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Chromium downregulates the expression of Acetyl CoA Carboxylase 1 gene in lipogenic tissues of domestic goats: a potential strategy for meat quality improvement

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ABSTRACT

Acetyl CoA Carboxylase 1 (ACC1) is a biotin-dependent enzyme that catalyzes the carboxylation of Acetyl CoA to form Malonyl CoA, the key intermediate metabolite in fatty acid synthesis. In this study, the mRNA expression of the ACC1 gene was evaluated in four different tissues (liver, visceral fat, subcutaneous fat, and longissimus muscle) of the domestic goat (*Capra hircus*) kids feeding on four different levels of trivalent chromium (0, 0.5, 1, and 1.5 mg/day) as food supplementation. RT-qPCR technique was used for expression analyses and heat shock protein 90 gene (HSP-90) was considered as reference gene for data normalization. Our results revealed that 1.5 mg/day chromium significantly reduced the expression of the ACC1 gene in liver, visceral fat, and subcutaneous fat tissues, but not in longissimus muscles ($P < 0.05$). We measured some phenotypic traits of kid's carcasses to detect their probable correlations with chromium-mediated downregulation of ACC1 expression. Interestingly, changes in ACC1 expression were accompanied with decreased accumulation of fats in adipose tissues such that the subcutaneous fat thickness and heart fat percentage decreased in kids feeding on chromium. By contrast, chromium supplemented kids showed higher percentage of muscles despite the fact that their total body weight did not differ from that of non-supplemented kids. Our study suggests that trivalent chromium alters the direction of energy accumulation towards muscles rather than fats and provides insights into application of chromium supplementation as a useful strategy for improvement of meat quality in domestic animals.

1. Introduction

The Fertile Crescent region, stretching from the southern Levant in southeastern Turkey and northern Syria to the high Zagros mountain pastures of Iran, is thought to be the center of domestication for some of the most important arrays of agricultural crops and livestock animals including barley, wheat, sheep, and goat (Zeder and Hesse, 2000). The domestic goats, *Capra hircus*, are one of the oldest domesticated animals (Zeder and Hesse, 2000) that are extensively reared throughout the world due to their excellent importance as sources of milk, meat, fiber and pelt (Dong et al., 2012). A recent study revealed that goat meat is the most widely consumed red meat eaten by more than 70% of the world population (www.agric.wa.gov.au).

The fat present in red meat supplies essential fatty acids and vitamins and plays an essential role in sensory perception of juiciness, flavor and texture. However, red meat is usually considered a food with excessively high fat concentration and is believed to cause a variety of human diseases because of its high proportion of detrimental fatty acids (Daley et al., 2010; Moloney, 2002; Wood et al., 2003). Hence, health professionals recommend a reduction in the overall consumption of inferior fats especially saturated fatty acids (Daley et al., 2010). This may be achieved by reducing the total fat content or improving the fat composition of meat (Wood et al., 2003).

Acetyl-CoA carboxylase (ACC) is a biotin-dependent enzyme that catalyzes the carboxylation of acetyl-CoA to produce malonyl-CoA substrate for the biosynthesis of fatty acids (Bengtsson et al., 2011). Mammalian ACC exists as two isoforms: ACC1 and ACC2 that are differently distributed across tissues (Oh et al., 2005). At energy surplus, ACC1 converts acetyl-CoA into malonyl-CoA for lipogenesis in the cytosol of lipogenic tissues such as liver and adipose tissue, while the ACC2, present typically on the mitochondrial surface, stimulates the same process to generate malonyl-CoA for the inhibition of carnitine palmitoyltransferase

Abbreviations: ACC1, Acetyl CoA Carboxylase 1; ACC2, Acetyl CoA Carboxylase 2; HSP-90, Heat shock protein 90 gene; ACC2, Acetyl CoA Carboxylase 1; CPT-1, Carnitine palmitoyltransferase 1; BW, Body Weight; H, Hour; RT-qPCR, Real-time Quantitative PCR; Q-RT-PCR, Quantitative Real-time PCR (Real-time Quantitative PCR); GLM procedure, General Linier Model procedure; cDNA, DNA complementary to RNA; rRNA, ribosomal RNA; DNase, deoxyribonuclease; dNTP, deoxyribonucleoside triphosphate.

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1 (CPT-1) and mitochondrial fatty acid oxidation (Bengtsson et al., 2011; Oh et al., 2005). Therefore, the two ACC isoforms play crucial roles in fatty acid biosynthesis in human and some other living organisms (Tong and Harwood, 2006). Because of its unique position in lipid metabolism (Oh et al., 2005), inhibition of ACCs has been proposed to reduce lipogenesis and favor lipid oxidation, thereby preventing deleterious lipids from accumulating in oxidative tissues such as muscles, heart, and liver (Bengtsson et al., 2011; Tong and Harwood, 2006). In recent years, the use of ACC enzymes as suitable targets for reducing tissue fatty acids and treating metabolic disorders (e.g. obesity, diabetes, and hyperlipidemia) has experienced increasing attention (Abu-Elheiga et al., 2003; Bengtsson et al., 2011; Bhadauriya et al., 2013; Savage et al., 2006). For example, it has been suggested that mice lacking ACC2 gene show high levels of fatty acid oxidation and accumulate 50% less fat in their adipose tissue when compared to wild-type mice (Abu-Elheiga et al., 2003). In another study, suppression of ACC1 and ACC2 gene expression using antisense oligonucleotides has been shown to stimulate fat oxidation in rat hepatocytes (Savage et al., 2006). These findings incline us to think about the idea that the modification of ACC or its encoding gene may potentially decrease the accumulation of fatty acids in muscles and improve meat quality.

Chromium has been widely applied as an essential supplemental nutrition for human and laboratory animals (Swanson et al., 2000). The trivalent form of chromium (Cr^{+3}) is an important structural element of glucose tolerance factor (GTF) which increases glucose tolerance by potentiating the action of insulin and so, shunts more energy towards growth and reproduction (Swanson et al., 2000). Therefore, it is a well-known ultra-trace element essential for normal metabolism of carbohydrates, lipids, proteins, and nucleic acids besides its extra roles in hormonal regulation, weight gain, improved dressing percentage and longissimus muscle area, and immunity against pathogens (Anderson, 1994; Emami et al., 2012; Haldar et al., 2009; Pechova et al., 2002). In a previous study carried out aimed at selection of a suitable reference gene in the domestic goats, we found unexpectedly that the gene encoding ACC1 enzyme (*ACC1*) showed high degrees of instability in expression status when different levels of chromium were added to their standard diet (unpublished data). We hypothesized that downregulation of *ACC1* expression by chromium may eventually leads to decrease in the overall fat content of goat carcass, a process that can be effectively applied to improve red meat quality for human consumption. To test this hypothesis, in the current study, we evaluated the expression status of *ACC1* in goat kids feeding on different levels of chromium.

2. Materials and methods

2.1. Animal husbandry and experimental design

This study was conducted at the Research Station of Department of Animal Science, College of Agriculture and Natural Resources, University of Tehran, Iran. The studied animals include twenty-four, 4 to 5-month old male goat kids belonging to the native Iranian breed, Mahabadi. Complete random design (CRD) with 4 treatments and 6 replicates in each treatment was the experimental design. All procedures of immunity and nutrition were conducted under protocols approved by this station.

After weighing (BW = 22 ± 2 kg), the kids were randomly allocated to one of the four following dietary treatments: standard diet plus 0, 0.5, 1, and 1.5 mg chromium per day as chromium-methionine (Availa®Cr 1000, Zinpro Corporation, USA). The standard diet was balanced and prepared using NRC computer software (see Table 1). Chromium supplementation was exposed to the kids before each morning nutrition meal as a pulverized powder mixed with 50 g barley. The kids were individually penned for 100 days (10 days for adaptation and 90 days for feeding period), with accessibility to enough water, and provided with the prepared diets twice a day (08:00 h and 17:00 h). The kids were weighed before the morning feeding meal triweekly (i.e. after 14–16 h of starvation) throughout the experiment period to determine changes in their body weight.

Table 1
Ingredient and chemical composition of basal standard diet fed to goat kids.

Ingredient	% of DM	Ingredient	% of DM
Alfalfa hay	16.49	Soybean meal	2.21
Corn silage	8.32	Calcium carbonate	1.3
Wheat straw	5.19	Mineral–vitamin supplement[a]	0.91
Barley grain	51	Sodium bicarbonate	0.78
Wheat bran	9.09	Salt	0.52
Canola meal	4.55		
Nutrient fractions			
DM (%)	80.78	ME (Mcal/kg DM)	36.6
CP (% DM)	13.5	Calcium (% DM)	0.89
Ether extract (% DM)	2.6	Phosphorus (% DM)	0.49
NDF (% DM)	36.6	Chromium (% DM)	0.83
Ash (% DM)	9		

[a] Containing per kg DM: calcium, 195 g; phosphor, 80 g; magnesium, 21,000 mg; sodium, 50 g; manganese, 2200 mg; iron, 3000 mg; copper, 300 mg; iodine, 120 mg; cobalt, 100 mg; zinc, 300 mg; selenium, 1.1 mg; antioxidant, 2500 mg; vitamin A, 600,000 IU; Vitamin D3, 200,000 IU; vitamin E, 200 mg.

2.2. Slaughtering and tissue sampling

Slaughtering, transport and invasive procedures on these animals include a statement indicating approval by the appropriate ethics/welfare committee confirming compliance with all requirements of the country in which the experiments were conducted. After feeding on the prepared diets for 90 days, the kids were transferred to the departmental abattoir, where they were kept for 12 h under starvation with free access to water. They were then slaughtered via decapitation and samples were taken from the liver, visceral fat, subcutaneous fat, and longissimus muscle. The samples were immediately frozen in liquid nitrogen (−196 °C) and transferred to the laboratory, where they were maintained at −80 °C until used.

2.3. Total RNA isolation, clean up and cDNA synthesis

Total RNA was extracted according to the method of Chomczynski and Sacchi (2006) using Trizol Reagent (Invitrogen Co., Carlsbad, CA, USA). The extracted RNA was then treated with RNase-free DNase I in order to remove the remnant genomic DNA from the samples (TaKaRa, Shuzo, Kyoto, Japan). RNA concentrations were estimated by Nanodrop spectrophotometry at 260 nm and their purities were checked by determining the absorption ratios at 260/280 nm. The quality of extracted RNA was assessed by electrophoresis at 1% agarose-gel containing Ethidium Bromide. First-strand cDNA was synthesized from 100 ng of total RNA using an oligo (dT) primer, random hexamers and a commercially available kit (AccuPower® RocketScript™ RT PreMix) according to the manufacturer's instructions. The process of cDNA synthesis initiates by connection of the primers at 37 °C for 1 min followed by cDNA synthesis at 50 °C for 60 min and terminates by inactivation of the reverse transcriptase enzyme at 90 °C for 5 min. The synthesized cDNA was incubated at −20 °C until used.

2.4. Reference gene

It is widely argued that some factors including RNA stability, RNA extraction, retrotranscription efficiency, PCR steps, etc., may negatively affect the accuracy and reliability of the results obtained for RT-qPCR and gene expression analysis studies (Han et al., 2012). To overcome this problem, a common procedure is to normalize the total amounts of RNA or a single internal reference gene known as HouseKeeping gene (Bustin, 2002). An ideal reference gene is expected to be stable in terms of expression level across various experimental conditions such as developmental stages, tissue types, treatments, and external stimuli