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Role of different sources of dietary PUFA supplementation on sheep welfare under high ambient temperature☆

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ABSTRACT

The purpose of this study was to evaluate the effects of different sources of polyunsaturated fatty acids (PUFA) supplemented in the diet on the welfare of lactating sheep exposed to high ambient temperature. The experiment was conducted during summer, it involved 32 ewes divided into 4 groups, and lasted 6 weeks. All groups were fed twice daily and received 1.8 kg/ewe/d of oat hay. The control group also received 1 kg/ewe/d of pelleted concentrate, whereas ewes in the experimental groups were supplemented with whole flaxseed (FS), seaweed *Ascophyllum nodosum* (Tasco®, AG), or their combination (FS + AG). At the beginning of the experiment, and weekly thereafter, the respiration rate, rectal temperature, body condition score (BCS) and body weight of the ewes were recorded. Milk samples were collected from each ewe weekly for the determination of the chemical composition, somatic cell count, and coagulating properties. Milk samples were also analyzed for their concentration of plasmin. Flaxseed diet during heat stress resulted in a reduction of the respiration rate, and in an increase of both milk and fat yield. The PUFA in the combined diet influenced the rectal temperature, and the respiration rate; also the milk yield and the concentration of plasmin (PL) increased in FS + AG diet. The lowest value of BCS recorded in ewes receiving the AG diet together with a rise in the respiration rate and rectal temperature suggested poor sheep welfare. Furthermore, sheep receiving the AG diet showed a consistent increase in the milk PL concentration that was responsible of the increase of milk clotting time and rate of clot formation.

1. Introduction

Sheep during exposition to high ambient temperature activate a series of physiological compensatory mechanisms to cope with extreme environmental condition and maintain vital functions. The first effect of increased ambient temperature on animals is the reduction of feed intake, followed by an alteration in water, protein and energy metabolism, mineral balance, enzymatic reactions, and hormonal secretion (Marai et al., 2007).

Heat stress in sheep arises when maximum air temperature exceeds 30 °C and the temperature–humidity index (THI) is over 80 (Sevi et al., 2001). Animal welfare of lactating ewes under heat stress is thwarted by the increased of respiration rate and rectal temperature, and the enhanced mobilization of body fat reserve for thermoregulation (Caroprese et al., 2012). Furthermore, during heat stress the milk composition is characterized by increased plasmin (PL) activity (Sevi and Caroprese, 2012), that is the main protease enzyme in milk. The increased PL activity has been associated with a worsening of coagulating properties of milk (Albenzio et al., 2004). The incidence of udder health problems in sheep also increases during summer consequently to the alteration of normal physiological functions of sheep, and to the concomitant depression of the immune system caused by heat stress (Caroprese et al., 2012; Sevi et al., 2002). Management and nutritional strategies may be used to sustain welfare and production performance of lactating animals under high ambient temperatures. Fat supplementation, based on polyunsaturated fatty acids (PUFA) has been investigated as a strategy to help dairy ewes to balance the negative effects of the heat stress on their physiological and immunological responses. Caroprese et al., (2011, 2012, 2014) demonstrated a positive effect

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of flaxseed supplementation on the immune responses of dairy sheep, sustaining their thermal balance and milk yield and quality under high ambient temperature. Nevertheless, in small ruminant nutrition a new field of investigation is developing with a growing interest in plant and plant extracts for their antioxidant and antibacterial activities (Sitzia et al., 2015).

The aim of the present study was to investigate the effects of different PUFA supplementation from flaxseed, seaweed and their combination, on the welfare, milk yield and milk quality of dairy ewes under heat stress.

2. Materials and methods

2.1. Experimental design and ewe feeding

The experiment lasted 6 weeks, and was conducted during the summer (July–August) of 2012 at Segezia research station of the Council for Research and Experimentation in Agriculture (CRA-ZOE); located approximately 15 km of Foggia, Apulia, southern Italy (latitude 41°27′6″ and longitude 15°33′5″north-east). Thirty-two late-lactation Comisana ewes (d 202.1 ± 5.3 of lactation, mean ± SD) were divided into four groups of eight, balanced for milk yield, body weight, and body condition score. All ewes were sheared in May and the groups were separately reared in external pens of 5 × 12 m bounded with mesh-fence; troughs and cribs were located in the external areas. During the trial, ambient temperature and relative humidity were monitored with thermo-hygrographs (LSI, I-20090 Settala Premenugo-Milano, Italy) placed at 1.5 m from the floor. The average temperature–humidity index (THI) was calculated using the Kelly and Bond (1971) formula. The maximum THI value registered was around 85 during the first week of the experiment, and remained around 76 until the end of the trial, whereas the minimun THI reached 73 at week 3 of the trial; mean of THI oscillated between 74 and 76.

The ewes were individually fed twice daily and received 1.8 kg/ewe/d of oat hay. The control group also received 1 kg/ewe/d of pelleted concentrate (Mangimificio Molino Gallo, Potenza, Italy), whereas ewes in the experimental groups were supplemented with whole flaxseed (Lin Tech, Tecnozoo srl, Torreselle di Piombino Dese, Italy), or *Ascophyllum nodosum* (Tasco®, Acadian Seaplants, Canada), or their combination according to Caroprese et al. (2014). Namely, ewes in the FS group received 750 g/ewe/d of pelleted concentrate, and 250 g/ewe/d of whole flaxseed; ewes in the AG group received 1 kg/ewe/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 (750 g/d) incorporating 5% *A. nodosum*. DMI was determined for each experimental group by weighing the refusals at 0800, 1200, 1600, and 2000 h. Water was available ad libitum for all groups from automatic drinking troughs at any time of day.

All procedures were conducted according to the guidelines of the EU Directive 2010/63/EU (2010) on the protection of animals used for experimental and other scientific purposes. Ewes were healthy and veterinarians carefully examined their conditions throughout the trial to exclude the presence of signs of diseases.

The chemical composition of pelleted concentrate, pelleted concentrate with 5% of *A. nodosum*, whole flaxseed, and oat hay was determined by standard procedures (AOAC, 1990).

The determination of methyl esters (FAME) of the diet ingredients was carried out according to O' Fallon et al. (2007). Briefly 1 g of the samples was pipetted into a screw-cap (16 × 25 mm) reaction tube. Methanol (0.5 mg) of C13:0/mL, 0.7 mL of KOH and 5.3 mL of MeOH was added into each tube, during incubation at 55 °C for 1 h and 30′, the tubes were inverted to mix for 5 s every 20 min. After cooling in a cold water bath, 3 mL of hexane were added into each tube and vortex for 5 min. The tubes were centrifuged at room temperature for 5 min at 500 × *g*; supernatant (1 mL) was taken from each tube and transferred into vials and stored at –20 °C to followed gas-chromatography (GC) analysis. Fatty acid profiles were quantified using Agilent Technologies, 6890 N GC equipped with a flame ionization detector (FID). Helium was the carrier gas and the gas flow rate was 175 kPa. The oven temperature (Eulitz et al., 1999) was initially held at 70 °C for 4 min, and then programmed to 175 °C at 13 °C/min increase and held isothermally for 45 min. A capillary column was used (HP88; 100 m × 0.24 mm i.d., 0.20 μm film thickness, Agilent Technologies Santa Clara, USA). Concentrations of FAME were analyzed utilizing a calibration curve with a mixture of standards of 50 fatty acid (GLC Reference standard 674, Nu-Check Prep, Inc., Elysian MN 56028, USA) with added CLA standards: C18:2 *trans*-8, *cis*-10; C18:2 *cis*-9, *trans*-11; C18:2 *cis*-11, *trans*-13; C18:2 *trans*-9, *cis*-11; C19:2 *cis*-8, *cis*-10; C18:2 *cis*-9, *cis*-11; C18:2 *trans*-10, *cis*-12; C18:2 *trans*-8, *trans*-10; C18:2 *trans*-9, *trans*-11; C18:2 *trans*-10, *trans*-12; C18:2 *trans*-11, *trans*-13 (GLC Reference standard UC–59 M, Nu-Check Prep, Inc., Elysian MN 56028, USA). Data on chemical composition and fatty acids profile is reported in Table 1.

2.2. Sampling and chemical analyses of milk

Ewes were milked twice daily (0700 and 0200 p.m.) in a parlor using a pipeline milking machine (Alfa Laval Agri, SE-147 21 Tumbas, Sweden). Milk samples from each ewe were collected at morning milking once a week, throughout the experiment. Fresh samples were used for the following chemical analysis: pH (GLP 21Crison, Spain), fat and protein content using an infrared spectrophotometer (MilkoScan™ FT120, Foss Electric, DK-3400Hillerød, Denmark) according to the International Dairy Federation standard (IDF, 1990), and SCC using a Fossomatic™ Minor (Foss Electric, DK-3400Hillerød, Denmark) (IDF, 1995). Evaluation of the renneting parameters (clotting time, rate of clot formation, and clot firmness after 30 min) was measured by a Foss Electric formagraph (Foss Electric, Hillerød, Denmark).

2.3. Weight gain and body condition score

Body weights and body condition scores (BCS, six-point scale 0 = thin, 5 = fat) of the ewes were recorded at the beginning of the experiment and then weekly throughout the trial. Body weight and BCS were measured in the morning after the milking and before feeding time. The body weight was measured by an electronic scale (METTLER MultiRange ID5, KC120/KC240).

2.4. Respiration rate and rectal temperature

Respiration rate (RR) was measured in all animals daily by counting the rate of flank movements. Subsequently, rectal temperature (RT) was measured by an electronic thermometer (LSI) with an accuracy of 0.1 °C.

2.5. Plasmin quantification of milk

Plasmin (PL) was determined in milk samples according to Albenzio et al. (2009) at week 3, 4, 5 and 6 in order to monitor the evolution of PL concentration in the last phase of the experiment. A standard curve was prepared to convert PL (Sigma Chemical Co., St. Louis, MO) activity to PL concentration by plotting changes in absorbance against concentrations of PL over a range from 0 to 16 mg/L. Plasmin concentration was reported as milligrams per liter of milk.