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Alterations in sheep peripheral blood mononuclear cell proliferation and cytokine release by polyunsaturated fatty acid supplementation in the diet under high ambient temperature

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ABSTRACT

The aim of this study was to investigate the effects of polyunsaturated fatty acid (PUFA) supplementation from different sources in the diet of dairy sheep under high ambient temperatures on ex vivo lymphocyte proliferation and inflammatory responses. The experiment was carried out during summer: 32 Comisana ewes were divided into 4 groups of 8. The FS group was supplemented with whole flaxseed, the AG group was supplemented with *Ascophyllum nodosum*, the FS+AG group was supplemented with a combination of flaxseed and *A. nodosum*. The fourth group (CON group) was a control and received a diet containing no supplement. The average maximum temperature was around 33°C during wk 2 and 3, whereas the mean temperature never decreased below 26°C. Following 15 d of treatment with respective diets, peripheral blood mononuclear cells (PBMC) from sheep who received a diet supplemented with *A. nodosum* had impaired cell proliferation responses and IL-6 production after mitogen stimulation compared with PBMC from FS+AG sheep. In addition, PBMC from AG sheep displayed impaired cell proliferation compared with cells from the CON group. The FS+AG cells produced lower levels of IL-10 than CON cells, and higher IL-6 than AG and CON cells. Results demonstrated that the supplementation with PUFA from different sources in a sheep's diet can influence their immunological responses under high ambient temperatures depending on the composition of fatty acid supplementation. In particular, synergistic effects of different PUFA from flaxseed and *A. nodosum*, simultaneously administrated in the sheep diet, were observed on activation of inflammation response.
Key words: cytokine, sheep, proliferation, polyunsaturated fatty acid, inflammation

INTRODUCTION

Farmed animals are subject to different types of stressors caused by both management procedures and environmental conditions. Stress can affect the immune system by altering immunological and inflammatory processes. In sheep, both physiological stress connected to gestation and lambing, and psychological stress, caused by isolation, increased in vivo plasma secretion of pro-inflammatory cytokines, including IL-6 (Caroprese et al., 2006, 2010). During an immunological challenge the balance between pro-inflammatory and anti-inflammatory cytokines is an important mechanism to regulate the ongoing pro-inflammatory processes and to avoid tissue damage resulting from excessive inflammation. Interleukin-10 is a well-known anti-inflammatory cytokine whose main role is to reduce the production of inflammatory mediators after immunological challenges (Murray, 2006). During the summer season in the Mediterranean basin, dairy animals are exposed to climatic conditions that often result in a depression of the immune system (Lacetera et al., 2005).

Interest is growing on the effects exerted by dietary fats on immune cell functions (Calder, 1996a,b, 1997, 1998). In dairy sheep and cows under heat stress, the dietary supplementation of whole flaxseed, rich in α-linolenic acid (C18:3n-3, **ALA**), resulted in an enhancement of humoral and cell-mediated responses, and in an alteration of plasma IL-10 secretion (Caroprese et al., 2009, 2012). *Ascophyllum nodosum* is a macroalgae rich in polysaccharides, PUFA, eicosapentaenoic acid (C20:5n-3, **EPA**), and antioxidants (Devi et al., 2008). Recently, *A. nodosum* was administrated in the diet of dairy cows and sheep to verify the effects on their hematological parameters and immunological responses. An increase of blood glucose and a decrease in sorbitol dehydrogenase in dairy cows was found (Karatzia et al., 2012; Novoa-Garrido et al., 2014). To the best of our knowledge, no studies have evaluated the effects of supplementation of *A. nodosum* to dairy sheep during

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summer, and on the activation of their inflammatory responses in terms of cytokine secretion.

Knowing the effects of high ambient temperatures on the activation of inflammatory response and its alteration after dietary supplementation of PUFA from different sources could be crucial to understanding sheep immune reactivity during the summer season to reduce the susceptibility to infectious diseases. We hypothesized that the supplementation of PUFA from whole flaxseed or from the macroalgae *A. nodosum* to dairy sheep during the summer season might influence the ex vivo activation of inflammatory response, in terms of pro-inflammatory and anti-inflammatory cytokine secretion and lymphocyte proliferation. This study, therefore, was undertaken to evaluate the effects of PUFA supplementation, administrated as whole flaxseed, *A. nodosum*, and a combination of flaxseed and *A. nodosum*, in the diet of dairy sheep under high ambient temperatures on ex vivo lymphocyte proliferation and inflammatory responses.

MATERIALS AND METHODS

Animals and Experimental Design

The experiment was conducted during the summer (June–July) of 2012 at Segezia research station of the Council for Research and Experimentation in Agriculture (CRA-ZOE). In the experimental design, 4 balanced groups each with 4 Cominsana ewes (d 202.1 ± 5.3 of lactation, mean ± SD) were assigned to 1 of 4 dietary treatments in a 2 × 2 factorial arrangement. Additionally, as a split-plot component, the blood samples taken from each ewe were treated differently ex vivo in the laboratory (see additional explanation below). The 4 dietary treatments were (1) control (**CON**) in which ewes received 1 kg/ewe per d of pelleted concentrate (Mangimificio Molino Gallo, Potenza, Italy); (2) supplemental whole flaxseed (**FS**; Lin Tech, Tecnozoo srl, Torreselle di Piombino Dese, Italy); (3) *A. nodosum* (**AG**; Tasco, Acadian Seaplants, Canada); or (4) the combination of FS and AG (**FS+AG**). The groups were balanced for milk yield (273.5 ± 7.9 g/d for the CON group, 268.2 ± 8.30 g/d for the FS group, 269.4 ± 9.41 for the AG group, and 273 ± 8.05 for the FS+AG group), BW (55.4 ± 1.2 kg for the CON group, 54.7 ± 0.9 kg for the FS group, 55.5 ± 1.1 kg for the AG group, and 55 ± 1.1 kg for the FS+AG group), and BCS (2.56 ± 0.1 for the CON group, 2.5 ± 0.2 for the FS group, 2.52 ± 0.1 for the AG group, and 2.53 ± 0.1 for the FS+AG group).

In the split-plot component of the experiment, an ex vivo study was performed involving isolations of sheep peripheral blood mononuclear cells (**PBMC**), and the evaluation of their proliferative response and cytokine production after stimulation with the mitogen phytohemagglutinin-(**PHA**) stimulated cells (**SC**), or not (**NSC**) was carried out. More details of sample collection and laboratory analysis are described subsequently.

Each animal received 1.8 kg/ewe per d of oat hay in 2 meals a day. Animal feeding procedures were described in Caroprese et al. (2014). In particular, ewes in the FS group received 750 g/ewe per d of pelleted concentrate, and 250 g/ewe per d of whole flaxseed; ewes in the AG group received 1 kg/ewe per d of pelleted concentrate in which 5% *A. nodosum* was incorporated; ewes in the FS+AG group were supplemented with both flaxseed (250 g/d) and pelleted concentrate incorporating 5% *A. nodosum*. Water was available ad libitum for all groups from automatic drinking troughs at any time of day.

During the trial, ambient temperature and relative humidity in indoor and outdoor areas were monitored with thermo-hygrographs (LSI, I-20090 Settala Premenugo-Milano, Italy) placed at 1.5 m from the floor. Data on maximum and mean ambient temperatures are reported in Figure 1.

All procedures were conducted according to the guidelines of the European Union Directive 2010/63/EU (EU Directive, 2010) on the protection of animals used for experimental and other scientific purposes. The ewes were healthy and their conditions were carefully examined by veterinarians throughout the trial to exclude the presence of any signs of disease.

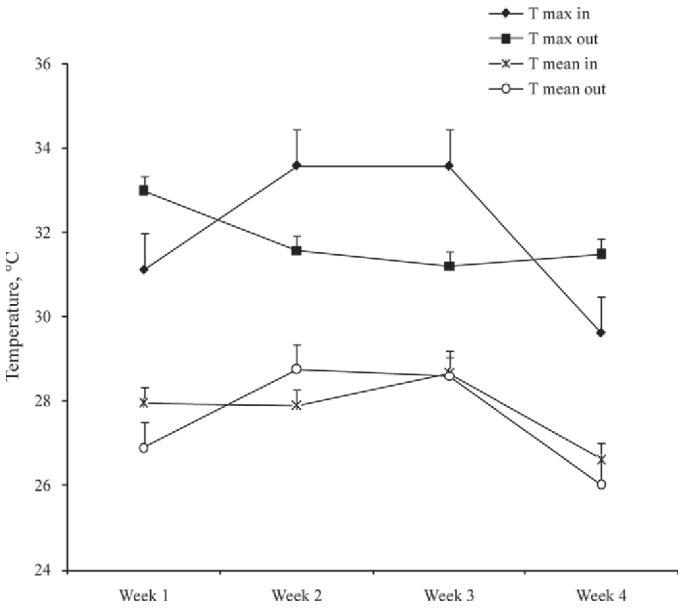

Figure 1. Means ± SE of maximum (T max) and mean ambient temperature (T mean) measured detected inside (in) and outside (out) the experimental pens during the weeks of the experimental period.