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Naringin and vitamin E influence the oxidative stability and lipid profile of plasma in lambs fed fish oil

R. Bodas *, N. Prieto, O. López-Campos, F.J. Giráldez, S. Andrés

Instituto de Ganadería de Montaña (CSIC-ULE), Finca Marzanas, s/n, E-24346 Grulleros, León, Spain

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ABSTRACT

Thirty two Merino lambs (15 weeks old) fed barley straw and fish oil enriched concentrate were used to assess the effect of vitamin E (6 g kg^{-1} DM) and naringin (1.5–3 g kg^{-1} DM) on plasma lipid peroxidation (TBARS), total antioxidant status (TAS), immune response, plasma cholesterol, and triglycerides. After 21 days feeding the experimental diets, lambs were subjected to a 4 h transportation stress period and then held 4 more hours without feed. TBARS values before stress were lower for animals consuming vitamine E and naringin when compared to control lambs ($P < 0.05$). However, after stress all groups presented similar levels of TBARS. TAS decreased ($P < 0.05$) in all groups in response to stress with values recovering ($P < 0.05$) to pre-stress values following 4 h of rest. A rise ($P < 0.05$) in serum concentrations of triacylglycerol following 21 d of fish oil supplementation was dampened in lambs consuming vitamin E or naringin. Both pre-stress TBARS and triacylglycerol-reducing effects of naringin added to fish oil enriched concentrate for fattening lambs are reported.

1. Introduction

Flavonoids are a group of naturally occurring compounds ubiquitous in the plant kingdom and known to have strong antioxidant effects (Kim et al., 2004). Thus, in order to improve meat and milk quality, supplementing ruminants with these compounds may be useful particularly when they are fed unsaturated fatty acids. For example, $n-3$ fatty acids are highly susceptible to peroxidation both in plasma and tissues. Transporting animals to the slaughterhouse can increase this susceptibility and, therefore, the values of TBARS and pigment oxidation in meat (Kannan et al., 2000; Renerre, 2000; Young et al., 2003). Furthermore, lipid peroxidation has been implicated in deterioration of physiological functions that include growth and reproduction, as well as immunity, leading to a higher susceptibility to infectious diseases (Gladine et al., 2007). Consequently, a supply of antioxidants is recommended to preserve both, the health of animals supplemented with oil (McDowell et al., 1996) and the oxidative stability of their products (Wood and Enser, 1997; Kannan et al., 2000). In this sense, different studies have demonstrated an insufficient ability of vitamin E to inhibit lipoperoxidation when $n-3$ fatty acids intake is increased (Miret et al., 2003). Nevertheless, the antioxidant effect of plant extracts rich in polyphenols (flavonoids) needs further investigation under the same conditions (Kim et al., 2004, 2006). This information has timely implications, since the European Commission may lift the ban on feeding fish meal and oils to ruminants (Stevenson, 2005).

Additionally, the incremental increase in plasma concentrations of cholesterol and triacylglycerols (TAG) when animals are fed fish oil might be counteracted by naringin, a type of grapefruit and citrus flavonoid. In fact, naringin is a potent plasma triacylglycerol and cholesterol-lowering agent in monogastrics (Casaschi et al., 2002; Jeon et al., 2004). It is unknown whether ruminants fed with fish oil can benefit from the same effect of naringin. Moreover, some authors suggest that polyphenol compounds may have antimicrobial activity and some other beneficial effects on certain immune parameters, such as inhibition of inflammation or modulation of the activation of B and T lymphocytes (Tripoli et al., 2007; Hamer, 2007).

The present study was conducted to assess the effect of naringin on the lipid peroxidation (TBARS), the total antioxidant status (TAS) and the immunological response of fattening lambs fed fish oil and subjected to transportation stress. In addition, biochemical parameters such as serum cholesterol (total, HDL, and LDL) and triglycerides (TAG) have been considered.

2. Materials and methods

2.1. Animals and diets

Thirty two Merino lambs (initial age 14–16 weeks) were used in this experiment. Lambs were kept with their mothers until weaning, allowing free access to a commercial starter concentrate,

* Corresponding author. Tel.: +34 987 307 054; fax: +34 987 317 161.
E-mail address: raul.bodas@eae.csic.es (R. Bodas).

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barley straw, and alfalfa hay, until the commencement of the trial. Animals were dewormed with Ivomec (Merial Labs., Spain) and vaccinated against enterotoxaemia (Miloxan, Merial Labs., Spain).

After random stratification on the basis of body weight (mean 28.4 kg), the lambs were allocated to one of four treatments (8 per treatment) prior to housing 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 5 days of adaptation to the basal diet (barley straw and basal concentrate feed), all the lambs were fed barley straw and the concentrate feed enriched with fish oil (30 g kg^{-1}) alone (control group) or supplemented with either vitamin E (6 g kg^{-1}, VitE group) or different doses of naringin (1.5 g kg^{-1}, Nar15 group; and 3 g kg^{-1}, Nar30 group) for 21 days. Concentrate and forage were supplied in separate feeding troughs at 9:00 a.m. every day, and fresh drinking water was always available. The ingredients and chemical composition of the feeds are shown in Table 1. The straw (200 g day^{-1}) and concentrate offered (30 g kg^{-1} BW day^{-1}) were weighed daily. The orts were also weighed daily with subsamples collected for subsequent analyses. Since guidelines for naringin supplementation of ruminants do not exist, two doses were selected based on previous research (Shin et al., 1999; Jeon et al., 2001; Gladine et al., 2007), where chronic doses of 1.0 and 0.5 g kg^{-1} and an acute single dose of 100 g kg^{-1} were used, respectively. In the present experiment interaction between ruminal bacterial community and naringin was expected (Gladine et al., 2007), so two chronic doses higher than those used in monogastric animals were selected (1.5 and 3 g kg^{-1}).

Three weeks later the animals were subjected to a 4 h transportation period and then held in pens four more hours with fresh drinking water to simulate preslaughter conditions.

2.2. Blood sample collection

All the animals were blood sampled by jugular venipuncture before supplying the experimental concentrate (day 0) and 3 weeks later before the transport period (day 21, 0 h), immediately after a 4 h transportation period (day 21, 4 h), and again 4 h later (day 21, 8 h). Blood samples were collected into two vacutainer tubes (10 ml) with and without sodium heparin.

Blood samples collected in the sodium heparin tubes (day 21) were immediately placed in iced water and centrifuged at 1000*g* for 10 min at 4 °C. Then plasma was separated and stored at −80 °C until required for antioxidant analyses. Those samples in tubes with no anticoagulant (day 0 and day 21) were allowed to clot for 30 min at room temperature and centrifuged at 2000*g* for 15 min at 4 °C. Thereafter, serum was stored at −20 °C until used to measure biochemical parameters.

2.3. Immune response

Fifteen days after having started this experiment, phytohemagglutinin (PHA, 1 mg, Sigma–Aldrich, Spain) dissolved in 1 ml of sterile saline solution was injected intradermally into a 2 cm wide circle marked on armpit. Skin-fold thickness was determined just before PHA injection and 24 h later with a calliper. The increase in skin-fold thickness for each animal was computed using these two measurements.

2.4. Antioxidant parameters

Lipid peroxidation was analysed in the plasma samples using the TBARS Assay Kit (Thiobarbituric Acid Reactive Substances) provided by Cayman Chemical (MI, USA), whereas total antioxidant status (TAS) was measured according to the Trolox-Equivalent Antioxidant Capacity (TEAC) assay (Cayman Chemical, MI, USA). Both analyses were performed in plasma samples according to manufacturer's instructions.

2.5. Biochemical parameters

Total cholesterol, LDL, HDL, and triacylglyceride (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).

Serum cortisol levels were determined by a solid-phase, competitive chemiluminescent enzyme immunoassay using a commercially available assay kit (Immulite® 1000 Cortisol, Siemens Healthcare, Munich, Germany; inter and intra-assay CV of 6.7% and 7.3%, respectively).

2.6. Statistical analyses

Data of feed intake and immune response were subjected to analysis of variance using the GLM procedure of SAS package (SAS, 1999). Data corresponds to antioxidant and biochemical parameters were analysed as a complete randomised, repeated measures design using the MIXED procedure of SAS (Littell et al., 1998) 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 $P < 0.05$. The tables show the residual standard deviation (RSD)

Table 1
Ingredients and chemical composition of the experimental feeds.

	Control	VitE	Nar15	Nar30	Barley straw
Ingredients (g kg^{-1} as feed)					
Barley	534	531	532.5	532	
Soy bean meal	214	213	214	213	
Corn	165	164	165	165	
Molasses	29	29	29	29	
Mineral vitamin premix	28	28	28	28	
Fish oil	30	30	30	30	
Vitamin E	0	6	0	0	
Naringin	0	0	1.5	3	
Chemical composition (g kg^{-1} DM)					
Dry matter (DM, g kg^{-1} fresh)	877	877	875	877	908
Crude protein	179	177	178	178	29
Neutral detergent fibre	168	161	163	163	846
Ash	65	61	62	64	46