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Thermographic variation of the udder of dairy ewes in early lactation and following an *Escherichia coli* endotoxin intramammary challenge in late lactation

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ABSTRACT

A total of 83 lactating dairy ewes (Manchega, n = 48; Lacaune, n = 35) were used in 2 consecutive experiments for assessing the ability of infrared thermography (IRT) to detect intramammary infections (IMI) by measuring udder skin temperatures (UST). In experiment 1, ewes were milked twice daily and IRT pictures of the udder were taken before and after milking at 46 and 56 d in milk (DIM). Milk yield was 1.46 ± 0.04 L/d, on average. Detection of IMI was done using standard bacterial culture by udder half at 15, 34, and 64 DIM. Twenty-two ewes were classified as having IMI in at least one udder half, the others being healthy (142 healthy and 24 IMI halves, respectively). Four IMI halves had clinical mastitis. No UST differences were detected by IMI and udder side, being 32.94 ± 0.04°C on average. Nevertheless, differences in UST were detected for breed (Lacaune – Manchega = 0.35 ± 0.08°C), milking process moment (after – before = 0.13 ± 0.11°C), and milking schedule (p.m. – a.m. = 0.79 ± 0.07°C). The UST increased linearly with ambient temperature (r = 0.88). In experiment 2, the UST response to an *Escherichia coli* O55:B5 endotoxin challenge (5 μg/udder half) was studied in 9 healthy Lacaune ewes milked once daily in late lactation (0.58 ± 0.03 L/d; 155 ± 26 DIM). Ewes were allocated into 3 balanced groups of 3 ewes to which treatments were applied by udder half after milking. Treatments were (1) control (C00, both udder halves untreated), (2) half udder treated (T10 and C01, one udder half infused with endotoxin and the other untreated, respectively), and (3) treated udder halves (T11, both udder halves infused with endotoxin). Body (vaginal) temperature and UST, milk yield, and milk composition changes were monitored by udder half at different time intervals (2 to 72 h). First local and systemic signs of IMI were observed at 4 and 6 h postchallenge, respectively. For all treatments, UST increased after the challenge, peaking at 6 h in T11 (which differed from that in C00, C01, and T10), and decreased thereafter without differences by treatment. Vaginal temperature and milk somatic cell count increased by 6 h postchallenge, whereas lactose content decreased, in the endotoxin-infused udder halves. Effects of endotoxin on lactose and somatic cell count values were detectable in the infused udder halves until 72 h. In conclusion, despite the accuracy of the camera (±0.15°C) and the moderate standard errors of the mean obtained for UST measures (±0.05 to 0.24°C), we were unable to discriminate between healthy and infected (subclinically or clinically) udder halves in dairy ewes.

Key words: dairy sheep, infrared thermography, mastitis detection, udder temperature

INTRODUCTION

Early diagnosis of IMI is a relevant topic in the dairy industry because of the effects of IMI on milk production and treatment-related costs. Animals respond to IMI locally (i.e., pain, heat, hardness, swelling) and systemically (i.e., antibody production, body temperature), but the response may vary according to the infectious agent (Rebhun, 1995; McGavin and Zachary, 2007). Thermal response to infection (fever) is a useful diagnostic indicator that can be observed locally in most IMI cases (Rebhun, 1995).

Infrared thermography (**IRT**) is a noninvasive technique that allows the temperature of a surface to be measured without contact. Infrared thermography can generate images of the amount of heat emitted by an object, which has been used to study the changes in udder surface temperature (**UST**) caused by IMI in dairy cows (Barth, 2000; Scott et al., 2000; Hovinen, 2009). Moreover, IRT is able to show changes in teat temperature according to milking parameters in dairy

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cows (Kunc et al., 2007; Vegricht et al., 2007) and dairy ewes (Murgia et al., 2008). Barth (2000) concluded that IRT showed promise for detecting clinical mastitis, although it was unlikely to be useful for detection of subclinical mastitis.

Berry et al. (2003) reported daily variations in UST of dairy cows measured by IRT as a consequence of exercise, ambient temperature, and circadian rhythm, concluding that UST can be predicted accurately and that the difference between actual and predicted temperature was useful for detecting mastitis. In this sense, Colak et al. (2008) and Polat et al. (2010) indicated that IRT had a predictive diagnostic ability similar to that of the California mastitis test (**CMT**) in dairy cows. Reported UST changes as a result of the IMI-like process induced by infusing *Escherichia coli* bacteria or the endotoxin LPS in dairy cows varied between 1.0 and 3.0°C (Hovinen et al., 2008; Pezeshki et al., 2011), similar to the range of accuracy of most IRT cameras. Nevertheless, Hovinen et al. (2008) stressed that UST changes may be affected by the vasoconstriction of the peripheral blood vessels observed during many IMI episodes.

The aim of this study was to evaluate the use of IRT to measure UST changes produced by dairy sheep breed and machine milking and to assess the use of IRT for detecting naturally occurring IMI and IMI induced by *E. coli* endotoxin infusion in early and late lactation, respectively.

MATERIALS AND METHODS

The treatment procedures and animal care conditions were reviewed and approved by the Ethical Committee on Animal and Human Experimentation of the Universitat Autònoma de Barcelona (reference CEEAH 2011/1056).

Experiment 1

Animal and Management Conditions. A total of 83 lactating dairy ewes (Manchega, n = 48; Lacaune, n = 35) from the Experimental Farm of the "Servei de Granges i Camps Experimentals" (SGCE) of the UAB (Bellaterra, Barcelona, Spain) were used after parturition. Ewes were kept in a semi-confinement system, allowed to graze for 6 h daily on an annual Italian ryegrass prairie, and supplemented indoors with alfalfa hay ad libitum (1.27 Mcal of NE_L/kg and 20.1% CP; DM basis) and concentrate pellets at a flat rate of 0.8 kg/d (1.75 Mcal of NE_L/kg and 16.5% CP; DM basis) distributed in 2 portions at milking time. After the weaning of the lambs (d 35), the ewes were milked by machine twice daily (0800 and 1700 h) in a double-12 stall parallel milking parlor (Amarre Azul I, DeLaval Equipos, Alcobendas, Madrid, Spain) with a central high milk pipeline, 12 DeLaval SG-TF100 milking clusters, and 12 MM25SG milk flow and recording units (both from DeLaval, Tumba, Sweden). Milking was performed at a vacuum of 40 kPa, 120 pulses/min, and 50% pulsation ratio. The milking routine included cluster attachment (without udder preparation), machine milking, and automatic cluster detachment (milk flow rate <0.1 L/min or milking time >3 min). Teat dipping with an iodine solution (P3-ioshield, Ecolab Hispano-Portuguesa, Barcelona, Spain) was done at the end of milking. On experimental days, teat dipping was done after taking the IRT pictures.

Milk Recording and Sampling. Milk yield of individual ewes was recorded at 60 DIM. Udders and milk were clinically examined for clinical signs of IMI (e.g., hypersensitivity, hardness, abnormal texture, swelling, and hyperthermia) and for physical milk changes (i.e., milk clots and color and consistency changes) according to NMC (1999). Detection of IMI by bacterial culture of foremilk samples were performed by udder half at 15, 34, and 64 DIM.

Milk samples were taken aseptically from each mammary gland before milking. Teats were dipped in an iodine solution (P3-ioshield), dried with disposable paper towels, and dipped in ethanol 70% before sampling. The initial milk squirts were discarded and approximately 5 mL was collected in sterile tubes with plastic caps, preserved under refrigeration (4°C), and processed on the same day. Milk samples were cultured using conventional methods, and 0.01 mL was streaked onto blood agar plates (Agar Sangre 90 mm, Lab. Conda, Torrejón de Ardoz, Madrid, Spain) and plates incubated at 37°C. Plates were examined for bacterial growth after 18, 24, and 48 h.

UST. The UST was measured using a handheld portable infrared imaging camera (IRI 4010, Irisys, Northampton, UK). The camera operated within the 8 to 14 μm spectral band and ±0.15°C thermal resolution (accuracy). Before each measurement, the camera was adjusted for the ambient temperature conditions of each scanning (9 to 26°C) to compensate the reflected temperature. The emissivity value was set to 0.98 according to the Irisys camera user's manual, which is the value commonly used for measuring the skin temperature in humans and in dairy cow udders (Hovinen et al., 2008).

The IRT pictures of each udder were taken immediately before and after milking at 46 and 56 DIM. Ewes were restrained in a standing position using the head locker of the milking parlor, and udder pictures were taken from a caudal view, placing the camera on a tripod at a distance of 0.5 m. Udders were free of debris or