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**Bodas R., Richardson R.I.**

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# Sunflower oil plus cholesterol and fish oil for fattening lambs: effects on plasmatic parameters

**R. Bodas* and R.I. Richardson****

*Instituto de Ganadería de Montaña (CSIC-ULE), 24346, Grulleros, León (Spain)
**Division of Farm Animal Science, University of Bristol, Langford, Bristol, BS40 5DU (UK)

**Abstract.** The objective of this work was to study the effects of oils rich in n–3 (fish oil) and n–6 (sunflower oil) polyunsaturated fatty acids on plasma lipid peroxidation (TBARS), plasma cholesterol and triglycerides (TAG), and white blood cells (WBC) count of fattening lambs. Fifteen Merino-cross lambs (initial weight 25 kg) were fed barley straw and concentrate (25 g/kg BW per day) either alone (Control group) or supplemented with either sunflower oil plus cholesterol (SFO+C group, sunflower oil 28 g and cholesterol 2 g/kg concentrate) or fish oil (FO group, 30 g/kg concentrate) for 21 days. From day 22 until the end of the trial (day 45), all the concentrates were supplemented with vitamin E (6 g/kg concentrate). Throughout the experimental period all the groups showed a decrease in plasma TBARS ($P<0.001$). There was a significant increase in LDL and total cholesterol with the SFO+C and FO diets ($P<0.05$), whereas HDL did not change ($P>0.10$). TAG concentration was lower in fish oil fed lambs ($P<0.05$). WBC count increased with time for Control and FO groups ($P<0.001$), but FO lambs showed greater WBC and lymphocytes counts than SFO+C and Control animals on day 21 and 45 ($P<0.001$).

**Keywords.** Triglyceride – Leukocytes – Vitamin E – TBARS.

***L'huile de tournesol avec le cholestérol et l'huile de poisson pour engraissement des agneaux: effets sur les métabolites sanguins***

***Résumé.*** *L'objectif de cette expérience a été d'étudier les effets des huiles riches en acides gras polyinsaturés n-3 (huile de poisson) et n-6 (huile de tournesol), sur la péroxydation des lipides du plasma (TBARS), les niveaux plasmatiques de cholestérol et les triglycérides (TAG) et le dénombrement des globules blancs (WBC) chez les agneaux d'engraissement. Quinze agneaux de race croisée Mérino (poids initial de 25 kg) ont été nourris à base de fourrage et de concentré (25 g/kg poids vif) seulement (Control), ou avec un apport d'huile de tournesol et de cholestérol (SFO+C, huile de tournesol 28 g and cholestérol 2 g/kg concentré) ou d'huile de poisson (FO, 30 g/kg concentré) pendant 21 jours. A partir du 22ème jour et jusqu'à la fin de l'expérience (45 jours), tous les concentrés ont été enrichis de vitamine E (6 g/kg). Les niveaux plasmatiques de TBARS ont accusé une chute importante ($P<0,001$) chez tous les agneaux. Les niveaux de LDL et cholestérol total dans le plasma des agneaux soumis aux traietements SFO+C et FO ont augmenté ($P<0,05$), tandis que le cholestérol HDL n'a pas changé ($P>0,10$). La concentration des TAG faible chez les agneaux FO ($P<0,05$). WBC a augmenté avec le temps chez les groupes Control et FO, mais chez les agneaux FO des nombres plus élevés ont été constatés les 21ème et 45ème jours ($P<0,001$), ce qui a été dû à l'augmentation du nombre des lymphocytes ($P<0,001$).*

***Mots-clés.*** *Triglycérides – Leucocytes – Vitamine E – TBARS.*

## I – Introduction

The addition of polyunsaturated fatty acids to the diet of lambs increases the PUFA content in lamb meat (Demirel *et al.*, 2004) and supplementing the diet with sunflower oil has also been shown to change the concentration of cholesterol in some tissues (Caputi Jambrenghi *et al.*, 2007).

The oxidative stability of lipids is associated with the number of double bonds they contain and the antioxidant concentration in the diet. Thus polyunsaturated fatty acids (PUFA) are more prone

to be oxidized than monounsaturated fatty acids (MUFA). Moreover, the process of lipid oxidation leads to vitamin losses (such as vitamin A, carotenoids, vitamin C, and vitamin E), due to the free radicals generated by the process that co-oxidizes these vitamins (Álvarez *et al.*, 2009). Deterioration in meat quality caused by lipid oxidation may be reduced by incorporating natural antioxidants into the animals diet. Antioxidants delay or inhibit the process of oxidation, even at low concentrations. Vitamin E, a natural antioxidant, functions as a lipid-soluble antioxidant in cell membranes and has been shown to protect PUFA from oxidation in body tissues, thus improving lipid and colour stability (Demirel *et al.*, 2004). Usually, the oxidation changes are described through the evaluation of the thiobarbituric acid reactive substances (TBARS).

Feeding diets that are intended to alter the fatty acid content of meat could affect other aspects of animal production. Nutritional status has a profound effect on immune function, and supplements provided for one physiological purpose, such as muscle lipid composition, may also have physiological effects that influence immune activity. The n-3 PUFA are considered as key components of immunonutrient formulations because of their antiinflammatory actions. Thus, fish oil supplementation of apparently healthy beef steers was seen to enhance in vitro lymphocyte proliferation (Wistuba *et al.*, 2005).

To our knowledge, there is a shortage of information related to the effects of supplementation of fish or sunflower oils in ruminant diets on immune characteristics. Therefore, the research reported here was conducted to study the effects of oils rich in n–3 (fish oil) and n–6 (sunflower oil) polyunsaturated fatty acids on plasma lipid peroxidation (TBARS), plasma cholesterol and triglycerides (TAG), and white blood cell (WBC) count of fattening lambs.

## II – Materials and methods

Fifteen Assaf lambs (initial weight 25.2 ± 0.76 kg) were used in this experiment. Lambs were kept with their mothers until weaning, allowing free access to a commercial starter concentrate, 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). Lambs were allocated to three groups (five lambs per group) and 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.

Animals were fed barley straw (200 g/day) and the concentrate (25 g/kg BW per day; ingredients: barley 556, soybean meal 210, corn 190, molasses 30, mineral vitamin premix 14 g/kg; chemical composition: DM 877 g/kg, CP 178, NDF 167, ADF 60 and ash 63 g/kg DM.) alone (Control group) or supplemented with either sunflower oil plus cholesterol (SFO+C group, sunflower oil 28 g and cholesterol 2 g/kg) or fish oil (FO group, 30 g/kg) for 21 days. As cholesterol is present in fish oil and is involved in lipid metabolism, it was added to sunflower oil. Concentrate and forage were supplied in separate feeding troughs at 9:00 a.m. every day, and fresh drinking water was always available. From day 22 till the end of the trial (day 45), all the concentrates were supplemented with vitamin E (6 g/kg concentrate as alpha-tocopherol acetate).

All the animals were blood sampled by jugular venipuncture before the administration of the experimental concentrate (day 0), three weeks later (before including vitamin E in the concentrate, day 21) and at the end of the trial (day 45). Blood samples were collected into Vacutainer tubes (10 ml) containing sodium heparin (for TBARS analysis), EDTA (for haematology analysis) and no anticoagulant at all (for biochemical analysis).

Lipid peroxidation was analyzed using the TBARS Assay Kit (Cayman Chemical, Ann Arbor, MI, USA). Total cholesterol, LDL, HDL and triacylglyceride (TAG) concentrations were determined with test kits from Roche Diagnostics on Cobas Integra 400 (Roche Diagnostic System, Basel,

Switzerland). Haematology analyses were performed in an electronic cell counter (Cellanalyzer CA530, Bromma, Sweden).

Data were analyzed as a complete randomized, 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 (SAS, 1999).

## III – Results and discussion

Mean values for TBARS, total, HDL and LDL cholesterol, triacylglyceride and white blood cell counts are presented in Table 1. Supplementing the diet with oils and cholesterol provides the animals with an extra source of this kind of nutrients. When these fatty acids reach the duodenum they are mainly adsorbed on feed particles, bile salts and lysolecithins solubilising them into micelles, which are then absorbed. Then, fatty acids are esterified and incorporated into chylomicrons and VLDL (Doreau and Chilliard, 1997). As a consequence of the incorporation of fatty acids in the diet, an increase in the plasmatic values of total cholesterol ($P<0.05$), mainly due to the greater LDL cholesterol levels ($P<0.01$) was observed.

The type of dietary fat is an important determinant of plasma lipid concentration both in man and animals, and altering fatty acid composition of foods, in particular increasing the ratio of polyunsaturated fatty acids (PUFA) to saturated fatty acids represents a useful factor in disease prevention and health (Caputi Jambrenghi *et al.*, 2007). However, the higher the proportion of unsaturated fatty acids in meat, the more susceptible it is to oxidation (Álvarez *et al.*, 2009). However, unexpectedly, in the current experiment, all diets showed decreased plasma levels of TBARS ($P<0.001$) at days 21 and 45 of feeding, which could be mainly due to the concentrate rich diet fed to the animals and the stress associated to the fattening period.

The lipid oxidation process may be delayed or inhibited by the addition of antioxidants to the diet (Lee *et al.*, 2006). In the current experiment, vitamin E was added, as a natural antioxidant, to all the concentrates from day 22 until the end of the trial (day 45). The dose used (6 g/kg concentrate) can be considered as supra-nutritional (Demirel *et al.*, 2004), however, no differences were observed attributable to the addition of vitamin E. Nevertheless, in a study conducted with lambs, Lee *et al.* (2006) demonstrates the importance of the form of adding vitamin E. Thus, when vitamin E was emulsified (in an oil and protein complex) before feeding to ruminants, its absorption was enhanced, it was more effective in raising the circulating blood serum levels and in impairing lipid oxidation (Lee *et al.*, 2006). This fact could help to explain our results, because, despite the dose used, it was added to the diet without any previous treatment.

As for white blood cells count, although leukocytes count increased with time for Control and FO groups ($P<0.001$), lambs in the latter group showed greater counts than SFO+C and Control group animals ($P<0.001$), which was due to the increase in the counts of lymphocytes ($P<0.001$). The effects of fish oil treatment on immune responses have been characterized as variable, and the responses seem to vary with species, age, and stressor being evaluated (Lewis *et al.*, 2008). Thus, fish oil has been proved to suppress immune function in healthy animals and enhance immune functions in immunosuppressed animals (Lewis *et al.*, 2008). The health status of the lambs in the current study did not seem to be compromised. On the other hand, differential white blood cell counts are often difficult to interpret, unless some specific event, such as bacterial challenge, can be identified (Lewis *et al.*, 2008). Nevertheless, in our experiment, fish oil induced a unexpected increase in WBC and lymphocytes count, this finding agrees with the results showed by Wistuba *et al.* (2005), who reported that fish oil supplementation of apparently healthy beef steers enhanced *in vitro* lymphocyte proliferation. As stated by these authors, these effects can be due to either a direct effect of n-3 PUFA or an effect of fat on the absorption of other sub-

stances important to the immune system. Therefore, the supplementation with fish oil could enhance immune status, but the decrease in plasma TBARS as well as the increase in cholesterol could impair this effect.

**Table 1. Effects of sunflower oil plus cholesterol (SFO+C) and fish oil (FO) on plasma parameters of intensively reared lambs at the beginning of the trial (day 0), on day 21 and after receiving vitamin E in the concentrate (6 g/kg concentrate from day 22) on day 45**

| | Day | Group | | | SED | P-value | | |
|---|---|---|---|---|---|---|---|---|
| | | Control | SFO+C | FO | | Group | Day | Group*Day |
| TBARS | 0 | 8.60$^{b}$ | 8.62$^{b}$ | 7.87$^{b}$ | 1.028 | 0.147 | <0.001 | 0.302 |
| (μM MDA) | 21 | 5.00$^{a}$ | 2.90$^{a}$ | 2.80$^{a}$ | | | | |
| | 45 | 4.08$^{a}$ | 3.30$^{a}$ | 4.20$^{a}$ | | | | |
| Total cholesterol | 0 | 1.24 | 1.30 | 1.61 | 0.274 | 0.035 | 0.228 | 0.490 |
| (mmol/l) | 21 | 1.22 | 1.64 | 1.40 | | | | |
| | 45 | 1.25$^{a}$ | 1.77$^{b}$ | 1.90$^{b}$ | | | | |
| LDL colesterol | 0 | 0.426 | 0.588 | 0.732 | 0.1679 | 0.009 | 0.257 | 0.606 |
| (mmol/l) | 21 | 0.510 | 0.794 | 0.666 | | | | |
| | 45 | 0.516$^{a}$ | 0.752$^{ab}$ | 0.966$^{b}$ | | | | |
| HDL cholesterol | 0 | 0.811 | 0.710 | 0.877 | 0.1426 | 0.366 | 0.278 | 0.334 |
| (mmol/l) | 21 | 0.713 | 0.849 | 0.732 | | | | |
| | 45 | 0.730 | 1.020 | 0.932 | | | | |
| Triacylglyceride | 0 | 16.3 | 11.5 | 16.2 | 3.74 | 0.027 | 0.439 | 0.143 |
| (mg/dl) | 21 | 21.4$^{b}$ | 16.4$^{ab}$ | 12.4$^{a}$ | | | | |
| | 45 | 20.6$^{b}$ | 19.9$^{b}$ | 11.5$^{a}$ | | | | |
| Leukocytes | 0 | 14.6$^{a}$ | 15.0$^{ab}$ | 14.1$^{a}$ | 3.76 | <0.001 | <0.001 | <0.001 |
| ($10^3$/μl) | 21 | 24.0$^{b}$ | 19.4$^{b}$ | 33.7$^{c}$ | | | | |
| | 45 | 21.8$^{b}$ | 18.7$^{b}$ | 45.5$^{d}$ | | | | |
| Lymphocytes | 0 | 9.04$^{a}$ | 8.54$^{a}$ | 7.14$^{a}$ | 3.91 | <0.001 | <0.001 | <0.001 |
| ($10^3$/μl) | 21 | 18.46$^{b}$ | 13.56$^{b}$ | 26.54$^{c}$ | | | | |
| | 45 | 17.16$^{b}$ | 12.56$^{b}$ | 43.80$^{d}$ | | | | |
| Granulocites | 0 | 4.80 | 5.74 | 6.08 | 0.936 | 0.012 | 0.785 | 0.865 |
| ($10^3$/μl) | 21 | 4.80 | 5.08 | 6.32 | | | | |
| | 45 | 3.92$^{a}$ | 5.34$^{ab}$ | 6.24$^{b}$ | | | | |
| Mid series | 0 | 0.800 | 0.760 | 0.880 | 0.138 | 0.121 | 0.928 | 0.858 |
| ($10^3$/μl) | 21 | 0.740 | 0.760 | 0.860 | | | | |
| | 45 | 0.680 | 0.800 | 0.960 | | | | |

$^{a, b, c}$ Within a column or a row, means that do not have a common superscript letter differ (P<0.05).

## IV – Conclusion

The results indicate that feeding lambs with sunflower oil plus cholesterol or fish oil causes an increase in plasma cholesterol. However, fish oil reduces plasma triglycerides and increases lymphocytes counts.


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