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 $32.94 \pm 0.04^\circ\text{C}$ on average. Nevertheless, differences in UST were detected for breed (Lacaune – Manchega = $0.35 \pm 0.08^\circ\text{C}$), milking process moment (after – before = $0.13 \pm 0.11^\circ\text{C}$), and milking schedule (p.m. – a.m. = $0.79 \pm 0.07^\circ\text{C}$). The UST increased linearly with ambient temperature ($r = 0.88$). In experiment 2, the UST response to an *Escherichia coli* O55:B5 endotoxin challenge ($5 \mu\text{g}$ /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 ($\pm 0.15^\circ\text{C}$) 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 μ m spectral band and $\pm 0.15^\circ\text{C}$ 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

Figure 1. Infrared thermographic images of clinically intramammary infected udder halves (left) and healthy udder halves (right) of dairy ewes in the milking parlor (indoor ambient temperature ranging between 14.1 and 15.9°C). Thermal histogram of the full image is shown under each image and udder skin temperature extreme values are indicated. Dotted rectangles on each udder half are the selected frame of 55 × 40 pixels in which the skin temperature was measured.

fecal materials. Infrared images were downloaded and the UST points analyzed with appropriate computer software (Irisys 4000 Series Imager v.1.0.0.17). Representative UST values of each udder half were obtained from the full udder IRT images (640 × 480 pixels) by selecting a rectangle of 55 × 40 pixels in the center of the cisternal part of each udder half (Figure 1). Special care was taken to avoid the influence of heat from the groin, leg, and median suspensory ligament, as they showed higher temperatures than the rest of the udder. Mean UST for each udder half was obtained using a 4× zoom by averaging the temperatures recorded in the center of each side of the selected rectangle.

Skin thickness of the udder was measured after the p.m. milking in duplicate in a random sample of 10 ewes of each breed with healthy udders. With this aim, the width of a fold of udder skin in 2 positions at the middle line of the udder perineal region (escutcheon) was measured at the end of the experiment and approximately 4 h after milking using a stainless steel electronic digital caliper (Yicheng, Beijing, China).

Experiment 2

Animal and Management Conditions. Nine healthy primiparous Lacaune dairy ewes (60.2 ± 0.8 kg of BW) at the end of lactation (155 ± 26 DIM and 0.58 ± 0.03 L/d) from the Experimental Farm of SGCE were used. Ewes were maintained indoors in straw-bedded pens ($1 \text{ m}^2/\text{ewe}$) and fed a mix of alfalfa and tall

fescue hays (2:1) ad libitum and the same concentrate mixture (0.6 kg/d, as fed) used in experiment 1. Once-daily milking was conducted (0800 h) in a 12-stall milking parlor (Westfalia-Surge Ibérica, Granollers, Spain) equipped with recording jars ($2 \text{ L} \pm 5\%$) and a low milk pipeline. Milking was performed at a vacuum of 42 kPa, 120 pulses/min, and a 50% pulsation ratio. The milking routine included cluster attachment, machine milking, machine stripping before cluster removal, and teat dipping in a iodine solution (P3-ioshield).

No signs of IMI were detected in any udder half by either clinical examination or CMT (Insvet, Esplus, Huesca, Spain) of foremilk before the experiment. Absence of IMI was also confirmed by bacterial culture as done in experiment 1.

Ewes were assigned to 3 balanced groups of 3 ewes to which the experimental treatments were randomly applied.

Endotoxin Challenge. Treatments consisted of intramammary challenges of *Escherichia coli* serotype O55:B5 LPS (Sigma-Aldrich, St Louis, MO) to induce an intramammary response similar to a mastitis-like process, and were as follows: (1) control (C00, both udder halves untreated; $n = 3$ ewes), (2) half udder treated (T10 and C01, one udder half was LPS infused and the other half was untreated, respectively; $n = 3$ ewes), and (3) both udder halves treated (T11, both udder halves were LPS infused; $n = 3$ ewes).

A solution of $5 \mu\text{g/mL}$ of *E. coli* O55:B5 LPS in physiological saline (0.9% NaCl; B Braun, Barcelona,

Spain) was aseptically prepared and preserved in 1-mL sterilized vials under refrigeration (4°C). The LPS challenge consisted of infusing 1 mL of LPS solution (5 µg/udder half) through the teat canal of the T10 (half udder) and T11 (both udder halves) ewe udders by using individual aseptic plastic cannulas (model J-2, Jorgensen Laboratories, Loveland, CO) cut to a length of 18 mm and with an outside diameter of 2.5 mm at approximately 30 min after the morning milking.

Clinical Observations and Milk Sampling. The experimental period lasted for 3 d, in which the clinical and productive stages of the udder halves were monitored after the challenge. Systemic (body temperature and udder appearance) and local (redness, hardness and UST) signs of reaction to the LPS challenge were monitored at 1 h, every 2 h from 2 to 12 h, every 12 h from 12 to 72 h, and additionally at 25, 28, 32, 49, and 73 h postchallenge.

Body temperature (systemic sign of reaction) was measured vaginally at the above indicated times using a clinical thermometer (Mini color, ICO Technology, Barcelona, Spain; range: 32.0 to 43.9°C; accuracy: $\pm 0.1^\circ\text{C}$). Udders and milk were also subjected to physical examination for IMI according to experiment 1 procedures.

Milk yield was recorded and milk samples collected (for CMT, milk composition, and SCC) by udder half during the preexperimental week and at each milking throughout the experiment (d 1 to 3). Additional milk samples were collected manually at 6, 24, 48, and 72 h postchallenge. For milk composition and SCC analyses, samples of approximately 50 mL were preserved with antimicrobial tablets (Bronopol, Broad Spectrum Micro-tabs II, D&F Control Systems Inc., San Ramon, CA) and kept at 4°C until processing. Both milk composition and SCC were determined in the interprofessional dairy laboratory of Catalonia (Allic, Cabrils, Barcelona, Spain), using an automatic somatic cell counter (Fossomatic 500, Foss-Electric, Hillerød, Denmark) previously calibrated for ewe milk.

UST. The UST was measured using the procedure described in experiment 1, taking 17 IRT images from each ewe's udder immediately before and at different times after the challenge (same times as for body temperatures). Images were obtained from the untouched udders, before udder examination and milk sampling, to avoid stress-induced hyperthermia (Bouwknicht et al., 2007).

Statistical Analyses

Data of both experiments were analyzed using the PROC MIXED (version 9.1; SAS Institute Inc., Cary, NC) and variation factors compared by classes. The mixed model used for experiment 1 data contained the

fixed effects of udder health (healthy and IMI), breed (Manchega and Lacaune), milking moment (before and after), and milking schedule (a.m. and p.m.), and the random effects of ewe (1 to 83) and error.

The model used in experiment 2 contained the fixed effects of treatment (C00, T11, C01, and T10), time postchallenge (0 to 73 h), and the random effects of ewe (1 to 9) and error. Performance data before the LPS challenge were used as a covariate to correct for individual initial differences, and results were presented as least squares means. Differences between least squares means were determined with the PDIF option of SAS. Logarithmic transformations ($\log_{10}$) of SCC values were used in the statistical analysis. Pearson correlations were used to determine the relationship between the studied variables. Significance was declared as $P < 0.05$, unless otherwise indicated, and tendencies were discussed at $P < 0.10$.

RESULTS AND DISCUSSION

Experiment 1

Milk Yield. Experimental ewes yielded 1.46 ± 0.04 L/d, on average, at 60 DIM. When classified according to bacterial culture, subclinical IMI ewes yielded 12% less milk than healthy ewes, although the difference was not significant (1.37 ± 0.08 vs. 1.49 ± 0.06 L/d; $P = 0.21$). A lower milk yield was expected in the IMI ewes according to different studies (Viguier et al., 2009; Pezeshki et al., 2011), although the difference from the healthy ewes may have been smaller because of the low incidence of udder halves having subclinical mastitis (12.1%) and the compensatory milk yield of the contralateral udder half (Leitner et al., 2004).

Subclinical Mastitis. From the 166 udder halves studied, 20 (12.0%) were classified as IMI by bacterial culture in at least 2 of the 3 tests at 15, 34, and 64 DIM. The subclinical IMI ewes did not present signs of mastitis and their milk was apparently normal but showed high SCC ($>300 \times 10^3$ cells/mL, according to McDougall et al., 2001). Reported values of the incidence of subclinical mastitis are markedly greater in dairy cows than in ewes (Lam et al., 2013; 22 to 23%).

No differences in UST values were detected between healthy and subclinical IMI udders of the same ewes throughout the experiment, but the IMI udders tended to show slightly lower mean UST values than the healthy udders (33.13 vs. 33.61°C , respectively; $\text{SEM} = \pm 0.28$; $P = 0.10$). These results are in contrast to those of Martins et al. (2013), who reported greater UST values in subclinical mastitis than in healthy udders of Santa Inês ewes (37.46 vs. 37.05°C , respectively), although the

Table 1. Effect of the experimental variation factors on the udder skin temperatures (UST, °C) of dairy ewes

Factor ¹	Class 1	Class 2	SEM	Effect ($P =$)
Breed	32.88	33.23	0.11	0.003
Milking schedule	32.66	33.45	0.06	0.001
Milking moment	32.99	33.12	0.05	0.01
Side of the udder	33.05	33.06	0.11	0.88
Health status of udder	33.11	33.00	0.16	0.48

¹Factor classes: breed (class 1, Manchega; class 2, Lacaune), milking schedule (class 1, a.m.; class 2, p.m.), milking moment (class 1, before; class 2, after), side of the udder (class 1, left; class 2, right) and health status of udder (class 1, healthy; class 2, subclinical IMI).

number of ewes (healthy, 16; subclinical mastitis, 8), size of the udder (meat sheep), and the cleaning process (using warm water) may have conditioned the results. Moreover, no differences were reported by Martins et al. (2013) for total udder mean temperatures (36.30 vs. 36.06°C, respectively).

Clinical Mastitis. A total of 4 udder halves (from 3 ewes, 3.6%) showed clinical mastitis and required antibiotic treatment. These IMI udders showed local and systemic mastitis clinical signs (severe swelling, tissue hardness, and redness) and marked changes in milk appearance (flakes, watery, and abnormal texture). Prevalence of clinical mastitis is generally $\leq 5\%$ in dairy sheep (Berger et al., 2004), in agreement with our data. The incidence of clinical mastitis is markedly greater in dairy cows compared with dairy sheep, ranging between 28 and 33.5% quarter cases as reported in Canada (Olde Riekerink et al., 2008) and the Netherlands (Lam et al., 2013).

Mean UST values for clinical IMI varied greatly and, on average, were slightly higher than for the healthy udder halves, although they did not differ significantly (33.51 vs. 33.47°C, respectively; SEM = ± 1.13 ; $P = 0.97$). Infrared thermography images of the only ewe presenting clinical IMI in both udder halves compared with a fully healthy udder are shown in Figure 1 (high-temperature pixels are shown by lighter gray color). Martins et al. (2013) reported lower UST values but no differences between clinical mastitis and healthy udders (36.67 vs. 37.05°C; $n = 13$); the lower UST values were thought to be a response to the decreased blood flow that occurred due to mammary gland loss of function. Moreover, Hovinen et al. (2008) indicated that UST changes may be affected by the vasoconstriction of the peripheral blood vessels observed during IMI episodes, producing a decrease in skin temperature.

Udder Temperature Variation Factors. Table 1 summarizes the effects of the main factors considered in the study to explain the variation of UST values in the experimental ewes. Overall mean UST ($32.94 \pm 0.04^\circ\text{C}$) was lower than the average body temperature reported for sheep (38.0 to 39.5°C; Adams and McKinley, 2009) and was affected by most of the studied factors (i.e.,

breed, milking schedule, and milking moment; $P < 0.05$ to 0.001). Values of UST were lower in Manchega compared with Lacaune ewes ($P < 0.01$; Table 1), which agrees with the lower milk yield observed for the healthy ewes in our study (1.25 vs. 1.72 L/d; SEM = ± 0.97 ; $P < 0.001$). No previous data on the relationship of milk yield and UST has been reported. Moreover, no differences in udder skin fold thickness were detected between Manchega and Lacaune ewes (15.82 ± 0.13 vs. 15.79 ± 0.07 mm, respectively; $P = 0.90$), but we noted that Manchega ewes more frequently had hair on their udders than Lacaune. According to Mitchell (2013), presence of hair may have decreased the UST values and contributed to the breed differences reported here.

The UST values before milking were lower at the morning milking than at the afternoon milking ($P < 0.001$; Table 1), which may be related to the circadian rhythm of body temperatures and exercise and feeding behavior (Berry et al., 2003; Piccione and Caola, 2003; D'Alterio et al., 2011). Moreover, UST correlated with ambient temperature ($y = 0.37x + 28.4$; $r = 0.88$; $P < 0.001$). Indoor ambient temperature was $12.18 \pm 0.74^\circ\text{C}$ on average, ranging between 9.1 and 16.6°C during the experiment. Loughmiller et al. (2001) also reported a correlation ($r = 0.98$) between ambient temperature (10 to 32°C) and body surface temperature measured by IRT in pigs; each 1°C increment in ambient temperature caused an increase of 0.40°C in the body surface temperature of pigs.

Additionally, UST values increased by effect of milking ($P < 0.05$; Table 1), which agreed with the conclusions of Kunc et al. (2007) and was attributed to the hyperemia caused by udder manipulation. Figure 2 shows the IRT images of the udder of the same ewe before and after milking. Nevertheless, Murgia et al. (2008) in sheep and Aljumaah et al. (2012) in camels reported a decrease in the temperature of the teats and the udder, measured by IRT, as a consequence of machine milking. Differences in the UST response to machine milking may also be due to the operating vacuum and milk yield (Kunc et al., 2007). Vegricht et al. (2007) reported smaller increments in UST in dairy cows with lower milking vacuums.

Figure 2. Infrared thermographic images of healthy udders of dairy ewes before (left) and after (right) milking in the milking parlor (indoor ambient temperature ranging between 14.3 and 16.0°C). Thermal histogram is shown under each image and udder skin temperature extreme values are indicated. Note the presence of a supernumerary teat in the left udder half.

No effects were detected in our ewes with respect to udder side or udder health status ($P > 0.05$; Table 1), allowing us to use pooled data and agreeing with the IMI data discussed above.

Experiment 2

Clinical Signs and Body Temperature. Local and systemic signs of IMI in the T10 and T11 mammary glands were clearly observed after LPS infusion, despite the low dose used per udder half (0.083 µg/kg of BW). None of the ewes had a severe reaction to the LPS challenge. The T10 and T11 ewes were calm after LPS infusion and did not present signs of pain or hypersensitivity, although 2 ewes of each treatment showed udders with moderate swelling and tissue hardness approximately 4 h after infusion. Clinical signs were similar to those observed in an acute IMI.

Body temperature, measured by vaginal temperature, of the T10 and T11 ewes increased after LPS infusion (Figure 3), peaking at 4 to 6 h after the challenge. In parallel, the vaginal temperature of C00 ewes slowly increased after milking as observed for ambient temperature and peaked 6 h after milking. Positive correlations of vaginal temperature (y) with ambient temperature (x) were detected for healthy ewes ($y = 0.072x + 37.02$; $r = 0.55$; $P < 0.001$), indicating that the observed increased temperature was unlikely due to the IMI response and could be a consequence of the increase in ambient temperature, agreeing with data of

experiment 1. Vickers et al. (2010) validated the use of vaginal loggers to measure body temperature; the technique has the advantage of not being affected by the presence of feces. Willard et al. (2007) also reported increased body temperatures after an endotoxin challenge in dairy cows, but the authors did not correlate this with the increased ambient temperature also recorded in their experiment.

The LPS dose used in our experiment was low compared with those used in dairy cows, which ranged between 0.02 and 2.60 µg/kg of BW of *E. coli* O55:B5 (Hovinen et al., 2008; Willard et al., 2007; respectively), but was sufficient to induce dramatic changes in milk appearance (milk flakes and yellow color) 6 h after the challenge; the 3 T11 ewes showed abundant flakes in their milk. A higher dose was used by Scott et al. (2000) in dairy cows but no information is available on the *E. coli* serotype used. Udder swelling disappeared gradually and was barely detectable 24 h after the challenge, as reported in dairy cows (Scott et al., 2000).

Milk Yield and Composition. Regarding milk production, all groups of ewes showed decreased milk yield on the day of treatment, which may have been a consequence of the experimental management and isolation of the ewes from the flock on the day before (Sufka and Hughes, 1991). Nevertheless, in agreement with the IMI signs of the T10 and T11 ewes, a greater milk yield decrease was observed in the LPS-treated udder halves after infusion (Figure 4). On average, the daily milk yield decreases in the treated (T10 and T11)

Figure 3. Changes in vaginal temperature of dairy ewes submitted to an *Escherichia coli* LPS challenge at late-lactation. Treatments by udder half were as follows: both udder halves untreated (—○—, C00, control), half udder treated (—▲—, T10 and C01, one udder half was LPS infused and the other half was untreated, respectively), and both udder halves treated (—●—, T11, both udder halves were LPS infused). Experimental details: *E. coli* challenge (0 h), once-daily milking (↓), and ambient temperature in the barn (---). Values are means with SEM indicated by error bars.

compared with the untreated (C00 and C01) udder halves at 24, 48, and 72 h postchallenge were -33.1 ($P < 0.05$), -41.4 ($P < 0.001$), and -36.6% ($P < 0.001$), respectively, compared with that at 0 h. All ewes showed

increased milk yield at 48 h except the T10 ewes, in which milk yield plateaued but recovered at 72 h. As a consequence, C00 and C01 ewes recovered their initial milk yield before the challenge, whereas the T10 and

Figure 4. Effect of the infusion of *Escherichia coli* LPS on the milk yield of dairy ewes at late-lactation by half udder (○, untreated; ●, LPS infused). Treatments were as follows: both udder halves untreated (---, C00, control), half udders treated (---, C01, untreated udder half; ---, T10, treated udder half) and both udder halves treated (---, T11, both udder halves infused). Experimental details: *E. coli* challenge (0 h) and once-daily milking (↓). Values are least squares means with SEM indicated by error bars.

Table 2. Effect of *Escherichia coli* LPS infusion treatments on milk composition by udder half of dairy ewes at the end of lactation

Item and time ¹	Treatment ²				SEM	Treatment effect (<i>P</i> =)
	Control		LPS infused			
	C00	C01	T10	T11		
Fat, %						
6 h	9.82 ^{ab}	9.33 ^b	7.96 ^a	8.26 ^{ab}	0.34	0.08
24 h	7.22	7.42	8.19	8.23	0.48	0.76
48 h	6.46	5.40	9.54	7.56	0.57	0.17
72 h	6.38	5.99	7.05	6.91	0.47	0.80
Fat yield, g/d						
6 h	—	—	—	—	—	—
24 h	22	21	17	18	2	0.62
48 h	25	22	16	21	3	0.65
72 h	24	29	23	20	2	0.12
Protein, %						
6 h	6.04 ^b	5.80 ^{ab}	5.60 ^{ab}	5.55 ^a	0.06	0.04
24 h	6.07	5.94	7.33	6.80	0.19	0.10
48 h	6.03	6.02	7.89	6.60	0.23	0.15
72 h	6.44	6.17	6.48	6.09	0.10	0.35
Protein yield, g/d						
6 h	—	—	—	—	—	—
24 h	20	17	14	14	2	0.50
48 h	25	23	14	18	2	0.20
72 h	26	31	24	18	2	0.05
Lactose, %						
6 h	4.46 ^c	4.51 ^c	2.73 ^a	3.47 ^b	0.12	0.001
24 h	4.29 ^c	4.48 ^c	2.02 ^a	2.92 ^b	0.10	0.003
48 h	4.13 ^c	4.42 ^c	2.90 ^a	3.11 ^a	0.13	0.003
72 h	4.12 ^{ab}	4.23 ^{bc}	3.59 ^a	3.73 ^{ac}	0.13	0.002
Lactose yield, g/d						
6 h	—	—	—	—	—	—
24 h	14	13	5	7	1	0.02
48 h	17	17	6	8	1	0.04
72 h	18	21	12	10	1	0.003
TS, %						
6 h	21.5 ^{bc}	20.6 ^c	17.5 ^{ab}	18.1 ^a	0.4	0.006
24 h	18.6	19.0	19.1	19.1	0.6	0.99
48 h	17.4	17.1	23.5	18.6	0.8	0.19
72 h	18.3	17.2	16.7	17.1	0.4	0.67
TS yield, g/d						
6 h	—	—	—	—	—	—
24 h	59	54	39	42	5	0.29
48 h	73	67	37	52	6	0.19
72 h	71 ^{bc}	84 ^c	62 ^{ab}	50 ^a	5	0.001
Log ₁₀ SCC						
6 h	5.66 ^a	5.88 ^a	7.22 ^b	7.27 ^b	0.06	0.001
24 h	5.46 ^a	5.91 ^a	6.24 ^a	7.32 ^b	0.17	0.005
48 h	5.32 ^a	5.49 ^a	7.28 ^b	7.13 ^b	0.05	0.001
72 h	5.24 ^a	5.27 ^a	6.37 ^b	6.69 ^b	0.07	0.001

^{a-c}Means within a row with different superscripts differ ($P < 0.05$).

¹Time after the LPS challenge.

²Treatments by udder half were as follows: both udder halves untreated (C00, control); half udder treated (T10 and C01, one udder half was LPS infused and the other half was untreated, respectively); and both udder halves treated (T11, both udder halves were LPS infused).

T11 ewes showed incomplete recuperation after 72 h. Lehtolainen et al. (2003) reported complete recovery of the udder of dairy cows on d 2 after the challenge, which was not observed in our results and may be a consequence of the involution stage of the mammary gland induced by the once-a-day milking at the end of lactation (Boutinaud et al., 2003).

Changes in milk components as a result of the LPS challenge varied according to the milk component considered (Table 2), being moderate in fat, protein, and TS, and dramatic in lactose and SCC. The effects of LPS challenge on fat, protein, and TS contents were only significant 6 h after the challenge, and no effects were detected in corresponding daily milk yields,

Figure 5. Changes in udder skin temperature (UST) of dairy ewes submitted to an *Escherichia coli* LPS challenge in late lactation. Treatments by udder half were as follows: both udder halves untreated ($\circ$, C00, control), half udder treated ($\blacktriangle$, T10 and C01, one udder half was LPS infused and the other half was untreated, respectively), and both udder halves treated ($\bullet$, T11, both udder halves were endotoxin infused). Experimental details: *E. coli* challenge (0 h), once-daily milking ($\downarrow$), and ambient temperature in the barn (---). Values are means with SEM indicated by error bars.

except in TS yield at 72 h, which was beginning to recover (Table 2; $P > 0.05$). Lactose content decreased dramatically in the T01 and T11 groups 6 h after the challenge ($P < 0.001$), showing a nadir at 24 h and signs of recovery at 72 h compared with the control groups (Table 2); similar effects were detected in lactose daily yield ($P < 0.05$). A decrease in lactose output was also reported by Leitner et al. (2004) in subclinical mastitis milk of dairy ewes, which was attributed to the local role of casein hydrolyzed peptides on the activity of mammary epithelial cells (Silanikove et al., 2000). Martins et al. (2013) also reported lower lactose levels in the milk of ewes with clinical mastitis, supporting the theory that the damage to secretory tissue leads to a decrease in lactose biosynthesis. On the other hand, $\log_{10}$ SCC increased in the milk of the LPS-treated groups (13 to 26%, $P < 0.005$; Table 1) and remained high during the experiment (Lehtolainen et al., 2003), the effect being consistent 72 h after the LPS challenge and with the decrease in milk lactose contents. These results confirm the adequacy of the LPS dose used in our ewes and the persistency of the mastitis clinical signs discussed above, agreeing with those reported by Hovinen et al. (2008) in dairy cows treated with a low dose of endotoxin.

UST. Dramatic changes in UST values were shown throughout the experimental period; UST peaked 2 to 6 h after milking. Indoor ambient temperature, also shown in Figure 5, was $23.00 \pm 0.38^{\circ}\text{C}$ on average, ranging between 20.6 and 26.6°C during the experiment, peaking at 36 h, and slowly declining thereafter.

A circadian rhythm pattern was observed daily for UST, with minimum and maximum values before and after milking, respectively (Figure 5). This pattern indicated the influence of the variation in the daily temperature and management conditions. Nevertheless, no differences between treatments were observed for mean UST values during the experiment ($P = 0.75$), with the exception of T11, which showed a peak 6 h after the challenge ($P < 0.001$).

The lack of difference in mean UST values by effect of treatment agreed with the fact that UST values largely varied between udder halves of the same animal despite the treatment applied. The range of differences between udder halves of the same animal were high for C00 (0.05 to 0.55°C) and T10 (0.05 to 0.85°C) and increased in the case of T11 (0.10 to 1.00°C), indicating large local variation in the UST values of healthy halves and in response to infection in the LPS-infused udder halves. These differences in response to an endotoxin

challenge between udder halves have not been reported previously. Our results agreed with those of Scott et al. (2000), Hovinen et al. (2008), and Pezeshki et al. (2011), which showed similar increases of UST in experimental and control halves, reflecting the systemic effect of the infused endotoxin. Moreover, positive correlations between vaginal and ambient temperature (indicated above), between UST (y) and vaginal (x) temperatures ($y = 2.241x - 49.51$; $r = 0.55$; $P < 0.001$), and between UST (y) and ambient (x) temperatures ($y = 0.504x + 25.72$; $r = 0.71$; $P < 0.001$) in the healthy ewes also supported the lack of effects of the experimental treatments.

Barth (2000) and Polat et al. (2010) reported significant UST differences (+0.5 and +2.35°C, respectively) in dairy cows between udder quarters with SCC below and above 100 and 400×10^3 cells/mL, respectively. Polat et al. (2010) also found a moderate positive correlation between UST and SCC ($r = 0.73$) that was higher than that in our ewe data ($r = 0.25$; $P = 0.03$). Additionally, Colak et al. (2008) and Polat et al. (2010) found positive correlations between UST and CMT values by udder in dairy cows ($r = 0.92$ and $r = 0.86$, respectively), but the correlation of UST with CMT was lower and not significant in our data ($r = 0.18$; $P = 0.50$). These results did not allow us to discriminate between the UST values of LPS-infused and healthy udder halves in our data, the variation observed being attributed to individual and environmental differences.

Our results disagree with those of Polat et al. (2010), who found 83.5% sensitivity and 100% specificity for a cutoff of 34.7°C when udder quarters were classified as healthy or infected with subclinical mastitis ($>200 \times 10^3$ cells/mL). Previous research showed that commercial thermal cameras are capable of detecting UST changes of 1.0 to 3.0°C in cows undergoing intramammary infusion with *E. coli* endotoxin (Hovinen et al., 2008; Pezeshki et al., 2011). In our experiment, the thermal camera was able to detect the changes in UST of 1 to 1.3°C associated with the *E. coli* LPS challenge in ewes.

CONCLUSIONS

Thermal imaging was a simple, fast and noninvasive technique to measure the skin temperature of the mammary gland in dairy ewes at milking. Nevertheless, despite the resolution of the camera used ($\pm 0.15^\circ\text{C}$) and the SEM obtained for UST measures (± 0.05 to $\pm 0.24^\circ\text{C}$), we were unable to discriminate between healthy, infected (subclinical or clinical), or LPS-infused udder halves in dairy ewes. Other factors (breed, ambient temperature, milking) can induce changes in UST greater than those observed after clinical or subclinical IMI.

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