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Short communication

The effects of chromium picolinate and simvastatin on pig serum cholesterol contents in swine muscular and adipose tissues

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ABSTRACT

In this study, the effects of two hypocholesterolemic drugs, simvastatin (a statin) and chromium dipicolinate, on serum cholesterol levels, and cholesterol contents in muscular and adipose tissues were tested. Although we find no significant effects of both drugs on cholesterol serum levels, we found a 25.5% and 18.9% reduction of cholesterol content in adipose tissue in statin and chromium dipicolinate-treated pigs, respectively, compared to controls. We also found a significant reduction in cholesterol content in *Longissimus thoracis* (9.6% reduction) and *Psoas major* (13.0% reduction) after statin administration. On the other side, chromium dipicolinate significantly reduced the cholesterol content only in *Longissimus thoracis* (10.0% reduction) but not in *Psoas major*. As a drawback, we have found that chromium dipicolinate significantly enhanced the ω6 fatty acid content in adipose tissue; simvastatin also enhanced PUFA ω6 fatty acid content to nearly significant levels.

1. Introduction

Cholesterol is an essential molecule for life. In humans, it is endogenously synthesized and it is also acquired from food. Meat is a major source of cholesterol in the human diet (Dinh et al., 2011). Cholesterol is of considerable interest for consumers due to its relation to disease; it can deposit on vascular epithelia, leading to atherosclerotic lesions that can cause cardiovascular diseases (Ross, 1999). As a consequence of the risks associated with cholesterol intake, the reduction of cholesterol content is one important goal in several food branches; indeed, if a reduction in cholesterol content is achieved in a particular food, whatever that means, it is one of the most used advertising slogans in those foods. Clearly, reducing cholesterol in meat could have a positive economic impact in the meat industry.

Cholesterol content varies greatly in pork, depending on several factors such as muscle type (Fernández et al., 1995), genetic background (Kellogg et al., 1977), fat level in the diet (Harris et al., 1993), and meat cut or processing (Dorado et al., 1999). Cholesterol level in pork ranges from 30 mg/100 g to 80 mg/100 g (Dinh et al., 2011). Cholesterol content in pig adipose tissue is larger, ranging from 70 mg/100 g to 140 mg/100 g (Dorado et al., 1999; Dinh et al., 2011). Reduction in cholesterol levels in pork has been tried by dietary and/or pharmacological means (Engeseth et al., 1992; Burgos et al., 2010). Chromium, mostly in the form of chromium dipicolinate but also as chromium propionate, chromium chloride or chromium nanocomposite, is one of the most frequently assessed pharmacological tools to reduce cholesterol levels (Press et al., 1990). This is because chromium picolinate (CrPi) was approved by the United States Department of Agriculture in 1996 as a source of chromium and because it also improves several productive traits, such as average daily gain and feed conversion index, and because it increases the lean content of the carcass (Lindemann et al., 1995). Indeed, the use of chromium picolinate in the swine industry was prompted by the observation that its delivery to humans resulted in the deposition of more muscle mass. However the effects of chromium on productive traits and carcass conformation are under debate as several studies have failed to confirm them (Mooney and Cromwell, 1999; Matthews et al., 2001). A recent meta-analysis with the most important studies has concluded that chromium supplementation decreases 10th-rib fat thickness and increases carcass lean and loin muscle area (Sales and Jančík, 2011).

The effects of chromium on serum cholesterol have been investigated by several authors. The results of these studies are also contradictory. Some articles have indicated that chromium did not affect serum cholesterol levels (Matthews et al., 2001; Kim et al., 2010), while others have reported an increase (Shelton et al., 2003) or a decrease (Wang et al., 2007) of this parameter.

Statins are drugs that have been used mostly in humans. They reduce cholesterol serum levels because they competitively inhibit one of the key enzymes of endogenous cholesterol synthesis,

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3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoA reductase) (Pedersen, 1994). Its use in pigs has had just the objective of answering fundamental questions of cholesterol metabolism (Dixon et al., 2002; Casani et al., 2005). To the best of our knowledge, there are no data available of the effect of statins on cholesterol content in pork products. In the present work, we have studied the separate effects of both chromium picolinate and simvastatin on serum levels of cholesterol during the last part of the feeding period. After slaughter, the fat content, cholesterol levels, and fatty acid composition were studied in two muscles, *longissimus thoracis* (LT) and *Psoas major* (*Ps. major*), as well as in subcutaneous adipose tissue mainly to assess if either of these two molecules could be used to reduce cholesterol content in pork in order to make it more attractive to consumers.

2. Materials and methods

Experiments have been carried out in accordance with University of Zaragoza rules and EU Directive 2010/63/EU for animal experiments.

2.1. Animal material

Forty three female pigs of a Pietrain × Large White cross were randomly selected from the offspring of 30 parities and randomly distributed in three adjacent pens. One pen housed the 14 animals of the control group, a second pen housed 15 animals from the statin group, and the third pen housed 14 animals of the chromium picolinate group. Three animals (two of the control group and one of the picolinate group) were discarded due to unrelated reasons and were not included in the experiment.

2.2. Animal management

Pigs were raised in a commercial farm under standard industrial conditions. At about 80 kg, blood samples were collected (in the morning after one night fasting) in Becton Dickinson blood collection tubes without any clotting or anticoagulant additive for cholesterol analysis to confirm that all animals had similar cholesterol levels that were comparable to published ones (Matthews et al., 2001; Page, 1993). Then, control feed, feed containing CrPi (200 ppb) or feed containing simvastatin (30 ppm) was given during 45 days to the three pig groups. One day before slaughter, a second blood sample was collected from each pig, also after one night fasting.

Pigs were slaughtered in a commercial slaughterhouse close to the farm. They were stunned with CO_2 and killed by exsanguination. Muscle and adipose tissue samples were collected about 60 min postmortem and frozen immediately. LT samples were taken at approximately the twelfth vertebrae level.

2.3. Serum levels of cholesterol

Immediately after the blood was collected, serum was obtained incubating upright the Becton Dickinson tubes for 45 min at room temperature to allow clotting and centrifuging them at 2000 g for 10 min. The clear serum supernatant was immediately divided in 1 ml aliquots. Aliquots were then stored at −20 °C until analyzed.

Total cholesterol concentrations were determined by an enzymatic–colorimetric procedure (BioSystems kit # 11539; BioSystems S.A., Barcelona, Spain). The LDL fraction was precipitated using a solution of polyvinyl sulfate/polyethylene glycol (BioSystems kits #11579; BioSystems S.A., Barcelona, Spain), and then the cholesterol in the supernatant was analyzed with a cholesterol kit (BioSystems kit # 11539; BioSystems S.A., Barcelona, Spain). The LDL fraction was the difference between the serum total cholesterol and the cholesterol in the supernatant after centrifugation. The HDL-cholesterol content was determined with the BioSystems cholesterol kit # 11523 (BioSystems S.A., Barcelona, Spain).

2.4. Fat extraction, fatty acids composition and cholesterol content analysis

Fat was extracted from 10 g of muscle homogenates or from 0.5 g of adipose tissue homogenate using the method of Bligh and Dyer (1959) as modified by Hanson and Olley (1963). Two aliquots of the chloroform phase were taken, one of 5 ml for gravimetric fat content analysis and later for fatty acid profile determination as described in Burgos et al. (2010), and the other (5 ml in the case of muscle samples or 1.5 ml in the case of adipose tissue samples) for cholesterol content analysis as described also in Burgos et al. (2010); but, in the case of adipose tissue, the colorimetric assay was carried out on the whole unsaponifiable fraction.

2.5. Statistical analysis

Analysis of variance (ANOVA) was carried out with R Core Team (2013), to assess the effect of dietary simvastatin and chromium picolinate on cholesterol serum levels, cholesterol and fat content and fatty acid composition in muscular and adipose tissue. The means of each treatment were compared for statistical significance using a Tukey's test. Significant differences were set at $P \leq 0.05$ and tendencies at $0.05 < P < 0.10$.

3. Results

3.1. Effects of statin or CrPi on cholesterol serum levels

The average weights at the beginning (81.3 ± 9.6, 85.6 ± 9.5, 82.5 ± 10.9 kg) and at the end of the experiment (100.9 ± 14.1, 114.3 ± 10.2 and 104.4 ± 11.7 kg) for the control, the statin and the CrPi group, respectively, showed no statistically significant differences; as weight and/or age strongly influence cholesterol serum levels (Kromhout, 1983), the absence of any significant weight difference between the experimental groups allows for the avoidance of weight influence in the discussion of the results.

All animals included in this work were normocholesterolemic at the beginning of the experiment (total cholesterol = 96.29 ± 1.93 mg/dl, LDL-cholesterol = 57.87 ± 1.57 mg/dl, HDL-cholesterol = 46.67 ± 1.23 mg/dl). Cholesterol levels after treatments administration are shown in Table 1.

There were no significant effects on any cholesterol serum parameter, although the 10.0% and 14.2% reductions in total and LDL-cholesterol levels observed in the statin-treated pigs are tendencies ($P = 0.064$ and $P = 0.096$, respectively).

Table 1
Effects of dietary simvastatin (S) and chromium dipicolinate (CrPi) on serum total cholesterol (total-C), LDL-cholesterol (LDL-C) and HDL- cholesterol (HDL-C).

	Control[a]	S[a]	CrPi[a]	SEM[b]	*P*-value	
					Control *vs* S	Control *vs* CrPi
Total-C (mg/dl)	97.2	87.5	92.3	1.73	0.064	0.497
LDL-C (mg/dl)	59.3	50.9	54.1	1.61	0.096	0.409
HDL-C (mg/dl)	43.4	46.0	42.2	0.83	0.421	0.827

[a] Data are presented as mean. Means with no common superscripts within the same rows differ significantly ($P \leq 0.05$).
[b] SEM, standard error of the mean.