Asian-Aust. J. Anim. Sci.
Vol. 23, No. 2 : 205 - 212
February 2010

www.ajas.info

Effects of Chromium on Energy Metabolism in Lambs Fed with Different Dietary Protein Levels*

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ABSTRACT : The effects of chromium (Cr), dietary crude protein (CP) level, and potential interactions of these two factors were investigated in term of energy metabolism in lambs. Forty-eight 9-week-old weaned lambs (Dorper×Small-tail Han sheep, male, mean initial body weight = 22.96 kg±2.60 kg) were used in a 2×3 factorial arrangement of supplemental Cr (0 μg/kg, 400 μg/kg or 800 μg/kg from chromium yeast) and protein levels (low protein: 157 g/d to 171 g/d for each animal, or high protein: 189 g/d to 209 g/d for each animal). Blood samples were collected at the beginning and end of the feeding trial. The lambs were then sacrificed and tissue samples were frozen for further analysis. Chromium at 400 μg/kg decreased fasting insulin level and the ratio of plasma insulin to glucagon, but these differences were not statistically significant; in contrast, chromium at 800 μg/kg increased the ratio significantly ($p<0.05$). Protein at the high level increased plasma tumor necrosis factor α (TNF-α) level ($p = 0.060$). Liver glycogen content was increased significantly by Cr ($p<0.05$), which also increased liver glucose-6-phosphatase (G-6-Pase) and adipose hormone-sensitive lipase (HSL) activity. At 400 μg/kg, Cr increased muscle hexokinase (HK) activity. High protein significantly increased G-6-Pase activities in both the liver ($p< 0.05$) and the kidney ($p<0.05$), but significantly decreased fatty acid synthase (FAS) activity in subcutaneous adipose tissue ($p<0.05$). For HSL activity in adipose tissue, a Cr×CP interaction ($p<0.05$) was observed. Overall, Cr improved energy metabolism, primarily by promoting the glycolytic rate and lipolytic processes, and these regulations were implemented mainly through the modulation by Cr of the insulin signal transduction system. High protein improved gluconeogenesis in both liver and kidney. The interaction of Cr×CP indicated that 400 μg/kg Cr could reduce energy consumption in situations where energy was being conserved, but could improve energy utilization when metabolic rate was increased. (**Key Words :** Lamb, Chromium, Protein Level, Energy Metabolism)

INTRODUCTION

Trivalent chromium (Cr) is an essential active component of glucose tolerance factor (GTF). It exerts its biological function at low concentrations in humans and animals primarily by increasing sensitivity to insulin and by improving glucose metabolism (Mertz et al., 1963; Anderson et al., 1998). The targets of Cr may also involve lipids and cholesterol, which interact with the effects of Cr on insulin signals (Brautigan et al., 2006; Pattar et al., 2006). Previous research had indicated that Cr effects might have some correlations with biological energy metabolism, but conclusive evidence for this is still lacking.

Glucose and lipids are the main energy sources for biological organisms. In livestock, some research has indicated that Cr may improve glucose metabolism by regulating insulin sensitivities in growing calves and lambs (Kegley et al., 2000; Yan et al., 2008). However, conflicting reports have been published (Ott et al., 1999). In terms of its role in lipid metabolism, the results of previous Cr supplementation studies are inconsistent. Most researchers have supported the conclusion that Cr decreases lipid deposition in carcasses and plasma of non-ruminants (Lien et al., 1999; Mooney et al., 1999; Króliczewska et al., 2004). In ruminants, however, less is known concerning the influence of Cr on lipid metabolism, and again there are equivocal reports regarding its effects (Kitchalong et al.,

* Supported by the programs of State Key Laboratory of Animal Nutrition "Initial Mechanism Exploration of Nutrients Modulating on Sheep Performance" (No. 2004DA 125184 (Group)), National Key Technologies R & D Program (No. 2006BAD14B07) and Shanxi Province-China Agricultural University Cooperative Project of Building Yanmenguan Ecological and Economic Animal Husbandry Area.

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Received June 8, 2009; Accepted August 21, 2009

1995; Williams et al., 2004).

In cell cultures, insulin signaling is strongly correlated with glucose and lipid metabolism and lipolysis (Anthonsen et al., 1998; Saltiel and Kahn, 2001). However, an effect of Cr on key enzymes involved in this metabolism has yet to be demonstrated. The present study was therefore conducted to clarify the precise effects of Cr on glucose and lipid metabolism in a ruminant animal. In our study, lambs were fed different levels of dietary protein (CP), and we investigated the interaction of Cr supplementation on the resulting energy metabolism, focusing on insulin levels and the activities of a number of key metabolic enzymes.

MATERIALS AND METHODS

Animals, diets, and experimental design

Forty-eight, 9-week-old, weaned Dorper×Small-tail Han breed autumn-born lambs (male, mean initial body weight = 22.96 kg±2.60 kg) were used in this study. All lambs were housed in individual steel pens (1.5×2.0 m^2) in an open-sided barn with a concrete floor. After a 2-week adjustment to the experimental feeding system, lambs were blocked by weight and randomly assigned to a 2×3 factorial arrangement for a 60-day feeding trial. Three levels (0 μg/kg, 400 μg/kg, or 800 μg/kg) of Cr were supplied using a Cr yeast supplement (1 g/kg Cr, Alltech, Nicholasville, KY). Two protein levels, low protein (157 g/d to 171 g/d for each animal) and high protein (189 g/d to 209 g/d for each animal) were maintained based on chemical analysis of the feed. In total, six dietary treatments were administered to six groups of eight animals. Two-phase lamb fattening diets were formulated to meet National Research Council guidelines (NRC, 1985) to provide nutrients for weight ranges of 20-30 kg (1.0 kg DMI/d, total mixed ration) and 30-40 kg (1.3 kg DMI/d, total mixed ration) and the rations were analyzed as containing an initial 834 to 858 μg/kg Cr and 707 to 719 μg/kg Cr, respectively. Supplemental Cr was first mixed with corn meal and then gradually completely mixed with the other dietary ingredients. The final ingredients and chemical composition of the diets are shown in Table 1. The animals were fed twice daily, in two equal amounts (0.5 kg from day 0 to day 30; 0.65 kg from day 30 to day 60, based on dry matter, DM) at 06:00 h and 18:00 h, with *ad libitum* access to water.

Dietary composition analyses

Feed samples were collected before the beginning of the experiment for the determination of DM, CP, calcium (Ca) and phosphorus (P), according to Association of Official Analytical Chemists procedures (AOAC, 1995). Neutral

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

Item	Day 0 to day 30		Day 30 to day 60	
	Low protein	High protein	Low protein	High protein
Feed ingredients				
Corn meal[1]	40	30	47.3	40.4
Soybean meal[1]	18.2	29.9	5	12
Wheat bran[1]	9	7.5	5	5
Alfalfa hay[1]	15	15	10	10
Oat hay[1]	15	15	30	30
Calcium hydrogen phosphate	0.3	0.1	0.2	0.1
Limestone	1	1	1	1
Salt	0.5	0.5	0.5	0.5
Vitamin-mineral premix[2]	1	1	1	1
Chemical composition				
Dry matter (DM)	88.21	88.76	88.49	88.65
Metabolizable energy (MJ/kg DM) 3	10.64	10.70	10.76	10.78
Crude protein	17.09	20.94	12.11	14.54
Neutral detergent fiber	29.55	29.86	36.19	36.44
Acid detergent fiber	16.69	16.92	18.42	18.67
Calcium	0.72	0.71	0.61	0.60
Available phosphorus 3	0.34	0.33	0.28	0.28
Chromium (μg/kg)	858	834	707	719

[1] Cr content: corn meal, 525 μg/kg; soybean meal, 689 μg/kg; wheat bran, 3,419 μg/kg; alfalfa hay, 879 μg/kg; Oat hay, 551 μg/kg.

[2] Provided per kilogram of the diet: 40 mg of Zn as $ZnSO_4 \cdot 7H_2O$; 30 mg of Mn as $MnSO_4 \cdot H_2O$; 1.2 mg of I as KI; 60 mg of Fe as $FeSO_4 \cdot 7H_2O$; 15 mg of Cu as $CuSO_4 \cdot 5H_2O$; 0.3 mg of Co as $CoSO_4 \cdot 7H_2O$, 0.2 mg of Se as $Na_2SeO_3 \cdot 5H_2O$; 2,000 IU of Vitamin A; 250 IU of Vitamin D and 25 IU of Vitamin E.

[3] Analyzed values except metabolizable energy and available phosphorus.

detergent fiber (NDF) in feed samples was measured according to Van Soest et al. (1991) without sodium sulfite or alpha-amylase, and acid detergent fiber (ADF) was measured according to Robertson et al. (1981). NDF and ADF are expressed with residual ash. The Cr content of the samples was determined using an Agilent 7500c ICP-MS mass spectrometer (Agilent Technologies, Japan).

Sample collection

At day 0 and day 60, lambs were fasted for 16 h, then blood samples were collected into plasma tubes containing EDTA-Na via jugular venipuncture. The samples were weighed, then plasma was separated by centrifugation at 1,500×g for 20 minutes at 4°C. Plasma samples were stored at -25°C for later hormone analyses.

Upon termination of the feeding trial, all lambs were electrically stunned and then slaughtered by exsanguination. Samples of subcutaneous adipose tissue, *longissimus* muscle adjacent to the 10th rib and the last lumbar vertebra, liver, and kidney were harvested from each animal and immediately immersed in liquid nitrogen, and then stored at -70°C for later enzyme activity analyses.

Plasma hormone assay

Levels of plasma insulin, glucagon, leptin, and tumor necrosis factor α (TNF-α) were measured with commercially available solid phase radioimmunoassay kits (Beijing Atom High Tech Co., Beijing, China).

Muscle and liver glycogen assay

Muscle and liver glycogen levels were detected with anthracenone coloirmetry using a glycogen commercial kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) following the manufacturer's instructions.

Tissue protein contents assay

Protein contents of liver, subcutaneous adipose, and skeletal muscle samples were measured with a commercial Coomassie blue protein kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) following the manufacturer's instructions.

Enzyme assays

Glucose-6-phosphatase (G-6-Pase) in liver and kidney tissue homogenates was assayed according to the method of Bradford (1976). G-6-Pase activity was expressed as the amount of inorganic phosphate liberated per 40 minute per gram tissue.

Hexokinase (HK) activities in liver and skeletal muscle were determined by the method of Crabtree et al. (1972). At pH 7.5 and 37°C, one unit of HK activity was defined as the formation of 1 nmol/L NADPH per minute in the reaction system and was expressed as units per gram tissue protein (U/g prot).

Fatty acid synthase (FAS) activities in liver and subcutaneous adipose tissue were assayed as described by Nepokroeff (1975) and Shillabeer (1990). One unit of FAS activity was defined as the oxidation of 1 nmol NADPH per minute, and activity was expressed as units per milligram tissue protein (U/mg prot).

Hormone-sensitive lipase (HSL) activity was measured using the procedures of Fredrickson et al. (1981) as modified by McNamara et al. (1987). One unit of HSL activity corresponded to the release of 1 μmol ^{3}H free fatty acids per minute at 37°C, and activity was expressed as units per gram tissue (U/g tissue).

All reagents utilized in enzyme assays were purchased from Sigma Chemical Co. (St. Louis, MO). ^{3}H triolein was obtained from China Isotope Co. (Beijing, China).

Table 2. Plasma hormone levels before feeding trial in fattening lambs

Items	Ins (μIU/ml)	Glucagon (pg/ml)	Leptin (nmol/ml)	TNF-α (ng/ml)
Mean	20.42	294.78	1.29	0.20
SEM	0.74	15.19	0.06	0.01

Ins = Insulin; TNF-α = Tumor necrosis factor α; SEM = Standard error of the mean.

Table 3. Influence of chromium supplementation and dietary protein level on plasma hormone levels of fattening lambs

Items	Low Protein			High Protein			Effects of Cr			Effects of CP		SEM	Probability levels		
	Cr 0	Cr 1	Cr 2	Cr 0	Cr 1	Cr 2	Cr 0	Cr 1	Cr 2	LP	HP		Cr	CP	Cr×CP
Ins (μIU/ml)	18.14	13.39	18.07	15.94	12.50	15.53	17.04	12.95	16.80	16.53	14.66	0.84	0.095	0.261	0.908
Glucagon (pg/ml)	243.48	203.86	216.18	249.14	214.18	204.18	246.31	209.02	210.18	221.17	222.50	11.29	0.376	0.857	0.926
Ins/glucagon	0.080	0.065	0.084	0.063	0.061	0.080	0.072^b	0.063^b	0.082^a	0.076	0.068	0.006	0.124	0.347	0.707
Leptin, (nmol/ml)	1.150	0.943	1.045	1.153	1.068	1.038	1.152	1.006	1.042	1.046	1.086	0.049	0.520	0.712	0.854
TNF-α (ng/ml)	0.145	0.138	0.150	0.175	0.153	0.168	0.160	0.146	0.159	0.144	0.165	0.005	0.441	0.060	0.821

Means in the same row with different small or capital letter superscripts differ significantly at 0.05 or 0.01 respectively ($p<0.05$ or $p<0.01$).

SEM = Standard error of the mean; Cr = Chromium supplementation: Cr0 = 0 μg/kg, Cr1 = 400 μg/kg, Cr2 = 800 μg/kg; CP = Protein level: LP = Low protein, HP = High protein; Cr×CP = Interactions of chromium and protein level; Ins = Insulin; TNF-α = Tumor necrosis factor α.

Statistical analysis

Data were analyzed by analysis of variance for a 2×3 factorial arrangement using the General Linear Models procedure of SAS (SAS Inst. Inc., Cary, NC).

RESULTS AND DISCUSSION

Plasma hormones

Plasma hormones measured at day 0 and day 60 are shown in Table 2 and Table 3. At day 0, no significant differences were observed.

After the feeding trial, no significant differences were observed in fasting insulin level ($p = 0.095$) or in the ratio of plasma insulin to glucagon ($p = 0.124$) in response to 400 μg/kg Cr, although there was a decreasing trend. However, at 800 μg/kg, the insulin/glucagon ratio increased significantly ($p<0.05$). An increased plasma TNF-α level ($p = 0.060$) was observed at day 60 in response to high protein. The other hormones were not affected by either Cr or CP supplementation.

Insulin and glucagon play major roles in hepatic carbohydrate metabolism and function in antagonistic ways. Consequently, a balance of these two hormones is important for glucose homeostasis (Bassett, 1975; Menuelle et al, 1991). Glucagon causes insulin release from the beta-cells and raises blood glucose, first by glycogenolysis and then by gluconeogenesis (Deetz et al., 1981). In muscle and adipose cells, insulin stimulates signal pathways, i.e. the insulin receptor substrate 1 (IRS-1) tyrosine phosphorylation system, in order to regulate glucose transmembrane transport (Sun et al., 1991).

Our previous trial had demonstrated that 400 μg/kg supplementation could increase average daily gain, decrease plasma insulin content and improve glucose utilization efficiency by improving insulin activity (Yan et al., 2008). For glucose homeostasis, an increased insulin activity means a decreased plasma insulin, so that the ratio of insulin to glucagon in the 400 μg/kg treatment in the present study was lower than in the control group. At 800 μg/kg Cr, this effect was not seen, which might be ascribed to a dose-effect relationship of Cr modulation on insulin activity (Kegley et al., 2000). The trend of a decrease in plasma insulin and glucagon contents in response to 400 μg/kg, although not significantly different in the current study, indicated that Cr improved glucose utilization efficiency primarily by strengthening IRS-1 tyrosine phosphorylation, rather than by influencing the secretory function of the pancreas. In the fasting state, equal amounts of amino acids are used by peripheral tissue and excellent glycogenic regulation is executed (Gentry et al., 1999; Van de Ligt et al., 2002), so that the plasma insulin and glucagon contents are maintained and the ratio of the two measurements were not obviously affected by different protein supplementation levels.

Leptin and TNF-α are both proteins secreted by adipose tissues. As an energy homeostasis factor, leptin secretion is secreted in direct proportion to adipose tissue mass and nutritional status, and its secretion can also be increased in response to insulin (Wajchenberg, 2000; Margetic et al., 2002). On the other hand, insulin may mediate the effect of caloric intake on leptin and could be a determinant of its plasma concentration (Saad et al., 1998). TNF-α clearly affects energy metabolic processes in adipose tissue and liver, as TNF-α has been shown to repress genes involved in uptake and metabolism of glucose and nonesterfied fatty acids (Ruan et al., 2002). TNF-α also impairs insulin signaling by activating serine kinases that increase serine phosphorylation of IRS-1 and IRS-2 (Hotamisligil, 2003).

In our trial, plasma leptin levels were not affected by either Cr or CP. This result might be closely associated with the plasma insulin content, which was also not influenced by either treatment, so that feedback regulation between leptin and insulin may not have been affected (Margetic et al., 2002). Although plasma TNF-α level following 400 μg/kg treatment was lower than for the other treatments, and although the difference was not statistically significant, this suggested that the response to Cr to decrease fatty deposition (Yan et al., 2008) has little correlation with the biological regulation of lipid metabolism by TNF-α. The response of plasma TNF-α to Cr was consistent with the result of Arthington et al. (2002), who reported that Cr supplementation using high-Cr yeast did not affect secretion of TNF-α in Holstein bull calves. Plasma TNF-α content might directly correlate with body weight and adipose tissue mass. Sheu et al. (2000) found that the TNF-α mRNA expression was increased in obese mice and in adults, but this declined when body weight was lost. This result suggests that TNF-α level changes may have been the result of increased body weight and adipose tissue mass in the lambs supplemented with high protein.

Tissue glycogen content

Glycogen content in liver and muscle are shown in Table 4. Cr significantly increased liver glycogen content. The difference between 0 μg/kg and 400 μg/kg treatments was highly significant ($p<0.01$), and the difference between 0 μg/kg and 800 μg/kg was significant ($p<0.05$). In muscle tissue, no significant differences were observed.

This finding is in agreement with the research of Rosebrough et al. (1981), who indicated that 20 mg/kg Cr increased liver glycogen levels and glycogen synthase activity in 3-week-old turkey poults, although they did not assess muscle glycogen levels. Campbell et al. (1989) also reported that rat liver glycogen concentration tended to be higher following Cr treatment and that liver glycogen

Table 4. Influence of chromium supplementation and dietary protein level on tissue glycogen contents of fattening lambs

Items	Low protein			High protein			Effects of Cr			Effects of CP		SEM	Probability levels		
	Cr 0	Cr 1	Cr 2	Cr 0	Cr 1	Cr 2	Cr 0	Cr 1	Cr 2	LP	HP		Cr	CP	Cr×CP
Glycogen (mg/g tissue)															
Liver	9.46	11.67	11.20	10.24	10.75	10.81	9.85^{bB}	11.21^{A}	11.01^{a}	10.78	10.60	0.23	0.020	0.579	0.136
Muscle	6.02	6.77	6.53	6.17	6.51	6.26	6.10	6.64	6.40	6.44	6.31	0.32	0.823	0.860	0.963

Means in the same row with different small or capital letter superscripts differ significantly at 0.05 or 0.01 respectively ($p<0.05$ or $p<0.01$).
SEM = Standard error of the mean; Cr = Chromium supplementation: Cr0 = 0 μg/kg, Cr1 = 400 μg/kg, Cr2 = 800 μg/kg; CP = Protein level: LP = Low protein, HP = High protein; Cr×CP = Interactions of chromium and protein level.

synthase and phosphorylase activities were dependent upon dietary chromium. However, glycogen concentration in the *gastrocnemius* and *biceps femoris* was not influenced by dietary Cr.

The metabolism of the storage polysaccharide glycogen is intimately linked to insulin action and blood glucose homeostasis (Lawrence et al., 1997). Insulin activates both glucose transport and glycogen synthase by tyrosine phosphorylation of IRS-1 (Myers et al., 1994). IRS-1 in turn mediates multiple downstream signals, including the direct activation of phosphatidylinositol 3'-kinase (PI-3K), SH2-containing protein tyrosine phosphatase (SH-PTP2), and pp70 S6 kinase (Backer et al., 1992; Cheatham et al., 1994; Sugimoto et al., 1994). Although plasma insulin content had not been clearly affected by Cr supplementation, the increases in liver glycogen reinforced the previous conclusion that Cr regulated glycogen metabolism, again mainly by modulating the insulin signal transduction system.

Enzyme activities

Cr increased liver G-6-Pase activity ($p = 0.15$) and adipose HSL activity ($p = 0.069$). At 400 μg/kg, Cr increased muscle HK activity ($p = 0.144$). High protein supplemention significantly increased G-6-Pase activities in both liver ($p<0.05$) and kidney ($p<0.05$). At the same time, FAS activity in subcutaneous adipose tissue was significantly increased ($p<0.05$). For adipose tissue HSL activity measurement, a Cr×CP interaction ($p<0.05$) was observed (Table 5).

Because dietary carbohydrate is fermented in the rumen, the glucose need of ruminant animals is dependent on gluconeogenesis (Bergman et al., 1970). G-6-Pase, an enzyme found primarily in the liver and the kidneys, plays an important role in blood glucose homoeostasis. Both gluconeogenesis and glycogenolysis result in the formation of glucose 6-phosphate, which has to be hydrolyzed by G-6-Pase before being liberated as glucose into the circulation (Schaftingen et al., 2002). The signaling pathway downstream of insulin and PI-3K is also involved in the regulation of G-6-Pase expression (Dickens et al., 1998). In this trial, the increased liver G-6-Pase activity in response to Cr was attributed to an improvement in insulin sensitivity, as shown previously (Yan et al., 2008). This indicated that Cr could promote metabolic rate by reinforcing gluconeogenesis and glycogenolysis; this agrees with the findings of Amoikon et al. (1995) and Matthews et al. (1997), who observed that Cr could improve the clearance rate of plasma glucose in pigs. High protein treatment also resulted in an increased G-6-Pase activity, which might be due to an improvement in gluconeogenesis. In this condition, propionic acid concentration was increased in the rumen, and the metabolism of amino acids to glucose in the liver and kidney was increased at the same time, so that gluconeogenesis was reinforced accordingly.

Table 5. Influence of chromium supplementation and dietary protein level on tissue enzyme activities of fattening lambs

Items	Low protein			High protein			Effects of Cr			Effects of CP		SEM	Probability levels		
	Cr 0	Cr 1	Cr 2	Cr 0	Cr 1	Cr 2	Cr 0	Cr 1	Cr 2	LP	HP		Cr	CP	Cr×CP
G-6-Pase, (mg P/g tissue•40 min)															
Liver	231.01	279.54	262.43	283.14	316.52	302.16	257.08	298.03	282.30	257.66^{b}	300.61^{a}	9.25	0.150	0.018	0.923
Kidney	39.57	44.17	41.66	43.13	52.53	54.11	41.35	48.35	47.89	41.80^{b}	49.92^{a}	2.00	0.256	0.043	0.629
HK (U/g prot)															
Liver	4.27	4.87	4.41	4.11	4.55	4.22	4.19	4.71	4.32	4.52	4.44	0.13	0.325	0.442	0.967
Muscle	1.30	1.54	1.45	1.23	1.46	1.29	1.27	1.50	1.37	1.43	1.33	0.05	0.144	0.278	0.913
FAS (U/mg prot)															
Adipose	313.09	281.29	370.39	161.02	195.35	190.39	237.05	238.32	280.24	321.49^{a}	182.25^{b}	21.83	0.758	0.023	0.766
HSL (U/g tissue)															
Adipose	81.45	185.15	117.42	85.39	78.54	151.96	83.42	131.85	134.69	128.01	105.30	9.57	0.069	0.244	0.017

Means in the same row with different small or capital letter superscripts differ significantly at 0.05 or 0.01 respectively ($p<0.05$ or $p<0.01$).
SEM = Standard error of the mean; Cr = Chromium supplementation: Cr0 = 0 μg/kg, Cr1 = 400 μg/kg, Cr2 = 800 μg/kg; CP = Protein level: LP = Low protein, HP = High protein; Cr×CP = Interactions of chromium and protein level; G-6-P = Glucose-6-phosphatase; HK = Hexokinase; FAS = Fatty acid synthetase; HSL = Hormone-sensitive lipase.

Under normal circumstances, glucose provides energy for organic metabolism through aerobic oxidation and anaerobic glycolysis. Intracellular glucose must first be phosphorylated by HK for further metabolism. As the first catalytic step in glycolysis, HK activity is implicated as a critical step in the control of glucose utilization (Gottlob et al., 2001; Liang et al., 2002). In response to insulin binding to its receptor on skeletal muscle, a signal cascade is initiated that culminates in the recruitment of the glucose transporter 4 (GLUT-4) to the plasma membrane of skeletal muscle (Cushman et al., 1980).

The increase in muscle HK activity indicated that 400 μg/kg Cr could promote glycolytic rate, increase the amounts of pyruvic acid participating in the tricarboxylic acid cycle in mitochondria, and elevate the production of ATP, thus increasing the fuel supply to muscular tissue. On the other hand, liver HK activity was not influenced by Cr treatments. This may indicate that the effect of Cr on improving glucose utilization efficiency is correlated exclusively with GLUT-4 activation in skeletal muscle cells. When an insulin signal is reinforced, GLUT-4 transporters are activated to translocate glucose more efficiently from intracellular storage compartments to the plasma membrane, thus accelerating glucose transportation into the muscles (Watson et al., 2001).

In ruminants, the adipose tissue is the important place where fatty acids are biosynthesized. In the cytoplasm of adipocytes, FAS catalyzes the utilization of acetyl-CoA and malonyl-CoA to synthesise triglycerides (Smith et al., 2003). Fatty deposition and lipolysis are contrary regulating processes that maintain homeostasis of lipid metabolism. HSL is the rate-limiting enzyme in the lipolytic pathway and is responsible for liberating the first 2 fatty acids from triacylglycerol following phosphorylation by protein kinase (Yeaman, 1994; Sumner et al., 2007). Either FAS or HSL can act as modulators of lipid and energy metabolism. In the current study, adipose FAS activity was not affected by Cr, but was significantly increased by low protein feeding. This finding is consistent with the results of Mildner et al. (1991), who reported that when the protein content of the diet was increased from 14% to 24%, the FAS mRNA abundance in pig adipose tissue tended to decrease by 50% ($p<0.10$). In a fasting stage, the low protein diet activated lipolysis of body fat to supply fuel more efficiently than did a high protein treatment. Lipid synthesis was induced accordingly, so the observation that low protein increased FAS activity might be a result of maximizing maintenance of metabolic energy balance through these two processes. Although no significant difference was found, the increased HSL activity in response to low protein supported the previous causal presumption of low protein effects on FAS activity.

Strålfors et al. (1984) found that addition of insulin rapidly decreased the extent of phosphorylation of HSL to the basal level, and that, after a short lag-time, the rate of lipolysis also was reduced. However, a long-term regulation of insulin plus glucose increased the rate of lipolysis by 40-65% and the amount of HSL protein by 40% (Botion et al., 1999). Kang et al. (1993) also reported that chronic exposure of adipose tissue to high concentrations of insulin increased the subsequent lipolytic response. Acute activation of HSL was controlled by phosphorylation and dephosphorylation events, which are regulated by the respective activities of a cAMP-dependent protein kinase and protein phosphatase situated in the modulating downstream of insulin (Sztalryd et al., 1995). The mechanisms responsible for the increased rate of FAS that are modulated by insulin sensitivity or that show time-dependence are not known. The modulating effects of Cr on FAS and HSL activities may be primarily influencing the lipolytic process rather than lipid synthesis.

The interaction between Cr and CP in response to a low protein diet showed that 400 μg/kg Cr had the ability to inhibit excessive lipolysis in adipose tissue, but that 400 μg/kg Cr decreased HSL activity in a high protein diet, which indicated that 400 μg/kg Cr reinforced the lipolysis. Therefore, at 400 μg/kg, Cr may have exerted special effects on regulating energy metabolism, which could result in a reduced energy consumption in a low energy conservation state, but may have improved energy utilization efficiency in a high energy conservation state. These findings supported our previous results that Cr has the potential for aiding energy redistribution (Yan et al., 2008). However, the exact mechanism of action of this influence is unclear and requires further investigation.

CONCLUSION

In the present study, Cr and dietary protein levels were shown to both affect the energy metabolism of lambs. A Cr supplementation level at 400 μg/kg in the feed ration was more efficient than a 800 μg/kg supplementation at modulating energy redistribution. The modulating effect apparently arose through regulation of an insulin signal transduction system, while high protein reinforced gluconeogenesis in the liver and kidney. A Cr×CP interaction suggested that, when supplemented at 400 μg/kg, Cr might give rise to a modulation of energy utilization in market lambs.

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