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The effect of naringin on plasma lipid profile, and liver and intramuscular fat contents of fattening lambs

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Abstract. Forty Assaf fattening lambs (initial age 14 weeks) fed barley straw (200 g/day) and concentrate feedstuff (30 g/kg BW per day) including 1.5 g naringin/kg DM (NAR group, n=10 males and 10 female lambs) or not (CONTROL group, n=10 males and 10 female lambs) were used to study the effect of this flavonoid on plasma triacylglycerol (TAG) levels, and liver and intramuscular fat content. All the animals were blood sampled on day 0 and thereafter with a weekly frequency. The experimental period lasted 7 weeks and 20 animals (ten per group) were slaughtered. During weeks 2 and 3 a significant (P<0.05) fall in plasmatic TAG levels of the male lambs corresponding to the NAR group could be observed when compared to the CONTROL ones. Thereafter until the end of the experimental period no more significant differences on plasmatic TAG levels were observed. The naringin effect could be related to an inhibitory effect of flavonoids on TAG synthesis. The lacking of effect of this flavonoid at the end of the experiment might be related to changes in ruminal bacterial community. With regard to liver and intramuscular fat content, these parameters were not statistically different (P>0.05) between NAR and CONTROL lambs. In summary, we report a transitory TAG-reducing effect of naringin added to the feedstuff for fattening lambs.

Keywords. Triacylglycerol – Flavonoid – Ruminant – Fat metabolism.

I – Introduction

The increasing demand of society for good animal welfare standards and high quality products for human consumption has opened the door to more innovative challenges in ruminant nutrition research, so the number of papers dealing with the beneficial effects of some minor components of the diet has been recently increased. In this context, special attention is being paid to the physio-
logical effects promoted by the flavonoids, a kind of polyphenols widely concentrated in citrus fruits. In human nutrition the flavonoids are suggested to be responsible for the prevention of chronic and degenerative diseases due to their antioxidant properties (Tripoli et al., 2007), but ruminant production may also benefit from these compounds. Naringin, one type of grapefruit and citrus flavonoid with proved antioxidant properties (Jeon et al., 2004) might be a good choice since it has been authorised as feed additive in ruminant nutrition.

In this sense, the stress suffered by the animal when transported to the slaughterhouse may jeopardize the oxidative stability of the animal and affect meat quality (Kannan et al., 2000). Therefore, a supply of flavonoids might be recommended for shelf life extension and colour stabilization of meat products.

Moreover, the enzymatic activity of some proteins related to the lipid metabolism (e.g. acyl CoA:diacylglycerol acyltransferase 1, DGAT1, and acyl-CoA: cholesterol acyltransferase, ACAT) seems to be also reduced by flavonoids (Casaschi et al., 2002; Jeon et al., 2004), so the chemical composition of meat produced might be modified according to the different requirements of the target market. Despite the reports on human and monogastric animals (rats, rabbit), the effects of plant flavonoids on ruminants has not been investigated previously.

Therefore, the aim of the present study was to examine the effects of naringin supplementation (1.5 g naringin/kg concentrate) on plasma triacylglycerol (TAG) levels, liver and intramuscular fat content.

II – Materials and methods

Forty Assaf lambs (20 male and 20 female) were used in this experiment. After random stratification on the basis of body weight (average BW, 24.8 ± 1.64 kg), lambs were allocated to two groups (ten lambs per group) and animals were housed individually. All handling practices followed the recommendations of European Council Directive 86/609/EEC for protection of animals used for experimental and other scientific purposes.

After 7 days of adaptation to the basal diet (barley straw and basal concentrate feed) all the lambs were fed barley straw and the concentrate feed (barley 550, soybean meal 210, corn 190, molasses 30, mineral vitamin premix 20 g/kg DM) alone (CONTROL group) or enriched with 0.15% of naringin (1.5 g/kg DM (NAR group)) for 7 weeks. Concentrate and forage were supplied in separate feeding troughs at 9:00 a.m. every day, and fresh drinking water was always available. The chemical composition of the concentrate was as follows: DM 876 g/kg, CP 177, NDF 160, ADF 45 and ash 58 g/kg DM. The straw (200 g/day) and concentrate (30 g/kg BW per day) offered to each lamb and the orts refused were weighed daily, and samples were collected for subsequent analyses.

All the animals were blood sampled by jugular venipuncture using Vacutainer tubes with no anticoagulant (10 ml) before the administration of the experimental concentrate (day 0) and thereafter with a weekly frequency until the final day of the experimental period (day 49). Blood samples were allowed to clot for 30 minutes at room temperature and centrifuged at 2000 x g for 15 min at 4°C. Thereafter, serum was stored at -20°C until TAG analysis. TAG concentrations in serum samples were determined by an automated enzymatic colorimetric principle with test kits from Roche Diagnostics on Cobas Integra 400 (Roche Diagnostic System).

Ten animals per group were weighed, stunned, slaughtered by exsanguination from the jugular vein, eviscerated and skinned. The hot carcass of each lamb was weighed and a sample of liver was taken. Carcass was chilled at 4°C for 24 h, halved carefully and the longissimus thoracis muscle was removed from the left carcass side.

The longissimus thoracis samples were trimmed to eliminate connective tissue and intermuscular fat, then freeze-dried and homogenized in a food processor (Moulinex®) in the same way as
the liver samples. Ether extract (EE) content analysis was performed in accordance with the methods described by the Association of Official Analytical Chemists (AOAC, 2003).

Data corresponding to TAG levels were analyzed as a repeated measures design using the MIXED procedure of SAS package (SAS 1999) with individual lamb as the experimental unit. Least square means were generated and separated using the PDIFF option of SAS for main or interactive effects, significance being determined at P<0.05. Data of liver and meat fat content were subjected to analysis of variance using the GLM procedure of SAS package (SAS, 1999).

III – Results and discussion

The inclusion of 1.5 g of naringin per kg of concentrate did not cause any food aversion, since the animals consumed the whole quantity of feed offered, thus reaching the established feed intake.

Regarding the effects of naringin supplementation on TAG content in plasma, these results are reported in Table 1. There was a decrease in plasmatic TAG levels of the NAR male lambs when compared to the CONTROL ones on day 14 and 28 (P<0.05). The administration of naringin has been demonstrated to result in a significant reduction in the plasma TAG and cholesterol. Thus, Jeon et al. (2007) reported that the supplementation with naringin lowered the plasma TAG in rats fed a high cholesterol diet. Likewise, Casaschi et al. (2002) observed that naringin markedly inhibited the TAG de novo synthesis. Flavonoids, such as quercitin, have been reported to decrease DGAT activity (Casaschi et al., 2002). Therefore, the effect of naringin could be related at least in part to this inhibitory effect of flavonoids on DGAT1 activity, one of the main enzymes involved in TAG synthesis.

Table 1. Least square means of TAG content in plasma (mg/dl)

<table>
<thead>
<tr>
<th>Day</th>
<th>Males</th>
<th></th>
<th>Females</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Nar</td>
<td>Control</td>
<td>Nar</td>
</tr>
<tr>
<td>0</td>
<td>22.6</td>
<td>25.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.6</td>
<td>18.3</td>
</tr>
<tr>
<td>7</td>
<td>20.7</td>
<td>17.0&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>17.1</td>
<td>18.1</td>
</tr>
<tr>
<td>14</td>
<td>22.7&lt;sup&gt;*&lt;/sup&gt;</td>
<td>16.9&lt;sup&gt;bc&lt;/sup&gt;&lt;sup&gt;*&lt;/sup&gt;</td>
<td>19.6</td>
<td>17.6</td>
</tr>
<tr>
<td>21</td>
<td>22.6&lt;sup&gt;*&lt;/sup&gt;</td>
<td>15.6&lt;sup&gt;c&lt;/sup&gt;&lt;sup&gt;*&lt;/sup&gt;</td>
<td>17.3</td>
<td>19.8</td>
</tr>
<tr>
<td>28</td>
<td>21.0</td>
<td>18.5&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>18.0</td>
<td>17.4</td>
</tr>
<tr>
<td>35</td>
<td>20.7</td>
<td>20.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20.0</td>
<td>17.3</td>
</tr>
<tr>
<td>42</td>
<td>19.3</td>
<td>17.3&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>16.1</td>
<td>15.2</td>
</tr>
<tr>
<td>49</td>
<td>20.1</td>
<td>19.8&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>16.3</td>
<td>17.0</td>
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</table>

P-value

<table>
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<tr>
<th></th>
<th>Diet</th>
<th></th>
<th>Day</th>
<th></th>
<th>Diet*Day</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0.171</td>
<td></td>
<td>0.012</td>
<td></td>
<td>0.059</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.740</td>
<td></td>
<td>0.371</td>
<td></td>
<td>0.749</td>
<td></td>
</tr>
</tbody>
</table>

Within each row, means with asterisk are significantly different (P<0.05).
Within each column, means with different subscripts are significantly different (P<0.05).

The effect of naringin seemed to disappear at the end of the experimental period. It should be pointed out that other authors also observed a lack of effect on plasma TAG levels when supplementing rabbits diet with naringin (Jeon et al., 2004). In our study, this lacking effect might be related to the adaptation of ruminal bacteria. Thus, López-Campos et al. (2009) reported changes in ruminal bacterial community of lambs consuming naringin. On the other hand, no plausi-
ble reason was found to explain why the TAG levels in female lambs were not affected by naringin. However, it is worthy to point out that TAG levels in females were lower than those observed in male lambs from the beginning of the experiment.

It has been reported that the administration of naringin could result in a significant reduction in hepatic TAG (Jeon et al., 2004), since the reduction of DGAT1 activity suggests that reduced availability and transfer of TAG to liver and muscle could happen. However, in the current experiment such effect was not observed. The effects of naringin supplementation on liver and meat fat content are shown in Table 2. Although male lambs showed greater liver fat content, neither meat nor liver fat content were statistically different (P>0.05) between NAR and CONTROL lambs. These data are in accordance with the lack of effect on plasmatic TAG levels in females and the time-dependant effect observed in male lambs.

### Table 2. Mean values of fat content (%) in liver and meat

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Nar</td>
<td>Control</td>
<td>Nar</td>
<td>RSD</td>
<td>Diet</td>
<td>Sex</td>
</tr>
<tr>
<td>Liver</td>
<td>8.35</td>
<td>6.99</td>
<td>6.43</td>
<td>6.59</td>
<td>1.032</td>
<td>0.213</td>
<td>0.024</td>
</tr>
<tr>
<td>Meat</td>
<td>10.6</td>
<td>9.6</td>
<td>13.8</td>
<td>9.2</td>
<td>4.63</td>
<td>0.201</td>
<td>0.503</td>
</tr>
</tbody>
</table>

**Acknowledgments**

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