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Effects of Non-protein Energy Intake on the Concentrations of Plasma Metabolites and Insulin, and Tissue Responsiveness and Sensitivity to Insulin in Goats

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ABSTRACT : A glucose clamp technique was used to investigate the effects of non-protein energy intake on tissue responsiveness and sensitivity to insulin for glucose metabolism in intact adults male goats. Three goats were fed diets at 1.0, 1.5 and 2.0 times of ME for maintenance, each for 21 d. Crude protein intake was 1.5 times of maintenance requirement in each treatment. Tissue responsiveness and sensitivity to insulin were evaluated using a hyperinsulinemic euglycemic clamp technique with four levels of insulin infusion, beginning at 13 h after feeding. Concentrations of plasma metabolites and insulin were also measured at 3, 6 and 13 h after feeding, for evaluating effects of non-protein energy intake on the metabolic status of the animals. Increasing non-protein energy intake prevented an increase in plasma NEFA concentration at 13 h after feeding ($p = 0.03$). Plasma urea-nitrogen and total amino-nitrogen concentrations decreased ($p<0.01$) and increased ($p = 0.03$), respectively, with increasing non-protein energy intake across time relating to feeding. Plasma insulin concentration was unaffected ($p = 0.43$) by non-protein energy intake regardless of time relating to feeding. In the glucose clamp experiment, increasing non-protein energy intake decreased numerically ($p = 0.12$) the plasma insulin concentration at half-maximal glucose infusion rate (insulin sensitivity), but did not affect ($p = 0.60$) maximal glucose infusion rate (tissue responsiveness to insulin). The present results suggest that an increase in non-protein energy intake may enhance insulin sensitivity for glucose metabolism, unlike responsiveness to insulin, in adult male goats. The possible enhancement in insulin sensitivity may play a role in establishing anabolic status in the body, when excess energy is supplied to the body. (**Key Words :** Energy Intake, Insulin Sensitivity, Insulin Action, Glucose Clamp, Feeding, Goat)

INTRODUCTION

The extent of effects of insulin on nutrient metabolism is changed by insulin concentration in blood and/or insulin action to tissues. Insulin action to tissue, which distinguishes between tissue responsiveness to insulin and insulin sensitivity (Kahn, 1978) is modified under various physiological conditions, such as pregnancy, lactation, obesity and cold exposure, in ruminants (Weekes, 1991).

Although nutritional conditions have effects on insulin action in human and rodents (Kelly, 2000) nutritional effects on insulin action are not well investigated in ruminants. Inconsistent results have been available on the effects of energy intake on insulin action for glucose metabolism in previous reports, which indicated that an increase in energy intake enhanced insulin action in goats supplemented starch (Fujita et al., 2000) reduced in the cold exposed sheep (Sano et al., 1999) and did not affect in male sheep (Terashima et al., 1991) or pregnant ewes (Petterson et al., 1993). The inconsistency may be partly associated with the fact that energy intake changed with intake of a whole diet in these studies except for that in goats, because changing intake of whole diet causes concurrent changes in intakes of dietary ingredients that would affect insulin action, such as CP (Sano et al., 2001) and starch (Fujita et al., 2000). Thus, although the effects of energy on insulin action should be distinguished from that of CP in ruminants, information is not available on the effects of dietary energy attributed to ingredients other than CP, which is defined as non-protein energy, on insulin action in ruminants, except for that of starch in goats described above.

The present study was designed to determine the effects of non-protein energy intake on tissue responsiveness to insulin and insulin sensitivity of glucose metabolism in goats, using a hyperinsulinemic euglycemic clamp technique with four doses of insulin infusion. Changes in concentrations of plasma metabolites and insulin in relation

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Table 1. The amounts of diets fed to goats in each treatment

Treatment[1]	The amounts of diet fed (g/kg BW/d)		
	Mixed hay	Ground corn	Ground soybean meal
1.0×M	5.49	7.47	3.52
1.5×M	8.24	14.60	1.86
2.0×M	10.98	21.74	0.20

[1] 1.0×M: energy intake for maintenance, 1.5×M: 1.5 times energy intake for maintenance, 2.0×M: 2.0 times energy intake for maintenance. Differences in energy intake were achieved by changing energy intake attributed to ingredients other than CP.

to feeding were also examined to evaluate the effects of non-protein energy intake on the metabolic status of the animals.

MATERIALS AND METHODS

Animals and management

Three Saanen intact male goats (3 yr, initial mean BW 37.8±6.4 kg SE) were surgically prepared under anesthesia with a skin loops enclosing the left carotid artery and kept individually in metabolic cages in a controlled environment chamber at air temperature of 20±1°C. Mixed hay (60% of reed canarygrass and 40% of orchardgrass; ME 1.73 Mcal/kg, CP 10.6%), ground corn (ME 2.78 Mcal/kg, CP 7.3%) and ground soybean meal (ME 2.77 Mcal/kg, CP 49.0%) were used as experimental diets. Each animal was offered three levels of ME, which were maintenance (1.0×M), 1.5 times maintenance (1.5×M) and 2.0 times maintenance (2.0×M) for requirement, by changing the amount of each experimental diet (National Research Council, 1985; Table 1). Crude protein intake and the percentage of mixed hay in each dietary treatment were 1.5 times maintenance for requirement (National Research Council, 1985) and 33%, respectively. This feeding regimen allowed the dietary treatments to have different levels of non-protein energy intake through different ME and the same CP intakes in each treatment. The period of each dietary treatment was 21 d. Animals were weighed in the morning of 1 and 16 d of each period. Two animals were allotted to a descending order of energy intake and the other to an ascending order. The diets were equally divided into two meals which were offered twice daily at 08:30 and 20:30 h. Water and mineral blocks were available free access. Animals completely ate the diets within half an hour after feeding. Surgery, management, and blood sampling were carried out according to the guidelines established by the Animal Care Committee of Iwate University.

Blood sampling with time course relating to feeding

On 17 d of each dietary treatment, three blood samples (10 ml) were taken via the left carotid artery by puncture at 10-min intervals between 2.5 and 3 h after feeding. Additionally, on 18, 19 and 20 d of each dietary treatment, daily blood samples were taken via the same vessel at 6 h after feeding. Blood samples were placed in heparinized tubes and stored in ice until centrifugation. Plasma was separated from blood by centrifuging at 8,000 g for 10 min at 4°C and stored at -20°C.

Hyperinsulinemic euglycemic clamp experiment

On 21 d of each dietary treatment, the hyperinsulinemic euglycemic clamp experiment was conducted over 8 h beginning at 10:00 h to evaluate tissue responsiveness to insulin and insulin sensitivity (Bergman et al., 1985). Catheters for infusion and for blood sampling were inserted into a jugular vein at 4 d before the experiment and into the left carotid artery at least 2 h before the initiation of the experiment, respectively. Both catheters were filled with a sterile solution of trisodium citrate (38 g/L). Animals were not given a morning meal on the day of experiment. Sterile saline (9 g sodium chloride/L) solutions of insulin (Novolin R, Novo Nordisk, Denmark) were continuously infused during four sequential 2-h periods at rates of 0.38, 0.96, 2.4 and 6.0 mU/kg BW/min, respectively, into the infusion catheter using a peristaltic pump (AC-2120, Atto Co. Ltd., Tokyo, Japan). The concentrations of insulin solution were 64 U/L of sterile saline for the two lower infusion doses and 400 U/L of sterile saline for the two higher infusion doses. A sterile solution of glucose (200 g/L) was infused with another peristaltic pump at variable rates through the same infusion catheter so as to maintain basal blood glucose concentrations. Ten milliliters of blood samples were taken at 10-min intervals over 30 min before the initiation of insulin infusion, and blood glucose concentrations were immediately determined with an automated glucose analyzer (GLU-1, Erma Optical Works, Tokyo, Japan). The basal blood glucose concentrations were determined by averaging glucose concentrations of these samples. Residual blood samples were placed in heparinized tubes and stored in ice until centrifugation, for assay of plasma metabolites and insulin. During the insulin infusion, 1 ml of blood samples were taken from the arterial catheter at 10-min intervals and additional 1 ml of blood samples were taken at 30-min intervals. Blood glucose concentrations were immediately determined by the same manner in order to maintain preinfusion glucose level by changing glucose infusion rates as required. Residual blood samples taken at 30-min intervals were placed in heparinized tubes and stored in ice until centrifugation, for insulin assay. The glucose infusion rates were recorded every 10 min during the insulin infusion. After the end of the clamp experiment, plasma was separated from blood by centrifuging at 8,000 g for 10 min at 4°C and stored at -20°C until used for metabolites or insulin assay.

Analysis

Glucose concentration in blood or plasma was measured with the automated glucose analyzer described above. Plasma lactate concentration was measured as described by Taylor (1996). Plasma urea-nitrogen concentration was determined by a modified diacetylmonoxim method (Coulombe and Favreau, 1963). Plasma NEFA concentration was enzymatically determined using a diagnostic kit (NEFA-C test wako, Wako, Osaka, Japan). Plasma total amino-nitrogen concentration was determined by a dinitrophenol method (Rapp, 1963). Plasma concentrations of individual VFAs were measured by GLC (HP5890A, Hewlett Packard, Avondale, USA) after separation by steam distillation (Sano et al., 1989). Plasma insulin concentration was measured using a RIA kit (IRI 'Eiken', Eiken Chemical Co. Ltd., Tokyo, Japan).

Calculations

The concentrations of plasma metabolites and insulin at 3 and 13 h after feeding were obtained as mean values of the three samples taken during 2.5 to 3 h after feeding on 17 d and before insulin infusion in the clamp experiment on 21 d, respectively. The concentrations at 6 h after feeding were obtained by averaging three values of samples taken on 18, 19 and 20 d. These values were used to evaluate the changes in the concentrations with time course relating to feeding.

In the hyperinsulinemic euglycemic clamp experiment, the mean concentrations of blood glucose and plasma insulin, and the mean glucose infusion rate (GIR) were calculated during the last hour of each 2-h period of insulin infusion. Tissue responsiveness to insulin and insulin sensitivity were evaluated on the basis of the maximal GIR (GIR_{max}) and the plasma insulin concentration at half-maximal GIR (EC_{50}, Bergman et al., 1985). These indices were calculated from logistic regression of individual dose-response curves for GIR vs. ln (insulin concentration) before the experiment and during the last hour of 0.38, 0.96, 2.4 and 6.0 mU/kg BW/min of insulin infusion (Clement et al., 1996).

Statistical analysis

All data were analyzed using the MIXED procedure of SAS (1996). The effects of energy intake on the plasma metabolites and insulin in relation to feeding were analyzed by a split-plot design with repeated measures for time relating to feeding. A main plot was energy intake, and sub-plots were time relating to feeding and an interaction between energy intake and the time, and a block was animal. Autoregressive order one was used as a covariance structure for repeated measures. In the hyperinsulinemic euglycemic clamp experiment, the mean concentrations of blood glucose and plasma insulin, and the GIR were analyzed by a split-plot design with repeated measures for insulin infusion levels, with energy intake as a main-plot, insulin infusion levels and an energy intake×insulin infusion levels interaction as sub-plots, and animal as a block. Autoregressive order one was used as a covariance structure for repeated measures. The GIR_{max} and EC_{50} were analyzed by a randomized block design with energy intake as a fixed effect and animal as a block. The difference of least square means with Tukey-Kramer adjustment was used to compare the differences between levels in each main effect. In any ANOVA, the main effect and interaction were considered significant at the $p<0.05$ level and as tendencies at the $0.05 \leq p < 0.10$ level. The significance of differences between each level within these effects was determined by Tukey-

Table 2. The effects of non-protein energy intake on the concentrations of plasma metabolites and insulin across time relating to feeding in adult goats[1]

	Treatment[2]			p-value[3]
	1.0×M	1.5×M	2.0×M	Energy
n	3	3	2	
Glucose (mg/dl)	62±2^{b}	59±2^{c}	66±2^{a}	<0.01
Lactate (mg/dl)	4.9±0.6^{b}	6.0±0.6^{ab}	8.3±0.8^{a}	0.08
NEFA (μEq/L)	179±27	134±27	62±32	0.11
Urea-nitrogen (mg/dl)	16.9±1.0^{a}	12.0±1.0^{b}	8.3±1.1^{c}	<0.01
Total amino-nitrogen (mg/dl)	4.6±0.2^{b}	5.0±0.2^{ab}	5.7±0.3^{a}	0.03
Insulin (μU/ml)	9.5±4.5	14.6±4.5	16.1±5.2	0.43
Acetate (μM)	672±113^{b}	885±113^{a}	816±117^{ab}	0.05
Propionate (μM)	10±2^{b}	16±2^{ab}	21±2^{a}	0.03
n-Butyrate (μM)	11±2	17±2	9±2	0.09
Isobutyrate (μM)	2±<1	3±<1	3±<1	0.49
Isovalerate (μM)	6±1	8±1	7±1	0.32

[1] Values represent least square means and SE. These values were obtained from statistical analysis (see the text). Different superscripts within a row are significantly different at $p<0.10$.

[2] 1.0×M: energy intake for maintenance, 1.5×M: 1.5 times energy intake for maintenance, 2.0×M: 2.0 times energy intake for maintenance. Differences in energy intake were achieved by changing energy intake attributed to ingredients other than CP.

[3] Energy: main effect of energy intake.

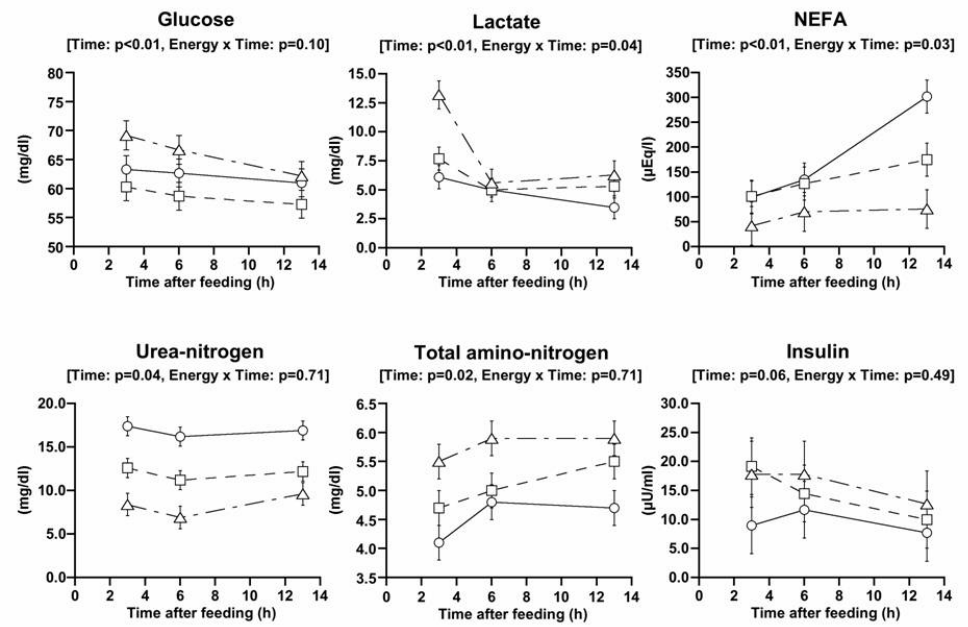

Figure 1. Changes in plasma concentrations of metabolites and insulin with time course relating to feeding in goats fed diets containing different metabolizable energy. Data are expressed as least square means and SE: energy intake for maintenance (O, n = 3), 1.5 times energy intake for maintenance (□, n = 3), 2.0 times energy intake for maintenance (Δ, n = 2). Differences in energy intake were achieved by changing energy intake attributed to ingredients other than CP. p-values of the main effect of time relating to feeding (time) and an interaction between energy intake and time relating to feeding (energy×time) are shown in brackets. Data were obtained from statistical analysis (see the text).

Kramer adjustment test at $p<0.10$, when the significant effect or the tendency were found.

RESULTS

Only two sheep were used for 2.0×M, because one of the animals refused food. Because the number of animals for 2.0×M was two, all data in tables and figures show the least square means calculated by the ANOVA with SAS.

The changes in plasma metabolites and insulin in relation to feeding

The effects of dietary non-protein energy intake on the concentrations of plasma metabolites and insulin across time relating to feeding are presented in Table 2. Figure 1 and 2 are also shown to facilitate evaluation for an interaction between non-protein energy intake and time relating to feeding in the concentrations.

Plasma glucose concentration was quadratically affected ($p<0.01$) by energy intake, and decreased linearly ($p<0.01$) with time course relating to feeding (Table 2, Figure 1). Increasing energy intake tended to increase linearly ($p = 0.08$) plasma lactate concentration, and decreased numerically ($p = 0.11$) plasma NEFA concentration. With transition of time relating to feeding, plasma lactate decreased quadraticallly ($p<0.01$), but plasma NEFA increased linearly ($p<0.01$). Energy×time interaction was found on these metabolites (lactate, $p = 0.04$; NEFA, $p = 0.03$). Plasma lactate was higher for 2.0×M than for 1.0×M and 1.5×M at 3 h after feeding, but was similar for each energy intake level at 6 and 13 h after feeding. Although plasma NEFA increased linearly with time course relating to feeding for 1.0×M, the low level of the metabolite was kept through the time course for 1.5×M and 2.0×M. Plasma concentration of urea-nitrogen decreased linearly ($p<0.01$) with increasing energy intake, but that of total amino-nitrogen increased linearly ($p = 0.03$). With time course relating to feeding, plasma urea-nitrogen changed quadratically ($p = 0.04$), and plasma total amino-nitrogen increased linearly ($p = 0.02$). Energy intake had no effects ($p = 0.43$) on plasma insulin concentration. Insulin tended to decrease quadratically ($p = 0.06$) with an advance of time relating to feeding.

Increasing energy intake tended to increase quadratically ($p = 0.05$) plasma acetate concentration, and increased linearly ($p = 0.03$) plasma propionate concentration (Table 2; Figure 2). These metabolites concentrations decreased linearly ($p<0.01$) with time course relating to feeding. Plasma propionate ($p = 0.03$), but not acetate ($p = 0.37$), was subjected to an interaction between energy intake and the time course. Plasma propionate increased linearly with increasing energy intake at 3 and 6 h after feeding, whereas was similar for each energy intake level at 13 h after feeding. Plasma n-butyrate concentration tended to be quadratically affected ($p = 0.09$) by energy intake, and decreased linearly ($p<0.01$) with transition of

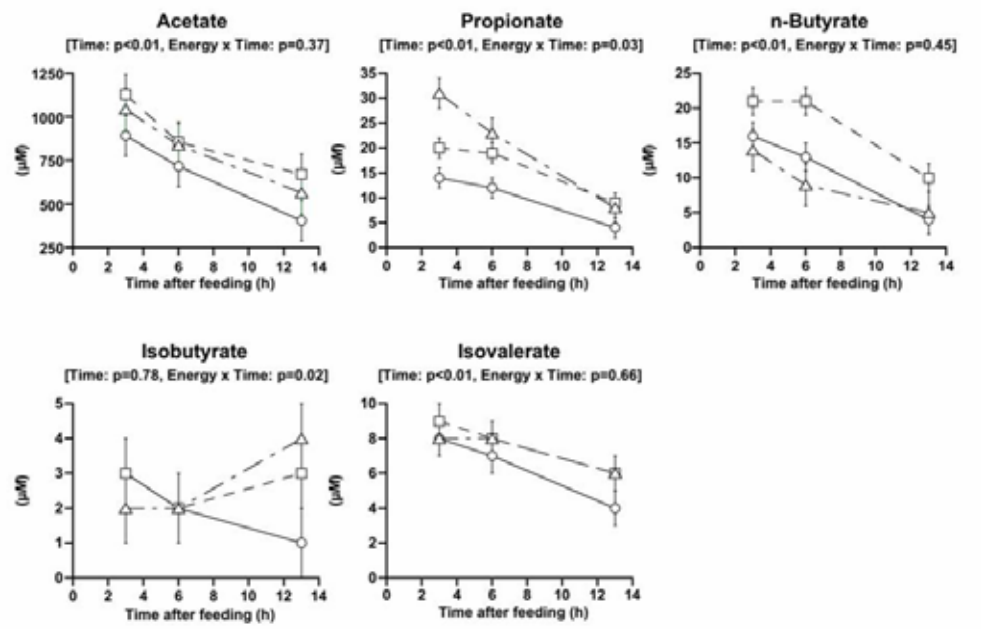

Figure 2. Changes in plasma VFAs concentrations with time course relating to feeding in goats fed diets containing different metabolizable energy. Data are expressed as least square means and SE: energy intake for maintenance (O, n = 3), 1.5 times energy intake for maintenance (□, n = 3), 2.0 times energy intake for maintenance (Δ, n = 2). Differences in energy intake were achieved by changing energy intake attributed to ingredients other than CP. P-value of the main effect of time relating to feeding (time) and an interaction between energy intake and time relating to feeding (energy×time) are shown in brackets. Data were obtained from statistical analysis (see the text).

time relating to feeding. A significant interaction (p = 0.02) between energy intake and time relating to feeding was found on plasma isobutyrate concentration, despite no significant effects of energy intake (p = 0.49) or time course relating to feeding (p = 0.78). The metabolite was similar for each energy intake level at 3 and 6 h after feeding, but was higher for 1.5×M and 2.0×M than for 1.0×M at 13 h after feeding. Plasma isovalerate concentration decreased quadartically (p = 0.01) with time course relating to feeding, but was not subjected to the effect of energy (p = 0.32).

Hyperinsulinemic euglycemic clamp

Blood glucose concentration was maintained at very close to the basal values by variable rates of glucose infusion in all experiments (Table 3). Plasma insulin concentration and GIR were relatively constant during the second hour of each 2-h period of insulin infusion (data not shown) and increased dose-dependently (p<0.01) with increasing rate of insulin infusion. The mean plasma insulin concentration (p = 0.82) and GIR (p = 0.27) during insulin infusion were unaffected by energy intake. There were no interactions between energy intake and insulin infusion in these measurements (plasma insulin concentration, p = 0.72; GIR, p = 0.10). The response curves of GIR to plasma insulin concentration in each dietary treatment are shown in Figure 3. Visually, GIR increased more rapidly with elevating plasma insulin concentration, as energy intake was greater, whereas it reached similar maximum values for each energy intake level. The GIR_{max} was similar (p = 0.60) for each energy intake level, but the EC_{50} decreased numerically (p = 0.12) with increasing energy intake.

DISCUSSION

In the present study, increasing energy intake increased plasma propionate, and tended to increase plasma lactate and acetate concentrations across time relating to feeding. However, a lack of differences in plasma lactate and propionate concentrations with increasing energy intake was found at 13 h after feeding. These results suggest that although carbohydrate fermentation in the rumen enhanced with an increase in non-protein energy intake, the extent of the enhancement should be greater at an earlier time relating to feeding. A numerical decrease in plasma NEFA concentration with increasing energy intake across time relating to feeding suggests enhancing supply of energy substrates to the body and thus inhibiting lipolysis in the adipose tissues with increasing non-protein energy intake. Additionally, a lack of change in the metabolite with time course relating to feeding for 1.5×M and 2.0×M, but not for 1.0×M, suggests that the enhancement in supply of energy substrates with increasing non-protein energy intake would be sustained through a day. The enhancement in energy supply at an earlier time relating to feeding would be attributed to increasing absorption of VFAs from the rumen

Table 3. The effects of non-protein energy intake on the concentrations of blood glucose and plasma insulin, and glucose infusion rate (GIR) in the hyperinsulinemic euglycemic clamp experiment in adult goats[1]

	Treatment[2]			p value[3]		
	1.0×M	1.5×M	2.0×M	Energy	Insulin	Interaction
n	3	3	2			
	---------- Glucose (mg/dl) ----------					
Basal	50	52	49			
0.38[4]	50	52	48			
0.96	50	54	50	0.13	0.08	0.34
2.4	50	52	49			
6	51	52	49			
SE	3	3	3			
	---------- Insulin (μU/ml) ----------					
Basal	8	10	30			
0.38	33	35	51			
0.96	80	82	89	0.82	<0.01	0.72
2.4	389	367	294			
6	1,301	1,300	1,190			
SE	50	50	60			
	---------- GIR (mg/kg BW/min)[5] ----------					
0.38	1.15	1.99	2.50			
0.96	2.73	3.50	4.96			
2.4	4.95	4.51	5.66	0.27	<0.01	0.10
6	5.66	5.64	6.01			
SE	0.55	0.55	0.64			
	---------- GIR_{max} (mg/kg BW/min)[6] ----------					
	5.81	5.28	5.71	0.60	-	-
SE	0.56	0.56	0.63			
	---------- EC_{50} (μU/ml)[7] ----------					
	104	53	34	0.12	-	-
SE	16	16	20			

[1] Values represent least square means and SE. These values were obtained from statistical analysis (see the text).

[2] 1.0×M: energy intake for maintenance, 1.5×M: 1.5 times energy intake for maintenance, 2.0×M: 2.0 times energy intake for maintenance. Differences in energy intake were achieved by changing energy intake attributed to ingredients other than CP.

[3] Energy: main effect of energy intake, Insulin: main effect of insulin infusion, Interaction: energy×insulin interaction.

[4] Insulin infusion rate, mU/kg BW/min.

[5] The mean glucose infusion rate during the last half of each 2-h of infusion period.

[6] The maximal glucose infusion rate.

[7] The plasma insulin concentration at half-maximal glucose infusion rate.

and also possibly glucose from the small intestine (Janes et al., 1985). However, at a later time relating to feeding, energy substrates other than these substrates seem to contribute the enhancement in energy supply. One of the substrates would be amino acid, because plasma total amino-nitrogen concentration increased significantly with increasing energy intake and with time course relating to feeding.

A linear decrease in plasma urea-nitrogen concentration with increasing energy intake would be largely associated with decreasing ammonia absorption from the rumen and increasing urea transfer from blood to the rumen (Norton et al., 1982; Obara et al., 1994; Sutoh et al., 1996). Plasma total amino-nitrogen concentration increased linearly with increasing energy intake, suggesting an increase in amino acid absorption with increasing non-protein energy intake. This is because supplementation with a readily fermentable carbohydrate, such as cereal starch and sucrose, has increased microbial protein synthesis (Norton et al., 1982; Obara et al., 1994) and non-ammonia-nitrogen flow into the lower digestive tracts (Obara et al., 1994; Sutoh et al., 1996). The effects of non-protein energy intake on these metabolites were not modified with transition of time relating to feeding, suggesting that the effects of non-protein energy intake on nitrogen metabolism, as well as on energy metabolism, in the rumen and body would be sustainable. The sustainable effects is possibly related with synchronization between energy and nitrogen metabolism in the rumen, because synchronizing the rate of dietary energy and nitrogen release has decreased concentrations of ruminal ammonia nitrogen and blood urea-nitrogen and tended to increase microbial protein synthesis in the rumen (Chumpawadee et al., 2006).

Regardless of the possible increase in absorption of acetate, propionate, lactate and amino acids, plasma insulin concentration was not significantly affected by energy

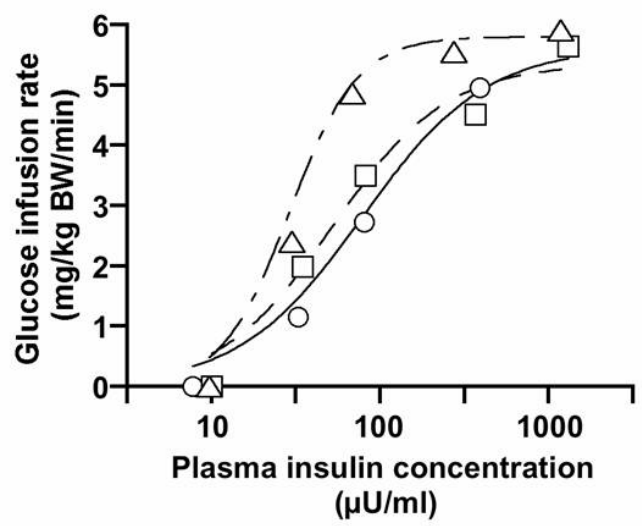

Figure 3. The responses of glucose infusion rate to plasma insulin concentration in the hyperinsulinemic euglycemic clamp experiment. Data are expressed as least square means: energy intake for maintenance (O, n = 3), 1.5 times energy intake for maintenance (□, n = 3), 2.0 times energy intake for maintenance (Δ, n = 2). Differences in energy intake were achieved by changing energy intake attributed to ingredients other than CP.

intake, although a numerical increase was observed. Previous results have also shown a lack of change in the concentration with increasing energy intake in sheep (Metcalf and Weekes, 1990; Terashima et al., 1991).

The GIR in the hyperinsulinemic euglycemic clamp experiment is an index of the overall effects of insulin on glucose metabolism because of stimulating peripheral glucose utilization and suppressing hepatic glucose production with insulin (Bergman et al., 1985). In the present results, the GIR_{max} did not respond to changing energy intake, but the EC_{50} decreased numerically with increasing energy intake. Although a careful interpretation is needed because of the small number of animals, these results indicate that the increase in non-protein energy intake may enhance insulin sensitivity without affecting tissue responsiveness to insulin. Inconsistent results for the effects of energy intake on insulin action were available in ruminants. Fujita et al. (2000) have shown that tissue responsiveness to insulin, but not insulin sensitivity, enhanced with increasing energy intake from 1.0 to 2.0 times of maintenance requirement in adult goats. In contrast, Sano et al. (1999) have reported that in cold exposed rams and ewes, the increase in energy intake from 0.82 to 1.37 times of maintenance requirement reduced tissue responsiveness to insulin without affecting insulin sensitivity. A lack of response of insulin action to different energy intakes has been also reported in pregnant ewes fed 0.5 and 1.0 times of maintenance requirement (Petterson et al., 1993) and in male sheep fed 1.3 and 1.8 times of maintenance requirement (Terashima et al., 1991). The inconsistency between these results may be associated with animal species, physiological condition, feed composition and feeding regimen.

In the previous researches described above, except for that of Fujita et al. (2000), which energy intake has been increased by starch supplementation, the increases in energy intake were accomplished by increasing intake level of same diets. This feeding regimen allows CP intake to increase with increasing energy intake and thus may induce a modified response of insulin action, because Sano et al. (2001) have shown that dietary CP level modified insulin action in response to cold exposure in sheep fed diets containing 70, 100 and 140% of CP for maintenance. In contrast, the results of the present experiment, as well as that of Fujita et al. (2000), indicate that energy intake independent of CP intake may modify insulin action in adult goats.

Unlike the present result, Fujita et al. (2000) have found the enhancement of tissue responsiveness to insulin, but not insulin sensitivity, with increasing supplemental starch. Although the mechanisms of tissue responsiveness to insulin and insulin sensitivity are associated with the postreceptor events, and the affinity and/or concentration of receptor in target cell, respectively (Kahn, 1978), it is unknown about the reason for the difference in mechanism resulting in enhancement of insulin action between these experiments.

In the present study, it appears that the sustainable changes in plasma concentrations of NEFA (decrease), urea-nitrogen (decrease) and total amino-nitrogen (increase) with increasing energy intake may be associated with the possible enhancement of insulin sensitivity. Although the relationship between NEFA concentration and insulin action is not reported in ruminants, an increase in NEFA concentration in blood would have been associated with the impairment of insulin action in rodent (Randle et al., 1963; Gilbert et al., 1993) and human (Roden et al., 1996). On the other hand, amino acids may also be related to the modification of insulin action because of the suggestion from Sano et al. (2001) as described above, although GIR in EGC has been unaffected by abomasal infusion of casein in dairy cows (Griinari et al., 1997) or i.v. infusion of amino acids mixture in lactating goats (Bequette et al., 2001). Possible effects of amino acids on insulin sensitivity were shown for L-carnitine, taurine and L-arginine in human or rodents (Kelly, 2000). In the present study, the changes in plasma urea-nitrogen and total amino-nitrogen concentrations may be associated with an increase in L-arginine in plasma, because plasma arginine is originated from absorbing from small intestine and synthesizing in urea cycle. Although the effects of arginine on insulin action were not investigated in ruminants, an enhancement

in insulin sensitivity with arginine has been shown in healthy subjects, obese patients and non-insulin-dependent diabetes patients with i.v. infusion of L-arginine (Wascher et al., 1997), and in type 2 diabetic patients with oral L-arginine administration for 30 d (Piatti et al., 2001). It was unknown whether plasma arginine concentration changed with increasing energy intake in the present study, because individual amino acids concentrations in plasma were not determined. In the previous reports (Koenig et al., 1982; Maltby et al., 2005), plasma arginine concentration was not largely affected by absorption of additional ammonia, but was increased by supply of additional arginine. These previous results led us to expect that the increase in plasma total amino-nitrogen concentration, which would result from increased amino acid absorption, may be associated with an increase in plasma arginine concentration in the present study.

In conclusion, an increase in non-protein energy intake may enhance insulin sensitivity, but not tissue responsiveness to insulin, for glucose metabolism in adult goats. Although a little change in plasma insulin concentration is often observed in ruminants even if excess energy is supplied, the possible enhancement in insulin sensitivity may play a role in establishing anabolic status in the body, when energy balance is positive.

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