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Total Cholesterol and its Fractions in the Blood of Finishing Pigs fed Diets with Different Levels of Canola Oil

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ABSTRACT

Background: Pork is an important source of protein and the most widely consumed meat throughout the world. Besides, search for healthy food over the years has been growing, which has stimulated research to improve animal health and consequently, the quality of the final product offered to consumers. People know that who eats a high-fat diet has greater risk of developing metabolism changes, such as high cholesterol levels and consequently, cardiovascular diseases such as atherosclerosis. Studies focusing handling of these lipid food components, both quantitative and qualitatively, can significantly contribute to increase acceptability of animal products by consumers because it can generate knowledge to produce meat with less cholesterol. Among the different cooking oils that can be used in animal feed, the canola oil has drawn the attention of researchers due to its high unsaturated fatty acid contents. The hypothesis is that canola oil modifies the plasmatic profile of the lipoproteins because α-linolenic acid (n-3) interfere the good functioning of LDL cholesterol (LDLc) receptors, i.e. interferes in the cholesterol turnover. Therefore, the aim of this study was to assess the effects of different levels of canola oil as sources of poly-unsaturated fats on modulation of total cholesterol and its fractions in the blood of pigs weighing 70 to 100 kg.

Materials, Methods and Results: The experiment was conducted in the Swine Experimental Center of the Federal University of Lavras (UFLA), Lavras, MG, Brazil. Thirty-two commercial hybrid barrows, with an initial weight of 73.65 (1.56) kg and final weight of 107.5 (2.37) kg, were used. The animals were distributed into four treatments and four replications, with two animals per experimental unit, in a complete casualized design. Diets were based on corn and soybean for finishing pigs and contained four levels of canola oil (2.0; 2.5; 3.0 and 3.5%). The consumption was measured at the end of the experimental period, which lasted 34 days. Five blood collections were performed from jugular vein with a 7 days interval from the beginning of the experiment until the slaughtering day. The samples were taken with EDTA 4% and centrifuged for separation of the plasma. These blood samples were used to assess the total cholesterol (CT), VLDL cholesterol (VLDLc), HDL cholesterol (HDLc), LDLc and LDLc/HDLc relationship. Total cholesterol in the blood was determined by the enzymatic method of cholesterol oxidase and the triacylglycerols (TG) were determined by a colorimetric enzymatic test. Lipoproteins were fractioned in the plasma by gel filtration chromatography with FPLC (Fast Protein Liquid Chromatography). Cholesterol was determined in the fractions by a colorimetric method using a 96-wells microplate assay. Data were analyzed using analysis of variance in a completely randomized blocks design. Regression analysis was performed to estimate the best level of canola oil. Total cholesterol and triglyceride levels did not differ among all treatments (P > 0.05).

Discussion: The fact that treatments have not had significant effect in the plasma lipoprotein profile might be associated to metabolic state of the animal prior to blood collection, since the animals were fasting, and this is probably why no specific VLDL peak was observed. The profiles were similar in the different treatments and in the two time periods studied, both from a qualitative and quantitative view. Similar Profiles were obtained for adult miniature pigs fed on a normal diet composed of corn and soybean meal. No results were found in the literature for animals fed on different fat sources and fat levels for comparison purposes.

Keywords: swine, lipoproteins, animal nutrition, metabolism, triglycerides.
INTRODUCTION

Pork is an important source of protein and the most consumed meat throughout the world, accounting for a little bit more than 42% of the total amount of meat produced in the World [18].

As well as growth of the pork market, consumer interest in healthy food has been increased over the years, which has stimulated researches to improve animal health and consequently, the quality of the product marketed. In this sense, it is known that people who eat a high-fat diet have greater risk of developing chronic diseases [16]. When there are problems with metabolism, cholesterol levels can be raised in the blood and consequently, cause cardiovascular diseases such as atherosclerosis [6]. Studies focusing handling of these lipid food components, both quantitative and qualitatively, can significantly contribute to increase acceptability of animal products by consumers [12]. Besides, rising the ratio of polyunsaturated fatty acids (PUFA): saturated fatty acids in humans can represents an important factor for disease prevention [9].

Among the different cooking oils that can be used in animal feed, canola oil has drawn the attention of researchers due to its high oleic (58%), linoleic (22%) and linolenic (10%) acid contents [14]. There were not found in the literature researches with this source of lipids, mainly involving lipoprotein separation techniques. The hypothesis is that canola oil modifies the plasmatic profile of the lipoproteins because α-linolenic acid (n-3) interfere the good functioning of LDL receptors, interfering in the cholesterol turnover.

MATERIALS AND METHODS

Local, animals and diets

The experiment was conducted in the Swine Experimental Center of the Federal University of Lavras (UFLA), Lavras, MG, Brazil. Thirty-two commercial hybrid barrows, with an initial weight of 73.65 (1.56) kg and final weight of 107.5 (2.37) kg, were used. The animals were distributed into four treatments and four replications, with two animals per experimental unit. The experimental period lasted 34 days.

The diets were based on corn and soybean for finishing pigs with a high genetic potential [11], and contained four levels of canola oil (2.0; 2.5; 3.0 and 3.5%) in a manner to not exceed diary requirements of energy (Table 1). The rations were isoenergetic, isoproteic and isolysinic and were given ad libitum. The consumption was measured at the end of the experimental period.

Blood samples were taken from the jugular vein of all animals. Five blood collections were performed with a 7 days interval from the beginning of the experiment until the slaughtering day. The samples were taken with EDTA 4% and then centrifuged for plasma separation.

Cholesterol and its fractions analysis

These blood samples were used to assess the total cholesterol (CT), VLDL cholesterol (VLDLc), HDL cholesterol (HDLc), LDL cholesterol (LDLc) and LDLc/HDLc relationship. The total cholesterol in the blood was determined by the enzymatic method of cholesterol oxidase [1] and the triacylglycerols (TG) were determined by a colorimetric enzymatic test [5]. Cholesterol in the HDLc, LDLc and VLDLc fractions was calculated by subtracting these values from the total cholesterol, after selective precipitation of the lipoproteins that contain Apo-B by the phosphotungstic acid. The LDLc was obtained by subtracting the VLDLc (VLDLc = TG x 0.25) from the total and HDLc [2], and according to the Friedwald modified equation [2]:

$$\text{LDLc} = \text{[total cholesterol]} - \text{[HDLc]} - \text{[TG x 0.25]}.$$  

The lipoproteins were fractioned in the plasma by gel filtration chromatography with FPLC (Fast Protein Liquid Chromatography) in a Waters 600 device, using a Superose 6 10/30 column [4]. An aliquot of 100 µL of serum was filtrated in a 0.45 µm membrane, injected in the column and separated with a buffer solution containing NaCl 0.15M, Na2HPO4 0.01M and EDTA 0.1 mM and pH 7.5, at a flow of 0.25 mL/min. Forty fractions of 0.5 mL were collected and the cholesterol was determined in each fraction. The cholesterol was determined in the fractions by a colorimetric method using a 96-wells microplate assay [4]. An aliquot of 100 µL of each fraction, separated by FPLC, was removed and mixed in a 1:1 ratio, with the color reagent for cholesterol. After an incubation period of 20 min at 37°C, the absorbance at 490 nm was read in a microplate reader.
Table 1. Experimental diets containing different levels of canola oil for finishing pigs.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Levels of canola oil (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.0</td>
</tr>
<tr>
<td>Corn (8.5%PB)</td>
<td>72.3</td>
</tr>
<tr>
<td>Soybean meal (45%PB)</td>
<td>22.6</td>
</tr>
<tr>
<td>Canola oil</td>
<td>2.0</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>1.2</td>
</tr>
<tr>
<td>Clay</td>
<td>0.7</td>
</tr>
<tr>
<td>Calcitic limestone</td>
<td>0.65</td>
</tr>
<tr>
<td>Commercial iodinated salt</td>
<td>0.3</td>
</tr>
<tr>
<td>Mineral premix A</td>
<td>0.1</td>
</tr>
<tr>
<td>Vitamin premix B</td>
<td>0.1</td>
</tr>
<tr>
<td>DL-Methionine (98%)</td>
<td>0.02</td>
</tr>
<tr>
<td>BHT</td>
<td>0.02</td>
</tr>
<tr>
<td>Lysine HCl (78%)</td>
<td>0.015</td>
</tr>
<tr>
<td>Total</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Calculated values:
- Raw protein (%) 16.52 16.51 16.51 16.51
- Digestible energy (kcal/kg) 3451 3450 3450 3451
- Calcium (%) 0.650 0.651 0.652 0.650
- Available phosphorus (%) 0.321 0.320 0.320 0.320
- Total lysine (%) 0.841 0.841 0.845 0.849
- Total threonine (%) 0.641 0.640 0.641 0.640
- Total Tryptophan (%) 0.195 0.196 0.197 0.197
- Total Met+Cis (%) 0.590 0.590 0.591 0.590

Table 2 shows growth performance of the animals. There was no difference among the treatments in any variable studied (P > 0.05).

Table 3 shows the profiles of the lipoproteins, separated by gel filtration chromatography. Generally, the first peak corresponds to the low-density lipoproteins, which, due to their greater size, are in the foreground in the column compared to the high-density lipoproteins. Then, the first peak was presumed to correspond to the LDL and the second peak to the HDL.
Table 2. Growth performance of finishing pigs fed diets containing 2.0, 2.5, 3.0, 3.5 % of canola oil.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Oil level (%)</th>
<th>CV %^A</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADG^B</td>
<td>1.03 1.08 0.97 1.05</td>
<td>7.54</td>
</tr>
<tr>
<td>FI^C</td>
<td>3.32 3.34 3.3 3.38</td>
<td>8.49</td>
</tr>
<tr>
<td>F:G^D</td>
<td>3.23 3.09 3.39 3.22</td>
<td>7.34</td>
</tr>
</tbody>
</table>

^CV - coefficient of variation. ^ADG - Average daily gain (kg). ^FI - Feed intake (kg). ^F:G - Feed:gain ratio (kg).

Table 3. Total cholesterol levels (mg/dL) in the blood of pigs weighing 70 - 100 kg, fed on different levels of canola oil.

<table>
<thead>
<tr>
<th>Cholesterol</th>
<th>Oil level (%)</th>
<th>CV (%)^A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>103.92 117.25 111.57 114.30</td>
<td>15.79</td>
</tr>
<tr>
<td>HDL</td>
<td>39.29 44.11 38.44 37.56</td>
<td>20.98</td>
</tr>
<tr>
<td>LDL</td>
<td>35.31 39.42 51.94 51.96</td>
<td>38.74</td>
</tr>
<tr>
<td>VLDL</td>
<td>29.37 33.72 21.18 24.78</td>
<td>34.90</td>
</tr>
</tbody>
</table>

^Coefficient of variation.

Figure 1. Lipoprotein profile of pigs fed until reaching 70 to 100 kg on a diet containing 2.0 (A), 2.5 (B), 3.0 (C) and 3.5% (D) of canola oil. The fractions from 16 to 23 correspond to the LDL and those from 24 to 31 correspond to the HDL.
DISCUSSION

The results observed for growth performance are according with those reported by Huang et al. [8] in 2008, that tested canola oil and some mixtures in chinook salmon Oncorhynchus tshawytscha and did not find any differences. So, it is safe to use canola oil in the diets of finishing pigs, but it not increases the growth performance.

The fact that treatments did not have significant effect in the plasma lipoprotein profile might be associated to metabolic state of the animal prior to blood collection, since the animals were fasting. The time period between feeding and blood collection strongly interferes with the total cholesterol levels and its fractions, which possibly affected the results of the present study. The levels of triglycerides in the blood are significantly reduced after 5 h of fast, but do not vary significantly approximately one hour after food intake [10]. On the contrary, it is not recommended the use of postprandial triglycerides for the calculation of cholesterol concentrations in lipoproteins due to the large variations in triglyceride concentrations [3].

The data were obtained from animals after a 12-h fasting, and this is probably why no specific VLDL peak was observed. The profiles were similar in the different treatments and in the two time periods studied, both from a qualitative and quantitative view.

Similar Profiles were obtained for adult miniature pigs fed on a normal diet composed of corn and soybean meal [7]. No results were found in the literature for animals fed on different fat sources and fat levels for comparison purposes. Milk fat, fish oil, olive oil and coconut fat, at a 4% level were tested previously [3]. The authors found several differences concerning the total cholesterol and lipoproteins, including results that corroborate the existence of important differences in the metabolism of lipoproteins between pigs and humans, i.e. the significant increase in the HDLc levels in animals fed on coconut fat, olive oil or milk fat (cream) compared to those fed on a control diet or a diet based on fish oil. A similar fact was observed by other authors, who obtained a quadratic effect for the total cholesterol and LDLc in pigs fed with different levels of coconut fat [13].

Another authors found a linear increase in the LDLc levels in pigs treated with growing levels (2%, 4%, 6%, 8%) of soybean oil after weaning [13], different of the present results. The referred author has not found any differences concerning the HDLc levels, however found higher levels of triacylglycerols in the blood of animals fed with coconut fat compared to those fed with soybean oil, which was in accordance to others [3].

CONCLUSION

Canola oil at 2.0%, 2.5%, 3.0% and 3.5% levels in diets for finishing pigs did not affect the blood cholesterol profile of these animals. Other studies must be done with higher concentrations of canola oil to verify the effects on plasmatic lipids.

REFERENCES