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Effects of Chromium Yeast on Performance, Insulin Activity, and Lipid Metabolism in Lambs Fed Different Dietary Protein Levels*

Xiaogang Yan, Wei Zhang, Jianbo Cheng, Runlian Wang[1], David O Kleemann[2]
Xiaoping Zhu and Zhihai Jia**
State Key Laboratory for Animal Nutrition, College of Animal Science and Technology
China Agricultural University, Beijing 100094, China

ABSTRACT : This experiment was conducted to study the effects of chromium (Cr), dietary crude protein (CP) level and potential interactions between these two factors on growth rate and carcass response, insulin activity and lipid metabolism in lambs. Forty-eight, 9-week-old weaned lambs (Dorper×Small-tail Han sheep, mean initial body weight = 22.96 kg±2.60 kg) were used in a 2×3 factorial arrangement of supplemental Cr (0 ppb, Cr0; 400 ppb, Cr1; or 800 ppb, Cr2 from chromium yeast) and CP levels (157 g/d to 171 g/d for each animal, LP; or 189 g/d to 209 g/d for each animal, HP). Growth data and blood samples were collected at the beginning and end of the feed trial, after which the lambs were killed. Both Cr additive groups and the HP group increased final weight and average daily gain, especially the Cr1 and HP group ($p<0.01$). HP increased pelvic fat weight ($p<0.05$), fat thickness of the 10th rib ($p<0.05$), longissimus muscle area ($p<0.01$) and rate of deposition of intramuscular fat ($p<0.01$). Supplemental Cr decreased the rate of deposition of intramuscular fat ($p<0.05$). Fasting insulin level and the ratio of insulin to glucose were lower with Cr1 than other groups, but with no significant difference. Glucose concentration was not affected by any treatment. Nonesterified fatty acids increased in the Cr1 ($p<0.05$) and HP ($p<0.05$) conditions and there was a significant Cr×CP interaction ($p<0.05$). Cr1 decreased triglycerides ($p<0.05$) and total cholesterol ($p = 0.151$) and HP increased high-density lipoprotein cholesterol ($p<0.05$). Cr1 decreased lipoprotein lipase activity in subcutaneous adipose tissue (aLPL, $p<0.05$) and the ratio of aLPL to lipoprotein lipase activity in skeletal muscle (mLPL, $p = 0.079$). mLPL and hepatic lipase (hHL) were not affected by any treatment. In the present study, Cr had limited effects on growth rate and carcass response, whereas Cr and CP had some notable effects on plasma metabolites and enzyme activities. Cr has a potential effect on energy modulation between lipid and muscle tissue. In addition, few Cr×CP interactions were observed. (**Key Words** : Lamb, Chromium Yeast, Protein Level, Performance, Insulin Activity, Lipid Metabolism)

INTRODUCTION

As the active substance in glucose tolerance factor (GTF), trivalent chromium (Cr) is a nutrient available at low concentrations in humans and animals. Cr exerts its biological function mainly by increasing sensitivity of insulin and improving glucose metabolism (Mertz et al., 1963; Anderson et al., 1998), though lipids and cholesterols may also be biological targets of Cr (Brautigan et al., 2006; Pattar et al., 2006). Whereas studies of Cr influence on insulin response and glucose and lipid metabolism in animals have reported varying results (Kegley et al., 2000; Sano et al., 2000; Sumner et al., 2007), these divergences also exist in growth measurements and carcass traits (Mooney et al., 1995; Gentry et al., 1999; Wang et al., 2004). The ambiguous effects of Cr are probably related to the contents of diets (Lukaski, 1999). Unfortunately, dietary Cr content was not measured in most of these trials for evaluating the effect of Cr.

Insulin action, glucose and cholesterol metabolism, and lipogenesis in both liver and adipose tissue correlate with dietary protein levels (Park, 1985; Santos et al., 2001; Van de Ligt et al., 2002; Sun et al., 2007). Previous research conducted in ruminants and nonruminants to evaluate the

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** Corresponding Author: Zhihai Jia. Tel: +86-10-6273-2728, Fax: +86-10-6273-2728, E-mail: jzh331@cau.edu.cn
[1] Department of Animal Science, Guangdong Ocean University, Zhanjiang 100037, China.
[2] Turretfield Research Centre, South Australian Research and Development Institute, SA 5350, Australia.
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potential of Cr in improving the growth measurements, carcass traits, and metabolic response at insufficient and sufficient protein levels has obtained conflicting results (Gentry et al., 1999; Van de Ligt et al., 2002), and had little focus on lipid metabolism.

The objective of this study was to investigate the effects of Cr yeast, dietary crude protein (CP) level, and potential interactions between these two factors on performance, insulin activity and lipid metabolism in fattening lambs based on dietary Cr determination, and to evaluate the feasibility of using Cr as an energy modifier by observing lipid metabolism systemically.

MATERIALS AND METHODS

Animals, diets and experimental design

Forty-eight, 9-week-old, weaned Dorper×Small-tail Han autumn-born lambs (mean initial body weight = 22.96 kg± 2.60 kg) were used in this study. All of the lambs were housed in individual steel pens (1.5×2.0 m^2) in an open-sided barn with concrete floor. After 2 weeks 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 ppb, Cr0; 400 ppb, Cr1; or 800 ppb, Cr2) of Cr supplementation from Cr yeast (1 g/kg Cr, Alltech, Nicholasville, KY) and two protein levels, low protein (157 g/d to 171 g/d for each animal, LP) and high protein (189 g/d to 209 g/d for each animal, HP) based on chemical analysis, were examined through 6 dietary treatments of eight animals each. 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 analyzed to contain 834 to 858 ppb Cr and 707 to 719 ppb Cr, respectively. Supplemental Cr was first mixed with corn meal and then gradually mingled completely with other dietary ingredients. 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. The weights of lambs were measured at day 0 and day 60.

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 procedures of the Association of Official Analytical Chemists (AOAC, 1995). Neutral 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 were expressed with residual ash. The Cr concentrations of the samples were determined using an Agilent 7500c ICP-MS mass spectrometer (Agilent Technologies, Japan).

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	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 (ppb)	858	834	707	719

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

[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.

Table 2. Influence of chromium yeast supplementation and dietary protein level on growth and carcass measurements 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
Growth performance															
ADG (g/d)	221	243	231	250	267	257	236	255	244	232^{B}	258^{A}	4.78	0.206	0.006	0.971
Finial weight (kg)	35.9	37.7	36.9	37.9	39.1	38.3	36.9	38.4	37.6	36.8	38.4	0.48	0.465	0.116	0.958
Carcass measurements															
Carcass wt (kg)	16.95	17.71	17.33	17.74	18.53	18.12	17.35	18.12	17.73	17.33	18.13	0.27	0.486	0.140	0.999
Dressing percentage (%)	47.15	46 98	47.04	46 79	47 34	47.30	46.97	47.16	47.17	47 06	47 14	0.35	0.939	0.868	0.826
Pelvic fat wt (kg)	0.40	0 34	0.37	0 44	0 41	0.43	0 42	0 38	0.40	$0\ 37^{b}$	$0\ 44^{a}$	0.01	0.313	0.030	0.893
Pelvic fat percentage (%)	1.13	0.89	0.99	1.17	1.04	1.13	1.15	0.97	1.06	1.00	1.11	0.04	0.493	0.414	0.907
Wall fat thickness (cm)	1.11	0 92	1.08	1.21	1.02	1.13	1.16	0.97	1.11	1 04	1 12	0.03	0.068	0.195	0.932
The 10^{th} rib fat thickness (cm)	0.61	0.48	0.50	0.65	0.60	0.62	0.63	0.54	0.56	0.53^{b}	0.62^{a}	0.02	0.157	0.027	0.628
LMA (cm)	14.48	15 05	14.78	16 65	18.15	17.01	15.57	16.60	15.90	$14\ 77^{B}$	$17\ 27^{A}$	0.41	0.452	0.002	0.823
Intramuscular fat rate (%)	5.66	4.88	4.91	7.22	5.70	6.53	6.44^{a}	5.29^{b}	5.72^{b}	5.15^{B}	6.48^{A}	0.21	0.019	<0.001	0.493

Means in the same row with different superscripts differ significantly ($p<0.05$).

SEM = Standard error of the mean; Cr = Chromium level; CP = Protein level; Cr×CP = Interactions of chromium and protein level.

ADG = Average daily gain; and LMA = Longissimus muscle area.

Sample collection and carcass quality evaluation

Blood samples were collected from each lamb at day 0 and day 60 before weighing and after fasting for 16 h. Samples were collected via jugular venipuncture and divided into two plasma tubes containing either EDTA-Na or potassium oxalate and sodium fluoride. Plasma samples were obtained by centrifugation at 1,500×g for 20 minutes at 4°C, and stored at -25°C after aliquotting for later analyses of several metabolites and hormones.

Upon termination of the feeding trial, all lambs were electrically stunned and then slaughtered by exsanguination. Subcutaneous adipose tissue, longissmus muscle adjacent to the 10th rib and the last lumbar vertebra and liver samples of each animal were harvested, immediately immersed in liquid nitrogen and then stored at -70°C for enzyme activity analyses. After removing the skin, head and feet, the carcasses were eviscerated and the pelvic fat separated from each lamb and weighed. Next, the hot carcass were weighed, chilled at 1°C for 24 h, and split longitudinally. Both sides of the cold carcass for each lamb were measured and the average value was calculated. Body wall fat at 8 cm from the lateral end of the longissmus muscle and the fat thickness of the 10th rib over the longissmus muscle area (LMA) were measured. LMA was obtained by tracing its surface area at the 10th rib and determining its area with a compensating polar planimeter (Model KP-27, Koizumi, Japan) (USDA, 1984).

About 5-cm-thick samples of longissimus muscle were removed from the 10th right rib of each carcass right side, frozen at -25°C and lyophilized (Model GT20, Leybold-Heraeus, Germany). The samples were ground with a food processor (Model BJ2-250B, Elizabeth, China) and the lipid contents were determined by the solvent extraction method introduced by the AOAC (1995).

Plasma insulin and metabolite assays

Plasma insulin was analyzed using the blood samples in the tubes containing EDTA-Na with a commercially available solid phase radioimmunoassay kit (Beijing Atom High Tech Co., Beijing, China). Plasma metabolites were analyzed using the blood samples centrifuged in tubes containing potassium oxalate and sodium fluoride. Glucose, nonesterified fatty acids (NEFA), triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) concentrations were measured with an auto analyzer (Hitachi 7600-020; Hitachi Co., Japan) using commercially available kits.

Enzyme assays

Homogenisation and assay of the lipoprotein lipase (LPL) activity in subcutaneous adipose tissue (aLPL) and skeletal muscle (mLPL) were performed as described by Faulconnier et al. (1994). Hepatic lipase (HL) activity in the liver was measured according to the methods described by Blay et al. (2002). LPL and HL activities were expressed as nanomoles of released fatty acid per minute per gram of adipose or muscle tissue. Glycerol tri [9, 10 (n)-^{3}H] oleate was obtained from China Isotope Co. (Beijing, China), and organic reagents were purchased from Sigma Chemical Co. (St. Louis, MO).

Statistical analysis

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

RESULTS

Growth rate and carcass response

Final weights in Cr supplemented and HP treatments were all higher than control groups and LP treatments; however, no significant difference was observed. Average daily gain (ADG) increased in the Cr1 and HP condition ($p<0.01$, Table 2).

HP increased pelvic fat weight ($p<0.05$), fat thickness of the 10th rib ($p<0.05$), LMA ($p<0.01$) and the rate of

Table 3. Plasma insulin and metabolites before feeding trial in fattening lambs

Items	Ins (μIU/ml)	Glu (mmol/L)	Ins/glu ratio	NEFA (mmol/L)	TG (mmol/L)	TC (mmol/L)	LDL-C (mmol/L)	HDL-C (mmol/L)
Mean	20.42	3.64	5.87	0.13	0.12	0.81	0.22	0.57
SEM	0.74	0.15	0.37	0.01	0.01	0.05	0.02	0.03

Ins = Insulin; Glu = Glucose; NEFA = Nonesterified fatty acid; TG = Triglycerides; TC = Total cholesterol; LDL-C = Low-density lipoprotein cholesterol; HDL-C = High-density lipoprotein cholesterol; and SEM = Standard error of the mean.

Table 4. Influence of chromium yeast supplementation and dietary protein level on plasma insulin and metabolites 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
Glu (mmol/L)	3.86	3.61	3.67	3.60	3.99	3.77	3.73	3.80	3.72	3.71	3.79	0.20	0.987	0.876	0.842
Ins/glu ratio	4.78	3.81	5.53	4.42	3.24	4.57	4.60	3.53	5.05	4.71	4.08	0.31	0.146	0.319	0.922
NEFA (mmol/L)[b]	0.13	0.13	0.10	0.08	0.25	0.18	0.11^{b}	0.19^{a}	0.14^{ab}	0.12^{b}	0.17^{a}	0.02	0.031	0.047	0.031
TG (mmol/L)[c]	0.15	0.09	0.13	0.19	0.12	0.13	0.17^{a}	0.11^{b}	0.13^{ab}	0.12	0.15	0.01	0.044	0.224	0.607
TC (mmol/L)	0.94	0.62	0.87	0.92	0.80	1.00	0.93	0.71	0.94	0.81	0.91	0.05	0.151	0.328	0.707
LDL-C (mmol/L)	0.29	0.24	0.26	0.24	0.20	0.28	0.27	0.22	0.27	0.26	0.24	0.02	0.452	0.574	0.727
HDL-C (mmol/L)	0.44	0.59	0.50	0.59	0.76	0.67	0.52	0.68	0.59	0.51^{b}	0.67^{a}	0.04	0.267	0.046	0.991

Means in the same row with different superscripts differ significantly ($p<0.05$).

SEM = Standard error of the mean; Cr = Chromium level; CP = Protein level; Cr×CP = Interactions of chromium and protein level; Ins = Insulin; Ins = Insulin; Glu = Glucose; NEFA = Nonesterified fatty acid; TG = Triglycerides; TC = Total cholesterol; LDL-C = Low-density lipoprotein cholesterol; and HDL-C = High-density lipoprotein cholesterol.

deposition of intramuscular fat ($p<0.01$). Cr additives decreased the rate of deposition of intramuscular fat ($p< 0.05$) and the Cr1 group was lowest. Wall fat was increased by Cr supplementation and HP without significant differences. Dressing percentage and pelvic fat percentage were not significantly different across treatments. For all growth and carcass measurements, no Cr×CP interaction was observed (Table 2).

Plasma insulin and metabolites

Plasma insulin and metabolites at day 0 and day 60 are shown in Table 3 and 4, respectively. All plasma indexes at day 0 had no significant difference.

After the feeding trial, fasting insulin level and the ratio of insulin to glucose in the Cr1 treatment group were lower than other groups without significant difference. Glucose concentration was not affected by any treatment. NEFA increased in the Cr1 ($p<0.05$) and HP ($p<0.05$) group, and showed Cr×CP interaction ($p<0.05$). Cr1 decreased TG ($p<0.05$) and HP increased HDL-C ($p<0.05$). There were no significant differences in TC or LDL-C across treatments.

Enzymatic activity

Cr1 decreased aLPL activity ($p<0.05$) and the ratio of aLPL to mLPL ($p = 0.079$). mLPL and hHL were not affected by any treatment. No Cr×CP interaction existed in enzyme activity values (Table 5).

DISCUSSION

Growth performance and carcass response

In the present research, Cr additives increased final weight and ADG of lambs, especially in Cr1 groups. These positive effects of Cr were also observed by Mooney et al. (1995), who indicated that the addition of 200 μg/kg chromium picolinate increased ADG in growing-finishing swine. Whereas some studies showed that Cr had no significant effects on growth performance (Van de Ligt et al., 2002; Mostafa-Tehrani et al., 2006), the ambiguous effects of Cr on growth probably relate to whether the contents of diets have been adequate to meet the physiological needs of animals (Lukaski, 1999).

Our finding that HP improves weight and ADG were

Table 5. Influence of chromium yeast 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
Lipoprotein lipase (nmol/min·g)															
aLPL	60.72	53.38	57.31	69.31	54.29	62.91	65.02^{a}	53.84^{b}	60.11^{a}	57.14	62.17	1.79	0.031	0.127	0.609
mLPL	16.63	18.76	17.23	20.39	19.58	19.33	18.51	19.17	18.28	17.54	19.77	0.65	0.854	0.113	0.674
aLPL/mLPL ratio	3.78	2.89	3.45	3.43	2.78	3.34	3.61	2.84	3.40	3.37	3.18	0.14	0.079	0.329	0.925
Hepatic lipase (nmol/min·g)															
HL	90.91	103.94	114.40	93.45	90.39	95.34	92.18	97.17	104.87	103.08	93.06	4.13	0.479	0.250	0.564

Means in the same row with different superscripts differ significantly ($p<0.05$).

SEM = Standard error of the mean; Cr = Chromium level; CP = Protein level; Cr×CP = Interactions of chromium and protein level; aLPL = Lipoprotein lipase activity in subcutaneous adipose tissue; mLPL = Lipoprotein lipase activity in longissimus muscle; and HL = Hepaticlipase activity in hepar.

consistent with the research of Van Heugten et al. (1994) in weaning pigs and Dabiri et al. (2004) in lambs. In their studies, linear increases were found in ADG with elevation of dietary protein required from 60% to 100% (Van Heugten et al., 1994) or from 13% to 17% (Dabiri et al., 2004). Jeon et al. (2006) found that HP feed could increase crude protein intake (CPI) of spotted deer. Although amino acid contents of the test diets were not analyzed in this study, Increasing CPI might provide more essential amino acids for protein utilization and deposition in lambs, and the increased weight may just be the result of improved ADG and CPI.

Our data indicate that Cr additives did affect lipid deposition and LMA. These results supported the findings of Lindemann et al. (1995), who observed that the addition of 200 ppb Cr reduced backfat and increased LMA of growing-finishing swine, and similar effects of Cr were also observed by Mooney et al. (1995) and Wang et al. (2004) in pigs. On the contrary, Evock-Clover et al. (1993) indicated that Cr did not affect backfat or LMA in growing pigs. As the cofactor of insulin, Cr acts on carcass traits mainly by influencing insulin sensitivity, which closely relates to carbohydrate and protein metabolism (Mertz et al., 1974; Anderson et al., 1998). The results of our research suggest that the chromium content in the test diet (<1,000 ppb) could not meet the requirements of fattening lambs. The findings that HP level increased lipid deposition in backfat and in longissimus muscle and enlarged LMA support the work of Anderson et al. (1988) who found that limited dietary CP resulted in decreased fattening of young bulls. The increased lipid deposition could be explained by the utilization of excess dietary protein as energy and the greater synthesis of fatty acids (Waghorn et al., 1987), and larger LMA from increased rates of carcass protein deposition in growing lambs when CP level increased (Willms et al., 1991). For growth and carcass traits, the effects of Cr yeast were more limited compared to dietary protein level.

Plasma hormone and metabolites

Although no significant difference was found, the lower insulin content and ratio of insulin to glucose with Cr1 showed the improvement of insulin activity. This result is consistent with Kegley et al. (2000) who reported that 400 μg/kg, but not 800 μg/kg, chromium-L-methionine increased the insulin response to an intravenous glucose challenge in growing calves with a functioning rumen. However, Ott et al. (1999) observed that chromium tripicolinate had no influence on insulin activity of yearling horses. These ambiguous results may also be attributed to the Cr status of the body, which is closely related to Cr content in feed. In our study, the action of 400 ppb Cr on insulin activity was more effective than 800 ppb, which may indicate that 800 ppb Cr was in excess of the requirements of lambs. The responses of plasma insulin and glucose to dietary protein were consistent with the results of Van de Ligt et al. (2002) who indicated that equal amounts of amino acids were used by peripheral tissue and excellent glucogenic regulation was executed by pigs in a fasting state. This ability for glucogenic regulation ability may be similarly possessed by ruminants.

Our study observed that the Cr1 and HP condition significantly increased NEFA in lambs fasting for 12 h ($p<0.05$). This finding supports the research of Gentry et al. (1999) who indicated that plasma NEFA was 50% higher in Suffolk wether lambs fed supplemental Cr tripicolinate than control lambs following 16 h of feed deprivation. However, Williams et al. (2004) observed that Cr had no effects either on heifers or on periparturient Holstein cows, while Sumner et al. (2007) obtained the opposite result. The increasing content of plasma NEFA in our study indicated that the degree of catabolism of lipid tissue was enhanced by Cr, which could explain the reduction of backfat. Increased NEFA also indicated a weakened β-oxidation in mitochondria. The discrepancies between our results and previous research, and between catabolism and β-oxidation cannot be explained at this time. It may be supposed that excess NEFA induced by Cr could not be completely oxidized for energy in a half-fasting stage, and when the energy balance developed for consumption, NEFA was produced more efficiently from adipose tissue. The fact that lambs fed supplemental Cr had plasma NEFA concentrations 22.6% higher than the control after 3 h of feed deprivation but 50% higher after 16 h (Williams et al., 2004), and that growing Holstein heifers had decreased NEFA 0-150 min after a glucose tolerance test (Sumner et al., 2007) supported this hypothesis. The finding that HP increased NEFA is consistent with Wilson et al. (1972) in lambs and Santos et al. (2001) in primigravid dairy cows. The increased supply of amino acid in the HP diet may have enhanced complete oxidation of fatty acids and reduced ketogenesis in the liver (Santos et al., 2001).The interaction of Cr×CP may be the result of Cr accelerating the lipolysis of adipose tissue more effectively when fat deposition was improved by HP.

The finding of decreased plasma TG levels in the Cr1 group is similar to the studies of Lien et al. (1999) in broiler chickens, but Mooney et al. (1997) found that TG was not changed by $CrCl_3$ in swine. The decreasing TG indicated that Cr could improve hepatic transport ability of TG and promote TG to catabolize, due to the increasing activity of LPL in plasma, but no direct evidence was found. As the inorganic form of the Cr additive, $CrCl_3$, could not have the same effective action as organic Cr, this may explain the

finding of Mooney et al. (1997). The lower TC and LDL-C and higher HDL-C contents in the Cr1 groups support the findings of Lien et al. (2005) in weaned pigs, which can probably be attributed to the increasing effect of insulin on liver LDL receptors, which reduces TC and LDL-C content and increases the proportion of HDL-C (Brindley et al., 1991; Lien et al., 1999). The improvement of insulin activity might explain the changeable trends of TC, LDL-C and HDL-C (Laakso et al., 1990). The result of increased HDL-C with HP is consistent with the research of Park (1985), who attributed this result to the increase of lecithin-cholesterol acyltransferase (LCAT) enzymatic activity.

Enzymatic activities

In mammals, insulin plays a critical role in modulating the activity of LPL. Some studies have shown that insulin stimulates LPL activity in adipose tissue (Ong et al., 1988; Faulconnier et al., 1994), but decreases LPL activity in skeletal muscle in humans and rats (Gorski et al., 1982; Farese et al., 1991). Our data indicate changeable trends in LPL activities in subcutaneous adipose tissue and in skeletal muscle, similar to previous findings. The lower aLPL/mLPL in Cr1 groups indicates that partial fatty acids are drawn away from adipose tissue towards muscle tissue and increase the availability of fatty acids to provide additional lipid substrates for working muscle, which reduces carbohydrate oxidation in the muscle (Ellis et al., 1994). It seems that plasma NEFA, TG and LPL have no relationship with LPL activity in adipose tissue or in muscle because most of the LPL released from adipose tissue or muscle is inactive and the small proportion of active LPL seems to be redistributed from muscle to adipose tissue (Karpe et al., 1998). Thus, the reason for higher plasma NEFA and lower plasma TG levels in Cr treatments needs further investigation. The lower mean LPL activity in LP treatments was consistent with Boualga et al. (2000) who found that a low-protein diet prevented increase in extrahepatic LPL activities in young rats and indicated that a low-protein diet limits lipid storage in adipose tissue due to reduced availability of serum very low-density lipoprotein cholesterol-triglycerides (VLDL-TG). These results were similar to the observations in our study of carcass traits.

The *in vivo* functions of HL are unclear, but previous studies have suggested that HL catalyzes the hydrolysis of triglycerides from IDLs and LDLs and takes part in the metabolism of HDLs and the uptake of remnant lipoproteins in the liver (Santamarina-Fojo et al., 1998). Our research shows that HL activity in the liver did not differ across treatments, and the relationships between liver HL activity and plasma HL activity and between plasma lipids and lipoproteins in ruminants need further investigation.

CONCLUSION

According to this experiment, the effects of Cr yeast on growth and carcass traits were limited compared with the effects of dietary protein level. The inconsistent trends of insulin activity and other plasma metabolites indicated that the sensitivity threshold of Cr was under 800 ppb (based on Cr addition). The lower aLPL/mLPL ratio suggested that Cr has the potential for aiding energy redistribution. Few Cr×CP interactions were observed.

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