendapatkan PBAT. Penelitian ketiga berupa penetapan kecernaan PBAT dan sintesis nitrogen mikroba (SNM) berbasis kandungan alantoin dalam urin domba yang mengonsumsi PBAT. Perbedaan indeks sinkronisasi pakan tidak merubah jumlah konsumsi nutrien tetapi meningkatkan kecernaan bahan kering, bahan organik, dan protein PBAT ($P<0,05$). Nilai pH rumen pasca konsumsi pakan menurun ($P<0,05$), tetapi konsentrasi TVFA, NH_3-N rumen serta level BUN pasca konsumsi pakan meningkat ($P<0,05$) akibat perlakuan indeks sinkronisasi pakan. Perlakuan indeks sinkronisasi pakan meningkatkan SNM ($P<0,05$), meskipun indeks sinkronisasi pakan sebesar 0,63 justru menurunkan SNM ($P<0,05$).

Kata kunci: ampas tebu, penyelarasan nutrien, sintesis protein mikroba, domba

ABSTRACT

The experimental research was conducted to clarify the effect of synchronization of ruminal carbohydrate and protein releases from sugarcane bagasse based diet (SBBD) on microbial protein synthesis in sheep. The first experiment was the formulation of three SBBD with similar nutrient content but differed in synchronization indexes (namely 0.36; 0.50 and 0.63). The in sacco nutrient degradability coefficient was used to calculate the synchronization index of each feedstuff. The second experiment was determination of post feeding ruminal pH, ruminal concentrations of total volatile fatty acids (TVFA) and ammonia nitrogen (NH_3-N), and blood urea nitrogen (BUN) level in sheep fed on experimental SBBD. The third experiment was determination of feed digestibility and estimation of microbial nitrogen synthesis (MNP) on the basis of excreted urinary allantoin. The alteration of dietary synchronization index did not change nutrient intake, but the digestibilities of DM, OM and CP were increased ($P<0.05$). The post feeding ruminal pH was decreased ($P<0.05$) but concentrations of post feeding ruminal TVFA and NH_3-N, and level of BUN were increased ($P<0.05$) by the treatment of dietary synchronization index. The treatment of dietary synchronization index improved MNP ($P<0.05$), although dietary synchronization index at 0.63 lowered the MNP ($P<0.05$).

Keywords: Sugarcane bagasse, nutrient synchronization, microbial protein synthesis, sheep

INTRODUCTION

Effects of synchronization of ruminal feed carbohydrate and nitrogen releases on some parameters of protein metabolism in ruminants were studied into some extents (Piao *et al.*, 2012; Seo *et al.*, 2010; Kaswari *et al.*, 2007; Chumpawadee *et al.*, 2006; Chanjula *et al.*, 2004; Kamel *et al.*, 2004; Richardson *et al.*, 2003). Effects of synchronization of carbohydrate and nitrogen in sugarcane bagasse based diet (SBBD) on some parameters of microbial protein synthesis in sheep remains to be elucidated. Balancing amounts of energy to protein in the diet is important to support an optimum level of sheep production. It is useful for further synchronizing feed rumen degradable protein and carbohydrate, thus the both can be utilized simultaneously by rumen microorganism (Karsli *et al.*, 2002). In general, dietary protein is more rapidly degraded in the rumen than dietary carbohydrate. The synchronization of ruminal carbohydrate and protein releases may also be a tool to achieve the friendly environment for ruminant animal industry, because excessive urinary nitrogen and losses of CO_2 and CH_4 from ruminal fermentation could be reduced (Yang *et al.*, 2010; Chumpawadee *et al.*, 2006). The Objective of this experimental research was to clarify effects of releases synchronization of ruminal carbohydrate and protein in the SBBD on microbial protein synthesis in sheep.

MATERIALS AND METHODS

Diet Formulation

This study used some agricultural wastes as feedstuffs, namely sugarcane bagasse, rice bran, sugarcane molasses, copra mill, palm frond mill, coffee seed shell, cassava waste, groundnuts shell, and soybean mill. The feedstuffs were purchased from some feed suppliers around Semarang city, province of Central Java. Samples of feedstuff (Table 1) were ground to pass through a 1 mm screen for the chemical analysis. Each feedstuff sample was also subjected on the study of ruminal nutrient degradability using in sacco technique.

Two local thin tail crossbred sheep with body weight average of 20 kg and aged at 2 years old were used in the in sacco technique. Experimental sheep were fitted with permanent rumen cannula and housed in individual cages. Sheep were fed on a diet containing 12% crude protein (CP); 62% total digestible nutrients (TDN); 55% neutral detergent fiber (NDF). The diet was offered at daily maintenance level and drinking water was available at all time. The nylon bag incubations were performed after two weeks adaptation to dietary and environmental experiment. Procedure of the in sacco technique was similar as described by Chumpawadee *et al.* (2006). The nylon bag technique tested ruminal organic matter (OM) and nitrogen (N) degradabilities of each feedstuff (Ørskov and McDonald, 1979). The synchronization index of ruminal releases of N to OM for each feedstuff was determined by using the data of ruminal OM and N degradabilities (Sinclair *et al.*, 1993). The synchronization index was calculated with assumption that 25 g of N per kg of OM is digested in the rumen (Czerkawski, 1986).

Table 1 shows the synchronization index and chemical composition of each feedstuff used in the formulation of experimental diets. Especially for urea and sugarcane molasses, at the first hour post feeding, 95% urea nitrogen was assumed to be degraded, and all OM and N of sugarcane molasses were assumed to be degraded completely (Chumpawadee *et al.*, 2006). The range of synchronization index is between zero and 1.00; the bigger synchronization index represents more perfect synchronization between ruminal nitrogen and energy supply throughout the day. Based on data of Table 1, three experimental SBBD were formulated to have synchronization indexes of 0.37; 0.50 and 0.63; respectively, but the contents of CP, TDN, and NDF were similar among them (Table 2).

Study of Ruminal Feed Fermentability

Two adult male sheep with body weight average of 23 kg and aged at 28 months old were used to study the ruminal feed fermentability. Sheep were fitted with permanent rumen cannula for sampling rumen fluid. In a cross manner, three experimental SBBD (Table 2) were offered randomly to the two sheep. Each experimental diet was offered to each sheep three times randomly for 7 days, with 7 days interval between feeding period of each experimental diet. About 10 ml of rumen fluid sample were collected at 1, 2, 3, 4, 5, and 6 h after feeding at the 7^{th} day of each feeding trial period. The pH of rumen fluid sample was determined immediately after the collection. The sample of rumen fluid was then filtered through four layers of cheesecloth. Rumen liquor sample from each collection was divided into two parts, and were used for determination of

Table 1. Chemical Compositions of Feedstuffs for Formulation of Experimental Diets

Feeedstuffs	SI	DM	Dry Matter Basis							
			OM	CP	EE	CF	NFE	TDN*	NDF	ADF
			---------- (%) ----------							
Sugarcane bagasse	0.09	93.09	96.66	1.75	2.06	38.22	54.63	49.49	89.07	53.98
Rice bran	0.67	91.30	87.19	6.00	8.15	24.44	48.60	66.63	61.20	28.39
Molasses	1.00	72.12	92.91	1.15	0.71	-	91.04	80.64	-	-
Copra mill	0.85	93.38	93.32	14.98	8.98	40.98	28.38	55.21	62.34	35.51
Urea	0.95	97.30	-	287.5	-	-	-	-	-	-
Palm frond mill	0.63	92.28	96.94	9.11	10.66	35.56	35.66	61.78	75.19	45.33
Coffee seed shell	0.56	89.98	90.13	7.37	3.93	42.61	36.21	47.68	72.58	51.69
Cassava waste	0.11	87.87	95.55	1.49	1.49	19.41	73.15	64.99	35.28	21.76
Wheat pollard	0.89	89.03	95.14	12.83	3.50	11.09	67.71	75.74	51.09	20.69
Groundnuts shell	0.29	91.41	91.47	5.59	1.16	54.98	29.74	34.38	69.68	56.89
Corn	0.53	88.54	98.79	8.84	2.85	5.04	82.06	80.06	51.07	17.56
Soybean mill	0.96	89.96	93.34	47.96	0.54	6.73	38.11	82.23	11.66	8.87

SI = synchronization index; DM = dry matter; OM = organic matter; CP = crude protein; EE = extract ether; NFE = nitrogen free extract; TDN = total digestible nutrients; NDF = Neutral detergent fiber; ADF = acid detergent fiber.
* Based on calculation (Harris *et al.*, 1972).

ammonia nitrogen (NH_3-N) and total volatile fatty acids (TVFA) concentrations, respectively. For determination of ruminal NH_3-N concentration, sample of rumen liquor was added preservative with a ratio of 1 ml rumen liquor : 1 ml NaCl 20%, and for determination of ruminal TVFA concentration, sample of rumen liquor was added preservative with a ratio of 1 ml of rumen fluid : 10 ml of $HgCl_2H_3PO_4$ (Widyobroto *et al.*, 2007).

A single jugular blood sample was also collected from each sheep at the same time of rumen fluid sample collection. About 5 ml of blood samples were collected using heparinized vacutainer in each sheep at 1, 3, and 6 h after feeding. The blood samples were then centrifuged at 2,500xg for 15 min, and plasma were for determination of blood urea nitrogen (BUN) concentration.

Feeding Trials

Fifteen male local thin tail cross bred sheep with body weight average of 18 kg and aged at 12 months were used in the feeding trials. Sheep were housed in the individual metabolic cages and drinking water was available at all time throughout the experimental period. The animals were divided randomly into three groups, and fed on the experimental SBBD (Table 2). The experimental diets were offered ad libitum in the form of total mixed ration.

After twelve weeks of adjustment to experimental diet, daily refusal feed, feces and urine were collected from each animal for ten days period of feeding trials. The daily refusal feed and feces respectively were weight and mixed well, and about 20% of sub samples were taken and stored at -20^{o}C. Daily feces sample was acidified using 0.1N H_2SO_4. The volume of daily urine sample was measured immediately after collection, and about 20% of sub samples were taken and stored at -20^{o}C. The daily samples of refusal feed, feces, and urine were accumulated at the end of collection period for each sheep. About 20% of each accumulated sample then was taken for analyses.

Chemical and Statistical Analysis

Concentrations of proximate and fiber components in feed and feces were determined according to proximate analysis (AOAC, 1995)

Table 2. Ingredients and Chemical Composition of Experimental Diets with Different Synchronization Index

	Synchronization index		
	0.37	0.50	0.63
Ingredient (%)			
Sugarcane bagasse	25.00	25.00	25.00
Rice bran	2.50	4.00	5.60
Molasses	2.00	4.00	7.00
Copra mill	2.50	6.00	16.30
Urea	0.70	0.50	0.20
Palm frond mill	16.50	9.00	1.00
Coffe seed shell	2.30	2.00	3.80
Cassava waste	30.20	15.50	2.20
Wheat pollard	1.10	15.50	23.00
Groundnuts shell	1.60	3.50	3.60
Corn	5.80	4.50	0.50
Soybean mill	11.30	10.00	11.30
Salt	0.50	0.50	0.50
Total	100.00	100.00	100.00
Chemical composition (%)			
Organic matter	94.44	94.03	93.48
Crude protein	12.06	12.17	12.95
Total digestible nutrients*	62.05	62.93	62.96
Extract ether	3.37	3.44	3.70
Carbohydrate	81.03	79.85	77.40
Non Structural Carbohydrate	26.22	23.89	21.43
Hemicellulose	21.95	23.85	24.31
Neutral detergent fiber	54.80	55.97	55.98

* Based on calculation (Harris *et al.*, 1972)

and detergent extraction method (van Soest *et al.*, 1991), respectively. Urinary allantoin concentrations was analyzed and used to estimate rumen microbial nitrogen synthesis (Chen and Gomes, 1992). Concentrations of TVFA and NH_3-N in rumen liquor were determined using method of gas chromatography (AOAC, 1995) and spectrophotometry (Chaney and Marbach, 1962), respectively. The blood plasma urea concentration (BUN) was assayed according to the method of Berthelot (AOAC, 1995).

In the study of ruminal feed fermentability, treatments of three experimental diets with different synchronization indexes were allocated according to a cross over design (two animals; three dietary synchronization index treatments) with four replicates for each treatment. Averages of after feeding ruminal pH, TVFA and NH_3-N concentrations and BUN level were used to discuss the effect of alteration of dietary

synchronization index on ruminal feed fermentability.

In the feeding trials, treatments of dietary synchronization indexe were allocated according to a completely randomized design with five replicates for each treatment. The nutrient digestibility of experimental diet was calculated based on amounts of nutrient intake and fecal nutrient excretion. The rumen degradable organic matter (RDOM) was calculated based on values of OM intake and digestibility (Liang *et al.*, 1994).

Data Analysis

All data were tested using analysis of variance and continued by the Tukey's test.

RESULTS AND DISCUSSION

Sugarcane bagasse is one of agricultural wastes that frequently used to sustain roughage availability for ruminant throughout the year. Previously, the maximum level of sugarcane bagasse in TMR for goat was determined to be 25% (Ariyani *et al.*, 2014). By synchronizing ruminal releases of energy and protein, the use of sugarcane bagasse in diet is expected to be more efficient. This study tested three diets containing similar portions of sugarcane bagasse with similar contents of CP, TDN, and NDF but differed in synchronization indexes (Table 2). In addition, another agricultural wastes were also included in the formulation of experimental diets, thereby values of dietary synchronization index were lower relatively compared with those of other studies (Piao *et al.*, 2012; Seo *et al.*, 2010; Kaswari *et al.*, 2007; Chumpawadee *et al.*, 2006; Biricik *et al.*, 2006).

Nutrient Intake and Digestibility

The alteration of synchronization index in SBBD did not effect on nutrient intake in sheep (Table 3). In this study, the experimental SBBD were designed to have similar levels of CP, TDN, NDF and ADF with different synchronization indexes (Table 2). The design of experimental diet was expected to result in similar intake amount of nutrients in sheep, thus avoiding any effect other than the treatment of dietary synchronization index on the microbial protein synthesis (Karsli *et al.*, 2002). The NDF concentration of the experimental diet was high relatively that may limit feed intake in sheep. If NDF concentration in forage based ration of dairy animal is higher than 35%, the feed intake may be limited by the rumen fill (Varga *et al.*, 1998). However, effect of NDF concentration in bagasse based diet on feed intake in sheep remains to be elucidated.

The feed digestibility is improved by the alteration of dietary synchronization index. The DM, OM and CP digestibilities of SBBD were increased ($P<0.05$) by the treatment of dietary synchronization index (Table 4). The synchronization of ruminal nutrients releases may contribute to the improvement in total tract of feed digestion, although the dietary synchronization index at 0.63 decreased ($P<0.05$) the DM, OM and CP digestibilities (Table 4). Although the dietary synchronization index was designed to be 0.95 the DM and OM digestibilities were not changed (Piao *et al.*, 2012). The nutrient digestibility is unchanged by synchronization of rumen nutrient release (Seo *et al.*, 2010). Eventhough the portions of corn meal and cassava chip were designed to be 75% in the diets, respectively, the DM and OM digestibilities remain unchanged (Chanjula *et al.*, 2004).

Ruminal Feed Fermentability

Figure 1 shows the changes of after feeding ruminal pH. The treatment of dietary synchronization index decreased ($P<0.05$) after feeding ruminal pH (Table 4). This result does not agree with others, the alteration of dietary synchronization index did not effect on after feeding ruminal pH (Piao *et al.*, 2012; Seo *et al.*, 2010; Chumpawadee *et al.*, 2006; Chanjula et al., 2004). The post feeding ruminal pH of sheep were ranging from 6.08 to 5.64. The decreasing post feeding ruminal pH may be caused by inclusions of some rumen readily available carbohydrate sources (Biricik *et al.*, 2006; Khezri *et al.* 2009). Portions of sugarcane molasses, rice bran, and copra mill were stepwise bigger to achieve the designed dietary synchronization indexes (Table 2). In addition, the post feeding ruminal pH of SI 0.63 group may be categorized as subclinical acidosis (Khezri *et al.* 2009). Therefore, this may contribute to the effect of the dietary synchronization index at 0.63 on the decreased DM, OM and CP digestibilities (Table 4), because more acidic ruminal condition may hamper the activity of digestive enzyme secreted by microbes. Although exact portion effect of fermentable carbohydrate in diet with different synchronization index on the changes of rumen pH in sheep remains to be clarified.

The changes of ruminal TVFA

Table 3. Averages of After Feeding Ruminal pH, Concentrations of Ammonia and Total VFA, and Blood Urea Nitrogen Level[1]

Parameters	Synchronization Index of Experimental Diet			SEM
	0.37	0.50	0.63	
Ruminal pH	6.08[a]	5.93[b]	5.64[c]	0.05
Ruminal NH_3-N, mg.dl^{-1}	9.92[c]	11.99[b]	16.59[a]	0.75
Ruminal TVFA, mmol.l^{-1}	69.59[b]	84.53[a]	86.33[a]	3.00
BUN, mg.dl^{-1}	78.27[b]	96.52[a]	96.81[a]	6.94

[1] Values are means of four observations.
SEM: Standard error of mean.
[a,b,c] $P<0.05$.

Table 4. Some Parameters of Protein Metabolism[1]

Parameters	Synchronization Index of Experimental Diet			SEM
	0.37	0.50	0.63	
Daily nutrient intake, g.kgBW$^{-0.75}$				
Dry matter	71.50	73.14	70.12	2.03
Organic matter	67.52	68.78	65.55	1.91
Crude protein	8.62	8.90	9.08	0.26
Total digestible nutriens	44.36	46.03	44.15	1.28
Neutral detergent fiber	39.18	40.94	39.25	1.14
Nutrient digestibility, %				
Dry matter	59.14[b]	62.96[a]	53.95[c]	1.02
Organic matter	61.88[b]	65.16[a]	56.98[c]	0.97
Crude protein	69.23[b]	72.06[a]	66.07[c]	0.71
Microbial protein synthesis				
Daily urinary allantoin, mM.d^{-1}	4.28[b]	6.86[a]	5.97[a]	0.39
Daily microbial N production, g.d^{-1}	3.25[b]	5.73[a]	4.90[a]	0.38
Efficiency of microbial N production*	11.76[b]	18.45[a]	20.04[a]	1.70

[1] Average values of 10 days observations in each sheep (n = 5).
SEM: Standard error of mean.
* Calculated from g of microbial N/kg of rumen degradable organic matter.
[a,b,c] $P<0.05$.

concentrations during post feeding period are shown in Figure 1. The treatment of dietary synchronization index increased ($P<0.05$) post feeding ruminal TVFA concentration, though there was no significant different between SI 0.50 and SI 0.63 groups (Table 4). The increased ruminal TVFA may be an indicative for the improved OM digestibility of dietary experiment

(Table 4). However, this result disagrees with some studies, who reported that post feeding ruminal TVFA is not affected by the treatment of dietary synchronization index (Piao *et al.*, 2012; Chumpawadee *et al.*, 2006; Richardson *et al.*, 2003). The characteristic of dietary components may explain the variations among these study results. The increased rumen TVFA concentration could contribute the decreased after feeding ruminal pH. There is a positive correlation between rumen pH and TVFA concentration (Seo *et al.*, 2010). The concentration of ruminal TVFA is highest when sheep fed on a diet that contained rapidly degradable starch and slowly degradable protein (Biricik *et al.*, 2006).

The post feeding ruminal NH_3-N concentrations increased ($P<0.05$) accordingly with the dietary synchronization indexes (Table 3). Some studies reported that after feeding ruminal NH_3 concentration is not affected by the treatment of dietary nutrients synchronization (Piao *et al.*, 2012; Seo *et al.*, 2010; Chanjula *et al.*, 2004; Biricik *et al.*, 2006). In addition, the pattern of ruminal NH_3-N concentrations were declining as the feeding time proceeded in all groups (Figure 1). The gradual decreasing ruminal NH_3-N throughout after feeding indicates the nitrogen capture for microbial protein synthesis (Kaswari *et al.*, 2007; Chumpawadee *et al.*, 2006).

Post prandial elevation of ruminal NH_3-N concentration increases urea level in blood circulation. After feeding concentrations of BUN were increased ($P<0.05$) by the treatment of dietary synchronization index, although the increased BUN concentrations were not accordance with dietary synchronization indexes (Table 3). The increased BUN levels were accordance with rised ruminal NH_3-N concentrations (Figure 2). The elevated post feeding ruminal NH_3-N is followed by the increased BUN levels in beef cattle (Chumpawadee *et al.*, 2006) and lactating dairy cattle (Chanjula *et al.*, 2004). However, there is no significant different in blood plasma urea of cattle when fed on synchronous or asynchronous diet (Sinclair *et al.*, 2000).

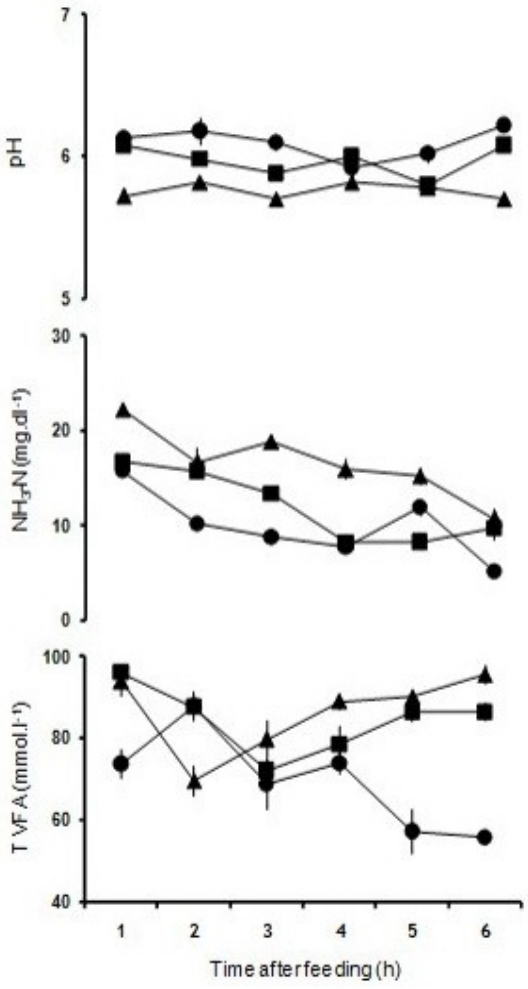

Figure 1. After Feeding Ruminal pH and Concentrations of NH_3-N and TVFA in Sheep Fed on SBBD with Synchronization Indexes of 0.37 (●); 0.50 (■); and 0.63 (▲). Values are means of four observations (± SEM).

Microbial Protein Synthesis

The elevated post feeding ruminal VFA and NH_3 productions are utilized partly for the growth of rumen microbes. Most microbial N flowing from the rumen is degraded and absorbed in the small intestine, and the waste is excreted via urine. Urinary excretion of allantoin could be an important indication of microbial nitrogen flowing from the rumen. The urinary nitrogen of purine derivatives (allantoin and uric acid) mostly from ruminal microbes because the majority of feed purine is degraded in the rumen (Moorby *et al.* 2006). The higher synchronization index of SBBD was postulated to improve the microbial protein synthesis in sheep, thus increases the dietary nutrient utilization. The treatment of dietary synchronization index increased ($P<0.05$) daily urinary allantoin, nitrogen microbial production (NMP), and efficiency of microbial nitrogen production (Table 4). This result agrees

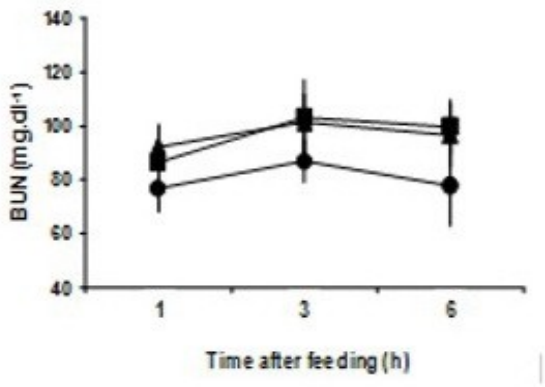

Figure 2. After Feeding Concentration of BUN in Sheep Fed on SBBD with Synchronization Indexes of 0.37 (●); 0.50 (■); and 0.63 (▲). Values are means of four observations (± SEM).

with some studies (Seo *et al.*, 2010; Kaswari *et al.*, 2007; Chumpawadee *et al.*, 2006; Richardson *et al.*, 2003). In this study, the daily microbial nitrogen synthesis that was estimated from the amount of urinary allantoin, ranging from 3 to 6 g. This amount of daily NMP is almost similar to the study of Srinivas *et al.* (2008), daily NMP were 6.36 and 3.28 g when daily CP intakes of sheep were 89.24 and 83.40 g, respectively. However, energy source availability is the one of main factor that effects on NMP (Richardson *et al.*, 2003).

The decreased NMP at the dietary synchronization index of 0.63 may be caused by the lowered ruminal pH. It is well known that the normal ruminal pH ranging from 6 to 7 supports an optimum microbial growth (Ørskov, 1986). The SBBD with synchronization index at 0.63 that lowered rumial pH at 5.64 (Table 3) may depress the growth of rumen microbes, although microbial population was not counted in this experiment. The depressed microbial growth could be related with the decreased nutrient digestibility of SBBD with synchronization index at 0.63 (Table 4). The increasing dietary synchronization index unaffects ruminal pH and increases population of rumen microbes which in turn improving in vivo feed digestibility (Chumpawadee *et al.*, 2006). In addition, the increased ruminal concentrations of TVFA and NH_3-N in SI 0.63 group (Table 3) may reflect the decreased capture of N for microbial protein synthesis. The decreased ruminal VFA and NH_3-N concentrations are associated with an increase in the use of both ruminal fermentation products for microbial protein synthesis (Seo *et al.*, 2010). However, the amount of duodenal nitrogen flow should be considered for clarifying the result of microbial protein synthesis estimation from excreted urinary allantoin (Yang *et al.*, 2010; Moorby *et al.* 2006).

CONCLUSION

The increasing concentrations of ruminal TVFA and NH_3-N and nutrient digestibility support the effect of dietary nutrient synchronization on an improvement of microbial protein synthesis in sheep. However, the dietary synchronization index at 0.63 resulted a decrease in rumen pH and lowered the microbial protein synthesis. It is suggested that rumen buffering agent should be included in the cellulolisic roughage based diet with higher index of nutrient synchronization.

REFERENCES

AOAC. 1995. Official Methods of Analysis. Association of Official Analytical Chemists, Washington, DC.

Ariyani, S.A., L.K. Nuswantara, E. Pangestu, F. Wahyono, J. Achmadi. 2014. Parameters of protein metabolism in goats fed diets with different portion of sugarcane bagasse. J. Indonesian Trop. Anim. Agric. 39:111-116.

Biricik, H., I.I. Turkmen, G. Deniz, B.H. Gulmez, H. Gencoglu, B. Bozan. 2006. Effects of synchronizing starch and protein degradation in rumen on fermentation, nutrient utilization and total tract digestibility in sheep. Ital. J. Anim. Sci. 5: 341-348.

Chaney, A.L. and E.P. Marbach. 1962. Modified reagent for determination of urea ammonia. Chem. Chemist. 8:130–132.

Chanjula, P., M. Wanapat, C. Wachirapaorn, P. Rowlinson. 2004. Effects of synchronizing starch sources and protein (NPN) in the rumen on feed intake, rumen microbial fermentation, nutrient utilization and performance of lactating dairy cows. Asian-Aust. J. Anim. Sci. 17:1400–1410.

Chen, X.B. and M.J. Gomes. 1992. Estimation of microbial protein supply to sheepand cattle on urinary excretion of purine derivatives. An overview of the technical details.

Occasional Publication. International Feed Resources, Rowett Research Institute, Aberdeen.

Chumpawadee, S., K. Sommart, T. Vongpralub, V. Pattarajinda. 2006. Effect of synchronizing the rate of dietary energy and nitrogen release on ruminal fermentation, microbal protein synthesis, blood urea nitrogen and nutrient digestibility in beef cattle. Asian-Aust. J. Anim. Sci. 19:181-188.

Czerkawski, J.W. 1986. An Introduction to Ruminal Studies. Oxford: Pergamon Press (Ed. H.J. Finlayson). The effect of pH on the growth and metabolism of *Streptococcus bovis* in continuous culture. J. Appl. Bacteriol. 61:201-208.

Harris, L.E., L.C. Kearl, P.V. Fonnesbeck. 1972. Use of regression equations in predicting availability of energy and protein. J. Anim. Sci. 35: 658–680.

Kamel, H.E.M., J. Sekine, A.M. El-Waziry, M.H.M. Yacout. 2004. Effect of *Saccharomyces cerevisiae* on the synchronization of organic matter and nitrogen degradation kinetics and microbial nitrogen synthesis in sheep fed Berseem hay (*Trifolium alexandrinum*). Small Rum. Res. 52: 211–216.

Karsli, M.A. and J.R. Russel. 2002. Effects of source and concentrations of nitrogen and carbohydrate on ruminal microbial protein synthesis. Turk. J. Vet. Anim. Sci. 26:201-207.

Kaswari, T., P. Lebzien, G. Flachowsky, U.T. Meulen. 2007. Studies on the relationship between the synchronization index and the microbial protein synthesis in the rumen of dairy cows. Anim. Feed Sci. Technol. 139: 1–22.

Khezri, A., K. Rezayazdi, M. Danesh.Mesgaran, M. Moradi-Sharbabk. 2009. Effect of different rumen-degradable carbohydrates on rumen fermentation, nitrogen metabolism and lactation performance of holstein dairy cows. Asian-Aust. J. Anim. Sci. 22: 651- 658.

Liang, J,B,, M. Matsumoto, B.A. Young. 1994. Purine derivative excretion and ruminal microbial yield in Malaysian cattle and buffalo. Anim. Feed Sci. Technol. 47:189-199.

Moorby, J.M., R.J. Dewhurst, R.T. Evans, J.L. Danelon. 2006. Effects of dairy cow diet forage proportion on duodenal nutrient supply and urinary purine derivative excretion. J. Dairy Sci. 89:3552-3562.

Ørskov, E. R. 1986. Starch digestion and utilization in ruminants. J. Anim. Sci. 63:1624-1633.

Ørskov, E.R. and I. McDonald. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agric. Sci. 92:499-504.

Piao, M.Y., H.J. Kim, J.K. Seo, T.S. Park, J.S. Yoon, K.H. Kim, J.K. Ha. 2012. Effects of synchronization of carbohydrate and protein supply in total mixed ration with Korean rice wine residue on ruminal fermentation, nitrogen metabolism and microbial protein synthesis in Holstein steers. Asian-Aust. J. Anim. Sci. 25:1568-1574.

Richardson, J.M., R.G. Wilkison, L.A. Sinclair. 2003. Synchronization of nutrient supply to the rumen and dietary energy source and their effects on the growth and metabolism of lambs. J. Anim. Sci 81:1332-1347.

Seo, J.K., J. Yang, H.J. Kim, S.D. Upadhaya, W.M. Cho, J.K. Ha. 2010. Effects of synchronization of carbohydrate and protein supply on ruminal fermentation, nitrogen metabolism and microbial protein synthesis in holstein steers. Asian-Aust. J. Anim. Sci. 23:1455-1461.

Sinclair, K.D., L.A. Sinclair, J.J. Robinson. 2000. Nitrogen metabolism and fertility in cattle: In adaptive changes in intake and metabolism to diet differing in their rate of energy release in the rumen. J. Anim. Sci. 78:2659-2669.

Sinclair, L.A., P.C. Garnsworhty, J.R. Newbold, P.J. Buttery. 1993. Effect of synchronizing the rate of dietary energy and nitrogen release on rumen fermerntation and microbial protein synthesis in sheep. J. Agric. Sci. (Camb.) 120: 251-263.

Srinivas, B., N. Swain, N.P. Singh. 2008. Disassociate bound protein in tree leaves by typsin hydrolysis and its effect on feed utilization, microbial protein and weight gain in sheep - an experimental study. Livestock Res. Rural Dev. 20, Article #12.

van Soest, P.J., J.B. Robertson, B.A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber and non starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74: 3583–3597.

Varga, G.A., H.M. Dann, V.A. Ishler. 1998. The use of fiber concentrations for ration formulation. J. Dairy Sci. 81:3063–3074.

Widyobroto, B.P., P.S. Budhi, A. Agus. 2007. Effect of undegraded protein and energy level on rumen fermentation parameters and microbial protein synthesis in cattle. J. Indonesian Trop. Anim. Agric. 32:194-200.

Yang, J.Y., J. Seo, H.J. Kim, S. Seo, J.K. Ha. 2010. Nutrient Synchronization: Is it a suitable strategy to improve nitrogen utilization and animal performance?. Asian-Aust. J. Anim. Sci. 23:972- 979.