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Influence of ventilation regimen on micro-environment and on ewe welfare and milk yield in summer

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ABSTRACT

The effects of ventilation regimen on air quality, and on the welfare and production performance of thirty-six Comisana ewes were assessed in a 6-week trial conducted during the summer of 2002. Animals were divided into three groups of 12, and subjected to the following treatments: low ventilation regimen providing a mean ventilation rate (VR) of 35 m³/h per ewe, split in 30 min ventilation cycles at an air speed of 2 m/s (LOV-30); moderate ventilation regimen (VR = 70 m³/h per ewe) split in 30 min ventilation cycles at an air speed of 4 m/s (MOV-30); moderate ventilation regimen (VR = 70 m³/h per ewe) split in 60 min ventilation cycles at an air speed of 2 m/s (MOV-60). Air concentrations of microorganisms, dust, and gaseous pollutants were measured twice weekly. Respiration rate (RR) and rectal temperature (RT) were monitored throughout the trial at 0830 and at 1400. Behavioral traits of ewes were recorded twice per week from 0900 to 1200 and from 1500 to 1800. Cell-mediated immune response to phytohemagglutinin (PHA) and humoral immune response to chicken egg albumin were determined. At d 37 ewes were injected with porcine ACTH, and subjected to blood sampling for evaluation of cortisol concentrations immediately before and 1, 2 and 4 h after ACTH injection. Milk yield was recorded daily. Individual milk samples were analyzed for composition, renneting parameters, somatic cell count (SCC), and bacteriological characteristics. Averages of maximum THI were about 3 points higher in the LOV-30 and the MOV-30 than in the MOV-60 room, whereas no differences emerged in the air concentrations of dust, gaseous pollutants and microorganisms. Significant interactions of treatment x time ($P < 0.05$) were found for respiration rate, and for the time the ewes spent lying, idling and eating in the afternoon during weeks 2 and 3 of the study period. Significant effects of ventilation regimen x time ($P < 0.05$) were also observed for milk yield and milk renneting parameters, the LOV-30 ewes giving smaller volumes of milk with a deteriorated coagulating behavior than those of the MOV-60 group during the second half of the trial. No significant differences emerged in ewe immune and endocrine responses. Results show that ventilation regimen had a moderate impact on ewe behavior, physiology and production performance. This experiment suggests that the length of ventilation cycles and air speed, together with ventilation rate, are critical for efficient ventilation regimens.

Key words: Dairy ewes, Ventilation, Respiration rate, Behavior, Milk coagulation properties

RIASSUNTO

INFLUENZA DEL REGIME DI VENTILAZIONE SUL MICRO-AMBIENTE DI ALLEVAMENTO
E SUL BENESSERE E LA PRODUZIONE LATTEA DELLA PECORA IN ESTATE

E' stato valutato l'effetto del regime di ventilazione estivo sulla qualità dell'aria in ovile e sul benessere e la produttività di 36 pecore Comisane. Gli animali sono stati suddivisi in 3 gruppi di 12 soggetti e sottoposti ai seguenti trattamenti spe-

rimentali: regime di ventilazione basso con una portata di ventilazione media (VR) di 35 m³/h per capo e suddiviso in cicli di ventilazione di 30 min ad una velocità dell'aria di 2 m/s (LOV-30); regime di ventilazione moderato (VR = 70 m³/h per capo), suddiviso in cicli di 30 min ad una velocità dell'aria di 4 m/s (MOV-30); regime di ventilazione moderato (VR = 70 m³/h per capo), suddiviso in cicli di 60 min ad una velocità dell'aria di 2 m/s (MOV-60). Nel corso della prova sono state monitorate le concentrazioni di polveri, di microorganismi e di gas nell'aria, nonché la frequenza respiratoria (RR) e la temperatura rettale (RT) delle pecore alle 8:30 ed alle 14:00. Il comportamento delle pecore è stato rilevato bisettimanalmente dalle 9:00 alle 12:00 e dalle 15:00 alle 18:00. Sono state determinate la risposta immunitaria cellulomediata alla fitoemagglutinina (PHA) e quella umorale alla ovalbumina. Il 37° giorno di prova alle pecore sono state iniettate 2 UI di ACTH/kg di peso metabolico. Sono stati quindi determinati i livelli cortisolemici subito prima ed 1, 2 e 4 ore dopo l'iniezione di ACTH. È stata rilevata la produzione lattea individuale e sul latte sono state determinate la composizione chimica, l'attitudine alla coagulazione, la conta delle cellule somatiche e la carica batterica. Le medie dei THI massimi sono risultate di circa 3 punti più elevate negli ambienti LOV-30 e MOV-30 rispetto all'ambiente MOV-60 (78,4 e 78,2 vs 75,7 rispettivamente), mentre non sono emerse differenze nelle qualità dell'aria. Interazioni significative del trattamento sperimentale x la settimana di prova ($P < 0,05$) sono state rilevate, durante il pomeriggio, a carico della frequenza respiratoria e dei tempi trascorsi in decubito, in inattività e in attività ingestive, in relazione ad una più intensa attivazione dei meccanismi di termoregolazione nei gruppi LOV-30 e MOV-30 rispetto al gruppo MOV-60 durante la seconda e la terza settimana di prova. Effetti significativi del regime di ventilazione x il tempo ($P < 0,05$) sono stati anche osservati per la produzione lattea e per i parametri lattodinamometrici, a motivo del fatto che le pecore LOV-30 hanno prodotto meno latte con una peggiore attitudine alla coagulazione rispetto a quelle del gruppo MOV-60 durante la seconda parte della prova. Differenze significative ($P < 0,05$) dell'indice di coagulazione del latte (CoI), sono emerse, tra questi 2 gruppi, durante la quarta (1,72 vs 3,14 mm/min), la quinta (1,96 vs 3,45 mm/min) e la sesta settimana di prova (2,02 vs 3,32 mm/min). Nel complesso, il regime di ventilazione ha avuto un impatto modesto sulle risposte comportamentali, fisiologiche e produttive delle pecore. I nostri risultati sembrano evidenziare che la durata dei cicli di ventilazione e la velocità dell'aria rappresentano, in aggiunta alla portata di ventilazione, altrettanti fattori critici per l'efficienza dei regimi di ventilazione.

Parole chiave: Pecore da latte, Ventilazione, Frequenza respiratoria, Comportamento, Attitudine del latte alla coagulazione

Introduction

The adverse effects of poor ventilation on the welfare, health and production performance of farmed livestock are well documented (Wathes, 1994). The effects on animal well-being are ascribed to excessive heat loads and overcrowding of cleaner and more ventilated areas of the livestock buildings, which may result in enhanced aggressive interactions (Smith *et al.*, 1996; Spoolder *et al.*, 2000). The effects on animal health are due to the worsening of air and surface hygiene and to high gaseous pollutant concentrations, which can impair immune function and increase morbidity in farmed livestock (Rylander, 1986; Sevi *et al.*, 2001b).

The efficacy of ventilation systems may largely depend on the choice of proper air speed and length of ventilation cycles. Indeed, low ventilation rates may fail to efficiently remove the moisture and gases which originate from the respiratory activity of animals and the decomposition and fermenta-

tion of manure. However, turbulent air currents generated by very rapid ventilation rates may disturb animals and result in greater amounts of dust entering the animal house, as well as in dust particles remaining suspended in the air for a longer time (Qi *et al.*, 1992; Sevi *et al.*, 2003b).

A previous experiment (Sevi *et al.*, 2002) demonstrated that a summer ventilation regimen programmed to operate over upper critical air temperature and relative humidity is economically unattractive in dairy sheep housing. In fact, it involved an almost three-fold increase in energy costs and did not lead to remarkable improvements in ewe welfare and productivity compared to an intermittent regimen split in 25 min ventilation cycles, at a fan speed of 4 m/s, during the warmest hours of the day. A further reduction of ventilation cycles to 12.5 min resulted in ewes displaying altered behavior, immune and endocrine responses and giving lower yields of milk.

This study was undertaken to investigate changes in the behavioral, immune and endocrine

responses, and production performance of lactating ewes as affected by: 1) two ventilation regimens varying in ventilation rate (35 vs 70 m³/h per ewe), being equal the length of ventilation cycles, and 2) two ventilation regimens which varied in air speed (2 vs 4 m/s) and the length of ventilation cycles (30 vs 60 min/cycle), being equal the ventilation rate.

Material and methods

Experimental design and animal management.

The experiment, which lasted 6 weeks, was conducted during the summer (June-July) of 2002. Thirty-six lactating Comisana ewes (d 176 ± 2.24 of lactation, mean ± SE) were used. Ewe health was checked at the start of the experiment and throughout the study period. The animals were divided into three groups of 12 each, which were balanced for age, parity, time of lambing, number of lambs suckled, body weight (55.19±2.44 kg), body condition score (2.31±0.14), milk yield (539±25 g/d), and milk protein (6.31±0.12%) and fat (7.56±0.18%) contents. Groups were separately housed on straw litter in 8m x 3m and 3.5m high rooms of the same building. The experimental rooms were adjacent, faced south, away from prevailing winds and were provided with transom windows (total glazed area = 6 m²), placed at a height of 2.5 m. Ewes could freely move within each room, which was provided with a negative-pressure mechanical system of ventilation, in which 0.28 m² suction fans (Vortice, 20067 Tribiano, Milan, Italy) were placed at 2.5 m from the floor and two 0.36 m² air inlets were placed at ground level on the opposite wall. In all rooms, fans provided 10 ventilation cycles per day; seven cycles were during daytime from 1000 to 2000 and three during night-time at 2100, at 0100 and at 0500. The three treatments were low ventilation (LOV-30), moderate ventilation with short ventilation cycles at high air speed (MOV-30) and moderate ventilation with long ventilation cycles at low air speed (MOV-60). In the LOV-30 room 30 min ventilation cycles were provided at a fan speed of 2 m/s. In the MOV-30 room 30 min ventilation cycles were provided at a fan speed of 4 m/s, while in the MOV-60 room 60 min ventilation cycles

were provided at a fan speed of 2 m/s. In all rooms, ventilation rate was checked daily by placing a hot wire anemometer (LSI, I-20090, Settala Premenugo, Milan, Italy) over the air outlet and converting readings to m³/h per ewe. The fans provided a mean ventilation rate of 35 m³/h per ewe in the LOV-30 room and 70 m³/h per ewe in the MOV-30 and MOV-60 rooms.

The air temperature and the relative humidity inside each room were continuously monitored through the trial, and during the week before the commencement of the experiment, in order to be sure that climatic conditions were the same in all rooms. Thermo-hygrographs TIG2-TH (LSI) were used, and were placed at a height of 1.5 m from the floor. Averages of air temperature and relative humidity were 27.6, 27.9 and 27.5 °C and 50, 52.3 and 53.4% in LOV-30, MOV-30 and MOV-60 rooms, respectively, during the pre-treatment week. Data from thermo-hygrographs and the Kelly and Bond's (1971) formula were used to calculate the temperature-humidity index (THI). In each pen, a layer of straw (about 0.4 kg/m²) was strewn on litter daily.

Each pen was provided with 2 mangers and a crib; feeder space per animal was about 0.45 m. The ewes were offered daily 1 kg of a pelleted concentrate and 1.8 kg vetch/oat hay, which were given in two meals a day (0730 and 1500). The chemical composition of dry matter was determined by standard procedures (AOAC, 1990). The pelleted concentrate contained 23.4% crude protein, 3.1% fat (by ether extraction), 30.3% NDF, 13.4% ADF, 2.3% ADL, 8.3% ash and 0.94 Milk Forage Unit/kg, while the hay contained 12.7% crude protein, 1.3% fat, 61.1% NDF, 37.7% ADF, 5.9% ADL, 9.3% ash and 0.52 Milk Forage Unit/kg. DM intakes were calculated daily as the difference between the amount of feed offered and feed refusals. The ewes consumed their daily ration of pelleted concentrate completely, while averages of daily DM intakes of vetch/oat hay were 0.98, 0.99 and 1.02 kg in the LOV-30, MOV-30 and MOV-60 groups, respectively. Mean daily energy and crude protein intakes were 1.39, 1.40 and 1.41 MFU/ewe and 344, 346 and 350 g/ewe, in the LOV-30, the MOV-30 and the MOV-60 group, respectively. Water was available from automatic drinking troughs.

Air sampling

Air sampling was performed twice weekly both in the morning, starting from 0900, and in the afternoon, starting from 1630. Air was sampled on the same day in each room at 0.6 m height above the floor and the sequence of air sampling in the three experimental rooms changed according to a prearranged program. Air concentrations of mesophilic microorganisms, coliforms, yeasts and molds, of total (particulate size > 5 µm) and respirable (particulate size = 2-5 µm) dust, and of carbon dioxide, hydrogen sulphide, ammonia and methane were measured, as described in a previous note (Sevi *et al.*, 2002).

Rectal temperatures and respiration rate

Respiration rate (RR) and rectal temperature (RT) were monitored in all animals throughout the trial at 0830 and at 1400. RR was recorded by a trained observer by counting the rate of flank movement and soon after RT was measured with TM46 electronic thermometers (LSI) accurate to 0.1 °C.

Behavioral observations

Behavioral observations were recorded twice weekly by two trained observers equipped with GR-AX 40 video cameras (JVC-Italia, Segrate-Milan, Italy). Scan samples were taken every 10 min from 0900 to 1200 and from 1500 to 1800, in order to assess ewe responses to rising temperatures and the thermal peak. At each observation period the number was recorded of animals engaged in each of two postures (standing or lying) and of eight behavior categories, which were eating, drinking, ruminating, walking, self-grooming, idling (i.e., animals were inactive and judged subjectively to be inattentive or phlegmatic), social and aggressive interactions. The number of animals involved in each behavior item was expressed as a percentage of the total number of animals in the group. Social activities (smelling, nuzzling and rubbing each other) and aggressive interactions (butting, threat jumping, shoulder pushing) are short lasting events; therefore their frequency of presentation was measured by continuous recording for the whole 3-hour periods.

Immune response

The phytohemagglutinin (PHA) skin test was performed to induce non-specific delayed-type hypersensitivity. At d 3, 21 and 42 of the experiment, 1 mg of PHA (Sigma-Aldrich Italia, Milan, Italy) dissolved in 1 ml of sterile saline solution was injected intra-dermally into the middle of two 2 cm wide circles stamped on shaved skin in the upper side of each shoulder. The skinfold thickness was determined before PHA injection and 24 h after with a caliper. For each animal, an average increase in skinfold thickness (24 h post-injection thickness - pre-injection thickness) was computed using the two measurements taken from each shoulder.

At d 2 of the study, 6 mg of chicken egg albumin (Sigma-Aldrich Italia) dissolved in 1 ml of sterile saline solution and in 1 ml of incomplete Freund's adjuvant (Sigma-Aldrich Italia) were injected subcutaneously in both shoulders of each ewe. A second injection without adjuvant was repeated 8 days later. Antibody titers were assayed by the ELISA method, as previously described (Sevi *et al.*, 2002). The inter- and intra-assay CV were 6.9 and 3.2%, respectively. The assay was optimized in our laboratory for concentrations of coating antigen, serum and detector antibody.

Cortisol levels

At d 37 ewes were intravenously injected with 2 IU porcine ACTH/kg body weight^{40.75} (Sigma-Aldrich Italia). Blood samples (10 ml) for evaluation of cortisol concentrations were collected in vacuum tubes from the jugular vein immediately before and 1, 2 and 4 h after ACTH injection. The ACTH dose was chosen on account of previous experiments conducted in beef heifers (Fisher *et al.*, 1997) and dairy ewes (Sevi *et al.*, 2002), while blood sampling times were chosen taking into account the expected peak of plasma cortisol concentration (Verkerk *et al.*, 1998). Hormone concentration was determined by a radioimmunoassay (ICN Biomedicals, Costa Mesa, CA). Validation for sheep plasma was performed as described by Fisher *et al.* (1997). Samples obtained from sheep and run in the same assay as the standard provided (human Plasma) yielded highly correlated results ($r = 0.97$). The sensitivity of the assay was

0.15 µg/dl. The inter-and intra-assay variation coefficients were 6.8 and 5.9 % respectively.

All procedures were conducted according to the guidelines of the Italian Legislative Decree 116 of 27 January 1992 in observance of the Council Directive 86/609/EEC of 24 November 1986 on the protection of animals used for experimental and other scientific purposes. In particular, for blood sampling each ewe was swiftly caught to minimize any excitement due to chasing and catching. There were always two trained persons involved in taking jugular blood samples. One person kept the animal and securely pulled the ewe's head to the side to stretch his neck gently. The second person obstructed jugular blood flow by applying some pressure with the thumb in order to engorge the vein before puncturing it with a sterile needle.

Sampling and analyses of milk

Ewes were milked twice daily (0700 and 1430) using pipeline milking machines (Alfa Laval Agri, SE-147 21 Tumbas, Sweden). Milk yield was recorded daily and individual milk samples were analyzed weekly for pH, total protein, fat and lactose content using an i.r. spectrophotometer (Milko Scan 133B; Foss Electric, Hillerød, Denmark) according to the IDF (1990) standard, casein content (IDF, 1964), renneting characteristics (clotting time, rate of clot formation and clot firmness after 30 min) using a Foss Electric Formagraph and the method of Zannoni and Annibaldi (1981), and somatic cell count (SCC) using a Foss Electric Fossomatic 90 cell counter (IDF, 1995). The milk coagulating index (CoI) was calculated as the clot firmness to clotting time + rate of clot formation ratio.

Table 1. Averages of air temperature, relative humidity and temperature-humidity index (THI) as affected by a low ventilation regimen (LOV-30), and moderate ventilation regimens providing short (MOV-30) and long ventilation cycles (MOV-60). Values are means \pm SD.

Item	Week	LOV-30 (35 m ³ /h/ewe)		MOV-30 (70 m ³ /h/ewe)		MOV-60 (70 m ³ /h/ewe)	
		Maximum	Minimum	Maximum	Minimum	Maximum	Minimum
Air temperature, °C	1	28.9 \pm 1.1	22.4 \pm 1.7	28.5 \pm 1.9	21.4 \pm 2.0	27.0 \pm 1.2	21.1 \pm 1.8
	2	31.5 \pm 1.7	22.8 \pm 1.1	31.1 \pm 1.5	21.4 \pm 1.2	28.9 \pm 1.5	21.6 \pm 0.9
	3	31.6 \pm 2.9	23.6 \pm 0.6	31.4 \pm 2.4	22.1 \pm 1.8	29.1 \pm 1.5	22.0 \pm 1.2
	4	30.6 \pm 2.7	23.6 \pm 1.4	30.6 \pm 2.8	22.4 \pm 0.6	28.8 \pm 2.5	22.9 \pm 0.9
	5	29.4 \pm 2.7	22.6 \pm 1.5	29.4 \pm 2.4	21.5 \pm 1.6	26.9 \pm 3.0	22.0 \pm 1.5
	6	25.0 \pm 4.2	21.7 \pm 1.9	24.7 \pm 4.3	20.6 \pm 1.5	24.3 \pm 3.4	21.0 \pm 1.6
Relative humidity, %	1	74.3 \pm 10.9	65.4 \pm 9.3	70.3 \pm 8.5	63.9 \pm 7.1	73.7 \pm 9.1	65.9 \pm 9.3
	2	68.6 \pm 6.1	50.6 \pm 4.2	67.4 \pm 4.0	48.7 \pm 2.2	66.3 \pm 3.9	50.6 \pm 3.7
	3	71.3 \pm 11.1	65.1 \pm 7.0	67.0 \pm 9.0	63.8 \pm 4.5	66.9 \pm 9.1	65.1 \pm 4.1
	4	56.5 \pm 10.0	41.0 \pm 6.8	52.1 \pm 12.1	39.4 \pm 4.4	53.1 \pm 2.7	42.1 \pm 7.3
	5	57.9 \pm 12.2	49.6 \pm 6.1	53.3 \pm 12.4	46.7 \pm 3.0	53.2 \pm 12.4	46.3 \pm 6.8
	6	67.2 \pm 12.1	59.3 \pm 5.8	61.3 \pm 10.2	56.9 \pm 5.6	61.1 \pm 10.2	56.4 \pm 8.3
THI ¹	1	79.1 \pm 1.4	69.6 \pm 2.7	78.6 \pm 1.3	68.0 \pm 3.0	76.5 \pm 1.3	67.8 \pm 2.7
	2	80.5 \pm 2.4	70.4 \pm 1.6	79.8 \pm 2.7	68.2 \pm 1.7	77.3 \pm 2.5	68.6 \pm 1.4
	3	83.2 \pm 2.0	71.4 \pm 1.0	82.0 \pm 1.5	69.2 \pm 2.5	79.0 \pm 1.8	69.0 \pm 1.7
	4	77.6 \pm 3.6	70.3 \pm 2.0	77.5 \pm 3.1	68.5 \pm 0.8	75.7 \pm 3.8	69.2 \pm 1.3
	5	77.1 \pm 3.6	68.6 \pm 2.1	77.8 \pm 3.2	66.9 \pm 1.9	74.2 \pm 4.3	67.6 \pm 2.0
	6	73.1 \pm 5.9	68.2 \pm 2.8	73.8 \pm 5.9	66.5 \pm 2.2	71.8 \pm 4.7	67.1 \pm 2.8

¹ Calculated using the Kelly and Bond's (1971) formula.

At the beginning of the trial, and fortnightly during the study period, the following bacteriological analyses were carried out on milk: enumeration of mesophilic bacteria (IDF, 1991b), psychrotrophs (IDF, 1991a), total and fecal coliforms (IDF, 1985).

The body weights and body condition scores of the ewes (a six-point scale with 0 = thin and 5 = fat) were recorded at the beginning, at d 21 and 42 of the study period, after the morning milking but before feeding.

Statistical analysis

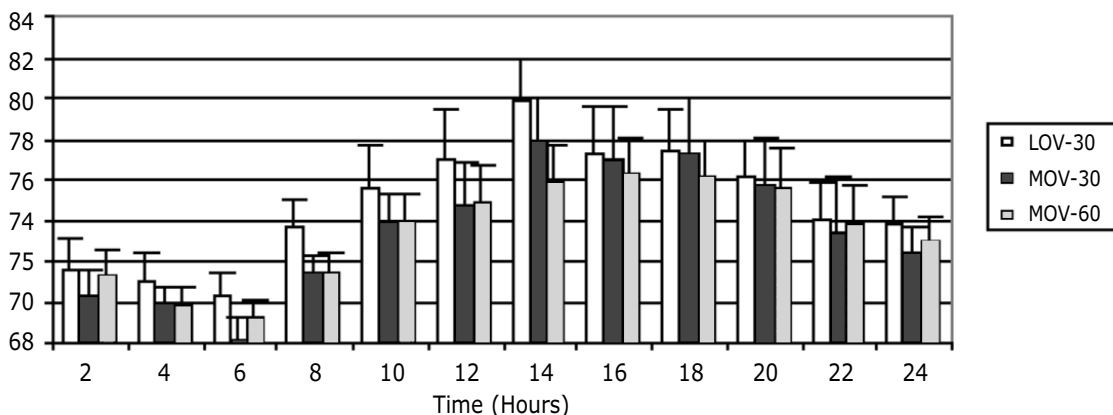
All the variables were tested for normal distribution using the Shapiro-Wilk test (Shapiro and Wilk, 1965). Behavioral data, antibody titers and milk SCC and air and milk microorganism counts were transformed into logarithmic form to normalize their frequency distributions before performing statistical analysis. Milk and air variables were processed using ANOVA for repeated measures (SAS, 1999). The variation due to treatment, trial week and their interaction was tested. Individual animal variation within treatment or sampling location within room was used as the error term. Rise in plasma cortisol levels after ACTH injection, body weights (BW), body weight changes and body condition scores (BCS) were analyzed using ANOVA with one factor (treatment). When significant effects ($P < 0.05$) were found the Student's *t* test was used to locate significant differences between means.

Results and discussion

Indoor climate and air quality

Averages of maximum air temperatures exceeded 30 °C during weeks 2, 3 and 4 in the LOV-30 and the MOV-30 rooms and were 2 to 3 °C higher than those recorded in the MOV-60 room during the first five trial weeks (Table 1). Instead, changes in weekly averages of minimum air temperature across treatments were very small; differences rarely exceeded 1 °C between the LOV-30 and the MOV-60 rooms and were always below this threshold between the LOV-30 and the MOV-30 rooms. Weekly averages of relative humidity were similar among rooms; averages values of the whole experimental period were 61.3, 58.9 and 59.9% in the LOV-30, the MOV-30 and the MOV-60 rooms, respectively. As a result, the more marked differences among treatments were observed in weekly averages of maximum THI. In particular, THI values very near or over 80 were recorded in the LOV-30 and the MOV-30 rooms during weeks 2 and 3, which were 3 to 4 points higher than those recorded in the MOV-60 room. Diurnal changes in THI (Figure 1) show that during the morning (0600 to 1200) about 2 point higher values were recorded in the LOV-30 than in the other two rooms, but, when air temperature peaked (around 1400), THI was about 3 and 4 points higher in the MOV-30 and the LOV-30 than in the MOV-60 room. This suggests that the MOV-60 treatment

Figure 1. Circadian changes in temperature-humidity index (THI) as affected by a low ventilation regimen (LOV-30), and moderate ventilation regimens providing short (MOV-30) and long ventilation cycles (MOV-60). Values are means \pm SD.



provided a more efficient control of air temperature and relative humidity as compared to both the LOV-30 and the MOV-30 regimens during the warmest part of the day.

Ventilation has direct and indirect effects on air quality in animal houses. The direct effect is that ventilation lowers air temperature and removes from the house air the moisture and gaseous pollutants arising from the respiratory activity of the animals and the decomposition and fermentation of manure (Wathes, 1992; Charles, 1994). The indirect effect is that increased air temperature and relative humidity, due to poor ventilation, enhance the growth and multiplication of microorganisms in the air and in the litter (Wathes *et al.*, 1983; Dodd *et al.*, 1984). The lack of significant differences in the concentrations of dust, gaseous pollutants and airborne microorganisms (Table 2) suggests that all the ventilation regimens provided a satisfactory control of the air quality. In fact, aerial dust and microorganism concentrations were quite low in all the experimental rooms, while average ammonia levels were near or little over 10 ppm, which is regarded as the safety threshold for farmed livestock (Verstegen *et al.*, 1994). Only traces of H₂S and CH₄ were found in all the experimental rooms (data not shown). However, it should be noted

that, although no significant differences were found, higher concentrations of dust, gaseous pollutants and microorganisms were recorded in the air of the LOV-30 and the MOV-30 rooms than in that of the MOV-60 room. Irrespective of ventilation regimen, the air concentrations of mesophilic bacteria increased during the last three trial weeks, probably due to the progressive reduction in the absorbing capacity of the litter associated with urine and feces accumulation in the bedding and the trampling by the ewes.

Respiration rate and rectal temperatures

The increase in evaporative heat loss through the respiratory tract is the first of a number of physiological mechanisms the sheep activate to cope with high air temperature (Habeeb *et al.*, 1992). Under these conditions, ventilation helps the animal to balance the increase in exogenous heat input by removing heat from its body surface through convection (Sevi *et al.*, 2002). If heat losses, due to thermoregulatory mechanisms carried out by the animal, fail to attain heat gain, heat is stored with a resultant increase in body temperature (Brosh *et al.*, 1998). The lack of differences in rectal temperature across treatments (Table 3) suggests that, in the present study, neither ventilation rate nor the length of ventilation cycles affect-

Table 2. Least squares means \pm SE of air dust, gaseous pollutants and microorganism concentrations as affected by a low ventilation regimen (LOV-30), and moderate ventilation regimens providing short (MOV-30) and long ventilation cycles (MOV-60).

Item		LOV-30	MOV-30	MOV-60	SE	Effects, P		
		(35 m ³ / h/ewe)	(70 m ³ / h/ewe)	(70 m ³ / h/ewe)		Treat- ment	Time	Treat. x Time
Total dust	mg/m ³	0.70	0.53	0.48	0.10	ns	ns	ns
Respirable dust	"	0.42	0.41	0.30	0.08	ns	ns	ns
NH ₃	ppm	11.7	11.7	8.1	1.81	ns	ns	ns
CO ₂	"	1054	1012	795	133.5	ns	ns	ns
Mesophilic bacteria	log ₁₀ (CFU/m ³)	2.44	2.29	2.35	0.06	ns	***	ns
Yeast and molds	"	1.81	1.84	1.71	0.08	ns	ns	ns

ns: not significant; ***P < 0.001.

ed the ability of ewes to maintain their thermal balance. Similarly, no effects of ventilation regimen on respiration rate were observed. Nevertheless, a treatment x time effect ($P < 0.05$) was found in the RR recorded during the afternoon, which may be

ascribed to the fact that respiration rate significantly increased ($P < 0.05$) in the LOV-30 and the MOV-30 compared to the MOV-60 ewes during week 3 and tended to increase ($P = 0.07$) in the LOV-30 compared to the MOV-60 group during

Table 3. Least squares means \pm SE of respiration rate (RR) and rectal temperature (RT) of ewes subjected to a low ventilation regimen (LOV-30), and to moderate ventilation regimens providing short (MOV-30) and long ventilation cycles (MOV-60).

Item	Week	LOV-30	MOV-30	MOV-60	SE	Effects, P		
		(35 m ³ /h/ewe)	(70 m ³ /h/ewe)	(70 m ³ /h/ewe)		Treat- ment	Time	Treat. x time
Respiration rate in the morning, breath/min	1	69	56	59	8.7	ns	***	ns
	2	51	50	42				
	3	55	51	51				
	4	55	50	50				
	5	50	47	44				
	6	48	46	44				
Respiration rate in the afternoon, breath/min	1	83	81	73	10.1	ns	*	*
	2	98	91	69				
	3	118a	116a	84b				
	4	83	80	77				
	5	85	80	76				
	6	62	63	59				
Rectal temperature in the morning, °C	1	39.0	38.9	38.9	0.14	ns	*	ns
	2	38.8	38.8	38.7				
	3	38.9	38.8	38.8				
	4	38.9	38.8	38.8				
	5	38.8	38.8	38.7				
	6	38.7	38.7	38.6				
Rectal temperature in the afternoon, °C	1	39.6	39.5	39.5	0.18	ns	***	ns
	2	39.6	39.6	39.4				
	3	39.9	39.8	39.6				
	4	39.6	39.5	39.5				
	5	39.5	39.4	39.4				
	6	39.0	39.0	39.0				

ns: not significant; * $P < 0.05$; *** $P < 0.001$. Means followed by different letters differ significantly at $P < 0.05$.

week 2 of the experiment. This suggests that, during the warmest part of the trial, the LOV-30, and the MOV-30 ewes to a lesser extent, had more difficulty maintaining their thermal balance compared to the MOV-60 ewes when air temperature and THI peaked in the early afternoon. Therefore, this study shows that, with a pending high heat load situation, the ventilation efficacy in removing heat from the animals' body surface was not improved by increasing air speed from 2 to 4 m/s, whereas ewes benefited from doubling the time the air flow lapped on their body surface. Significant effects of time were found for RR and RT both in the morning and in the afternoon. In particular, a higher RR was recorded in the first week than in the fifth and the sixth week ($P < 0.05$) during the

morning, and in the second ($P < 0.01$) and the third ($P < 0.001$) weeks than in sixth week during the afternoon. Relative to RT, higher values were recorded in the first week ($P < 0.05$) than in sixth week during the morning, and in the third week compared to the fifth ($P < 0.05$) and the sixth weeks ($P < 0.001$) during the afternoon.

Behavior

No differences were found in the behavioral activities during the morning (Table 4), except for a significant increase ($P < 0.05$) in the time spent lying in the LOV-30 compared to the MOV-60 ewes. Significant interactions of treatment x time ($P < 0.05$) were found in the time the ewes spent lying, idling, eating and drinking during the after-

Table 4. Least squares means \pm SE of time spent in each of behavioral categories in the morning (0900-1200) and in the afternoon (1500-1800) by ewes when subjected to a low ventilation regimen (LOV-30), and to moderate ventilation regimens providing short (MOV-30) and long ventilation cycles (MOV-60). Data are presented as % of animals involved in each behavior item on the total number of animals in the group.

Item	Day period	LOV-30	MOV-30	MOV-60	SE	Effects, P		
		(35 m ³ /h/ewe)	(70 m ³ /h/ewe)	(70 m ³ /h/ewe)		Treatment	Time	Treatment x Time
Lying	Morning	34a	22	19b	4	*	**	ns
	Afternoon	22	21	17	5	ns	***	*
	Mean	28	22	18	4	ns	***	ns
Idling	Morning	64	62	54	6	ns	ns	ns
	Afternoon	52	50	43	6	ns	***	*
	Mean	58	56	48	6	ns	**	ns
Eating	Morning	27	31	38	7	ns	ns	ns
	Afternoon	39	44	52	6	ns	***	*
	Mean	33	37	45	6	ns	***	ns
Drinking	Morning	2	2	2	1	ns	**	ns
	Afternoon	2	2	1	1	ns	***	*
	Mean	2	2	2	1	ns	***	ns
Ruminating	Morning	2	2	2	1	ns	ns	ns
	Afternoon	4	2	2	1	ns	***	ns
	Mean	3	2	2	1	ns	*	ns
Walking	Morning	3	2	3	1	ns	ns	ns
	Afternoon	1	1	1	1	ns	***	ns
	Mean	2	2	2	1	ns	***	ns

ns: not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Means followed by different letters differ significantly at $P < 0.05$.

noon. When the combined effects of ventilation regimens and time of observation were investigated, a marked increase was found, during the third trial week, in the time the LOV-30 and the MOV-30 ewes spent lying and idling compared to the MOV-60 ewes (43 and 32% vs 8.3%; 83 and 81% vs 43%, respectively), which was associated with a marked reduction in the time the LOV-30 and the MOV-30 ewes spent eating (8 and 11% vs 52%, respectively). Given that DMI was substantially similar across treatments, the reduction in eating time observed in the LOV-30 and the MOV-30 groups during afternoon may be ascribed to the change in the time of feeding to evening and night the LOV-30 and MOV-30 ewes used as a strategy to reduce their heat load during the warmest hours of the day (Brosh *et al.*, 1998). Increase in inactive behaviors, such as lying and idling, may be also interpreted as a strategy by which the LOV-30 and the MOV-30 animals aimed to reduce their heat production under high air temperatures. Indeed, decreased levels of activity have been found to have a definite thermoregulatory purpose in sheep (Sevi *et al.*, 2001a; Sevi *et al.*, 2002). The fact that, irrespective of ventilation regimen, the ewes had longer eating times, and shorter lying and idling times, in the afternoon than in the morning may seem conflicting with previous considerations. However, the fact that the afternoon observations started soon after feed administration, and the morning ones about 1 hour and a half after the feed had been offered, could account for the ewes being observed more active in the afternoon than in the morning. Therefore, also behavioral data suggest that, irrespective of ventilation rate, the ewes subjected to shorter ventilation cycles tried harder to cope with heat loads during the warmest period of the trial as compared to the ewes which benefited from longer ventilation cycles.

No differences emerged in social and aggressive interactions, which were only sporadically recorded in all groups.

Time effects were found for almost all the behavioral traits recorded, which were mainly due to a significant increase in the time the ewes spent lying, idling, drinking and ruminating during the middle of the study period (primarily in weeks 3

and 4), and to a concurrent reduction in the time the animals spent eating and walking.

Immune and cortisol responses

The generation of inflammatory mediating lymphocytes in response to different mitogens and antigens is regarded as a convenient indicator of an animal's ability to mount active humoral and cell-mediated immune responses (Burton *et al.*, 1989). A suppressive effect of stressful conditions on immunological competence in farmed livestock is well documented (Mallard *et al.*, 1982; Minton, 1994). As well, the increase in the plasma cortisol levels, as a consequence of the activation of the hypothalamic-pituitary-adrenal axis, is one of the best-known and consistent neuroendocrine responses to stress (Hashizume *et al.*, 1994). In particular, in the welfare assessment of farmed animals, the administration of exogenous ACTH aims to stimulate the adrenal secretion of cortisol, whose release may be strengthened by the existence of concurrent stressful events (Sevi *et al.*, 2002). Data in Table 5 show that ventilation regimen did not significantly affect the humoral and cell-mediated immune responses of the ewes throughout the experiment. However, when subjected to the PHA injection at the end of the third and warmest week of the study period, the LOV-30 ewes displayed a 30% reduction in their skinfold thickness compared to the values recorded at the beginning of the trial, while only a 15% decrease in the cell-mediated immune response was observed in the MOV-30 and the MOV-60 ewes.

A significant effect of time ($P < 0.001$) was observed for antibody titers to OVA (Table 5) as a consequence of the fact that IgG concentrations increased in all groups after the second antigen injection performed at 10 d.

Pre-ACTH cortisol levels were 6.64, 8.65 and 6.78 $\mu\text{g/dl}$ in the LOV-30, MOV-30 and MOV-60 groups. Data in Table 5 show that the hormone concentration peaked 60 min after the ACTH injection. At this time plasma cortisol levels increased by 70% more ($P < 0.001$) in the blood of the LOV-30 ewes compared to the MOV-60 animals (Table 5). Cortisol concentrations then slightly declined in all groups, but the LOV-30 group continued to exhibit a greater rise ($P < 0.01$) in the

hormone levels compared to the MOV-60 group 120 min after the ACTH injection. The MOV-30 ewes had intermediate values at each sampling time. This suggests that the hypothalamic-pituitary-adrenal axis of all ewes was activated by ACTH administration, and by capture, handling, and venipuncture, but the physiological disturbance from reduced ventilation acted as an additional stress in the LOV-30 ewes, which resulted in a higher cortisol level and a slower decrease to baseline values.

Milk yield and quality

A significant treatment x time effect was found for ewe milk yield (Table 6). The significant reduction (-22%, $P < 0.05$) in the milk production, which was observed in the LOV-30 compared to the MOV-60 group during week 3, can account for this, together with the less marked, and not significant, drop (about -10%) of milk yield observed in the LOV-30 and the MOV-30

compared to the MOV-60 ewes during weeks 4 and 6 of the study period. Depression in appetite and increased energy demand for thermoregulation are regarded as the main factors of milk yield reduction in animals exposed to high ambient temperatures (Habeeb *et al.*, 1992). However, feed intake did not substantially change among groups during the present trial. Hence, the lowering of milk production in the LOV-30, and in the MOV-30 ewes to a lesser extent, has to be primarily ascribed to a greater energy waste for thermoregulation at the expense of milk synthesis (Bauman and Currie, 1980). Indeed, high heat loads may lead to energy deficit, even when they do not induce a marked reduction of feed intake in animals, because it has been estimated that only the rise in respiration rate may result in a 7 to 25% increase in energy requirements for maintenance in animals exposed to high air temperatures (NRC, 1981).

Differences in milk yield were inversely relat-

Table 5. Least squares means \pm SE of skinfold thickness after PHA injection, of antibody response to chicken egg albumin injection (OVA), and of rise in plasma cortisol levels after porcine ACTH injection in ewes subjected to a low ventilation regimen (LOV-30), and to moderate ventilation regimens providing short (MOV-30) and long ventilation cycles (MOV-60).

Item	Sampling time	LOV-30 (35 m ³ / h/ewe)	MOV-30 (70 m ³ / h/ewe)	MOV-60 (70 m ³ / h/ewe)	SE	Effects, P		
						Treatment	Time	Treatment x time
Skinfold thickness, mm/d	3 d	6.82	6.96	6.85				
	21 d	4.76	5.88	5.76				
	42 d	6.27	6.32	6.15	0.36	ns	***	ns
Antibody titer to OVA, log ₁₀ (x+1)	2 d	0.18	0.16	0.17				
	10 d	0.25	0.23	0.21				
	21 d	0.31	0.32	0.30				
	31 d	0.30	0.29	0.29				
	42 d	0.29	0.28	0.28	0.02	ns	***	ns
Rise in plasma cortisol levels after ACTH-injection, μ g/dl	60 min after	24.15a	18.81	14.03b	2.3	*		
	120 min after	8.55a	5.34	3.25b	1.2	*		
	240 min after	2.12	0.30	0.21	0.7	ns		

ns: not significant; * $P < 0.05$; *** $P < 0.001$.

ed to changes in milk fat content (Table 6). In fact, the MOV-60 milk had significantly lower fat contents than the LOV-30 milk during week 3 ($P < 0.01$) and than the LOV-30 and the MOV-30 milk during week 6 of the study period ($P < 0.001$). This supports the hypothesis that the reduction in milk fat content observed in the MOV-60 group can be

the outcome of a dilution effect, due to the greater volumes of milk yielded.

No differences, instead, emerged in the milk protein and casein contents (Table 6). It is likely that the relatively high energy and protein intakes of the ewes, enhancing the protein flow to the udder, minimized the effect of different productive

Table 6. Least squares means \pm SE of yield and protein, casein and fat content of ewe milk as affected by a low ventilation regimen (LOV-30), and moderate ventilation regimens providing short (MOV-30) and long ventilation cycles (MOV-60).

Item	Week	LOV-30 (35 m ³ / h/ewe)	MOV-30 (70 m ³ / h/ewe)	MOV-60 (70 m ³ /h /ewe)	SE	Effects, P		
						Treatment	Time	Treatment x time
Milk yield, g/d								
	1	753	747	760				
	2	705	712	748				
	3	648b	767	833a				
	4	717	737	795				
	5	820	827	843				
	6	767	772	860	50.5	ns	***	*
Protein content, %								
	1	6.06	5.80	5.99				
	2	6.20	5.96	6.14				
	3	6.04	5.74	5.97				
	4	5.94	5.68	5.83				
	5	5.92	5.72	5.75				
	6	6.45	6.01	6.10	0.29	ns	***	ns
Casein content, %								
	1	4.71	4.69	4.73				
	2	5.25	4.49	5.13				
	3	4.93	4.50	4.59				
	4	4.62	4.42	4.52				
	5	4.94	4.41	4.57				
	6	4.97	4.54	4.88	0.25	ns	***	ns
Fat content, %								
	1	6.19	6.10	6.06				
	2	5.88	5.57	5.39				
	3	5.81a	5.25	4.89b				
	4	6.43	6.18	5.87				
	5	6.31	6.03	6.00				
	6	6.79a	6.57a	5.42b	0.21	**	**	ns

ns: not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Means followed by different letters differ significantly at $P < 0.05$.

levels on milk protein and casein contents.

Significant interactions of treatment x time ($P < 0.05$) were found in the clot formation time, the rate of clot formation and the clot firmness of milk (Table 7). This depended on the fact that the milk from the LOV-30 ewes underwent a significant worsening of coagulating behavior compared to the MOV-60 milk during the second half of the trial (data not shown). Indeed, when the renneting parameters were gathered in the milk coagulating index (CoI), significant differences ($P < 0.05$) were found between the LOV-30 and the MOV-60 group during week 4 (1.72 vs 3.14 mm/min), 5 (1.96 vs 3.45 mm/min) and 6 (2.02 vs 3.32 mm/min) of the study period (Figure 2). This event is not easy to interpret in light of the higher milk fat content observed in the less ventilated group, and the lack of differences in milk protein and casein contents, and in the casein to protein ratio. A tentative explanation could be found in a possible reduction of milk calcium and phosphorus contents, which play a prominent role in milk coagulation (Remeuf, 1994). Sevi *et al.* (2003a) observed a decrease of calcium and phosphorus contents in ewe summer milk, which was associated to a marked worsening of the milk coagulating behavior.

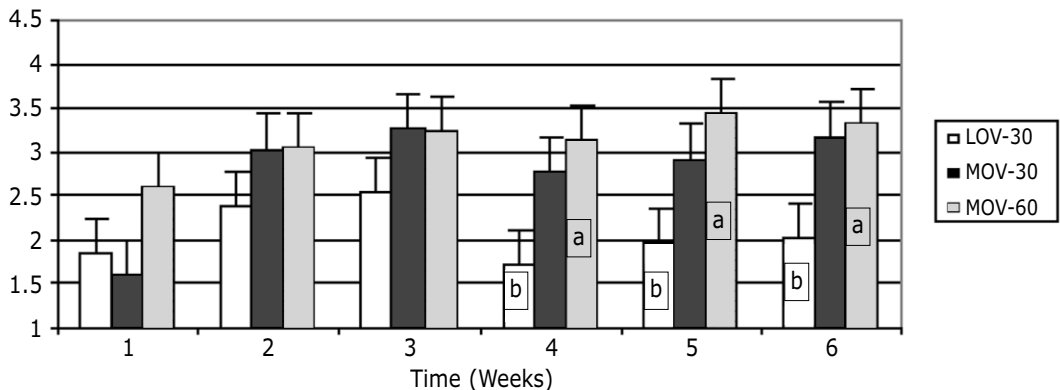
In a previous experiment (Sevi *et al.*, 2002), keeping the air speed constantly at 4 m/s, and

varying the length of ventilation cycles from 12.5 to 25 min, in order to provide ventilation rates of 33 and 66 m³/h per animal during summer, a deleterious effect of the lower ventilation regimen on ewe behavioral, immune and endocrine responses and on milk yield was observed. In the present study, in spite of the differences in ventilation rate (35 vs 70 m³/h) and air speed (2 vs 4 m/s), the ewes in the LOV-30 and the MOV-30 groups displayed comparable response. On the other hand, the MOV-60 ewes were found to benefit from long ventilation cycles, which help them to maintain their thermal balance more easily and sustain their production performance, suggesting that the longer the time the air flow removes heat from ewe body surface the greater is the benefit for its well-being.

Ventilation regimen did not affect the hygienic quality of milk (Table 7), except for the mesophilic count, which was significantly higher ($P < 0.05$) in the LOV-30 than in the MOV-30 and the MOV-60 ewes. This was probably related partly to the slightly higher air concentrations of mesophilic bacteria and partly to the smaller volume of milk yielded by the less ventilated group.

No significant differences were found in body weight, body weight changes and body condition scores of the ewes, although the MOV-60 animals were the quickest and the LOV-30 ones the slowest in restoring their body reserves. In fact, aver-

Figure 2. Least squares means \pm SE of coagulating index (CoI)¹ in ewe milk as affected by a low ventilation regimen (LOV-30), and moderate ventilation regimens providing short (MOV-30) and long ventilation cycles (MOV-60).



¹ Calculated as the clot firmness to the clot formation time + rate of clot formation ratio.

Table 7. Least squares means \pm SE of somatic cell count (SCC), renneting parameters and bacteria count in ewe milk as affected by a low ventilation regimen (LOV-30), and moderate ventilation regimens providing short (MOV-30) and long ventilation cycles (MOV-60).

Item		LOV-30	MOV-30	MOV-60	SE	Effects, P		
		(35 m ³ / h/ewe)	(70 m ³ / h/ewe)	(70 m ³ / h/ewe)		Treatment	Time	Treatment x time
SCC	log ₁₀ cells/ml	5.53	5.53	5.31	0.18	ns	ns	ns
pH		6.62	6.58	6.57	0.04	ns	***	ns
Casein to protein ratio		0.80	0.78	0.79	0.02	ns	***	ns
Clot formation time	min	21.1	18.2	17.2	1.5	ns	***	*
Rate of clot formation	min	3.7	3.2	2.8	0.4	ns	*	*
Clot firmness	mm	40.2	49.6	53.4	4.6	ns	***	*
Mesophilic count	log ₁₀ (CFU/ml)	5.18a	4.89b	4.94b	0.08	*	***	ns
Psychrotroph count	"	4.03	4.22	4.07	0.11	ns	***	ns
Total coliforms	"	2.78	2.80	2.84	0.09	ns	***	ns
Fecal coliforms	"	1.69	1.88	1.85	0.08	ns	***	ns

ns: not significant; *P < 0.05; ***P < 0.0001. Means followed by different letters differ significantly at P < 0.05.

ages of weight gains were 86, 104 and 128 g/d in the LOV-30, the MOV-30 and MOV-60 groups, while body condition scores, at the end of the study period, ranged from 2.52 in the LOV-30 to 2.73 in the MOV-60 group.

Conclusions

Ventilation regimen did not affect the air quality and had a moderate impact on ewe behavior, physiology and production performance. However, the ewes were in their late lactation, as generally occurs in summer in the Northern hemisphere due to sheep reproductive seasonality. In addition, the weather was not very hot during the study period. Both these events probably contributed to minimize the differences across treatments. On the other hand, it should also be noted that doubling air speed from 2 to 4 m/s and ventilation rate from 35 to 70 m³/h per animal did not lead to any significant improvement of ewe well-being and performance in the groups subjected to 30 min venti-

lation cycles. Instead, the ventilation rate being kept at 70 m³/h per animal, the group subjected to short ventilation cycles displayed a more intense activation of thermoregulatory mechanisms during the warmest part of the study period compared to the group benefiting from 60 min ventilation cycles. Therefore, results suggest that, aside from ventilation rate, the choice of proper air speed and length of ventilation cycles are required for optimizing the efficacy of ventilation regimen.

The paper must be attributed equally to the authors.

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REFERENCES

- AOAC, 1990. Official Methods of Analysis, 15th ed. Association of Official Analytical Chemists, Washington, DC, USA.
- BAUMAN, D.E., CURRIE, B.A., 1980. Partitioning nutrients during pregnancy and lactation: a review of mechanism involving homeostasis and homeorhesis. *J. Dairy Sci.* 63: 1514-1529.
- BROSH, A., AHARONI, Y., DEGEN, A.A., WRIGHT, J., YOUNG, B.A., 1998. Effects of solar radiation, dietary energy and time of feeding on thermoregulatory responses and energy balance in cattle in a hot environment. *J. Anim. Sci.* 76: 2671-2677.
- BURTON, J.L., BURNSIDE, E.B., KENNEDY, B.W., WILKIE, B.N., BURTON, J.H., 1989. Antibody responses to human erythrocytes and ovalbumin as marker traits of disease resistance in dairy calves. *J. Dairy Sci.* 72: 1252-1265.
- CHARLES, D.R., 1994. Comparative climatic requirements. In: C.M. Wathes and D.R. Charles (eds.) *Livestock Housing*. CAB International, Wallingford, UK, pp 3-24.
- DODD, F.H., HIGGS, T.M., BRAMLEY, A.J., 1984. Cubicle management and coliform mastitis. *Vet. Record* 114: 522-523.
- EUROPEAN COMMUNITIES 1986. Council Directive 86/609/EEC of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes, pp. 1-28. Official Journal L 358, European Communities Publication office: Luxembourg.
- FISHER, A.D., CROWE, M.A., PRENDIVILLE, D.J., ENRIGHT, W.J., 1997. Indoor space allowance: Effects on growth, behaviour, adrenal and immune responses of finishing beef heifers. *Anim. Sci.* 64: 53-62.
- HABEEB, A.A. M., MARAI, I.F.M., KAMAL, T.H., 1992. Heat Stress. In: C. Phillips and D. Piggins (eds.) *Farm Animals and the Environment*. CAB International, Wallingford, UK, pp. 27-47.
- HASHIZUME, T., HAGLOF, S.A., MALVEN, P.V., 1994. Intracerebral methionine-enkephalin, serum cortisol, and serum β -endorphin during acute exposure of sheep to physical or isolation stress. *J. Anim. Sci.* 72: 700-708.
- IDF, 1964. Determination of the casein content of milk. FIL-IDF Standard no. 29, Brussels, Belgium.
- IDF, 1985. Enumeration of coliforms. FIL-IDF Standard no. 73A, Brussels, Belgium.
- IDF, 1990. Determination of milk fat, protein & lactose content - Guide for the operation of mid-infrared instruments. FIL-IDF Standard no. 141B, Brussels, Belgium.
- IDF, 1991a. Enumeration of psychrotrophic microorganisms - Colony count at 6.5 °C. FIL-IDF Standard no. 101A, Brussels, Belgium.
- IDF, 1991b. Enumeration of microorganisms - Colony count at 30 °C. FIL-IDF Standard no. 100B, Brussels, Belgium.
- IDF, 1995. Enumeration of somatic cells. FIL-IDF Standard no. 148A, Brussels, Belgium.
- KELLY, C.F., BOND, T.E., 1971. *Bioclimatic Factors and their Measurement: a Guide to Environmental Research on Animals*. National Academy of Sciences, Washington, DC, USA.
- MALLARD, B.A., BURNSIDE, E.B., BURTON, J.H., WILKIE, B.N., 1982. Variation in serum immunoglobulins in Canadian Holstein-Friesian. *J. Dairy Sci.* 66: 862-866.
- MINTON, J.E., 1994. Function of the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system in models of acute stress in domestic farm animals. *J. Anim. Sci.* 72: 1891-1898.
- NRC, 1981. Effect of environment on nutrient requirements of domestic animals. National Academy Press, Washington, DC, USA.
- QI, R., MANBECK, H.B., MAGHIRANG, R.G., 1992. Dust net generation rate in a poultry layer house. *Trans. Am. Soc. Agric. Eng.* 35: 1639-1645.
- REMEUF, F., 1994. Relationship between physico-chemical characteristics and cheesemaking properties of milk. *Rec. Med. Vet.* 170: 359-365.
- RYLANDER, R., 1986. Lung diseases caused by organic dusts in the farm environment. *Am. J. Ind. Med.* 10: 221-227.
- SAS. 1999. SAS/STAT User's Guide (Version 8.1). Statistical Analysis System Inst, Cary, NC, USA.
- SEVI, A., ALBENZIO, M., ANNICCHIARICO, G., CAROPRESE, M., MARINO, R., TAIBI, L., 2002. Effects of ventilation regimen on the welfare and performance of lactating ewes in summer. *J. Anim. Sci.* 80: 2349-2361.
- SEVI, A., ALBENZIO, M., MARINO, R., SANTILLO, A., MUSCIO, A., 2003a. Effects of lambing season and stage of lactation on ewe milk quality. *Small Rum. Res.* (In press).
- SEVI, A., ANNICCHIARICO, G., ALBENZIO, M., TAIBI, L., MUSCIO, A., DELL'AQUILA, S., 2001a. Effects of solar radiation and feeding time on behavior, immune response and production of lactating ewes under high ambient temperature. *J. Dairy Sci.* 84: 629-640.
- SEVI, A., TAIBI, L., ALBENZIO, M., ANNICCHIARICO, G., MUSCIO, A., 2001b. Airspace effects on the yield and quality of ewe milk. *J. Dairy Sci.* 84: 2632-2640.
- SEVI, A., TAIBI, L., ALBENZIO, M., CAROPRESE, M., MARINO, R., MUSCIO, A., 2003b. Ventilation effects on air quality and on the yield and quality of ewe milk in winter. *J. Dairy Sci.* (In press).
- SHAPIRO, S.S., WILK, M., 1965. An analysis of variance test for normality. *Biometrika.* 52:591-601.
- SMITH, J.H., WATHES, C.M., BALDWIN, B.A., 1996. The preference of pigs for fresh air over ammoniated air. *Appl. Anim. Behav. Sci.* 49: 417-424.

- SPOOLDER, H.A.M., EDWARDS, S.A., CORNING, S., 2000. Aggression among finishing pigs following mixing in kennelled and unkennelled accommodation. *Liv. Prod. Sci.* 63: 121-129.
- VERKERK, G.A., PHIPPS, A.M., CARRAGHER, J.F., MATTHEWS, L.R., STELWAGEN, K., 1998. Characterization of milk cortisol concentrations as a measure of short-term stress response in lactating dairy cows. *Anim. Welfare.* 7: 77-86.
- VERSTEGEN, M., TAMMINGA, S., GREERS, R., 1994. The effect of gaseous pollutants on animals. In: I. Ap Dewi, R.F.E. Axford, I.F.M. Marai and H. Omed (eds.) *Pollution in livestock production systems.* CAB International, Wallingford, UK, pp. 81-96.
- WATHES, C.M. 1992. Ventilation. In: C. Phillips and D. Piggins (eds.) *Farm Animals and the Environment.* CAB International, Wallingford, UK, pp. 83-91.
- WATHES, C.M., 1994. Air and surface hygiene. In: C.M. Wathes and D.R. Charles (eds.) *Livestock Housing.* CAB International, Wallingford, UK, pp 123-148.
- WATHES, C.M., JONES, C.D.R., WEBSTER, A.J.F., 1983. Ventilation, air hygiene and animal health. *Vet.Rec.* 113: 554-559.
- ZANNONI, M., ANNIBALDI, S., 1981. Standardisation of the renneting ability of milk by Formagraph. *Sci. Tec. Latt. Cas.* 32:79-94.