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Research Article

Effects of replacing soybean meal with urea and negative energy balance on ruminal fermentation characteristics, kinetics of plasma glucose and urea in sheep

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Abstract

The objective of this study was to determine effects of replacing soybean meal by urea and negative energy balance (NEB) on nitrogen balance, ruminal fermentation characteristics and the kinetics of plasma glucose and urea in sheep. A crossover design with two different types of isonitrogenous (crude protein 5 g/ ($kg^{0.75}$·day) source, either soybean meal or replaced with urea, was assigned to six sheep. High energy dietary treatment (669 kJ/ ($kg^{0.75}$·day) following with low energy dietary treatment (222 kJ/ ($kg^{0.75}$·day) was nested in each isonitrogenous source for NEB induction. Plasma glucose and urea turnover rates were determined by the primed continuous infusion of [U-^{13}C] glucose and [$^{15}N_2$] urea simultaneously. NEB induction was modest with indicated by increased ($P<0.01$) of serum non-esterified fatty acid concentration, while β-hydroxybutyric acid concentration did not change. Nitrogen retention was lower ($P<0.01$) in NEB. Rumen pH and ammonia concentration were higher with urea replacement ($P=0.04$) and NEB ($P<0.01$). Almost all rumen volatile fatty acids concentration and plasma free amino acid concentrations were lower ($P<0.01$ and $P<0.05$) in NEB. Plasma glucose and ammonia concentrations were not influenced by urea replacement and NEB. The glucose turnover rate was lower ($P<0.01$) in NEB. However, plasma urea concentration and turnover rate were higher ($P<0.01$) in NEB. In conclusion, ruminal fermentation characteristics are affected by urea replacement and NEB. Regardless of urea replacement effect and interaction of urea × energy, NEB itself has strong influence on both of glucose and urea kinetics.

Keywords: negative energy balance; urea; glucose; kinetic; sheep

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Introduction

Urea could be economically substituted for rumen degradable protein in ruminant diet because ammonia from urea can be utilized by rumen microbes to form microbial protein. Urea is commonly hydrolyzed by microbes into ammonia and carbon dioxide in rumen (Johnson et al., 1979). When urea is used instead of rumen degradable protein, the rate of ammonia release from urea is faster (Cherdthong and Wanapat, 2010). Sharp rise in rumen ammonia concentration results in elevated concentrations of ammonia and urea in blood (Barley et al., 1981) and ammonia when carried to liver is usually converted into urea (Hammond, 1997).

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However, previous *in vitro* studies showed negative effect of ammonia concentration on hepatic gluconeogenesis (Demigné et al., 1991; Overton et al., 1999). Furthermore, ammonia loading by either infusion into the mesenteric vein or supplemental urea to feed (Wilton et al., 1988; Maltby et al., 1991) caused a decrease in the net hepatic production of glucose.

It is well known that animals experience the negative energy balance (NEB) during the peak of production. NEB condition is associated with depressed capacity of hepatic gluconeogenesis (Rukkwamsuk et al., 1999) and also decreased capacity of ureagenesis (Strang et al., 1998; Zhu et al., 2000; Overton, 2003). Hence, use of urea during NEB may be contradicted as it compromises the hepatic gluconeogenesis and ureagenesis under practical feeding condition. However, to the author's knowledge there is no report regarding the use of dietary urea during NEB on hepatic gluconeogenesis and ureagenesis. This prompted us to determine the effects of urea replacement on glucose and urea kinetics during NEB by deficiency of energy supply. It was hypothesized in this study that the isonitrogenous replacement of soybean meal by urea during NEB condition would be more depressible to the rate of gluconeogenesis and ureagenesis.

Because, glucose is absorbed from the gastrointestinal tract of ruminants is relatively small, most of the glucose is supplied from gluconeogenesis (Widiawati et al., 2014). Moreover, almost all of urea in ration is rapidly hydrolyzed to ammonia in the rumen (Cherdthong and Wanapat, 2010) and urea by itself very slowly transported across the rumen wall for direct absorption (MooNey and O'Donovan, 1970). Therefore, the glucose and urea absorption are limited. The rates of glucose and urea turnover could be used to assess gluconeogenesis and ureagenesis in the current study. In addition, nitrogen balance, ruminal fermentation characteristics, plasma free amino acids concentration and interaction of urea × energy were within concurrent examination.

Materials and Methods

Animals, experimental design and diets

The experiment was carried without any noticeable stress to the animals under approval of the Laboratory Animal Care and Use Committee of Iwate University (approval no: A201255). All sheep had been keeping inside the free stall farm in Iwate University before the study was started. Four rams and two wethers healthy crossbred (Corriedale x Suffolk) sheep with average age of 3.5 – 4 years. The trial was carried out in crossover design with two isonitrogenous diets of either soybean meal or urea replaced diet. Each experimental period lasted for 24 days, consisted of 14 days adaptation period and 10 days experimental period. The sheep were randomly assigned to each sequence of trial.

The diet composition is stated in Table 1. The basal diet consisted of mixed hay (orchard grass and reed canary grass), cornstarch and soybean meal. The trial was preceded by 669 kJ/ ($kg^{0.75}$·day) metabolizable energy (ME) of high energy diet and crude protein 5 g/ ($kg^{0.75}$·day) contained either soybean meal or replaced with 0.8 g/ ($kg^{0.75}$·day) (Wako, Japan) urea in isonitrogenous during the adaptation period. The same dietary treatments were continued for the first 5 days of the sample collection period, then ME intake was suddenly reduced to 222 kJ/ ($kg^{0.75}$·day) for the last 5 days (low energy). The nitrogen source was still unchanged and crude protein level was balanced with the basal diets in each sequence of trial. Cornstarch, soybean meal and urea were weighed according with the ration treatments, then homogenous mixed and mashed together with mixed hay prior feeding time. Diets were offered in equal proportion twice a day at 08:30 and 20:30 hours and had free access to drinking water during the whole experimental period.

The animals were housed in individual pens during the adaptation period. The sheep were moved to individual metabolic cages in a controlled environmental room with 23±1°C, 70% relative humidity and 08:00 – 22:00 hours of lighting during last 10 days in each treatment period.

Samples collection

The sheep were weighed weekly and feed were adjusted accordingly. On day 1-3 of each high and low energy diets, urine and faeces were collected for 3 days from each sheep for nitrogen (N) balance study. The urine bucket contained 50 ml of 6 N H_2SO_4. The 24 h urine samples were measured and a subsample of 50 ml was stored at -30°C until analysis. Feed and each 24 h of faeces samples were collected, dried at 60°C for 48 h, ground and stored in a plastic box at room temperature until analysis.

Daily serum sample was collected at last 2 day of high energy diet and day 1-5 of low energy diet. Before the morning meal, blood was sampled from jugular vein puncture into serum separating tube (Vacutainer® SST™, BD, USA). Serum was separated by centrifuging at 3500*g* for 15 min at 4°C (H-3R, Kokusan, Japan) and subsequently analyzed the serum non-esterifies fatty acid (NEFA) and β-hydroxybutyric acid (BHBA) for NEB monitoring on the same day.

On day 4 of each high and low energy diet, rumen liquid samples (approximately 50 ml) were taken at pre-feeding (0 h) and at 1.5, 3 and 6 h post-feeding. Rumen fluid pH was immediately measured (F-51, Horiba Ltd., Japan) and the rumen liquid samples were centrifuged at 8000*g* for 10 min at 4°C (RS-18IV, Tomy, Japan). The mixture of 1 ml of the supernatant from the rumen

liquid samples and 1 ml of 0.1 mol/l HCl was used for ruminal ammonia analysis. A 5 ml of supernatant of rumen liquid samples were used for ruminal volatile fatty acid (VFA) analysis. All of the rumen samples were stored at -30°C.

Table 1: Dietary treatment formulation and chemical composition of diets

g (dry matter)/ ($kg^{0.75}$·day)	Non-urea		Urea	
	High energy	Low energy	High energy	Low energy
Dietary treatment formulation				
Mixed hay	21.5	7.2	21.8	7.6
Cornstarch	31.1	2.8	36.3	7.8
Soybean meal	5.2	8.9	0.1	3.9
Urea	0.0	0.0	0.8	0.8
Chemical composition				
Dry matter intake	57.8	18.9	59.0	20.1
Crude protein	5.3	5.0	5.3	5.1
Metabolizable energy[A] (kJ/ ($kg^{0.75}$·day)	671	221	674	223

[A] estimated from AFRC, 1993.

Isotope infusion procedure and collection

On day 5 of each high and low energy diet, the primed continuous infusion of [U-^{13}C]glucose and [$^{15}N_2$]urea was applied to determine plasma glucose and urea kinetics simultaneously. Polyvinyl catheters were inserted in both of left and right jugular veins at pre-feeding. A mixed saline solution containing 2.9 μmol/$kg^{0.75}$ of [U-^{13}C]glucose (D-glucose-$^{13}C_6$, 99 atom% excess; Cambridge Isotope Laboratories, USA) and 10.2 μmol/$kg^{0.75}$ of [$^{15}N_2$]urea ($^{15}N_2$urea, 98 atom% excess; Cambridge Isotope Laboratories, USA) was injected into the right jugular catheter as a priming dose at 12:00 hours. Same mixed saline solution was continuously infused for 4 h through the same catheter by using a multichannel peristaltic pump (AC-2120, Atto, Japan) at rate of 2.9 and 10.2 μmol/ ($kg^{0.75}$·h).

For stable infusion attainment, the infusion rate of tracer solution was monitored every 30 min throughout the infusion period. A 10 ml of blood sample was taken from the left jugular catheter into heparinized tubes at before priming dose infusion (0 h) and 6 ml at 2.5, 3, 3.5 and 4 h during infusion. The blood samples were centrifuged at 10000*g* for 10 min at 4°C and plasma was collected and stored at -30°C until further analysis.

Chemical analysis

Nitrogen contents of diets, faeces and urine were determined with the Kjeldahl method (AOAC, 1990) by using Tecator 2520 and Kjeltec 2300 (FOSS, Sweden). A factor of 6.25 was used to convert N into crude protein. N absorption, N retention and N digestibility were calculated by (N intake – faecal N), (N intake – (faecal N + urinal N) and (N absorption/ N intake) x 100, respectively. Rumen ammonia concentration was analyzed with a colorimetric method (Weatherburn, 1967) by using a spectrophotometer (V-630, JASCO, Japan). The VFA concentration was measured by using gas chromatography (HP-5890, Hewlett Packard, USA) after stream distillation as described by Sano et al. (2009).

Serum NEFA and BHBA were analyzed by using automatic chemistry analyzer (Toshiba TBA 40FR Accute, Diamond Diagnostics, USA) based on ACS-ACOD method (NEFA-HAII) and cyclic enzymatic method (autokit 3-HB). The plasma glucose concentration was determined by the glucose oxidase method (Huggett and Nixon, 1957). The plasma free amino acids and ammonia concentrations at pre-infusion period were determined by using an automated amino acid analyzer (JLC-500/V, JEOL, Japan).

The plasma [U-^{13}C]glucose enrichment was determined according to Fujita et al. (2006) by using gas chromatography mass spectrometry (QP-2010, Shimadzu, Japan). The derivatives were analyzed with ionized monitoring as m/z 314 and m/z 319 for plasma [U-^{13}C]glucose. The [$^{15}N_2$]urea enrichment was analyzed according to Wood et al. (2006) by using gas chromatography mass spectrometry. The derivatives were analyzed with ionized monitoring as m/z 231 and m/z 233 for plasma [$^{15}N_2$]urea. The plasma isotope enrichments were obtained by comparing their peak areas and peak area abundances with standard curve. The plasma urea concentrations were calculated from the ratio of unlabeled urea and [^{13}C,$^{15}N_2$]urea as internal standard (^{13}C, 99%,·$^{15}N_2$, 98% atom excess; Cambridge Isotope Laboratories, USA) with m/z 231 and m/z 234.

Calculations and statistical analysis

The glucose and urea turnover rates were calculated according to the equation described by Wolfe (1984), as follows:

Turnover rate (mmol/ ($kg^{0.75}$·h) = I x (1 / E-1)

Where I represents the infusion rate of [U-^{13}C]glucose or [$^{15}N_2$]urea. E represents the isotope enrichment of the plasma [U-^{13}C]glucose or [$^{15}N_2$]urea at the steady state condition, respectively.

The mean values and standard error of means (SEM) were generated for all data. All data were analyzed by using MIXED procedure in SAS (1996). The model composed of the fixed effects as periods (first and second), urea (soybean meal and urea), energy (high and low energy) and the interaction of urea × energy. Sheep were randomized as random effect. Ruminal fermentation characteristics were subjected to repeated measurement analysis. Tukey-Kramer adjustment was used to identify diets with different effects on the variable involved. The level of significance was set at $P<0.05$ throughout the analysis unless otherwise mentioned.

Results

Negative energy balance induction

Effects of replacing soybean meal with urea and NEB on average daily gain (ADG) and serum metabolites are presented in Table 2. No significant difference (P>0.05) of ADG with urea replacement was observed. However, ADG was lower (P<0.01) in low energy when compared to high energy. Interaction effect of urea × energy was not found (P>0.05). Serum NEFA concentration was not affected (P>0.05) by urea replacement but was higher (P<0.01) during low energy. Interaction effect of urea x energy was not found (P>0.05). Serum BHBA concentrations had no difference (P>0.05) either in urea replacement or low energy.

Nitrogen balance

Effects of replacing soybean meal with urea and NEB on N balance are presented in Table 3. N intake was influenced (P<0.01) by urea replacement as well as energy. Faecal N, N absorption and digestibility were also affected (P<0.05) by urea replacement and low energy. Urinary N excretion was not affected by urea replacement (P>0.05) but it was higher (P<0.01) in low energy. Consequently, the lower (P<0.01) N retention during low energy was accompanied by a significantly increase in urinary N excretion. However, urea replacement did not affect (P>0.05) N retention. Urea and energy interaction effect was not found in any of the N balance parameters.

Ruminal fermentation characteristics

Average (0-6 h post-feeding) of rumen fermentation characteristics are presented in Table 4. Rumen pH and ammonia concentration were higher with urea replacement (P=0.04) and low energy (P<0.01). Total VFA, acetate, propionate and butyrate concentrations were not affected (P>0.05) by urea replacement but were higher (P<0.01) in high energy. Interaction effect of urea × energy was found in concentration of acetate and butyrate (P<0.05), and acetate: propionate ratio (P<0.01). Acetate concentration was higher (P<0.05) in high energy while butyrate concentration was lowest (P<0.05) in non-urea low energy diet.

Plasma free amino acids, glucose, ammonia and urea concentration

Plasma free amino acids, glucose, ammonia and urea concentrations at pre-isotope infusion are presented in Table 5. Plasma free amino acid concentrations were not influenced (P>0.05) by urea replacement, except for glutamine. Plasma threonine, valine, methionine, isoleucine, leucine, phenylalanine, serine, asparagine, glutamic acid, glutamine, glycine, alanine, tyrosine and proline concentrations were higher (P<0.05) in high energy. Interaction effect of urea × energy was not found (P>0.05).

Plasma glucose and ammonia concentrations did not differ (P>0.05) at pre-infusion levels in all dietary conditions. Plasma urea concentration was not affected (P>0.05) by urea replacement while it was higher (P<0.01) in low energy. There was no interaction effect of urea and energy (P>0.05).

Plasma glucose and urea kinetics

Plasma glucose concentration and [U-^{13}C] glucose enrichment almost reached in a steady state during 2.5 - 4 hours of isotope infusion in all dietary treatments [Fig 1]. Plasma glucose kinetics is presented in Table 6. During primed continuous infusion period, plasma

Table 2: Effects of replacing soybean meal with urea and negative energy balance on average daily gain and serum metabolites in sheep

	Non-urea		Urea		SEM	P value		
	High energy	Low energy	High energy	Low energy		Urea	Energy	Urea × Energy
ADG (kg/day)	-0.05	-0.40	0.05	-0.60	0.06	0.63	<0.01	0.13
Serum metabolites (mmol/l)								
NEFA	0.14	0.46	0.08	0.52	0.05	0.93	<0.01	0.30
BHBA	0.27	0.30	0.44	0.40	0.03	0.06	0.90	0.57

ADG - Average daily gain, NEFA - Non-esterified fatty acid, BHBA - β-hydroxybutyric acid

Table 3: Effects of replacing soybean meal with urea and negative energy balance on nitrogen (N) balance in sheep

N balance	Non-urea		Urea		SEM	P value		
	High energy	Low energy	High energy	Low energy		Urea	Energy	Urea *x* Energy
N intake g/ ($kg^{0.75}$·day)	0.84	0.80	0.85	0.80	<0.01	<0.01	<0.01	0.36
Fecal N g/ ($kg^{0.75}$·day)	0.46	0.20	0.36	0.14	0.02	0.02	<0.01	0.46
Urinary N g/ ($kg^{0.75}$·day)	0.23	0.64	0.30	0.65	0.03	0.23	<0.01	0.29
N absorption g/ ($kg^{0.75}$·day)	0.39	0.60	0.50	0.66	0.02	0.01	<0.01	0.43
N retention g/ ($kg^{0.75}$·day)	0.16	-0.04	0.20	0.02	0.03	0.31	<0.01	0.88
N digestibility (%)	46	75	58	82	2.50	0.02	<0.01	0.45

Table 4: Effects of replacing soybean meal with urea and negative energy balance on average (0-6 h post-feeding) of ruminal fermentation characteristics in sheep

Ruminal fermentations (mmol/l)	Non-urea		Urea		SEM	*P* value		
	High energy	Low energy	High energy	Low energy		Urea	Energy	Urea x Energy
pH	6.35	6.99	6.72	7.01	0.1	0.04	<0.01	0.06
Ammonia	2.54	8.66	3.76	9.88	0.7	0.04	<0.01	0.99
Total VFA	64.4	35.4	60.2	38.2	3.1	0.59	<0.01	0.14
Acetate	40.7[a]	21.2[b]	36.1[a]	24.6[b]	1.9	0.72	<0.01	0.03
Propionate	11.9	6.7	14.6	5.3	1.0	0.68	<0.01	0.17
Butyrate	8.6[a]	4.4[c]	8.2[a]	6.5[b]	0.4	0.12	<0.01	0.02
Isobutyrate	1.0	1.2	0.3	0.7	0.1	0.01	0.15	0.83
Valerate	0.6	0.6	0.5	0.4	<0.1	0.08	0.33	0.18
Isovalerate	0.9	1.3	0.4	0.8	0.1	<0.01	0.01	0.71
A/P ratio	4.0[a]	3.6[ab]	2.6[b]	4.7[a]	0.2	0.73	0.04	<0.01

a, b, ab, c – means with different letters in a row differ significantly (P<0.05); A/P - Acetate: Propionate ratio

Table 5: Effects of replacing soybean meal with urea and negative energy balance on plasma free amino acids, glucose, ammonia and urea concentrations at pre-isotope infusion in sheep

Plasma concentrations	Non-urea		Urea		SEM	*P* value		
	High energy	Low energy	High energy	Low energy		Urea	Energy	Urea x Energy
Amino acids (µmol/l)								
Threonine	161	79	197	76	13	0.32	<0.01	0.24
Valine	195	135	208	111	11	0.73	<0.01	0.24
Methionine	17	12	19	11	1	0.87	<0.01	0.53
Isoleucine	80	62	83	58	3	0.89	<0.01	0.54
Leucine	108	84	102	71	5	0.27	<0.01	0.68
Phenylalanine	46	28	48	28	3	0.86	<0.01	0.75
Histidine	42	40	48	42	2	0.18	0.20	0.51
Lysine	107	88	127	113	6	0.06	0.15	0.79
Aspartic acid	5	N/D	7	4	1	N/D	N/D	N/D
Serine	140	79	225	105	17	0.05	<0.01	0.30
Asparagine	84	48	92	48	6	0.78	<0.01	0.79
Glutamic acid	75	55	73	47	6	0.51	0.02	0.14
Glutamine	248	171	335	259	20	<0.01	0.04	0.76
Glycine	772	416	1157	508	76	0.26	<0.01	0.66
Alanine	236	141	251	169	24	0.15	<0.01	0.42
Tyrosine	60	40	80	37	8	0.13	<0.01	0.09
Arginine	123	124	123	124	5	0.41	0.77	0.40
Proline	95	53	112	54	6	0.28	<0.01	0.36
Glucose (mmol/l)	4.5	4.6	4.3	4.7	0.2	0.92	0.57	0.77
Ammonia (µmol/l)	144	143	122	137	9	0.96	0.75	0.80
Urea (mmol/l)	3.4	5.7	3.7	5.5	0.3	0.66	<0.01	0.25

N/D - not detected

glucose concentration did not change (P>0.05). Urea replacement did not influence (P>0.05) plasma glucose turnover rate. However, lower (P<0.01) plasma glucose turnover rate was found in low energy but no interaction effect (P>0.05) of urea × energy was observed.

Plasma urea concentration and [$^{15}N_2$] urea enrichment almost reached in a steady state during 2.5 - 4 hours of isotope infusion in all dietary treatments [Fig. 1]. Plasma urea kinetics is presented in Table 6. Plasma urea concentration and turnover rate were not affected (P>0.05) by urea replacement. Nevertheless, higher (P<0.01) plasma urea concentration and turnover rate were found in low energy but no interaction effect (P>0.05) of urea × energy was found.

Discussion

Effects of urea and NEB on glucose kinetic

Both urea replacement and low energy treatments had no effect on basal glucose concentration at pre-isotope infusion. The basal glucose concentration (4.3-4.7 mmol/l) was slightly above than standard serum glucose concentration of 1.7-3.6 mmol/l in sheep (Jackson and Cockcroft, 2002). This might be because of blood samples were collected 3 h post-feeding and rumen VFA concentrations peaked at that time.

Table 6: Effects of replacing soybean meal with urea and negative energy balance on plasma glucose and urea kinetics during 2.5-4 h of primed continuous infusion in sheep

Plasma kinetics	Non-urea		Urea		SEM	P value		
	High energy	Low energy	High energy	Low energy		Urea	Energy	Urea ×Energy
Glucose concentration (mmol/l)	4.4	3.8	4.8	4.5	0.2	0.08	0.09	0.60
Glucose turnover rate (mmol/ ($kg^{0.75}$·h)	1.8	1.2	1.6	1.0	0.1	0.13	<0.01	0.83
Urea concentration (mmol/l)	2.9	5.4	3.0	5.2	0.3	0.64	<0.01	0.57
Urea turnover rate (mmol/ ($kg^{0.75}$·h)	1.9	5.4	1.5	3.3	0.4	0.06	<0.01	0.15

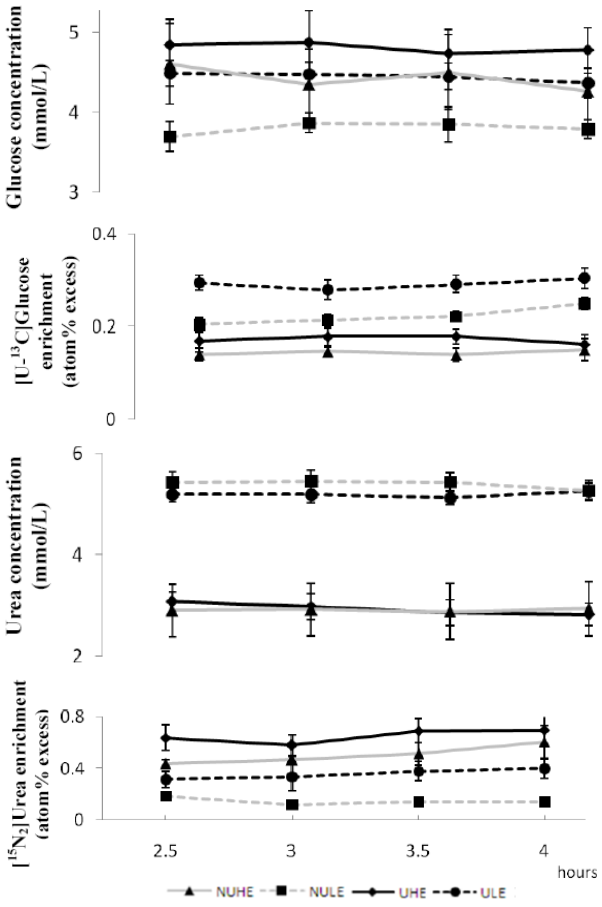

Fig. 1: Time course changes on plasma glucose concentration, [U-^{13}C]glucose enrichment, plasma urea concentration and [$^{15}N_2$]urea enrichment during 2.5- 4 h of primed continuous infusion with four dietary treatments; Non-Urea High Energy (NUHE), Non-Urea Low Energy (NULE), Urea High Energy (UHE) and Urea Low Energy (ULE) in sheep.

However, blood glucose concentration is subjected by hormonal regulation (Weekes, 1991).

In our study, urea replacement had no effect on plasma glucose turnover rate. This result did not accord with the hypothesis that isonitrogenous replacement of soybean meal by urea could impair glucose turnover rate. Low plasma ammonia concentration might be responsible for that. Even rumen ammonia concentration was higher with urea replacement diet and also higher during NEB. Our results showed lower plasma ammonia concentration (<0.15 mmol/l) than previous studies that successfully decrease glucose production (Weekes et al., 1978; Demigné et al., 1991). Because the amount of urea is rapidly hydrolyzed to ammonia in the rumen (Golombeski et al., 2006; Highstreet et al., 2010) and ammonia utilization was intrinsically related to energy supply from rumen carbohydrate availability (Delgado-Elorduy et al., 2002; Reynolds, 2006).

Plasma glucose turnover rate was lower during NEB. The result stated that glucose production rate was lower with low availability of energy according to our expectation that NEB condition would depress rate of glucose turnover. In contrast, glucose turnover rate is higher with high energy intake and gluconeogenic supply to the liver (Brockman, 1993; Danfaer et al., 1995).

Ortgues-Marty et al. (2003) who reviewed that the effect of intake level are unanimously considered as being highly determinant for glucose turnover.

Total VFA concentration was higher during high energy diet, because high dietary intake increases VFA production (Doreaua et al., 2003). In addition, the rumen pH was lower in high energy diet because of higher VFA production. High energy diet improved N retention under isonitrogenous intake, with decreases in digestible N. These changes in N metabolism might be associated with increased ammonia utilization as well as improved microbial N supply. Superior microbial N supply with high energy level could be due to more efficient synchronizing between dietary energy and N supply (Sinclair et al., 1995).

Besides that, faecal N excretion was higher in high energy diet. Cornstarch was a major composition in high energy diet. Even cornstarch was highly fermented in the rumen but a part of unfermented could escape from ruminal fermentation (Ørskov, 1986). The escape starch was fermented in the large intestine and increased microbial protein production. Therefore, faecal N excretion loss would be expected (Owens and Soderlund, 2006).

Effects of urea and NEB on urea kinetic

Plasma ammonia concentration at pre-isotope infusion did not differ among treatments. However, plasma urea concentration at pre-isotope infusion was higher in low energy. Plasma urea concentration was 1.5 times higher in low energy (5.5-5.7 mmol/l) than high energy (3.4-3.7 mmol/l) but both remained in normal serum concentration (3–10 mmol/l) in sheep (Jackson and Cockcroft, 2002). Plasma urea turnover rate was higher in low energy with no effects by urea replacement and interaction. Similar results were observed by Cocimano and Leng (1967) who found a linear relationship between plasma urea concentration and urea turnover rate in sheep. The increase of plasma urea turnover rate was a contrary result to our hypothesis that the NEB condition would be associated with a depressed capacity of hepatic ureagenesis. The possible reasons might be due to lack of severe NEB induction in our study and also lack of high lipid accumulation in liver cells to impair liver function (Zhu et al., 2000; Overton, 2003).

Usually the NEFA concentration above than 0.4 mmol/l affects hepatic function in sheep (Cynthia, 2010). The slightly higher NEFA concentration (0.46-0.52 mmol/l) in our study might not enough to affect liver function during NEB. Moreover, serum BHBA concentration remained at the normal range (<0.8 mmol/l) in healthy sheep (Cynthia, 2010). ADG during NEB was lower (-0.40 to -0.60 kg/day) than previous negative energy induction (-0.05 to 0.05 kg/day) as well. Taken together, it reveals that the animal lost their body weight during NEB which might be indicated the fat mobilization from adipose tissues. This phenomenon may have satisfied energy balance while ketogenesis might not be activated. As N retention is largely depended on amount of fermentable carbohydrate in the diet (Sarwar and Ajmal Khan, 2003). Low N retention was found in low energy diet in the present study. The result agrees with the observation of EL-Sabagh et al. (2009) who reported that N balance was negative at the low energy intake level.

Plasma urea turnover rate was more pronounced during NEB condition. Though propionate is a major gluconeogenic substrate, amino acids may also act as gluconeogenic substrates during increased metabolic demand of glucose, such as during NEB (Bergman and Heitmann, 1978; Overton et al., 1999). When liver increases hepatic catabolism of amino acids, ammonia production will be increased (Overton et al., 1999). The liver would compensate this increased amount of ammonia by detoxifying ammonia into urea and accordingly resulted in increased urea turnover rate (Nolan and Leng, 1970). Moreover, almost all plasma free amino acid concentrations were lower during NEB.

Nozière et al. (2000) showed similar findings of decreased concentration of almost all essential and non-essential amino acids, when dietary intake was reduced. However, the potential of amino acids as gluconeogenic substrate during NEB in our experiment were unclear due to study design. In addition, only plasma glutamine concentration was higher in urea replacement diet. Glutamine is a major amino acid to carry ammonia from peripheral tissues to corporate into urea at the liver or excrete in the urine at the kidney. The urea usage in the diets for ruminants enhanced synthesis of glutamine, which inhibited the synthesis of nitric oxide from arginine in urea cycle (Wu, 2013).

Urinary N was also higher during NEB. This data showed inefficient ruminal utilization of ammonia that was reflected by the urinary nitrogen excretion (Henning et al., 1993). Similarly Lindberg and Jacobsson (1990) reported that the proportion of urea N excretion increased in low energy diet and decreased in high energy diet.

Finally, from our result high plasma urea turnover was simultaneous with low plasma glucose turnover during NEB. This might be resulted from the competition for cytoplasmic oxaloacetate between ureagenesis and gluconeogenesis pathways (Krebs et al., 1979). Moreover, after ammonia addition in the incubated liver cells, Meijer et al. (1978) found decreased gluconeogenesis because of decreased flux through phosphoenolpyruvate carboxykinase (PEPCK) and a fall of malate concentration which in turn cause a fall of oxaloacetate concentration. However, our finding in this experiment is unclear for this mechanism and may need further study for more understanding.

Conclusion

The isonitrogenous replacement of soybean meal with urea does not impair rates of plasma glucose and urea turnover. On the other hand, NEB (222 kJ/($kg^{0.75}$·day) has strong influence on both plasma glucose and urea turnover rates without any interaction with urea replacement.

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