

## Airspace Effects on the Yield and Quality of Ewe Milk

A. Sevi,\* L. Taibi,† M. Albenzio,\* G. Annicchiarico,†  
and A. Muscio\*

\*Istituto di Produzioni e Preparazioni Alimentari, Facoltà di Agraria,  
via Napoli, 25, 71100 Foggia, Italy

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

### ABSTRACT

Three groups of 12 midlactating Comisana ewes were housed in separate rooms of the same building and assigned to treatments of low (LV, 4.1 m<sup>3</sup>), medium (MV, 5.6 m<sup>3</sup>), or high (HV, 7.3 m<sup>3</sup>) airspace/animal. The concentrations of airborne microorganisms in the experimental rooms were measured twice weekly at 0930 and 1630. Ewe milk yield was recorded daily. Individual milk samples were analyzed weekly for milk composition, coagulating properties, somatic cell concentration (SCC), and polymorphonuclear neutrophil leukocyte count (PMNLC), and fortnightly for bacteriological characteristics; samples with more than 10<sup>6</sup> somatic cells/ml were cultured for mastitis-related pathogens. The LV and MV treatments resulted in higher relative humidity and air concentrations of staphylococci than the HV treatment. Greater amounts of air mesophilic bacteria were also found in the LV than in the HV room. Ewes in the HV group gave greater yields of milk than those in the LV and MV groups. LV milk also had a lower casein content than HV milk. Significant interactions of treatment × time were found for milk protein and fat content as well as for clotting time and clot firmness, with LV milk having the poorest composition and deteriorated renneting ability during the last 3 wk of the trial. The HV ewes had lower SCC and PMNLC and psychrotroph counts in their milk than LV and MV ewes and smaller amounts of mesophilic bacteria and fecal coliforms than LV animals. Subclinical mastitis occurred in two ewes of the LV and one of the MV groups, while no cases were recorded in the HV group. Results suggest that airspace is a critical factor in dairy sheep housing and indicate that a volume allocation of less than 7 m<sup>3</sup>/animal may adversely affect the performance and health of the lactating ewe.

**(Key words:** sheep, airspace, milk yield, milk quality)

**Abbreviation key:** HV = high volume, LV = low volume, MV = medium volume, PMNLC = polymorphonuclear neutrophil leukocyte count.

### INTRODUCTION

Poor hygiene of the air and of building surface is potentially a serious limitation to high efficiencies of production and good health in intensive systems of animal husbandry (Wathes, 1994). A relationship between the concentration of airborne microorganisms and the hygienic quality of milk and udder health has been demonstrated for dairy cows and sheep (Barkema et al., 1999; Sevi et al., 1999, 2000).

Airspace has been recognized as one of the most important factors that influence the concentration of airborne particulates in animal houses (Hartung, 1989). In housed calves, Wathes et al. (1983) found that doubling air space allowance results in a reduction of airborne microorganisms equivalent to that achievable by quintupling air change rate. This may be of practical interest for sheep housing, because sheep are generally raised in warm climates and do not benefit from efficient ventilation systems.

Recommendations for cubic capacity and ventilation rate are available for poultry (Charles, 1981), pigs (Bruce, 1981), cattle (Webster, 1988), and horses (Wathes, 1989).

Little is known about the effects of airspace on the production performance of sheep because extensive production systems are predominant for this species. However, the gradual increase of intensive housing in sheep, as a consequence of the increased size of specialized dairy flocks, especially in the northern countries of the Mediterranean basin, necessitates specifications for space requirements of sheep and also design, materials, and structures of sheep houses.

In the present study, the concentrations of airborne microorganisms and the milk yield and udder health of ewes stocked at a different volume/animal were monitored.

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Corresponding author: A. Sevi; e-mail: agostinosevi@tiscalinet.it.

## MATERIALS AND METHODS

### Experimental Design and Animal Management

The experiment, which lasted 6 wk, was conducted during the spring (April and May) of 2000. Thirty-six midlactating Comisana ewes ( $106 \pm 1.3$  d in lactation), with no history of mastitis, were used. The animals were divided into three groups of 12 each, which were balanced for age, parity, time of lambing, number of lambs suckled, BW ( $56.48 \pm 1.16$  kg, means  $\pm$  SE), BCS ( $2.23 \pm 0.08$ ), milk yield ( $811.7 \pm 30.3$  g/d), and milk protein ( $5.92 \pm 0.08\%$ ) and fat ( $6.75 \pm 0.13\%$ ) contents. Groups were housed on straw litter in separate rooms of the same building, which were (length  $\times$  breadth  $\times$  height)  $8.1 \times 3 \times 2$  m,  $8.35 \times 3 \times 2.7$  m, and  $8.3 \times 3 \times 3.5$  m, respectively. The three groups were designed low, medium, and high volume/animal (**LV**, **MV**, and **HV**); each individual had 4.05, 5.64, and 7.26 m<sup>3</sup>, respectively. Volume allocation in dairy sheep housing varies widely because of missing reference values and because sheep are often kept in makeshift houses. We selected room volumes knowing that a space allocation of 2 m<sup>2</sup>/animal has been recommended to sustain productivity and udder health in the lactating ewe (Sevi et al., 1999). Hence, differences in airspace allowance across treatments were mainly obtained by changing room heights. The experimental rooms were bounded by polyethylene sheets framed by wooden plank and beams. They were adjacent, faced south, away from prevailing winds, and were provided with transom windows (total glazed area = 4.8 m<sup>2</sup>) placed at a height of about 1.5 m. A natural ventilation system was used. In all rooms, ventilation rate was measured daily at 2-h intervals from 0800 to 2000 by placing a thermo hot wire anemometer (LSI, I-20090, Settala Premenugo, Milan, Italy) over the air outlet and converting readings to m<sup>3</sup>/h per ewe. On average, ventilation rate was  $22.1 \pm 0.7$  m<sup>3</sup>/h per ewe. The ambient temperature and the relative humidity inside each room were continuously monitored throughout the trial by means of thermo-hygrographs (LSI) placed at a height of 1.5 m from the floor. In each pen, a layer of straw (about 0.3 kg/m<sup>2</sup>) was strewn on litter daily.

Each pen was provided with two mangers; feeder space per animal was about 0.45 m. The ewes were fed on a diet composed of a pelleted concentrate and ryegrass hay (37.5 and 62.5% of total diet, respectively), which was offered as a TMR twice daily. The chemical composition of dry matter was determined by standard procedures (AOAC, 1990) and contained 14.2% CP, 2.2% fat (by ether extraction), 24.9% crude fiber, and 7.9% ash. Water was available from automatic drinking troughs.

### Air Sampling

Air sampling was performed twice a week, at 0930 and 1630, at a height of 0.6 m from the floor. Samples were taken about 2.5 h after machine milking and 2 h after food administration and straw spreading on the litter. Each time the order in the sequence of air sampling in the experimental rooms was rearranged according to a preestablished program. The concentrations of mesophilic and psychrotroph bacteria, coliforms, staphylococci, *Pseudomonas* spp. and yeasts and molds were determined from 720 L of air (flow rate = 1.5 L/s). Air was sampled (Surface Air System pump, PBI International, Milan, Italy) directly onto plates containing plate count agar (Oxoid, Basingstoke, UK), violet red bile lactose agar (Biolife, Milan, Italy), Baird Parker agar (Oxoid) supplemented with egg yolk tellurite emulsion, *Pseudomonas* selective agar (Oxoid), and sabouraud dextrose agar (Oxoid), respectively. All measurements were made 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 4°C for a week for psychrotrophs, at 37°C for 24 to 36 h for coliforms, staphylococci, and *Pseudomonas* spp., and at 25°C for 96 h for yeasts and molds.

### Sampling and Analyses of Milk

Ewes were milked with pipeline milking machines (Alpha Laval Agri, SE-147 21 Tumbas, Sweden). Animals were moved twice daily (0700 and 1400 h) to a milking parlor that was about 30 m away from the experimental building. Daily milk yield was recorded 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 disinfecting of teats (0.7 ethyl alcohol) and discharging the first streams of foremilk. Samples were brought to our laboratory by means of transport tankers at 4°C. Upon arrival, the following measurements were carried out on milk: pH, total protein, fat and lactose content (IDF, 1990) using an infrared spectrophotometer (Milko Scan 133B; Foss Electric, Hillerød, Denmark), casein content (IDF, 1964), SCC (IDF, 1995) with a Foss Electric Fossomatic 90-cell counter, polymorphonuclear neutrophil leukocyte count (**PMNLC**), by means of direct microscopic count in milk smears stained with May-Grünwald-Giemsa, and 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).

All ewes were examined daily to detect the presence or confirm the absence of clinical signs of mastitis being

fever, pain, or gland swelling. A small quantity of milk was checked visually for signs of mastitis. Milk samples containing more than  $10^6$  somatic cells/ml were cultured for mastitis-related pathogens. Presumptive *Escherichia coli*, *Pseudomonas aeruginosa*, staphylococci, and streptococci were determined and identified at species level as described earlier (Sevi et al., 1999, 2001a). Samples were considered to be bacteriologically positive when at least  $10^3$  cfu/ml of the same type were isolated (Watkins et al., 1991). If two bacterial species were isolated, they were treated as a case of either species. If three or more bacterial species were cultured from a sample, the sample was considered contaminated. Sheep whose udders were without clinical abnormalities, and whose milk was apparently normal but bacteriologically positive (with SCC >  $10^6$ /ml and PMNLC > 20% of total somatic cells) were considered to have subclinical mastitis when the same bacterial species was isolated from milk samples at least in two of three consecutive samplings (Andrew et al., 1983).

The following bacteriological analyses were also carried out on milk fortnightly: enumeration of mesophilic microorganisms (IDF, 1991), psychrotrophs (IDF, 1991), total coliforms (IDF, 1985), and fecal coliforms on violet red bile agar (Biolife) incubated at  $44.5 \pm 0.5^\circ\text{C}$  for 24 h.

The BW and BCS of the ewes (in a six-point scale with 0 = thin and 5 = fat) were recorded at the beginning, at d 21 and at the end of the study period, after the morning milking but before feeding.

### Calculations and Statistical Analysis

Milk yield was corrected for fat content using the Cannas' (1999) equation. The energy content of the milk was calculated using the equation of Šebek and Everts (1992): 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 AFRC (1993). The net energy of the ration was calculated as the ratio between NE output (milk energy + maintenance energy + BW gain energy) and DMI. Data from ewes considered to have subclinical mastitis were excluded from statistical analysis of milk production data. All the variables were tested for normal distribution by the Shapiro-Wilk test (Shapiro and Wilk, 1965), 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, 1990). The variation due to treatment, trial week and their interaction

was tested. Individual animal variation within treatment was used as the error term. Pretreatment values of airborne microorganisms, and milk yield and quality were collected twice during the week before the commencement of the trial, which were used as covariates for air and milk variables. The data on bacteriologically positive milk samples and on mastitis prevalence were subjected to the  $\chi^2$  test. Body weights, BW changes, and feed intake and efficiency data were analyzed using ANOVA with one factor (treatment). Results are presented as the least square 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.  $P < 0.05$  was considered significant, unless otherwise noted.

## RESULTS

### Airborne Microorganisms

Ambient temperature was not changed by the experimental treatment (Table 1), whereas relative humidity was significantly lower in the HV than in the LV room during wk 3 ( $P < 0.01$ ) and in the HV than in the MV and LV rooms during wk 4 ( $P < 0.01$  and  $P < 0.001$ , respectively), 5 ( $P < 0.05$ ) and 6 ( $P < 0.01$  and  $P < 0.001$ , respectively). Significant effects of treatment and of treatment  $\times$  time were found for the air concentrations of microorganisms, with the LV room having higher mean mesophilic counts than the HV room (2.98 and 2.67  $\log_{10}$  of cfu/m<sup>3</sup>, respectively,  $P < 0.01$ ), as well as greater amounts of coliform bacteria ( $P < 0.05$ ) at wk 6, and of staphylococci ( $P < 0.05$ ) at wk 3 and 5 of the experiment. No differences were found for the air concentrations of psychrotroph bacteria, *Pseudomonas* spp. and yeast/molds.

### Milk Yield and Composition

The HV group gave greater yields of milk than the MV and the LV groups (943.5, 804.6, and 769.4 g/d, respectively,  $P < 0.001$ ) (Table 2), due to higher milk productions during wk 2 ( $P < 0.01$ ), 3 ( $P < 0.05$ ), 4, 5 ( $P < 0.05$  and  $P < 0.01$ , respectively), and 6 ( $P < 0.05$ ) of the study period. Ewes in the LV group also had a lower mean casein content in their milk than those in the HV group (4.32 and 4.61%, respectively,  $P < 0.01$ ), as well as lower ( $P < 0.05$ ) protein and fat contents than the other two groups during wk 5 and 6 of the experiment. Both the LV and MV groups had a lower ( $P < 0.05$ ) milk fat content than the HV group at wk 2.

Milk pH was significantly lower in the HV than in the LV group (6.58 and 6.72, respectively,  $P < 0.01$ ) (Table 3). Significant interactions of treatment  $\times$  time were found for renneting parameters, with the HV milk

**Table 1.** Ambient temperature, relative humidity, and air concentrations of microorganisms ( $\log_{10}$  of cfu/m<sup>3</sup>) in low (LV), medium (MV), and high (HV) volume rooms.

Item	Trial wk	LV (4.05 m <sup>3</sup> /ewe)	MV (5.64 m <sup>3</sup> /ewe)	HV (7.26 m <sup>3</sup> /ewe)	SE	Effects, <i>P</i>		
						Treatment	Time	Treatment × time
Temperature, °C	1	18.4	17.2	18.7				
	2	21.2	21.8	20.2				
	3	19.7	19.2	20.2				
	4	21.4	20.3	21.6				
	5	24.2	24.1	23.7				
	6	22.1	23.0	23.0				
	Mean	21.2	20.9	21.2	0.2	NS <sup>1</sup>	**	NS
Relative humidity, %	1	67.7	63.8	61.8				
	2	68.8	65.6	62.8				
	3	73.6 <sup>b</sup>	68.5 <sup>b</sup>	64.4 <sup>a</sup>				
	4	76.9 <sup>b</sup>	73.1 <sup>b</sup>	64.1 <sup>a</sup>				
	5	71.5 <sup>b</sup>	70.8 <sup>b</sup>	63.4 <sup>a</sup>				
	6	70.2 <sup>b</sup>	66.8 <sup>b</sup>	57.4 <sup>a</sup>				
	Mean	71.5 <sup>b</sup>	68.1 <sup>b</sup>	62.3 <sup>a</sup>	1.0	***	***	***
Mesophilic count	1	2.74	2.54	2.44				
	2	2.57	2.64	2.34				
	3	3.08 <sup>b</sup>	2.81	2.52 <sup>a</sup>				
	4	3.04 <sup>b</sup>	2.70	2.61 <sup>a</sup>				
	5	3.17	3.08	3.03 <sup>a</sup>				
	6	3.26	3.15	3.07				
	Mean	2.98 <sup>b</sup>	2.82 <sup>a</sup>	2.67 <sup>a</sup>	0.06	**	***	NS
Coliform count	1	1.11	1.12	1.10				
	2	1.16	1.14	1.11				
	3	1.19	1.18	1.13				
	4	1.26	1.27	1.20				
	5	1.57	1.46	1.38				
	6	1.98 <sup>b</sup>	1.89	1.62 <sup>a</sup>				
	Mean	1.38	1.35	1.26	0.06	NS	***	*
Staphylococci count	1	2.81	3.02	2.74				
	2	3.16	3.27	2.86				
	3	3.52 <sup>b</sup>	3.38	3.01 <sup>a</sup>				
	4	3.45	3.25	3.06				
	5	3.48 <sup>b</sup>	3.26	3.04 <sup>a</sup>				
	6	3.31	3.34	3.01				
	Mean	3.29 <sup>b</sup>	3.26 <sup>b</sup>	2.94 <sup>a</sup>	0.07	**	NS	*

<sup>a,b</sup>Means with a different superscript differ at  $P < 0.05$ .

<sup>1</sup>NS, not significant; \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

having a shorter ( $P < 0.05$ ) clotting time and a higher ( $P < 0.05$ ) clot firmness than the LV milk at wk 4 and 5. No differences were found for rate of clot formation across treatments.

Reduced airspace had a deleterious effect on the hygienic quality of milk (Table 4). Somatic cell counts were higher ( $P < 0.01$ ) in the LV group during wk 4 and in the LV and the MV groups during wk 5 and 6 compared with the HV group. Hence mean PMNLC were significantly higher ( $P < 0.001$ ) in the LV and MV groups than in the HV group (4.84, 4.59, and 4.25  $\log_{10}$  of cells/ml, respectively). The LV milk also had greater ( $P < 0.05$ ) amounts of mesophilic bacteria and of fecal coliforms than the HV milk on average (5.67 and 5.40, 2.65 and 2.38  $\log_{10}$  of cfu/ml, respectively). Psychrotroph counts were higher in the milk yielded by LV ( $P < 0.01$ ) and LV ewes ( $P < 0.05$ ) than in that of HV ewes (5.12, 5.00, and 4.72  $\log_{10}$  of cfu/ml, respectively).

### Incidence of Subclinical Mastitis

There were no cases of clinical mastitis during the study period (Table 5). Two cases of subclinical mastitis were detected in the LV group, infection being considered to have set in at d 14 and 28, respectively. One case was detected in the MV group at d 28 of the trial, whereas infected ewes were not found in the HV group. The number of bacteriologically positive milk samples was greater in the LV ( $P = 0.006$ ) and the MV ( $P = 0.042$ ) groups than in the HV group (14, 11, and 4 samples, respectively). Streptococci and coagulase-negative staphylococci were predominant (30 and 26%, respectively) in the bacteriologically positive milk samples followed by *Pseudomonas* spp. (23%) and *E. coli* (13%). *Staphylococcus aureus* was detected in four milk samples, whereas *Streptococcus agalactiae* and *Pseudomonas aeruginosa* were not found in any milk sample.



**Table 2.** Yield and protein, casein, and fat content of milk in ewes stocked at either a low (LV), a medium (MV), or a high (HV) volume/animal.

Item	Trial wk	LV (4.05 m <sup>3</sup> /ewe)	MV (5.64 m <sup>3</sup> /ewe)	HV (7.26 m <sup>3</sup> /ewe)	SE	Effects, <i>P</i>		
						Treatment	Time	Treatment × time
6.5% FCM, g/d	1	895.2	907.2	916.6				
	2	833.1 <sup>b</sup>	821.2 <sup>b</sup>	1031.9 <sup>a</sup>				
	3	779.3 <sup>b</sup>	767.3 <sup>b</sup>	960.9 <sup>a</sup>				
	4	763.7 <sup>b</sup>	825.1 <sup>b</sup>	991.3 <sup>a</sup>				
	5	671.7 <sup>b</sup>	823.6 <sup>b</sup>	940.5 <sup>a</sup>				
	6	673.9 <sup>b</sup>	683.1 <sup>b</sup>	819.9 <sup>a</sup>				
	Mean	769.4 <sup>b</sup>	804.6 <sup>b</sup>	943.5 <sup>a</sup>	23.2	***	***	***
Protein content, %	1	6.05	5.90	5.90				
	2	6.16	6.15	6.18				
	3	6.30	6.57	6.21				
	4	6.33	6.48	6.39				
	5	5.28 <sup>b</sup>	6.15 <sup>a</sup>	5.97 <sup>a</sup>				
	6	5.39 <sup>b</sup>	6.26 <sup>a</sup>	6.13 <sup>a</sup>				
	Mean	5.92	6.25	6.13	0.14	NS <sup>1</sup>	***	**
Casein content, %	1	4.40	4.41	4.69				
	2	4.34	4.52	4.55				
	3	4.41	4.44	4.54				
	4	4.42 <sup>b</sup>	4.73	4.93 <sup>a</sup>				
	5	4.11 <sup>b</sup>	4.39	4.58 <sup>a</sup>				
	6	4.29	4.29	4.36				
	Mean	4.32 <sup>b</sup>	4.46	4.61 <sup>a</sup>	0.06	**	***	*
Fat content, %	1	6.59	6.67	6.71				
	2	6.56 <sup>b</sup>	6.34 <sup>b</sup>	7.22 <sup>a</sup>				
	3	6.78	6.74	7.07				
	4	6.11	6.35	6.18				
	5	5.76 <sup>b</sup>	6.63 <sup>a</sup>	6.58 <sup>a</sup>				
	6	5.02 <sup>b</sup>	6.17 <sup>a</sup>	6.12 <sup>a</sup>				
	Mean	6.13	6.48	6.66	0.14	NS	***	*

<sup>a,b</sup>Means with a different superscript differ at  $P < 0.05$ .

<sup>1</sup>NS, not significant; \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

Body weights, BW changes, and DMI of ewes remained substantially unchanged during the study period and were unaffected by airspace allowance (Table 6). In contrast, the NE of the ration was higher in the HV ( $P < 0.01$ ) and MV ( $P < 0.01$ ) groups compared with the LV group during the 0- to 21-d period (4.32, 4.17, and 3.97 MJ/kg) and in the HV group than in the MV ( $P < 0.01$ ) and LV ( $P < 0.001$ ) groups during the 22- to 42-d period (4.12, 3.94, and 3.59 MJ/kg).

## DISCUSSION

Data in Table 1 show that relative humidity increased and air quality deteriorated as the volume allotted to ewes decreased, suggesting that airspace has direct and indirect effects on air and surface hygiene in animal houses. The direct effect is that, other things being equal, the cleanliness of the air with regard to airborne microorganisms and dusts is proportional to the volume of air into which those wastes are dispelled, while the indirect effect is that reduced air space results in increased moisture content of the house air and condensation on internal surfaces, both events enhancing

the growth and multiplication of microorganisms in the air and in the litter (Wathes et al., 1983; Dodd et al., 1984). Previous experiments have shown that high ambient microbial populations have a detrimental effect on feed efficiency and production performance in farmed livestock (Weawer and Meijerhof, 1991; Sevi et al., 2001b). Accordingly, data in Table 6 indicate that, in the present trial, the NE density of the ration decreased along with the yield and quality of milk as the airborne microorganisms concentrations increased when going from the LV to the MV and the HV treatments. The gradual increase observed in the microbial content of the milk with the reduction in housing airspace suggests a causal relationship. This indicates that increased microbial concentrations in the bedding may be regarded as the most insidious effect of inadequate volume allocation, especially in ewes whose udders are closer to the ground and may be more affected by the degree of litter pollution than those of other species. The greater bacterial load in the LV and MV milk can account for higher numbers of bacteriologically positive milk samples as well as for increased SCC and number of subclinical mastitis cases. However, a predisposing

**Table 3.** Coagulating behavior of milk in ewes stocked at either a low (LV), a medium (MV), or a high (HV) volume/animal.

Item	Trial wk	LV (4.05 m <sup>3</sup> /ewe)	MV (5.64 m <sup>3</sup> /ewe)	HV (7.26 m <sup>3</sup> /ewe)	SE	Effects, <i>P</i>		
						Treatment	Time	Treatment × time
pH	1	6.73	6.67	6.62				
	2	6.82	6.73	6.68				
	3	6.77	6.71	6.62				
	4	6.66	6.57	6.51				
	5	6.67	6.62	6.54				
	6	6.68	6.58	6.47				
	Mean	6.72 <sup>b</sup>	6.65	6.58 <sup>a</sup>	0.04	*	***	NS <sup>1</sup>
Clotting time, min	1	24.5	24.3	24.4				
	2	21.3	21.6	21.2				
	3	23.9	23.3	23.7				
	4	25.7 <sup>b</sup>	22.6	20.4 <sup>a</sup>				
	5	26.0 <sup>b</sup>	22.0	19.9 <sup>a</sup>				
	6	28.6	27.3	28.5				
	Mean	25.0	23.5	23.0	1.08	NS	***	**
Rate of clot formation, min	1	4.7	4.5	3.9				
	2	3.6	3.6	4.0				
	3	4.2	4.1	3.7				
	4	4.7	3.8	3.8				
	5	4.9	3.7	3.5				
	6	5.3	4.9	5.2				
	Mean	4.6	4.1	4.0	0.32	NS	***	NS
Clot firmness, mm	1	27.9	27.8	34.0				
	2	30.1	38.6	38.9				
	3	34.5	36.0	36.3				
	4	24.0 <sup>b</sup>	35.8	41.5 <sup>a</sup>				
	5	23.2 <sup>b</sup>	39.7	51.9 <sup>a</sup>				
	6	21.5	25.4	26.2				
	Mean	26.8	33.9	36.3	4.19	NS	***	*

<sup>a,b</sup>Means with a different superscript differ at  $P < 0.05$ .

<sup>1</sup>NS, not significant; \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

effect of reduced defense mechanisms to bacterial penetration into the udder was not excluded because it is known that the progress of mammary infection depends on the ability of bacterial pathogens to adapt to milk and udder tissues, the various virulence factors they activate and the efficiency of animal defense mechanisms (Saran and Leitner, 2000). Evidence shows that inadequate housing conditions may be stressful for animals (Hughes and Curtis, 1997) and that high concentrations of airborne wastes may impair the immune function in farmed livestock (Rylander, 1986) and enhance the risk for udder infections in lactating ewes (Sevi et al., 1999). Bacterial penetration into the udder, and the subsequent activation of immune defense mechanisms by the mammary gland, may have a number of adverse effects on the yield and quality of milk. Indeed, PMNL recruitment, which generally represents the first immune defense line of the mammary gland, can cause extensive epithelium secretory cell damage with the same mechanisms used to face invading bacteria (Burvenich et al., 2000). Enzymes produced by the bacterial flora, in turn, exert a postsecretory proteolytic and lipolytic action in milk (Auld et al., 1996; Sevi

et al., 2001b), and may act as plasminogen activators (Fajardo-Lira and Nielsen, 1998). Products of bacterial metabolism may activate some bioactive compounds (such as prostaglandins and cytokines), that induce an increase in capillary permeability, resulting in a breakdown in the blood-milk barrier, which in turn results in transudation of serum components into the lacteal secretion (Kehrli et al., 2000). These components include a range of hydrolytic enzymes, which further modify milk composition via the breakdown of casein and fat (Grieve and Kitchen, 1985). Data in Table 3 show that the gradual decrease in milk casein and fat contents when going from the LV to the MV and the HV treatments affected the renneting ability of milk. This could be expected, because clot formation involves the aggregation of casein micelles into a network within which the fat is entrapped (Dalglish, 1993).

## CONCLUSION

Airborne microorganism concentrations increased, and yield, nutritional properties, renneting ability, and hygienic quality of milk deteriorated as the volume al-

**Table 4.** Log<sub>10</sub> of somatic cell and bacteria counts (cfu/mL) in milks of ewes stocked at either a low (LV), a medium (MV), or a high (HV) volume/animal.

Item	Trial wk	LV (4.05 m <sup>3</sup> /ewe)	MV (5.64 m <sup>3</sup> /ewe)	HV (7.26 m <sup>3</sup> /ewe)	SE	Effects, <i>P</i>		
						Treatment	Time	Treatment × time
Somatic cell count	1	5.65	5.46	5.18				
	2	5.45	5.48	5.35				
	3	5.64	5.50	5.36				
	4	5.81 <sup>b</sup>	5.51	5.23 <sup>a</sup>				
	5	5.81 <sup>b</sup>	5.74 <sup>b</sup>	5.16 <sup>a</sup>				
	6	5.87 <sup>b</sup>	5.78 <sup>b</sup>	5.36 <sup>a</sup>				
	Mean	5.71 <sup>b</sup>	5.57 <sup>b</sup>	5.27 <sup>a</sup>	0.06	***	**	***
Mesophilic count	2	5.56	5.40	5.30				
	4	5.70	5.48	5.37				
	6	5.75	5.72	5.53				
	Mean	5.67 <sup>b</sup>	5.53	5.40 <sup>a</sup>	0.07	*	NS <sup>1</sup>	NS
Psychrotroph count	2	4.88	4.92	4.72				
	4	5.09	5.04	4.74				
	6	5.41 <sup>b</sup>	5.06	4.71 <sup>a</sup>				
	Mean	5.12 <sup>b</sup>	5.00 <sup>b</sup>	4.72 <sup>a</sup>	0.08	*	NS	NS
Total coliform count	2	3.02	2.84	2.97				
	4	3.26	3.14	2.88				
	6	3.91	3.90	3.85				
	Mean	3.39	3.29	3.24	0.13	NS	***	NS
Fecal coliform count	2	2.28	2.38	2.13				
	4	2.37	2.51	2.18				
	6	3.30 <sup>b</sup>	2.90	2.82 <sup>a</sup>				
	Mean	2.65 <sup>b</sup>	2.60	2.38 <sup>a</sup>	0.08	*	***	NS

<sup>a,b</sup>Means with a different superscript differ at  $P < 0.05$ .

<sup>1</sup>NS, not significant; \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

lotted to ewes decreased. This suggests that reduced airspace may be a limitation to high efficiencies of production and good health in farmed livestock, especially when animals cannot benefit from efficient ventilation systems, as generally occurs in sheep husbandry. Our results indicate that an airspace allocation of 7 m<sup>3</sup>/

animal can be recommended to sustain production performance and udder health in the lactating ewe. If dairy livestock cannot be allocated an adequate airspace/animal, most scrupulous control of housing sanitation is necessary, with special regard to efficient litter management.

**Table 5.** Cases of subclinical mastitis, number of bacteriologically positive milk samples, and bacteria isolated from the milk of ewes stocked at either a low (LV), a medium (MV), or a high (HV) volume/animal.

	Day of sampling	LV (4.05 m <sup>3</sup> /ewe)	MV (5.64 m <sup>3</sup> /ewe)	HV (7.26 m <sup>3</sup> /ewe)
No. of cases of subclinical mastitis	14	1	0	0
	28	2	1	0
	42	2	1	0
No. of bacteriologically positive milk samples	14	2	3	1
	28	5	4	1
	42	7	4	2
Bacteria isolated and no. of milk samples from which isolated				
<i>Escherichia coli</i>		4	2	1
<i>Streptococcus agalactiae</i>		0	0	0
<i>Enterococcus faecium</i>		3	3	0
Other streptococci		5	3	2
<i>Staphylococcus aureus</i>		1	2	1
<i>Staphylococcus xylosum</i>		2	1	0
<i>Staphylococcus chromogenes</i>		1	0	0
Other CN-staphylococci		5	3	2
<i>Pseudomonas aeruginosa</i>		0	0	0
<i>Pseudomonas</i> spp.		7	3	2

**Table 6.** Body weights, BW changes, DMI, and net energy of the ration in ewes stocked at either a low (LV), a medium (MV), or a high (HV) volume/animal.

Item	Day	LV (4.05 m <sup>3</sup> /ewe)	MV (5.64 m <sup>3</sup> /ewe)	HV (7.26 m <sup>3</sup> /ewe)	SE	Effects, <i>P</i>
						Treatment
BW, kg	0	56.80	56.02	56.61	1.61	NS <sup>1</sup>
	21	57.06	56.67	56.84	1.55	NS
	42	57.26	57.09	57.20	1.48	NS
BW changes, g/d	0 to 21	12.3	30.9	11.1	12.4	NS
	22 to 42	9.5	19.8	17.1	10.9	NS
DMI, kg/d	0 to 21	2.35	2.35	2.40	0.02	NS
	22 to 42	2.27	2.31	2.34	0.03	NS
Net energy of the ration <sup>†</sup> , MJ/kg	0 to 21	3.97 <sup>b</sup>	4.17 <sup>a</sup>	4.32 <sup>a</sup>	0.08	*
	22 to 42	3.59 <sup>c</sup>	3.94 <sup>b</sup>	4.12 <sup>a</sup>	0.07	**

<sup>a,b,c</sup>Means with a different superscript differ at  $P < 0.10$ .

<sup>1</sup>NS, not significant; \* $P < 0.10$ ; \*\* $P < 0.05$ .

<sup>†</sup>Calculated as the net energy output (milk energy + maintenance energy + BW gain energy) to DMI ratio.

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