Small Ruminant Research 106S (2012) S21–S28

Contents lists available at SciVerse ScienceDirect

Small Ruminant Research

journal homepage: www.elsevier.com/locate/smallrumres

Extruded linseed supplementation in the diet of dairy sheep: The effects on immune response and oxidative stress as affected by the physiological state☆

G. Acuti[a], M. Trabalza-Marinucci[a,*], M. Cagiola[b], M. Pela[b], G. Curina[b], L. Moscati[b]

[a] Dipartimento di Patologia, Diagnostica e Clinica Veterinaria, Università degli Studi di Perugia, via S. Costanzo, 4, 06126 Perugia, Italy
[b] Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, via G. Salvemini 1, 06126 Perugia, Italy

ARTICLE INFO

Keywords:
Transition ewe
Extruded linseed
Polyunsaturated fatty acids
Immune response
Oxidative state

ABSTRACT

Several investigations have studied the influence of polyunsaturated fatty acids on the immune function of ruminants but many contradictory observations have been reported in this field. The aim of this study was to evaluate the influence of an extruded linseed dietary supplementation on the immune response and oxidative stress in the transition dairy ewe. The experiment was performed using 58 Sarda pluriparous ewes, 42 pregnant (T) and 16 non-pregnant and non-lactating (NP). Both groups were fed an isoenergetic and isonitrogenous diet from 60 d before to 15 d after lambing of the T ewes. Within each physiological state, one sub-group received a commercial pelleted concentrate (CTR) and the other an extruded linseed-enriched (10 g/100 g) concentrate (EL). Blood samples were collected from all animals at −28, −21, −14, −7, +3 and +15 d to parturition, based on expected date of lambing of the T ewes, to evaluate the innate immunity (serum lysozyme, haemolytic complement and bactericidal activity), the acquired immunity and the oxidative stress. The dietary supplementation with extruded linseed did not markedly influence the innate immunity response of the animals. The antibody production against *Salmonella enterica* subsp. *enterica* serovar *Abortusovis* was shown to be increased by the linseed-enriched diet in the T ewes (2.37 vs. 2.19 $\log_{10}$ for the T and the NP ewes, respectively). As for the cell-mediated immunity, no differences between dietary treatments were observed. The extruded linseed-enriched diet was able to induce a transitory overproduction of oxidants in the NP ewes, whereas no effects were observed in the T ewes. In conclusion, under the adopted experimental conditions, the use of extruded linseed in the diet did not greatly affect the oxidative state and the immune response of the transition ewe.

1. Introduction

Several investigations have studied the influence of polyunsaturated fatty acids (PUFAs) on the immune function of ruminants, monogastric species and experimental animals (Agazzi et al., 2004; Calder, 1998; Calder et al., 2002; Caroprese et al., 2009; Harbige, 2003; Healy et al., 2000; Lessard et al., 2003; Parmentier et al., 1997, 2002; Savoini et al., 2010).

Many contradictory observations have been reported in this field. Studies in humans have indicated that an increased intake of omega-3 PUFA with the diet can inhibit the immune system and the inflammatory response (Tricon et al., 2004). This effect is probably due to a decreased level of circulating proinflammatory cytokines, such as IL1β, IL6, and TNFα, and a decreased proliferation of peripheral blood

☆ This paper is part of a supplement titled "SIPAOC Congress 2010" Guest Edited by Antonello Carta, Maria Teresa Manfredi, Giuseppe Moniello and Massimo Trabalza-Marinucci.

* Corresponding author at: Dipartimento di Patologia, Diagnostica e Clinica Veterinaria, via S. Costanzo, 4, 06126 Perugia, Italy.
Tel.: +39 0755857707; fax: +39 0755857764.
E-mail address: massimo.trabalza@unipg.it (M. Trabalza-Marinucci).

0921-4488/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.smallrumres.2012.04.029

mononuclear cells (macrophages, T lymphocytes, plasma cells), without inhibition of the T and B cells' immune response (Kelly, 2001). On the contrary, an improvement in cellular immune reactivity to phytohaemoagglutinin, an increased anti-OVA IgG concentration and a reduction of IL-10 secretion was observed in dairy cows (Caroprese et al., 2009). Lessard et al. (2003) have demonstrated that the immunomodulatory effects of omega-3 PUFA in cattle are directed towards the response of lymphocytes and monocytes/macrophages, whereas the antibody response to ovalbumin and the in vitro production of interferon γ do not seem to be affected by the PUFA dietary supplementation.

An appropriate PUFA concentration in the diet, together with a balanced omega-6/omega-3 ratio, is therefore important. This ratio is estimated to be approximately 15–20 in the current Western diet, and should decrease to below 5 or 4 (British Nutrition Foundation, 1992; Wood et al., 2004) to avoid the prothrombotic and proaggregatory state induced by a high level of n-6 PUFA.

Furthermore, a balanced omega-6/omega-3 ratio in the diet is essential for normal growth and to decrease cardiovascular disease and other chronic diseases (Simopoulos, 1999).

Dietary lipids, such as supplemental oil seeds rich in PUFA, can be significant contributors to the load of free radicals in animals (Andrews et al., 2006). Oxidative stress leads to peroxidative damage to lipids and other macromolecules, with a consequent alteration of cell membranes and other cellular components (Toyokuni, 1999). In addition, oxidative stress can be related to alterations of physiology and pathologies in man and in other animals (Miller et al., 1993).

Most published studies on the effects of dietary PUFA have focused on the bovine species, while the knowledge of small ruminants is limited and discordant (Caroprese et al., 2006, 2009; Thanasak et al., 2005). It is not clear whether the role of PUFA may be influenced by the physiological state of the animal and the transient states of depressed immune function. The peripartum is considered the most critical period for dairy ruminants in consideration of the feeding- and management-related diseases associated with the depression of the immune system. The aim of this research was to verify the effects of extruded linseed supplementation in the dairy ewe's diet on the innate and adaptive immune response and on the oxidative stress during two different physiological states, the transition and the dry period, which are characterised by different immunological conditions.

2. Materials and methods

2.1. Animals and dietary treatments

The experiment was performed using 58 Sarda pluriparous ewes, 42 pregnant (T, two months before parturition) and 16 non-pregnant (ultrasonography diagnosis) and non-lactating (NP), homogeneous for age (4–5 years), body condition score (BCS) and milk yield at previous lactation. The ewes had been subjected to oestrus synchronisation and ovulation induction using a combination of commercially available (Cronogest®) progestogen sponges plus PMSg injections (Intervet Srl, Milano, Italy) and then subjected to natural mating. The sheep were housed in a stable with an outdoor paddock. The animal care procedures followed the European directives for the protection of experimental animals (Directive 2010/63/EU).

In order to provide the same amount of nutrients, the nutritional plan and protocol were the same for the T and NP ewes. The animals were group-fed. Both T and NP ewes were randomly allocated into two subgroups of equal size and fed mixed (pregnancy) or alfalfa (lactation) hay ad libitum and either a commercial pelleted concentrate (CTR) or an extruded linseed-enriched (10 g/100 g as fed) concentrate (EL). The experimental diets were fed from 60 d before to 15 d after lambing of the T ewes. The two concentrates were formulated to be isoenergetic and isonitrogenous and were administered at a rate of 200, 400 and 800 g/d at the beginning of the trial, during the last 30 d of pregnancy and at early lactation of the T ewes, respectively.

Two months before, 5 d before and 15 d after delivery in the T ewes, the total dry matter intake (TDMI) was assessed over a 3-d period by weighing the residual feed intake. A 0–5 scale (Russel et al., 1969) was used by four trained operators to assess the BCS of the sheep (60 and 15 d before and 15 d after lambing).

Samples of the feeds were collected weekly during the trial and analysed for chemical composition (Table 1). The dry matter, crude protein, ether extracts and ash were determined according to AOAC procedures (AOAC, 1990, 2000). The methods of Van Soest et al. (1991) were used for the analyses of the neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin. Sodium sulphite was used in the NDF procedure. Both the NDF and ADF are expressed inclusive of ash. The calcium and phosphorous concentrations were determined according to Julshamn et al. (1998) and AOAC (1996), respectively.

Fatty acid extractions were performed according to the method of Folch et al. (1957). Methylation of feed fat was conducted using the sodium methoxide methylation procedure (Christie, 1982). Fatty acid methyl esters were quantified using a gas-chromatograph Perkin-Elmer-8410 (Perkin-Elmer, Norwalk, CT, USA) fitted with a fused silica capillary column [SP150-2380, 100 m × 0.25 mm (internal diameter) with 0.2 μm film thickness; Supelco, Inc., Bellefonte, PA, USA]. Hydrogen was used as the carrier gas with a pressure of 120 kPa. The injector and detector temperature was 250 °C. Gas-chromatography conditions were as follows: an initial oven temperature of 70 °C was maintained for 1 min, then ramped up at a rate of 5 °C/min to a final temperature of 100 °C and maintained for 2 min; the temperature was then increased to 175 °C at 10 °C/min and held at 175 °C for 28 min; the temperature was ramped up to 225 °C at a rate of 5 °C/min and maintained for 25 min. The split ratio was 40:1. Fatty acid peaks were identified by comparing the retention times of sample peaks with those of the standard mixture (37-Component FAME Mix, Supelco, Bellefonte, PA, USA).

The metabolisable energy (ME) content of feeds was estimated according to Andrieu et al. (1981) and Sauvant (1981).

2.2. Blood analytical determinations

Blood samples were collected from the T ewes during pregnancy (28, 21, 14 and 7 d before delivery) and during early lactation (3 and 15 d after delivery), at 8:00 am via jugular venipuncture using vacuum tubes (Vacuette, Greiner, Austria) containing lithium heparin or no anti-coagulant. At the same time, blood samples were also taken from the NP ewes. The samples without anti-coagulant were incubated at room temperature for 2 h and then centrifuged at 3520 × *g* for 16 min at 4 °C; the aliquots of serum were stored at −80 °C under sterile conditions until analysis. The serum samples were tested to evaluate the innate immunity [serum lysozyme (SL), haemolytic complement (HC) and serum bactericidal activity (SBA)], the acquired immunity (antibodies against *Salmonella enterica* subsp. *enterica* serovar *Abortusovis*, hereinafter referred as to *S. Abortusovis*) and the oxidative stress [antioxidant power of plasma (AOP) and reactive oxygen metabolites (ROMs)]. The samples collected with lithium heparin were used to evaluate the cellular immunity (total lymphocytes, CD4+ and CD8+ lymphocytes). For this determination, a follow-up sample was collected at 90 d after lambing.

To eliminate systematic error arising from inter-assay variability, all the samples from each animal were analysed within the same assay session, which included an equal number of animals belonging to the experimental groups.

The SL was measured using a lyso-plate assay (Osserman and Lawlor, 1966), performed at 37 °C for 18 h in a humidified incubator. The method was based on the lyses of *Micrococcus lysodeikticus* in 1% (w/v) agarose. The diameters of the lysed zones were measured with a ruler and compared