Ventilation Effects on Air Quality and on the Yield and Quality of Ewe Milk in Winter

A. Sevi,* L. Taibi,† M. Albenzio,* M. Caroprese,* R. Marino,* and A. Muscio*

*Dipartimento PRIME, Facoltà di Agraria, Via Napoli, 25, 71100 Foggia, Italy

†Istituto Sperimentale per la Zootecnia, Via Napoli, 71020 Segezia-Foggia, Italy

ABSTRACT

The effects of ventilation on air quality, and on the welfare and production performance of dairy ewes were assessed in a 6-wk trial conducted during the winter of 2002. Thirty-six midlactation Comisana ewes were divided into three groups of 12, which were randomly balanced for parity, time of lambing, and number of lambs suckled. Treatments were low (LOV), moderate (MOV), and programmed ventilation regimen (PROV). In LOV and MOV rooms, fans provided 10 ventilation cycles of 40 min each at a fan speed of 1 and 2 m/s, respectively. In the PROV room, the fan was programmed to maintain a 70% relative humidity. Mean ventilation rates were 23, 47, and 73 m³/h per ewe in LOV, MOV, and PROV rooms, respectively. Air concentrations of microorganisms and dust, and of gaseous pollutants were measured twice weekly. Cell-mediated immune response to phytohemagglutinin at d 1, 21 and 42, and humoral response to chicken egg albumin at d 11, 21, 30, and 40 were determined. At d 39, ewes were injected with 2 IU of porcine adreno-corticotrophic-hormone/kg body weight^{0.75}, and subjected to blood sampling for evaluation of cortisol concentrations immediately before and 1, 2, and 4 h after adreno-corticotrophic-hormone injection. Milk yield was recorded daily. Individual milk samples were analyzed weekly for composition, renneting parameters, and somatic cell count. The LOV treatment resulted in higher air concentrations of NH₃ and CO₂ than the MOV and PROV treatments. Greater amounts of total and respirable dust were found in the PROV room than in the LOV and the MOV rooms. The LOV ewes had lower milk yield than the PROV ewes, lower milk casein content, and higher rate of clot formation than the MOV and PROV ewes. The ventilation regimen did not affect the immune and endocrine responses of the ewes. Results suggest that an intermittent ventilation regimen, providing a mean ventilation rate of 47 m³/h per ewe at a

fan speed of 2 m/s, is required to sustain the yield and cheese-making ability of ewe milk during the winter season.

(Key words: air quality, milk renneting parameters, dairy ewe, ventilation)

Abbreviation key: CoI = milk coagulating index, LOV = low ventilation, MOV = moderate ventilation, NE = net energy; PHA = phytohemagglutinin, PROV = programmed ventilation.

INTRODUCTION

Ventilation is recognized as playing a key role in sustaining the welfare and performance of farmed livestock in summer (Charles, 1994; Sevi et al., 2002). The importance of ventilation during the winter season is, instead, often underestimated, and this may result in seriously limiting high efficiencies of production and good health in intensive systems of animal husbandry. In fact, inadequate ventilation systems may lead to high concentrations of noxious gases in the air of livestock buildings, and to increased moisture content of the house air and surfaces, which enhances the growth and multiplication of microorganisms in the air and in the bedding (Sevi et al., 2001). In addition, inefficient ventilation systems result in animals avoiding more contaminated areas of livestock buildings (Smith et al., 1996). This may lead to an uneven use of space between very low and very high densely stocked areas in animal housing.

A minimum ventilation rate of 30 m³/animal per hour has been recommended for housed sheep in winter (Chiumenti, 1987). However, little is known about the effects of ventilation rate and regimen on the welfare and performance of the lactating ewe. Almost all sheep housing relies on natural ventilation because extensive production systems are predominant for this species. Nevertheless, the gradual increase of intensive housing in sheep, as a consequence of the increased size of specialized dairy flocks, requires more efficient control of the indoor climate, which can be achieved with mechanical ventilation systems.

A variety of indicators are required to assess animal welfare because single physiological functions may be

Received December 2, 2002

Accepted April 22, 2003

Corresponding author: A. Sevi; e-mail: a.sevi@unifg.it.

not sensitive to peculiar stressors. Sevi et al. (2002), using such an integrated approach, found a reduction in milk yield, depressed immune reactivity, and an increased cortisol release in ewes subjected to a low ventilation rate (33 m³/h per ewe) during the summer season.

The present study was undertaken to assess whether a programmed ventilation regimen operating at 70% relative humidity during the winter season could improve air quality and ewe welfare and production performance compared with intermittent ventilation regimens providing a low (23 m³/h per ewe) and a moderate ventilation rate (47 m³/h per ewe).

MATERIALS AND METHODS

Experimental Design and Animal Management

The experiment, which lasted 6 wk, was conducted during the winter (February-March) of 2002 at Segezia research station of the Italian Istituto Sperimentale per la Zootecnia (latitude: 41° 27' 6" and longitude: 15° 33' 5"). The climate of this area is Mediterranean, with a winter rainfall of about 130 mm, and an 8.3°C mean winter temperature over the last 20 yr.

Thirty-six midlactation Comisana ewes (d 106 ± 1.87 of lactation, mean ± SE) with no history of clinical mastitis were used. The animals were housed in a prefabricated building provided with external paddocks before the experiment. Ewe health was checked at the start of the experiment and throughout the study period. In particular, all ewes were examined daily to detect the presence or confirm the absence of signs of clinical mastitis, such as fever, pain, or gland swelling. A small quantity of milk was checked visually for signs of mastitis. The animals were divided into three groups of 12 each, which were in a low (**LOV**), moderate (**MOV**), and programmed ventilation regimen (**PROV**). Groups were balanced for parity, time of lambing, and number of lambs suckled. Mean BW (± SE) were 54.2 ± 2.1, 55.1 ± 1.5 and 53 ± 2.2 kg, in the LOV, MOV, and PROV groups, respectively; BCS were 2.33 ± 0.07, 2.25 ± 0.07, and 2.21 ± 0.08; milk yield was 998 ± 73, 940 ± 70, and 990 ± 76 g/d; and milk protein and fat contents were 6.25 ± 0.19, 6.23 ± 0.17, and 6.11 ± 0.17%, and 5.91 ± 0.29, 6.08 ± 0.33, and 5.73 ± 0.31%, respectively. Groups were separately housed on straw bedding in 8 × 3 m and 3.5 m 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 an underpressurized mechanical system of ventilation. In each room a circular suction fan of 0.3 m diameter (Vortice, Tribiano-Milan, Italy) was 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 LOV and MOV rooms, fans provided 10 ventilation cycles per day. Each cycle duration was 40 min/h; five cycles were during daytime from 0700 to 1800 h and five during nighttime from 2100 to 0500 h. Fan speed was kept constant at 1 m/s in the LOV room and at 2 m/s in the MOV room. In the PROV room, the fan was connected to a relative humidity sensor, which provided an on/off two-stage control function switching power to the fan. Ventilation system was programmed to maintain a 70% relative humidity with a fan speed of 1 m/s. The relative humidity threshold was chosen on account of previous reports about critical humidity for sheep welfare and productivity (Casamassima et al., 1991a; Sevi et al., 2001). In all rooms, ventilation rate was checked daily by placing a hot wire anemometer (LSI, Settala Premenugo, Milan, Italy) over the air outlet and converting readings to m³/h per ewe. The fans worked 6 h and 40 min in the LOV and the MOV rooms and 20 h and 50 min in the PROV room, providing a mean ventilation rate of 23, 47, and 73 m³/h per ewe, respectively.

The air temperature and the relative humidity inside each room were continuously monitored during the trial, and during the week before the start of the experiment, to be sure that climatic conditions were the same in all rooms. TIG2-TH thermo-hygrographs (LSI) were used, which were placed at a height of 1.5 m from the floor. In each pen, a layer of straw (about 0.4 kg/m²) on bedding was provided daily.

Each pen was provided with two mangers; feeder space per animal was about 0.45 m. The ewes were fed a diet composed of a pelleted concentrate, oat grains, and ryegrass hay (32, 6, and 62% of total diet, respectively), which was offered as a TMR twice daily. The chemical composition of DM was determined by standard procedures (Association of Official Analytical Chemists, 1990) and contained 16.1% CP, 2.4% fat (by ether extraction), crude fiber 19.1%, 9.4% ash, and 8.4 MJ/kg of metabolizable energy. Dry matter intakes were calculated daily as the difference between the amount of feed offered and feed refusals. Averages of DMI were 2.80, 2.81, and 2.83 kg/ewe per day during the 0- to 21-d period and 2.65, 2.69, and 2.68 kg/ewe per day during the 22- to 42-d period in the LOV, MOV, and PROV groups, respectively. Water was available from automatic drinking troughs.

The BW and BCS 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.

Air Sampling

Air sampling was performed twice weekly both in the morning, starting from 0900 h, and in the afternoon,

starting from 1630 h. Air was sampled on the same day in each room at 0.6 m height above the floor. The sequence of air sampling in the three experimental rooms changed according to a prearranged program.

The concentrations of mesophilic microorganisms, coliforms, and yeasts/molds were recorded from 720 L of air (flow rate = 1.5 L/s) sampled using a Surface Air System pump (PBI International, Milan, Italy) directly onto plates containing plate count agar (Oxoid, Basingstoke, UK), violet red bile lactose agar (Oxoid) and Sabouraud dextrose agar (Oxoid), respectively. The air concentrations of microorganisms were measured at six locations within each room. After sampling, the plates were immediately incubated at 30°C for 24 to 36 h for mesophilic bacteria, at 37°C for 24 to 36 h for coliforms, and at 25°C for 96 h for yeasts and molds.

Air concentrations of total (particulate size > 5 μm) and respirable (particulate size = 2 to 5 μm) dust were recorded using DIGIT ISO pumps (Zambelli, Bareggio, Milan, Italy). Dust was gravimetrically collected on cellulose nitrate filters with a pore size of 0.8 μm . A Lippman cyclone was used for selecting respirable dust by centrifugation. Samples were taken in two different locations in each room, and the amount of air collected per room for each of the two parameters was 1 m³ (flow rate = 30 L/min). The dust collected was being weighed on an analytical scale. The filters were dried in an oven at 50°C for 8 h before weighed and used for air sampling.

Air concentrations of gaseous pollutants were recorded from six locations within each room, using a Gas Detection Pump (Dräger-Italia, Corsico, Milan, Italy). The concentrations of carbon dioxide, hydrogen sulphide, ammonia, and methane were colorimetrically determined in graduate detection tubes.

Immune Response

Skin tests were performed on all the animals by injecting 0.7 mg of phytohemagglutinin (**PHA**) dissolved in 1 ml of sterile saline solution into shaven spots on each shoulder to evaluate the effect of ventilation regimen on their cell-mediated immunity. A total of three skin tests were performed—one at the beginning, one at the end, and one at mid-trial (21 d). Responses were evaluated by measuring the ewes' skinfold thickness with a caliper before PHA injection and 24 h after.

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 9 d later. Antibody titers were determined in blood samples collected in heparinized vacuum tubes (Becton Dickinson, Plymouth, UK) im-

mediately before the first antigen injection (2 d) and then at 11, 21, 30, and 40 d of the study period. An ELISA was performed in 96-well U-bottomed microtiter plates. Wells were coated with 100 μl of antigen (10 mg of chicken egg albumin/ml of PBS) at 4°C for 12 h, washed and incubated with 1% skimmed milk (200 μl) at 37°C for 1 h to reduce nonspecific binding. After washing, the serum (1:1000 dilution in PBS; 100 μl per well) was added and incubated at 37°C for 1 h. The extent of antibody binding was detected using a horseradish peroxidase-conjugated donkey anti-sheep IgG (Sigma-Aldrich Italia) (1:20,000 dilution in PBS; 100 μl per well). Optical density was measured at a wavelength of 450 nm using a microtiter plate reader (Anthos 2020, DIESSECHEM, Milan, Italy). The inter- and intra-assay CV were 6.9 and 2.5%, respectively. The assay was optimized in our laboratory for concentrations of coating antigen, serum, and detector antibody.

Cortisol Levels

At d 39 ewes were intravenously injected with 2 IU porcine ACTH/kg BW^{0.75} (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. Hormone concentration was determined by a radioimmunoassay (Radim, Rome, Italy). Validation for sheep plasma was performed as described by Fisher et al. (1997). The sensitivity of the assay was 0.9 ng/ml. The inter- and intraassay variation coefficients were 6.5 and 4.1%, respectively.

All procedures were conducted according to the guidelines of the Council Directive 86/609/EEC of November, 24, 1986, on the protection of animals used for experimental and other scientific purposes.

Sampling and Analyses of Milk

Ewes were milked using a highline milking machine (Alfa Laval Agri, SE-147 21, Tumba, Sweden) twice daily at 0800 and 1500 h. Milk yield was recorded daily by means of graduated measuring cylinders attached to individual milking units. Milk samples, consisting of proportional volumes of morning and evening milk, were individually collected weekly in 200-ml sterile plastic containers after cleaning and disinfection of teats (70% ethyl alcohol) and discharging the first streams of foremilk. Milk samples were carried to our laboratory by means of transport tankers at 4°C. The following measurements were carried out on milk: pH, total protein, fat, and lactose content using an infrared spectrophotometer (Milko Scan 133B; Foss Electric, Hillerød, Denmark) according to the International

Dairy Federation (1990) standard, casein content (International Dairy Federation, 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 SCC using a Foss Electric Fossomatic 90 cell counter (International Dairy Federation, 1995). In addition, the milk concentrations of chloride, urea, and lactic acid were determined enzymatically at 14-d intervals using a Foodlab spectrophotometer (CDR, Florence, Italy). Optical density was then measured at wavelengths of 505, 700, and 545 nm for chloride, urea, and lactic acid, respectively. Milk conductivity was measured using a CONMET 2 conductivity meter (Hanna Instruments, Padua, Italy).

After the morning milking but before feeding, the BW and BCS 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, to evaluate the effect of ventilation regimen on ewe BW changes and mobilization of body fat reserves.

Calculations and Statistical Analysis

Milk yield was corrected for fat content using the Cannas (1999) equation: fat-corrected milk = milk yield $\times$ [0.3688 + (0.0971 % fat)]. The energy content of the milk was calculated using the Šebek and Everts (1992) equation: milk energy content (MJ/kg) = $0.0419 \times F + 0.0159 \times P + 0.0214 \times L$, where F, P, and L are grams of fat, protein, and lactose per kilogram of milk, respectively. Maintenance requirements and the energy content of BW gain were calculated according to Agriculture and Food Research Council (1993). The net energy (NE) of the ration was calculated as the ratio between NE output (milk energy + maintenance energy + BW gain energy) and DMI, to assess the effect of ventilation regimen on the efficiency of utilization of energy from feed. The milk coagulating index (CoI) was calculated as the clot firmness to clotting time + rate of clot formation ratio. All the variables were tested for normal distribution using the Shapiro-Wilk test (Shapiro and Wilk, 1965). Antibody titers, plasma cortisol levels, and milk SCC and air microorganism counts were transformed into logarithmic form to normalize their frequency distributions before performing statistical analysis. Skinfold thickness and cortisol levels were processed using ANOVA for repeated measures (SAS, 1990). The variation due to treatment, time of sampling, and their interaction was tested. Individual animal variation within treatment was used as the error term. Antibody titers, and air and milk variables were tested, with treatment, time of sampling, and their interaction as main factors. Pretreatment values of airborne micro-

organisms, and milk yield and quality—collected twice during the week before the commencement of the trial—were used as covariates for air and milk variables. Pre-antigen injection values were used as covariates for antibody titers. Body weight, BCS, and the NE density of the ration were analyzed using the GLM procedure (SAS, 1990) with one factor (treatment), having initial BW and BCS as covariates. Results are presented as the least squares means of the ewes in each treatment, and variability of the data is expressed as the SE of the mean response over the whole experimental period.

RESULTS

Microenvironment

Ventilation regimen had a minor impact on air temperature. Weekly averages of mean temperatures ranged from 10.8 to 13.1°C through the trial, and differences among the experimental rooms were less than 1°C (Table 1). Weekly averages of relative humidity were 3 to 9% lower in the PROV room than in the rooms provided with intermittent ventilation regimens.

The experimental treatment markedly affected the air concentrations of gaseous pollutants and dust (Table 2). Higher levels of total dust were recorded in the PROV room than in the LOV room ($P < 0.01$) during wk 3 and compared to the MOV room ($P < 0.05$) during wk 6. The PROV treatment also resulted in higher mean concentrations of respirable dust compared to the LOV ($P < 0.01$) and MOV treatments ($P < 0.05$) (0.26 vs. 0.16 and 0.19 mg/m³, respectively).

Conversely, higher concentrations of aerial NH₃ were found in the LOV room than in PROV room ($P < 0.05$) during wk 5 and in the LOV than in the MOV and PROV rooms ($P < 0.001$) during wk 6 (Table 2). Higher aerial CO₂ levels were found in the LOV room than in the PROV room ($P < 0.05$) during wk 6 of the trial. Only traces of H₂S and CH₄ were found in the air of all the experimental rooms (data not shown).

A significant effect of time ($P < 0.001$) was found for the air concentrations of microorganisms, which increased significantly ($P < 0.001$) in all groups with the advancement of the trial, whereas no differences were observed across treatments. The concentrations of mesophilic bacteria were 1.95 ± 0.07 , 1.78 ± 0.10 , and 1.86 ± 0.08 log₁₀ cfu/m³ of air in the LOV, MOV, and PROV rooms, respectively, while yeast counts were 1.16 ± 0.10 , 1.08 ± 0.08 , and 0.98 ± 0.10 log₁₀ cfu/m³ of air, and mold counts were 1.43 ± 0.12 , 1.49 ± 0.10 , and 1.36 ± 0.12 log₁₀ cfu/m³ of air, respectively (data not shown). Coliforms were sporadically found in all the experimental rooms.

Table 1. Averages of air temperature and relative humidity in rooms provided with a low (LOV), moderate (MOV), and programmed ventilation regimen (PROV). Values are means $\pm$ SD.

Item	Trial wk	LOV (23 m ³ /h/ewe)	MOV (47 m ³ /h/ewe)	PROV (73 m ³ /h/ewe)
		Mean ¹	Mean	Mean
Air temperature, °C	1	12.2 $\pm$ 1.2	12.7 $\pm$ 1.1	11.7 $\pm$ 0.9
	2	10.4 $\pm$ 2.4	11.8 $\pm$ 1.4	10.2 $\pm$ 1.8
	3	11.1 $\pm$ 2.3	12.1 $\pm$ 2.1	11.6 $\pm$ 2.1
	4	12.4 $\pm$ 1.1	12.9 $\pm$ 1.0	12.6 $\pm$ 1.2
	5	12.3 $\pm$ 1.1	12.6 $\pm$ 0.9	12.4 $\pm$ 1.1
	6	13.1 $\pm$ 4.1	13.3 $\pm$ 3.5	12.8 $\pm$ 4.1
	1 to 6	11.9 $\pm$ 0.8	12.6 $\pm$ 0.7	11.9 $\pm$ 0.8
Relative humidity, %	1	86 $\pm$ 9.5	83 $\pm$ 6.2	77 $\pm$ 9.0
	2	79 $\pm$ 9.4	76 $\pm$ 7.0	75 $\pm$ 8.1
	3	70 $\pm$ 5.3	69 $\pm$ 3.1	66 $\pm$ 5.1
	4	90 $\pm$ 7.2	85 $\pm$ 7.1	83 $\pm$ 6.6
	5	77 $\pm$ 8.2	76 $\pm$ 5.6	76 $\pm$ 5.8
	6	76 $\pm$ 4.5	75 $\pm$ 2.8	71 $\pm$ 3.9
	1 to 6	79.7 $\pm$ 2.9	77.3 $\pm$ 3.3	74.6 $\pm$ 3.2

¹Based on hourly measured data.

Immune Response

Ewe immune response to PHA injection was similar across treatments and remained substantially unchanged through the trial (Table 3).

No differences were found in the ewe antibody response to chicken egg albumin. A significant effect of time ($P < 0.01$) was observed that might be attributed to the fact that IgG concentrations increased in all groups after the second antigen injection (Table 3).

Plasma Cortisol Levels

Cortisol response was similar across treatments (Table 3). A significant effect of time of sampling ($P < 0.001$) was observed because in all groups cortisol levels peaked 60 min after the ACTH injection, were found to be halved at 120 min and decreased below preinjection values ($P < 0.05$) at 240 min.

Milk Yield and Quality

The PROV ewes had a significantly higher milk yield than the LOV ewes, on average (891 vs. 807 g/d, $P < 0.01$), whereas the MOV animals displayed intermediate productive levels (Table 4). In particular, a significantly higher milk production was recorded in the PROV group than in the MOV group ($P < 0.05$) during wk 3, and in the PROV and MOV groups than in the LOV group ($P < 0.05$) during wk 5 of the experiment.

No differences emerged for the fat and protein contents of milk (Table 4), whereas significantly lower milk casein contents were found in the LOV than in the MOV and PROV groups, on average (4.57 vs. 4.75%, $P < 0.01$).

Clotting time, clot firmness, pH, and SCC of milk were unchanged by the ventilation regimen (Table 5).

The casein-to-protein ratio and rate of clot formation, instead, deteriorated in the milk yielded by the LOV ewes ($P < 0.05$). In general, the milk from the less ventilated group showed a general worsening of coagulating ability compared with the two other groups. As a result, when renneting parameters were gathered in the milk-coagulating index (CoI), values showed a tendency to decrease in the LOV compared with the PROV milk ($P = 0.07$).

The ventilation regimen did not affect the milk conductivity, and the milk concentrations of urea, chloride, and lactic acid (Table 5). However, a significant interaction of treatment $\times$ time ($P < 0.01$) was found for the chloride concentration with the LOV milk having a higher chloride concentration ($P < 0.01$) than the PROV milk at d 42 of the trial (301 vs. 230 mg/dl, respectively) (data not shown).

BW, BCS, and Feed Efficiency

The BW and BCS of the ewes were unaffected by the experimental treatment throughout the study period (Table 6). However, the NE of the ration tended to increase in the PROV ($P < 0.10$) compared with the LOV group during the 22- to 42-d period, due to greater volumes of milk yielded.

DISCUSSION

Both the LOV and PROV treatments provided less efficient control of air pollution than the MOV treatment. In fact, the low ventilation regimen resulted in increased relative humidity and higher air concentrations of ammonia and carbon dioxide that may be ascribed to failure in removing efficiently the moisture and gases that originated from the respiratory activity

Table 2. Least square means $\pm$ SEM of air dust and gaseous pollutants concentrations as affected by low (LOV), moderate (MOV), and programmed ventilation regimen (PROV).¹

Item	Trail wk	LOV (23 m ³ /h/ewe)	MOV (47 m ³ /h/ewe)	PROV (73 m ³ /h/ewe)	SEM	Effects, <i>P</i>		
						Treatment	Time	Treatment $\times$ time
Total dust, mg/m ³	1	0.28	0.22	0.39				
	2	0.29	0.44	0.36				
	3	0.28 ^b	0.56 ^{ab}	0.78 ^a				
	4	0.50	0.45	0.56				
	5	0.66	0.61	0.78				
	6	1.01 ^{ab}	0.71 ^b	1.09 ^a				
	1 to 6	0.50 ^b	0.50 ^b	0.66 ^a	0.05	*	***	NS ¹
Respirable dust, mg/m ³	1	0.13 ^{ab}	0.11 ^b	0.25 ^a				
	2	0.11	0.14	0.18				
	3	0.12	0.12	0.24				
	4	0.22	0.29	0.33				
	5	0.22	0.25	0.20				
	6	0.16 ^b	0.24 ^b	0.38 ^a				
	1 to 6	0.16 ^b	0.19 ^b	0.26 ^a	0.02	**	**	NS
NH ₃ , ppm	1	7	5	6				
	2	5	6	5				
	3	11	8	6				
	4	9	8	6				
	5	12 ^a	9 ^{ab}	6 ^b				
	6	25 ^a	9 ^b	10 ^b				
	1 to 6	11.5 ^a	7.5 ^b	6.5 ^b	0.8	***	***	*
CO ₂ , ppm	1	800	675	525				
	2	800	690	575				
	3	625	625	650				
	4	800	600	600				
	5	800	500	650				
	6	1325 ^a	925 ^{ab}	850 ^b				
	1 to 6	858 ^a	669 ^b	642 ^b	60	*	*	NS

^{a,b}Means within a row with different superscript letters are significantly different at $P < 0.05$.

¹NS, not significant.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

of animals and the decomposition and fermentation of manure.

The programmed ventilation regimen, instead, resulted in higher air dust concentrations. Undoubtedly, this partly depended on the lower humidity levels observed in the PROV room, because water molecules drag the dust particles suspended in the air to the ground. As a consequence, the dryer the air the higher the ambient dustiness. However, it is very likely that other factors also contributed to the rise in the air dust concentrations observed in the PROV room. In fact, the programmed fan system worked almost continuously, and this probably resulted in dust particles remaining suspended in the air for a longer time. In caged layers, Qi et al. (1992) observed that generation rates of respirable and total dust was faster at rapid ventilation rates, probably due to turbulent air currents suspending dust from cage surfaces. However, the amount of airborne microorganisms, which mainly originate from feces, feed, and the animal's body surface (Hartung, 1994),

were not changed by the ventilation regimen. This suggests that the raising of dust from the bedding, the mangers and the animals' skin, due to ascending flow of the air entering the building, contributed little to the increase of the aerial particulate in the PROV room. In general, the concentrations of total and respirable dust in all the experimental rooms air were relatively low when compared to the values reported previously for pig and poultry house (Muller and Wieser, 1987; Hartung, 1994). Mean ammonia concentrations, instead, were near or over the safety threshold of 10 mg/kg (Verstegen et al., 1994) in the LOV room during the last four trial wks. This may partly account for the reduction in milk yield and the deterioration of milk quality observed in the less ventilated group. There is evidence that high concentrations of air pollutants may have a general performance-reducing effect on farmed animals (Verstegen et al., 1994). In particular, high ammonia levels have been recognized as responsible for reduced growth in pigs, sheep, and poultry (Drummond et al., 1980;

Table 3. Least square means $\pm$ SEM of skinfold thickness after phytohemagglutinin injection, of antibody response to chicken egg albumin injection (OVA), and of plasma cortisol levels after porcine adreno-corticotrophic-hormone injection in ewes subjected to a low (LOV), moderate (MOV), and programmed ventilation regimen (PROV).

Item	Sampling time	LOV (23 m ³ /h/ewe)	MOV (47 m ³ /h/ewe)	PROV (73 m ³ /h/ewe)	SEM	Effects, <i>P</i>		
						Treatment	Time	Treatment $\times$ time
Skinfold thickness, mm	1 d	6.67	6.98	7.31				
	21 d	6.08	7.21	7.21				
	42 d	6.64	7.57	8.20				
	Mean	6.46	7.25	7.57	0.45	NS ¹	NS	NS
Antibody titer to OVA, log ₁₀ (x + 1)	11 d	0.03	0.04	0.03				
	21 d	0.09	0.09	0.11				
	30 d	0.09	0.10	0.10				
	40 d	0.09	0.09	0.10				
	Mean	0.07	0.08	0.09	0.02	NS	***	NS
Plasma cortisol levels, log ₁₀ [(ng/ml) + 1]	Pre-ACTH injection	0.49	0.44	0.25				
	60 min after	1.63	1.68	1.72				
	120 min after	0.83	0.94	0.72				
	240 min after	0.10	0.06	0.15				
	Mean	0.76	0.78	0.71	0.07	NS	***	NS

¹Not significant.

****P* < 0.001.

Casamassima et al., 1991b; Wathes et al., 1997). Furthermore, Bauman and Currie (1980) suggested that the activation of homeostatic regulatory mechanisms in response to environmental challenge can easily impair homeorhetic mechanisms operating in support of galactopoiesis. The lower NE of the ration observed in the LOV ewes supports the hypothesis of an increased energy demand for the maintenance of their physiological equilibrium under conditions of poor air quality, which gives rise to a waste of energy otherwise available for milk synthesis.

The air quality in all the experimental rooms deteriorated with the advancement of the study period. The progressive reduction in the absorbing capacity of the bedding associated with growing amounts of urine and feces per unit volume of bedding and the trampling by the ewes were probably concurrent factors for this. The marked worsening in the air quality observed during the last week of the trial also suggests that had the study period been prolonged further the adverse effect of a low ventilation regimen on gaseous pollutant concentrations could have strongly emphasized.

The LOV ewes had reduced milk yields with lower milk casein contents and casein to protein ratios than the MOV and PROV ewes. Given that the milk protein content was not changed by the experimental treatment, it may be argued that, although the low ventilation regimen did not reduce the nitrogen availability in the mammary gland, it impaired the efficiency of casein synthesis. Among the milk constituents, casein is the

most susceptible to reduced efficiency in the secretory activity of the mammary epithelium, because milk fat is only partially synthesized in the mammary gland (Kaufmann and Hagemester, 1987) and lactose concentration in milk tends to remain constant, as lactose is the main osmotically active component in the milk (Sevi et al., 2000). The lower milk casein content resulted in curd firming rate taking a longer time in the LOV than in the other two groups. On the whole, all renneting parameters tended to deteriorate in the less-ventilated group to such an extent that milk coagulating index was significantly lower in the LOV than in the PROV group. The adverse effects of the low ventilation regimen on the milk coagulating behavior may be ascribed to the fact that, after the milk coagulation has started, the rate of clot formation depends on the amount of casein available for rennet activity. Clot firmness, as well, is directly dependent on the milk casein content, because milk coagulation involves the aggregation of casein micelles into a network within which the fat is entrapped (Dalgleish, 1993). In all groups, the milk fat content was relatively scarce and lower than the milk protein content. This may be because high-yielding ewes were used in this experiment, and it is known that the greater the volume of milk yielded the lower the concentration of milk constituents. Probably such a dilution effect was slighter for the milk protein than for the milk fat, because ewes were given a high energy level diet with a relatively high protein content, which enhanced the protein flow to the udder.

Table 4. Least square means $\pm$ SEM of yield and protein, casein, and fat content of ewe milk as affected by a low (LOV), moderate (MOV), and programmed ventilation regimen (PROV).

Item	Trail wk	LOV (23 m ³ /h/ewe)	MOV (47 m ³ /h/ewe)	PROV (73 m ³ /h/ewe)	SEM	Effects, <i>P</i>		
						Treatment	Time	Treatment $\times$ time
6.5% FCM yield, g/d	1	893	944	911				
	2	863	907	872				
	3	792 ^{ab}	729 ^b	909 ^a				
	4	756	790	874				
	5	732 ^b	916 ^a	905 ^a				
	6	806	818	877				
	1 to 6	807 ^b	851 ^{ab}	891 ^a	23	*	NS ¹	NS
Fat content, %	1	5.26	4.95	5.15				
	2	5.58	5.64	4.96				
	3	5.70	4.99	5.07				
	4	5.70	5.04	4.96				
	5	5.41	5.44	4.76				
	6	6.49	5.78	6.02				
	1 to 6	5.69	5.31	5.15	0.24	NS	***	NS
Protein content, %	1	6.12	6.10	5.98				
	2	6.14	6.47	6.28				
	3	6.10	5.96	5.97				
	4	6.32	6.00	6.03				
	5	6.06	6.09	6.03				
	6	6.38	6.17	6.21				
	1 to 6	6.19	6.13	6.08	0.15	NS	*	NS
Casein content, %	1	4.64	4.87	4.77				
	2	4.44 ^b	4.79 ^a	4.78 ^a				
	3	4.56	4.67	4.67				
	4	4.65	4.66	4.77				
	5	4.51	4.80	4.75				
	6	4.63	4.74	4.80				
	1 to 6	4.57 ^b	4.75 ^a	4.75 ^a	0.09	*	NS	NS

^{a,b}Means within a row with different superscript letters are significantly different at $P < 0.05$.

¹NS, not significant.

* $P < 0.05$.

*** $P < 0.001$.

Table 5. Least square means $\pm$ SEM of pH, casein to protein ratio, renneting parameters, SCC, conductivity, and urea, chloride, and lactic acid content in ewe milk as affected by a low (LOV), moderate (MOV), and programmed ventilation regimen (PROV).

Item	LOV (23 m ³ /h/ewe)	MOV (47 m ³ /h/ewe)	PROV (73 m ³ /h/ewe)	SEM	Effects, <i>P</i>		
					Treatment	Time	Treatment $\times$ time
pH	6.59	6.58	6.56	0.01	NS ²	***	NS
Casein-to-protein ratio	0.74 ^b	0.78 ^a	0.78 ^a	0.01	*	***	NS
Clotting time, min	21.6	21.7	21.4	0.50	NS	***	NS
Rate of clot formation, min	4.1 ^a	3.5 ^b	3.4 ^b	0.18	*	***	NS
Clot firmness, mm	40.8	41.3	43.5	1.97	NS	***	NS
Coagulating index (Col) ¹ , mm/min	1.57 ^b	1.63 ^{ab}	1.76 ^a	0.07	†	***	NS
SCC, log ₁₀ cells/ml	5.05	5.10	5.16	0.05	NS	NS	NS
Conductivity, ms/cm	4.34	4.34	4.33	0.03	NS	***	NS
Urea, mg/dl	43.2	41.6	43.9	0.80	NS	***	NS
Chloride, mg/dl	250	238	237	7.5	NS	***	**
Lactic acid, ppm	2.88	2.85	2.77	0.5	NS	NS	NS

^{a,b}Means within a row with different superscript letters are significantly different at $P < 0.10$.

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

²NS, not significant.

† $P < 0.10$.

* $P < 0.05$.

*** $P < 0.001$.

Table 6. Body weight, BCS and net energy of ration of ewes as affected by a low (LOV), moderate (MOV), and programmed ventilation regimen (PROV).

Item		LOV (23 m ³ /h/ewe)	MOV (47 m ³ /h/ewe)	PROV (73 m ³ /h/ewe)	SEM	Effects, <i>P</i> Treatment
Bw, kg	0 d	54.16	55.10	53.01	1.98	NS ²
	42 d	57.26	56.86	56.27	0.81	NS
Bw changes, kg/d	0 to 42 d	0.07	0.04	0.08	0.02	NS
BCS	0 d	2.33	2.25	2.21	0.07	NS
	42 d	2.84	2.72	2.80	0.09	NS
BCS changes	0 to 42 d	0.51	0.47	0.59	0.09	NS
NE of the ration, MJ/kg ¹	0 to 21 d	3.80	3.75	3.78	0.05	NS
	22 to 42 d	3.89 ^b	3.95 ^{ab}	4.04 ^a	0.06	†

^{a,b}Means within a row with different superscript letters are significantly different at $P < 0.10$.

¹Calculated as the net energy output (milk energy + maintenance energy + BW gain energy) to DM intake ratio.

²NS, not significant.

† $P < 0.10$.

Apparently the ventilation regimen did not affect ewe udder health. In fact, no cases of clinical mastitis were detected and milk SCC, which is regarded as the most reliable indicator of ongoing clinical and subclinical mastitis (Droke et al., 1993) was relatively low and very similar across treatments.

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). 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 most consistent neuroendocrine responses to stress (Hashizume et al., 1994). There is evidence that the graded immune and cortisol responses to stress can be attributed to the relative stressfulness and the cumulative action of each stressor (Mears and Brown, 1997). Indeed, in the welfare assessment of farmed animals, the administration of exogenous ACTH is aimed at stimulating the adrenal secretion of cortisol, whose release may be strengthened by the existence of concurrent stressful events. Thus, the lack of effects of the experimental treatment on the cortisol, and the cell-mediated and humoral immune responses of the ewes suggests that the stress arising from poor winter ventilation is not severe enough to activate the ewe endocrine apparatus and to impair their immune reactivity. The peak of the cortisol levels was reached 60 min after the ACTH injection, but, at 240 min, the hormone concentrations in ewe plasma were lower than those recorded before the ACTH administration. This trend may be explained with a moderate activation of the hypothalamic-pituitary-adrenal axis of the ewes following capture, handling, and venipuncture. The cortisol release was magnified by the

ACTH administration, and then slowly declined to basal values, probably because the ACTH effect waned and the ewes became accustomed to the testing procedure.

The lack of clear signs of physiological distress in the LOV group suggests that the low ventilation regimen does not severely impair the ewes' well being, even though it lowered their production performance. In a previous trial (Sevi et al., 2002), conducted during the summer season, we found a depressed immune reactivity and an increased cortisol release in ewes subjected to a low ventilation rate. In that experiment, the less-ventilated ewes had to cope with two concurrent stressful situations, such as poor air quality and excessive heat load. In the present trial, instead, high gaseous pollutant levels were not associated to concomitant conditions of physical discomfort; the LOV ewes were managed very carefully and allotted adequate volume and surface area. Similarly, Quaranta et al. (2002), assessing the effect of prolonged exposure to noise in lambs, found a marked reduction in growth rate, but no adverse effects on their behavioral, immune, and endocrine responses. These authors argued that, other sources of physiological disturbance being absent, noise is not per se a sufficiently strong source of stress and danger for sheep.

CONCLUSIONS

The air concentrations of dust, and of ammonia and carbon dioxide, were affected by the ventilation regimen. In fact, the low ventilation system providing ventilation cycles of 40 min each at an air speed of 1 m/s resulted in significantly higher gas concentrations than the programmed ventilation system, and the moderate ventilation system providing 40-min ventilation cycles

at an air speed of 2 m/s. Programmed ventilation resulted in higher dust levels than moderate ventilation. The low ventilation regimen led to reduced yield and deteriorated coagulating behavior of milk compared with the programmed and the moderate ventilation regimens. Though the low ventilation regimen caused a significant reduction of ewe performance, the animals showed no clear signs of distress, and their immune and endocrine responses were unchanged by the experimental treatment. The present experiment suggests that the choice of a proper ventilation regimen is critical for optimizing the yield and cheese-making ability of ovine milk during the winter season.

AKNOWLEDGMENTS

The authors would like to thank C. Perilli and S. D'Urso for their expert technical assistance.

REFERENCES

- Agriculture and Food Research Council. 1993. Energy and Protein Requirements of Ruminants. CAB International, Wallingford, UK.
- Association of Official Analytical Chemists. 1990. Official Methods of Analysis. Vol. I. 15th ed. AOAC, Arlington, VA.
- Bauman, D. E., and W. B. Currie. 1980. Partitioning nutrients during pregnancy and lactation: A review of mechanism involving homeostasis and homeorhesis. *J. Dairy Sci.* 63:1514–1529.
- Burton, J. L., E. B. Burnside, B. W. Kennedy, B. N. Wilkie, and J. H. Burton. 1989. Antibody responses to human erythrocytes and ovalbumin as marker traits of disease resistance in dairy calves. *J. Dairy Sci.* 72:1252–1265.
- Cannas, A. 1999. Adaptation of the CNCPS to small ruminant. Ph.D. thesis. Cornell University. Ithaca, NY.
- Casamassima, D., O. Montemurro, and A. Sevi. 1991a. Effect of relative humidity on production performance of fattening lambs. Pages 1841–1845 in Proceedings of the 45th Convegno Nazionale SISVet., Altavilla Milicia, Palermo, Italy.
- Casamassima, D., A. Sevi, and L. Carosielli. 1991b. Effects of air change rate on production performance of intensively managed Sopravissana lambs. *Selezione Vet.* 32:1105–1113.
- Charles, D. R. 1994. Comparative climatic requirements. Pages 3–24 in *Livestock Housing*, C. M. Wathes and D. R. Charles, ed. CAB International, Wallingford, UK.
- Chiumenti, R. 1987. Rural Buildings. Edagricole, Bologna, Italy.
- Dagleish, D. G. 1993. Bovine milk protein properties and the manufacturing quality of milk. *Livest. Prod. Sci.* 35:75–93.
- Droke, E. A., M. J. Paape, and A. L. Di Carlo. 1993. Prevalence of high somatic cell counts in bulk tank goat milk. *J. Dairy Sci.* 76:1035–1039.
- Drummond, J. G., S. E. Cuiris, J. Simon, and H. W. Norton. 1980. Effects of aerial ammonia on growth and health of young pigs. *J. Anim. Sci.* 50:1085–1091.
- 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. Pages 1–28 in *Official Journal L 358*, European Communities Publication office, Luxembourg.
- Fisher, A. D., M. A. Crowe, D. J. Prendiville, and W. J. Enright. 1997. Indoor space allowance: Effects on growth, behaviour, adrenal and immune responses of finishing beef heifers. *Anim. Sci.* 64:53–62.
- Hartung, J. 1994. The effect of airborne particulate on livestock health and production. Pages 55–69 in *Pollution in Livestock Production Systems. I*. Ap Dewi, R. F. E. Axford, I. F. M. Marai, and H. Omed, eds. CAB International, Wallingford, UK.
- Hashizume, T., S. A. Haglof, and P. V. Malven. 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.
- International Dairy Federation. 1964. Determination of the casein content of milk. FIL-IDF Standard no. 29, Brussels, Belgium.
- International Dairy Federation. 1990. Determination of milk fat, protein & lactose content. Guide for the operation of mid-infrared instruments. FIL-IDF Standard no. 141B, Brussels, Belgium.
- International Dairy Federation. 1995. Enumeration of somatic cells. FIL-IDF Standard no. 148A, Brussels, Belgium.
- Kaufmann, W. and H. Hagemester. 1987. Composition of milk. Pages 107–171 in *Dairy-Cattle Production*. H. O. Gravert, ed. Elsevier Science Publishers, Amsterdam, The Netherlands.
- Mears, G. J., and F. A. Brown. 1997. Cortisol and β -endorphin responses to physical and psychological stressors in lambs. *Can. J. Anim. Sci.* 77:689–694.
- Muller, W., and P. Wieser. 1987. Dust and microbial emissions from animal production. Pages 47–89 in *Animal Production and Environmental Health*. D. Strauch, ed. Elsevier, Amsterdam, The Netherlands.
- Quaranta, A., A. Sevi, A. Nardomario, G. E. Colella, and D. Casamassima. 2002. Effects of graded noise levels on behavior, physiology and production performance of intensively managed lambs. *Ital. J. Anim. Sci.* 1:217–227.
- Qi, R., H. B. Manbeck, and R. G. Maghirang. 1992. Dust net generation rate in a poultry layer house. *Trans. Am. Soc. Agric. Eng.* 35:1639–1645.
- SAS. 1990. SAS/STAT User's Guide. Version 6, 4th ed. Statistical Analysis System Inst, Cary, NC.
- Šebek, J. B. J., and H. Everts. 1992. Prediction of the gross energy of ewe milk. *Anim. Prod.* 56:101–106.
- Sevi, A., M. Albenzio, G. Annicchiarico, M. Caroprese, R. Marino, and L. Taibi. 2002. Effects of ventilation regimen on the welfare and performance of lactating ewes in summer. *J. Anim. Sci.* 80:2349–2361.
- Sevi, A., L. Taibi, M. Albenzio, G. Annicchiarico, and A. Muscio. 2001. Airspace effects on the yield and quality of ewe milk. *J. Dairy Sci.* 84:2632–2640.
- Sevi, A., L. Taibi, M. Albenzio, A. Muscio, and S. Dell'Aquila. 2000. Effect of parity on milk yield, composition, somatic cell count, renneting parameters and bacteria counts of Comisana ewes. *Small Rum. Res.* 37:99–107.
- Shapiro, S. S., and M. Wilk. 1965. An analysis of variance test for normality. *Biometrika* 52:591–601.
- Smith, J. H., C. M. Wathes, and B. A. Baldwin. 1996. The preference of pigs for fresh air over ammoniated air. *Appl. Anim. Behav. Sci.* 49:417–424.
- Verstegen, M., S. Tamminga, and R. Greers. 1994. The effect of gaseous pollutants on animals. Pages 81–96 in *Pollution in Livestock Production Systems. I*. Ap Dewi, R. F. E. Axford, I. F. M. Marai, and H. Omed, ed. CAB International, Wallingford, UK.
- Wathes, C. M., M. R. Holden, R. W. Sneath, R. P. White, and V. R. Phillips. 1997. Concentrations and emission rates of aerial ammonia, nitrous oxide, methane, carbon dioxide, dust and endotoxin in UK broiler and layer houses. *Br. Poultry Sci.* 38:14–28.
- Zannoni, M., and S. Annibaldi. 1981. Standardization of the renneting ability of milk by Formagraph. *Sci. Tecnica Lattiero-Casearia* 32:79–94.