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in

Ranilla M.J. (ed.), Carro M.D. (ed.), Ben Salem H. (ed.), Morand-Fehr P. (ed.).
Challenging strategies to promote the sheep and goat sector in the current global context

Zaragoza : CIHEAM / CSIC / Universidad de León / FAO
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 99

2011
pages 223-226

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.

Effet de la naringine sur le profil lipidique du plasma et le contenu gras hépatique et intramusculaire des moutons d'engraissements

Résumé. *Quarante agneaux de race Assaf en engraissement (âge initial de 14 semaines) ont reçu de la paille d'orge (200 g/jour) et un concentré (30 g/kg poids vif) renfermant 1,5 g de naringine/kg (NAR) ou sans cet additif (TEMOIN) pour étudier de l'effet de ce flavonoïde sur les niveaux plasmatiques de triglycérides (TAG) et sur le contenu dans le gras intramusculaire et hépatique. Une prise de sang a été effectuée sur tous les animaux le jour 0 puis une fois par semaine. L'expérience a duré 7 semaines, après quoi, dix animaux par groupe ont été abattus. Pendant la 2ème et la 3ème semaine on a observé une diminution significative des TAG dans les niveaux plasmatiques des agneaux mâles correspondants au groupe NAR et comparés au groupe témoin ($P<0.05$). A partir de ce moment-là et jusqu'à la fin de l'expérience nous n'avons plus observé de différences dans les niveaux des TAG. L'effet de la naringine semble être en rapport avec un effet inhibiteur des flavonoïdes sur la synthèse des TAG. L'absence d'effet de ce flavonoïde à la fin de cette expérience pourrait être la cause des changements dans la population bactérienne du rumen. Le contenu de gras du muscle et du foie n'ont pas été différents ($P>0.05$) entre les agneaux NAR et témoin. En résumé, nous obtenons un effet réducteur transitoire des TAG quand on ajoute la naringine au concentré pour les agneaux d'engraissement.*

Mots-clés. *Triglycérides – Flavonoïde – Ruminant – Métabolisme des gras.*

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 physiological 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 stabilisation 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 4ºC. Thereafter, serum was stored at -20ºC 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)

	Males		Females	
Day	**Control**	**Nar**	**Control**	**Nar**
0	22.6	25.8^{a}	19.6	18.3
7	20.7	17.0^{bc}	17.1	18.1
14	22.7*	16.9^{bc}*	19.6	17.6
21	22.6*	15.6^{c}*	17.3	19.8
28	21.0	18.5^{bc}	18.0	17.4
35	20.7	20.3^{b}	20.0	17.3
42	19.3	17.3^{bc}	16.1	15.2
49	20.1	19.8^{bc}	16.3	17.0
P-value				
Diet	0.171		0.740	
Day	0.012		0.371	
Diet*Day	0.059		0.749	

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 plausible 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

	Males		**Females**		**P-value**			
	Control	**Nar**	**Control**	**Nar**	**RSD**	**Diet**	**Sex**	**Diet x Sex**
Liver	8.35	6.99	6.43	6.59	1.032	0.213	0.024	0.119
Meat	10.6	9.6	13.8	9.2	4.63	0.201	0.503	0.397

Acknowledgments

This investigation was funded by the CSIC "Intramural" Project 200840I116 and carried out by the "Grupo de Investigación de Excelencia de Castilla y León" GR158 "Nutrición y Alimentación de Rumiantes", supported by the Department of Education of the Junta de Castilla y León, Spain.

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