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Effects of dietary protein level on ewe milk yield and nitrogen utilization, and on air quality under different ventilation rates

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The experiment, which lasted 53 d, was conducted during the winter (February and March) of 2004 and used 48 Comisana ewes in mid lactation. A 2×2 factorial design was used, with ewes receiving two levels of dietary crude protein (CP) (moderate, 16% CP *v.* low, 13% CP) in the dry matter (DM) and being exposed to two ventilation rates (moderate, 47 m^3/h *v.* low, 23·5 m^3/h per ewe) for each dietary treatment. Air concentrations of NH_3 and of microorganisms were measured twice weekly. Milk yield was recorded daily. Individual milk samples were analysed weekly for composition and fortnightly for bacteriological characteristics. After the last milk sampling (day 49 of the study period), four animals from each group were placed in a metabolism box and their individual faeces and urine were collected for three consecutive days. Amounts of urine and faeces excreted, and urinary and faecal N outputs were measured. The 16% CP diet resulted in a lower milk casein content and a higher milk urea concentration than the 13% CP diet, as well as in a reduced gross efficiency of utilization of dietary N, a greater amount of N excreted and a higher total coliform concentration in milk. The moderate ventilation rate resulted in higher yields of milk, irrespective of CP content. Significant interactions of CP level × ventilation rate were found for the amounts of urine, of total water and of faecal N, and for mesophilic concentration in milk, the highest values being displayed by the ewes fed the 16% CP diet and exposed to the low ventilation rate. The moderate dietary CP level and low ventilation rate had a deleterious effect on air concentrations of microorganisms and ammonia. Results suggested that a reduction of dietary CP level from 16 to 13% of DM had no detrimental effect on ewe milk yield in mid lactation and could even improve some of its nutritional and hygienic characteristics. Our findings also indicate that the choice of a proper ventilation rate is critical for high efficiency of production in the lactating ewe, especially in intensively managed flocks receiving diets high in CP.

Keywords: Dairy ewes, dietary protein level, milk yield, nitrogen emission, ventilation rate.

The nutritional and processing properties, and thereby the market value of milk, largely depend on its protein content. The efficiency of utilization of dietary N for milk protein synthesis in dairy animals is quite low (15–35%) (NRC, 1988; Tamminga, 1992) so farmers are driven to use high protein level diets to sustain milk production. This can result in a number of deleterious events for animals, farmers and the environment, such as an increase in the milk production cost; an overloading of renal draining activity; an increase of N output to the environment; and high levels of ammonia pollution in animal houses originating from animals' urine and faeces and which can be harmful to the health of both livestock and stock persons.

Previous experiments show that an increase in the protein level of the ration from 13 to 16% (Jaime & Purroy, 1995) and from 13 to 19% (Gonzalez et al. 1984) has a beneficial effect on ewe milk yield and protein content, and also results in higher blood urea concentrations (Jaime & Purroy, 1995) and increased N excretion in urine (Gonzalez et al. 1984). Recently, Cannas (2002) proposed a dietary CP content not lower than 17–18% for both early and mid-lactating ewes, in order to meet requirements for synthesis of milk and wool. Conversely, Cowan et al. (1981) did not find any difference in the yield and protein

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content of the milk from ewes receiving diets with CP concentrations of 116 and 143 g/kg dry matter (DM). Sevi et al. (1998) found that the efficiency of utilization of dietary N in the lactating ewe increases with decreasing protein levels in the diet from 16 to 13%, especially when limiting amino acids (lysine and methionine) in the ration are encapsulated to prevent bacterial deamination in the rumen.

Increased N excretion in urine and faeces, as a consequence of poor utilization of dietary N, can result in high microorganism and ammonia concentrations in animal houses which, in turn, demands a high ventilation rate for sustaining the performance and health of farmed livestock. Increased ammonia concentrations were found in sheep houses with poor ventilation (Sevi et al. 2002, 2003), which were associated with lowered milk yield and casein content, and with altered immune and endocrine responses of ewes. In particular, Sevi et al. (2003) recorded ammonia concentrations near or over the safety threshold of 10 ppm (Verstegen et al. 1994) after exposure of lactating ewes to a mean ventilation rate of 23 m^3/h during the winter season, and recommended a mean ventilation rate of 47 m^3/h per ewe for sustaining the yield and quality of ovine milk in winter.

In the light of these considerations, the present study was undertaken to assess: (i) the possibility of reducing N losses from ewes in mid lactation receiving high energy diets, without lowering the yield and quality of milk, via the reduction of dietary protein levels from 16 to 13% of DM, and thereby (ii) the possibility of reducing the winter ventilation rate in sheep houses from the recommended value of 47 to 23·5 m^3/h per ewe via the reduction of microbial proliferation and ammonia release from the manure.

Material and Methods

Experimental design and animal management

The experiment was conducted during the winter (February and March) of 2004 at the research station of the Italian Istituto Sperimentale della Zootecnia of Segezia-Foggia and used 48 Comisana ewes in mid lactation (92 ± 0·8 d in lactation at the beginning of the experiment). A 2 × 2 factorial design was tested with ewes receiving two dietary CP levels and being exposed to two ventilation rates for each dietary treatment. Treatments were: (1) low dietary CP level (13% of DM) at a low ventilation rate (23·5 m^3/h per ewe) (LPLV); (2) low dietary CP at a moderate ventilation rate (47 m^3/h per ewe) (LPMV); (3) moderate dietary CP (16% of DM) at a low ventilation rate (MPLV); and (4) moderate dietary CP level diet at a moderate ventilation rate (MPMV). The animals were housed in a prefabricated building provided with external paddocks before the experiment began. 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. No cases of clinical mastitis were recorded throughout the study period. The experimental phase, which lasted 53 d, was preceded by a 10-d adjustment period, during which time all ewes were fed on a diet of (kg/d) 0·25 wheat straw, 1·4 vetch and oat hay and 0·75 pelletted concentrate containing 20·4% CP in the DM (metabolizable energy (ME) 9·8 MJ/kg DM). At the end of this adjustment period, the ewes were divided into the four experimental groups, which were balanced for parity, time of lambing and number of lambs suckled. Body weights (BW ± SE) were 49·6 ± 2·0, 51·1 ± 2·2, 50·6 ± 1·7 and 49·5 ± 1·9 kg, in the LPLV, MPLV, LPMV and MPMV groups, respectively; corresponding values for milk yield were 620 ± 37, 578 ± 36, 615 ± 44 and 602 ± 40 g/d; and for milk protein content, 5·92 ± 0·22, 6·07 ± 0·21, 5·97 ± 0·15 and 5·98 ± 0·23 g/kg.

To avoid excessive fattening, ewes were not fed *ad libitum*; however, in order to avoid a limiting effect of feed intake on ewe performance, the amount of DM available to animals was 5% of their body weight, which is the maximum level of voluntary feed intake predicted for lactating ewes (INRA, 1988). So, during days 1–49 of the experimental period, the LP ewes received a diet containing 13% CP in DM containing 0·8 kg of vetch and oat hay, 0·8 kg of barley hay and 1·2 kg of a pelletted concentrate containing 15·6% CP in the DM, while the MP ewes were given a diet containing 16% CP in the DM and containing 1·6 kg of vetch and oat hay and 1·2 kg of a concentrate containing 20·4% CP in the DM. Daily rations were completely consumed by the ewes in all groups.

Concentrate was composed of barley flour, durum wheat shorts, sunflower meal, maize flour, 44% CP extruded soybean meal, dehydrated alfalfa meal, beet molasses, brewer's yeast, $CaCO_3$, Na_2CO_3, bentonite, lignum sulphite and vitamin mix. Such ingredients were mixed in different amounts to obtain two different CP levels and the same nutritional value of concentrates. Both diets contained 0·85% rumen-protected lysine (Overlysin, containing 300 g/kg L-lysine encapsulated in hydrogenated vegetable oils) and 0·28% rumen-protected methionine (Overmet, containing 400 g/kg DL-methionine encapsulated in hydrogenated vegetable oils), which were supplied by Ascor Chimica (Capocalle di Bertinoro, Italy). Hay and concentrate were offered twice daily as a total mixed ration. The chemical composition and nutritional value of total diets and of single diet components are given in Table 1. Water was available at all times from automatic drinking troughs.

Groups were separately housed on straw bedding in 8 m × 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 m^2), placed at a height of 2·5 m. In each room a circular suction fan of 0·28 m^2 (Vortice, Tribiano-Milan, Italy) was placed at 2·5 m from

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Table 1. Chemical composition and nutritional value of forage and concentrates and of the experimental diets used in the experiment (values on a dry matter basis)

	Vetch/oat hay	Barley hay	Low protein concentrate†	Moderate protein concentrate‡	Low protein diet	Moderate protein diet
Dry matter, %	89·88	91·62	87·91	88·07	89·53	89·19
Crude protein (CP), %	12·37	9·69	15·55	20·40	12·97	15·95
Fat by ether extract, %	1·41	1·24	3·09	2·96	2·08	2·07
Neutral detergent fibre, %	51·49	64·96	24·22	27·28	43·65	41·11
Acid detergent fibre, %	33·39	39·05	11·16	13·68	25·48	24·94
Acid detergent lignin, %	3·51	3·31	3·87	4·37	3·61	3·88
Ash, %	10·04	6·93	8·63	9·11	8·55	9·64
Non Structural Carbohydrates, %	24·69	17·18	48·51	40·25	37·86	31·35
Metabolizable energy (ME), MJ/kg§					11·9	12·0
Fermentable metabolizable energy (FME), MJ/kg§					10·0	9·4
Metabolizable protein (MP), %§					10·4	11·5
ME to CP ratio					0·92	0·75
FME to MP ratio					0·96	0·81

† Ingredients g/kg: 370 barley flour, 202 durum wheat shorts, 155 corn flour, 40 sunflower meal extracted by solvent, 25 soybean meal extracted by solvent, 102 alfalfa hay dehydrated, 20 beet molasses, 17 calcium carbonate, 7 di-calcium phosphate, 6 sodium bicarbonate, 10 lignum sulphite, 10 Bentonite, 5 brewer's yeast, 5 vitamin mix, 20 rumen-protected lysine, 6 rumen-protected methionine

‡ Ingredients g/kg: 293 barley flour, 226 durum wheat shorts, 90 corn flour, 140 sunflower meal extracted by solvent, 70 soybean meal extracted by solvent, 75 alfalfa hay dehydrated, 20 beet molasses, 17 calcium carbonate, 7 di-calcium phosphate, 6 sodium bicarbonate, 10 lignum sulphite, 10 Bentonite, 5 brewer's yeast, 5 vitamin mix, 20 rumen-protected lysine, 6 rumen-protected methionine

§ Values calculated and estimated on the basis of AFRC (1993)

the floor and two 0·36 m^2 air inlets were placed at ground level on the opposite wall. Fans provided 10 ventilation cycles/d and fan speed was kept constant at 1 m/s. Each cycle duration was 40 min in the LV rooms and 80 min in the MV rooms; five cycles were during daytime from 07.00 to 19.00 (07.00, 09.40, 12.20, 15.20 and 17.40) and five during night-time from 21.00 to 06.00 (21.00, 23.00, 01.00, 03.00 and 04.40). 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 m^3/h per ewe. 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, in order 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/m^2) on bedding was provided daily. Averages of ambient temperature and relative humidity inside the experimental rooms ranged from 9·3 to 10 °C and from 73·3 to 74·7%, respectively.

Sampling and analysis of milk

Ewes were milked using a highline milking machine (Alfa Laval Agri, SE-147 21 Tumba, Sweden) twice daily at 08.00 and 14.30. 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 disinfecting the teats with 70% ethanol and discharging the first streams of foremilk. Milk samples were carried to our laboratory by means of transport tankers at 4°. The following measurements were made on milk: 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, and somatic cell count (SCC) using a Foss Electric Fossomatic 90 cell counter (IDF, 1995). At weeks 1, 3, 5 and 7 milk casein content (IDF, 1964) was determined. Milk urea concentration was also recorded using a Foodlab spectrophotometer (CDR, Florence, Italy), and optical density was then measured at a wavelength of 700 nm. Enumeration of mesophilic bacteria (IDF, 1991) and total coliforms (IDF, 1985) were also carried out on milk fortnightly throughout the study period.

Sampling and analyses of air, urine and faeces

Air sampling was performed twice weekly in the morning, starting from 09.00 (fans switched off) and in the afternoon, starting from 15.30 (fans switched on). Air was sampled 0·6 m from the floor and the sequence of air sampling in the four experimental rooms changed according to a prearranged programme.

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Concentrations of mesophilic microorganisms, coliforms and N-fixing bacteria were measured from 240 l of air (flow rate = 1·5 l/s). Air was sampled using a Surface Air System pump (PBI International, Milan, Italy), directly onto plates containing plate count agar (Oxoid, Basingstoke, UK), violet red bile agar (Oxoid), and the Azotobacter Medium (DSMZ, Braunschweig, Germany), respectively. All measurements were made at six locations within each room. After sampling, the plates were immediately incubated at 30 °C for 24–36 h for mesophilic bacteria, at 37 °C for 24–36 h for coliforms (under anaerobiosis), and at 37 °C for 48 h for N-fixing bacteria.

Air concentration of NH_3 was recorded from ten locations within each room, using a Gas Detection Pump (Dräger-Italia, Corsico-Milan, Italy); NH_3 concentration was determined colorimetrically in graduated detection tubes.

After the last milk sampling and during three consecutive days (day 50–53 of the experimental period), four animals from each experimental group were placed in metabolism boxes, which were allocated inside the four experimental rooms. Ewes in each group were exposed to the same ventilation regime as during the day 1–49 period and received a 2·4 kg daily feed ration, keeping the same hay to concentrate ratio as during the day 1–49 period. The amount of feed given to the ewes was reduced during the day 50–53 period taking into account decreased energy requirements due to enclosure of animals in metabolism crates. After a 1-d adjustment period, individual faeces and urine were collected for three consecutive days in stainless steel containers fixed to the slatted floor of each box. Individual feed intakes were recorded as the difference between feed given in the trough and feed refusals. The total amount of individual urine was measured and then collected in a plastic bin containing 2 M-H_2SO_4 to avoid fermentation and losses of ammonia, as described by Colombani et al. (1988). NH_4-N was distilled with MgO and titrated using 0·1 M-HCl; total N was determined using the Kjeldahl method. Every 24 h, the total amount of faeces was collected in a plastic bin, weighed and dried in an oven to determine their moisture content; total N was then determined by the Kjeldahl method.

After the morning milking but before feeding, the BW of the ewes was recorded at the beginning, and at days 21 and 49 of the study period.

Calculations and statistical analysis

The gross efficiency of utilization of dietary N was calculated as the ratio (%) between milk N output and dietary N intake. All variables were tested for normal distribution using the Shapiro-Wilk test (Shapiro & Wilk, 1965). Milk SCC and air and milk microorganism counts were transformed into log form to normalize their frequency distributions before performing statistical analysis. Milk, air and manure variables were subjected to ANOVA for repeated measures and were processed using the GLM procedure (SAS, 1999). Variations due to dietary protein level, ventilation rate, trial week and their interactions were tested. Individual animal variations within dietary protein level and ventilation rate or sampling location within room were used as error terms. BW and BW changes were analysed using ANOVA with two categorical factors (dietary protein level and ventilation rate). When significant effects ($P<0·05$) were found, Student's *t* test was used to locate significant differences between means.

Results and Discussion

Air quality

Concentrations of ammonia and microorganisms in the air were lower than those reported for cattle, pig and poultry houses (Muller & Wieser, 1987; Hartung, 1994) but in line with our earlier findings for sheep houses (Sevi et al. 2001, 2002). In particular, daily averages of N-fixing bacteria and coliform counts were $(0·62–0·88) \times 10^3$ cfu/m^3 air and 2·1–6·1 cfu/m^3 air in the LPLV and MPLV groups, respectively, while mesophilic counts were $(0·99–1·35) \times 10^3$ cfu/m^3 air in the MPMV and LPMV groups, respectively.

No significant differences emerged among groups in overall mean values for all the air parameters recorded. However, Sevi et al. (2002) showed that even temporary rises in air concentrations of gaseous pollutants and microorganisms may have a deleterious effect on animal welfare and efficiency of production. Indeed, when maximum and minimum air measurements were separately subjected to ANOVA, a significant interaction of protein level × ventilation rate ($P<0·01$) was found for averages of maximum NH_3 concentrations (recorded in the morning, fans being switched off), with the LPLV room having the highest and the LPMV room the lowest NH_3 levels, and for maximum total coliform concentrations, with the MPLV room displaying about 2-fold higher values than all the other three rooms (Table 2). An effect of ventilation rate ($P<0·01$) was observed for averages of maximum N-fixing bacteria, which were about 15% higher in the LV than in the MV rooms (4·1 *v.* 3·56 $\log_{10}$ cfu/m^3). No differences emerged between groups in averages of minimum NH_3 and bacterial loads.

A significant effect of time was found for maximum ammonia concentrations ($P<0·05$) and for mesophilic and total coliform counts ($P<0·01$) due to a general increase of values during the last three trial weeks (results not shown).

Milk yield and composition

Milk yield per ewe was not affected by dietary protein level; averages of milk production ranged from 785 to 798 g/d in the LP and MP groups (Table 3). The marked rise in milk production, which was observed in both LP and MP groups compared with pretreatment period (~200 g/d), may be explained by the increase in energy

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Table 2. Least square means ± SEM† of air concentrations of ammonia and microorganisms in the rooms containing ewes fed on a low and moderate dietary CP level and exposed to a low (LPLV and MPLV) or moderate ventilation rate (LPMV and MPMV)

							Effects, *P*		
Item		LPLV	MPLV	LPMV	MPMV	SEM	CP level	Ventilation rate	CP level × ventilation rate
NH_3, ppm	Max	9·6b‡	7·0ab	6·3a	7·4ab	0·9	NS§	*	**
	Min	3·0	4·0	2·9	3·8	0·7	NS	NS	NS
Mesophilic bacteria, log_{10} cfu/m^3	Max	4·13	4·46	4·39	3·93	0·21	NS	NS	NS
	Min	1·81	1·54	1·57	1·79	0·11	NS	NS	NS
Total coliforms, log_{10} cfu/m^3	Max	0·16a	0·37b	0·21a	0·18a	0·05	NS	NS	**
	Min	0·10	0·13	0·14	0·20	0·04	NS	NS	NS
N-fixing bacteria, log_{10} cfu/m^3	Max	3·82ab	4·38b	3·91ab	3·21a	0·19	NS	**	NS
	Min	1·60	1·36	1·52	1·24	0·33	NS	NS	NS

† SEM, standard error of the mean response over the 7 weeks for each treatment
‡ In rows, means followed by different letters are significantly different at $P<0·05$
§ NS, not significant, * $P<0·05$, ** $P<0·01$

intake and reduction in locomotion due to confinement in pens. The failure to find significant effects of dietary protein level on ewe milk yield is consistent with the results of a previous experiment with Comisana ewes fed rumen-protected lysine and methionine at two levels (13 *v.* 16%) of dietary CP (Sevi et al. 1998). Cowan et al. (1981) obtained similar results when assessing the impact of 11·5 and 14·5% CP diets on milk yield in Finnish Landrace × Dorset Horn ewes. Gonzalez et al. (1984), giving lactating ewes rations containing 13, 16 and 19% CP at ME levels of 19, 23 and 27 MJ/d, observed a milk yield response to protein supplementation only at relatively high energy intakes. Conversely, Jaime & Purroy (1995), keeping ME intake constant at 12·5 MJ, found an increase in ewe milk yield when passing from 13 to 16% CP, irrespective of the protein source (faba beans, soybean cake and fish meal).

A significant effect ($P<0·001$) of ventilation rate on milk production was observed, with the MV groups giving 13% higher average yields of milk than the LV groups (840 *v.* 744 g/d). In particular, significantly lower milk yields ($P<0·001$) were found in the LPLV and MPLV groups than in the LPMV and the MPMV groups during the first week of the trial and during weeks 5 and 6 in the LPLV than in the in LPMV and the MPMV groups. These results may seem surprising given that changes in air pollution between the LV and MV rooms were not very marked. However, one of our previous experiments (Sevi et al. 2003) showed that dairy sheep are very susceptible to poor air quality and that a proper ventilation rate is also required during the winter season to sustain high efficiencies of milk production. In fact, ewes have high productive levels in winter because they are in early or mid lactation and so the adverse effects of a poor ventilation rate on milk yield can be even more marked than in summer.

Neither the fat nor the protein content of milk was changed by dietary CP level, in agreement with the results of Jaime & Purroy (1995) in Rasa Aragonesa and of Cowan et al. (1981) in Finnish Landrace × Dorset Horn ewes. Similar results were also found by Frank & Swensson (2002) in Swedish Holstein receiving diets with of 13% and 17% CP in DM. Gonzalez et al. (1984) found an increased protein content, but not fat content of Finnish Landrace × Dorset Horn ewes' milk when increasing dietary CP level from 13% to 16% and 19% of DM.

MPMV ewes displayed a significantly lower ($P<0·05$) milk casein content than LPLV ewes during week 1 and than the LPLV and the LPMV animals during week 7 of the study. As a consequence, averages of milk casein content were lower ($P<0·05$) for MPMV than for LPLV and LPMV groups, while the MPLV group showed intermediate values. Such differences are not easy to interpret. A tentative explanation may be the imbalance between fermentable and degradable protein in the rumen, as documented by the lower FME to MP ratio associated with a lower proportion of non-structural carbohydrates (NSC). However, Landau et al. (2005) when assessing the effects of diets differing in the provision of rumen degradable organic matter and degradable CP, found that the highest organic matter to CP ratio had a depressive effect on milk protein but not on casein content. So further research is needed on this topic, which is of great practical interest given that sheep milk is almost totally made into dairy products.

Milk urea concentrations and gross efficiency of utilization of dietary N (Fig. 1) lend support to this hypothesis. In fact, a significant CP level effect ($P<0·001$) was observed for milk urea concentrations, which depended on the fact that the LPMV group displayed lower urea levels in milk than both MP groups throughout the study, and so did the LPLV group with the exception of the first week of the trial. Indeed, evidence exists (Jaime & Purroy, 1995; Cannas et al. 1998; Frank & Swensson, 2002) that milk and blood urea concentrations are strictly correlated with dietary CP levels, especially when the ME to CP ratio

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Table 3. Least square means ± SEM† of milk yield and chemical composition of ewes fed low and moderate dietary levels of CP and exposed to a low (LPLV and MPLV) or moderate ventilation rate (LPMV and MPMV)

							Effects, *P*		
Item	Wk	LPLV	MPLV	LPMV	MPMV	SEM	CP level	Ventilation rate	CP level × Ventilation rate
Milk yield, g/d	1	711a‡	738a	867b	878b				
	2	799	808	832	848				
	3	742	763	870	858				
	4	782	729	852	803				
	5	675a	779ab	847b	857b				
	6	673a	772ab	852b	825b				
	7	693	753	803	752				
	Mean	725a	763a	846b	833b	21·2	NS§	***	NS
Fat content, %	1	6·05	5·80	5·75	5·78				
	2	5·77	5·74	5·59	5·81				
	3	5·47	5·31	5·23	5·39				
	4	5·91	5·89	6·14	6·33				
	5	5·95	5·53	5·83	5·74				
	6	5·72	5·84	6·16	6·23				
	7	5·97	5·59	5·72	5·80				
	Mean	5·83	5·67	5·77	5·87	0·22	NS	NS	NS
Protein content, %	1	5·73	5·61	5·62	5·64				
	2	5·43	5·64	5·65	5·55				
	3	5·83	5·95	5·87	5·73				
	4	5·75	5·74	5·78	5·61				
	5	5·93	5·73	5·83	5·68				
	6	5·80	5·75	5·73	5·49				
	7	5·68	5·73	5·62	5·63				
	Mean	5·74	5·73	5·73	5·62	0·12	NS	NS	NS
Casein content, %	1	4·53b	4·37ab	4·27ab	4·00a				
	3	4·37	4·43	4·81	4·38				
	5	4·64	4·30	4·52	4·45				
	7	4·48b	4·28ab	4·55b	4·00a				
	Mean	4·51b	4·34ab	4·54b	4·21a	0·10	**	NS	NS
Urea, mg/dl	1	34·45b	36·05b	26·86a	38·18b				
	3	35·33a	44·01b	35·21a	43·64b				
	5	36·00a	42·52b	37·87a	45·29b				
	7	38·14a	45·54b	38·99a	46·99b				
	Mean	35·98a	42·03b	34·73a	43·52b	0·84	***	NS	NS

† SEM, standard error of the mean response over the 7 weeks for each treatment
‡ In rows, means followed by different letters are significantly different at $P<0·05$
§ NS, not significant, $^{**}P<0·01$, $^{***}P<0·001$

and the dietary NSC are relatively low. Indeed, both events can result in lower availability of fermentable energy in the rumen, which in turn leads to high ruminal ammonia concentrations and, consequently, to a higher urea production by the liver (Cannas et al. 1998). The MP groups, and especially that exposed to the low ventilation rate, required more dietary N to synthesize milk protein than the LP groups. In fact, a dietary CP effect ($P<0·05$) was also found for the gross efficiency of utilization of dietary N during the day 0–21 period (13·45, 11·64, 10·86 and 9·84%, respectively, in the LPMV, the LPLV, the MPMV and the MPLV groups) and the day 22–49 period (13·25, 11·26, 10·13 and 9·71%, respectively). Sevi et al. (1998) in Comisana ewes, and Kaufmann & St-Pierre (2001) in Holstein and Jersey cows observed a substantial drop in the efficiency of converting absorbed N to milk N as the CP level in the diet increased. Dietary CP level did not affect pH and the lactose content of milk.

Ventilation rate did not affect the concentration of any milk constituent. A time effect ($P<0·001$) was found for the urea concentration in milk, which can be ascribed to a progressive increase of milk urea levels with the advancement of the study period (33·9, 39·5, 40·4 and 42·4 mg/dl at weeks 1, 3, 5 and 7 of the trial) probably due to a gradual reduction in N requirements for milk protein synthesis.

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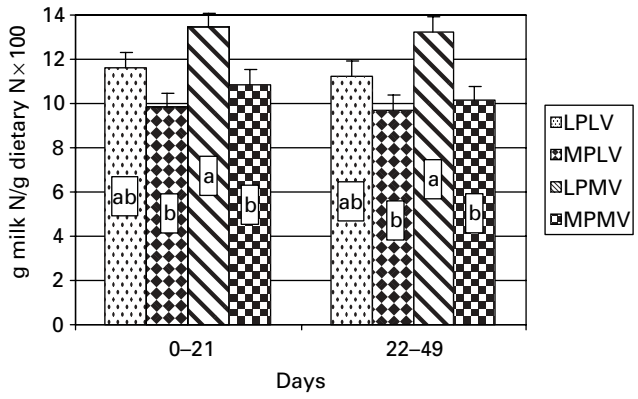

Fig. 1. Gross efficiency of utilization of dietary N in ewes fed diets of low and moderate CP level and exposed to a low (LPLV and MPLV) or moderate ventilation rate (LPMV and MPMV).

Values are least square means ± SE of the milk N output to dietary N intake ratio × 100

Daily production of milk constituents reflected differences in milk yield between LV and MV groups. In fact, the MV groups displayed higher averages ($P<0·001$) of milk protein yield (47·6 *v.* 42·6 g/d), of fat yield (48·8 *v.* 42·7 g/d) and of casein yield (36·7 *v.* 32·9 g/d) compared with the LV groups.

No significant differences were observed among treatments for average daily gains, which were 20, 34, 43 and 48 ± 17 g/d in the LPMV, LPLV, MPLV and MPMV groups, respectively.

Nitrogen excretion in urine and faeces

DM intake of ewes during confinement in the metabolism cages was 2·27, 2·28, 2·31 and 2·26 kg/d, and N intake was 47·2, 58·2, 48 and 57·7 g/d in LPLV, MPLV, LPMV and MPMV groups, respectively. Significant interactions ($P<0·01$) of dietary CP level × ventilation rate were found for total water and urinary excretion, with the MPLV group excreting 40–64% higher volumes of urine and 40–79% greater amounts of total water than the other experimental groups (Table 4). MP ewes excreted significantly more N than LP animals (41·57 *v.* 34·72 g/d; $P<0·05$), in agreement with previous findings in Swedish Holstein cows (Frank & Swensson, 2002). A combined effect of CP level × ventilation rate was also observed for faecal N emission, with MPLV ewes excreting about 15% more N than the LPMV and MPMV ewes ($P<0·05$) and about 30% more N than the LPLV animals ($P<0·01$). The low CP level resulted in a lower amount of faeces ($P<0·05$) and in less wet faeces ($P<0·05$) and led to higher NH_3 release in the urine ($P<0·001$) compared with the moderate CP level, irrespective of ventilation rate.

Giger-Reverdin & Gihad (1991) showed that higher dietary protein levels diets are responsible for higher water intakes in goats. Unfortunately, water intakes were not recorded in the present study because the experimental rooms were provided with automatic drinking troughs, but it is likely that the higher urine and total water outputs, which were observed in the groups receiving the moderate level of dietary CP, were related at least partly to the greater volumes of water consumed. A reduced efficiency of water resorption via the intestine and kidney was not excluded, especially for the ewes fed the moderate CP level and exposed to the low ventilation rate, which displayed the highest volumes of urine and water excreted.

The increase in the urinary NH_3 content in the LP groups may be ascribed to a concentration effect owing to their lower urine and total water output. However, Swensson (2003) suggested that the concentration of urea, rather than the total amount of urea in the urine, is responsible for higher NH_3 release, and argued that the increase of urine volume may be regarded as a sustainable strategy to reduce NH_3 emissions.

Release of NH_3 from urine (predominantly) and from faeces is one of the most important sources of environmental pollution associated with dairy production: the magnitude of NH_3 emission depends on the pH of urine, and on the physical factors related to air conditions, such as temperature and wind speed (Monteny et al. 2002). This explains why the highest NH_3 levels were recorded in the room housing the LPLV ewes, which had the highest NH_4-N in their urine and were exposed to the low ventilation rate. Similarly, the highest faeces and faecal N excretion from the MPLV ewes may be claimed for the most elevated air concentrations of total coliforms and N-fixing bacteria recorded in the MPLV room. The highest faecal output and the relatively high weight gains, which were displayed by the MPLV ewes, suggest that both the use of excess N for body protein synthesis and reduced nutrient digestibility were responsible for the lowered efficiency of dietary N utilization for milk protein synthesis in this group. Indeed, it has been postulated that poor welfare conditions can modify digestive tract physiology, producing a modification in mobility and nutrient utilization and, as a result, a higher amount of incompletely digested material (Bertoni, 1999). Therefore, an adverse effect of poor air quality on dietary N utilization cannot be excluded as an explanation of the higher faecal N outputs that were recorded in the ewes fed the higher CP level diets and exposed to the low ventilation rate.

Hygienic quality of milk

Somatic cell count (SCC) and bacteria counts were low in all the experimental groups. Averages of leucocyte concentration in milk ranged from 148 000 cells/ml and 250 000 cells/ml milk in the MPMV and LPLV groups, respectively, and never exceeded the threshold of 500 000 cells/ml milk, which has been suggested as a criterion of normality for ovine milk for the purpose of making an early diagnosis of mastitis (Sevi et al. 1999). Averages of mesophilic count ranged from 116 000 in LPMV to

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Table 4. Least square means ± SEM† of N intake, and urinary and faeces excretion of ewes fed low and moderate levels of CP and exposed to a low (LPLV and MPLV) or moderate ventilation rate (LPMV and MPMV)

						Effects, *P*		
Item	LPLV	MPLV	LPMV	MPMV	SEM	CP level	Ventilation rate	Protein level × Ventilation rate
Urine, l/d	0·936a‡	1·531b	1·100a	0·986a	0·119	NS§	NS	**
Faeces, kg/d	0·897a	1·627b	0·990a	1·310b	0·126	*	NS	NS
Faeces moisture, %	63a	71b	60a	71b	2	*	NS	NS
Total Water excreted, l/d	1·501a	2·686b	1·694a	1·916a	0·167	**	NS	**
Urinary NH_4-N, g/d	1·99b	0·79a	1·86b	0·55a	0·18	***	NS	NS
Urinary N, g/d	20·39	25·11	21·98	26·33	2·06	NS	NS	NS
Faecal N, g/d	13·00a	16·81b	14·07a	14·91a	0·57	°	NS	*
Total N excreted, g/d	33·39a	41·92b	36·05ab	41·22b	2·17	*	NS	NS

† SEM standard error of the mean response over the 3 d for each treatment
‡ In rows, means followed by different letters are significantly different at $P<0·05$
§ NS, not significant, ° $P<0·10$, * $P<0·05$, ** $P<0·01$, *** $P<0·001$

Table 5. Least square means ± SEM of somatic cell count (SCC) and microbial counts in the milk of ewes fed low and moderate levels of CP and exposed to a low (LPLV and MPLV) or moderate ventilation rate (LPMV and MPMV)

							Effects, *P*		
Item	Week	LPLV	MPLV	LPMV	MPMV	SEM	CP level	Ventilation rate	CP level × Ventilation rate
SCC, log_{10} of cells/ml	1	2·24b†	1·85a	1·86a	1·76a				
	2	1·90	1·84	1·96	1·86				
	3	2·06b	1·90ab	2·02b	1·65a				
	4	1·91ab	2·12b	2·13b	1·79a				
	5	2·34c	1·79ab	2·03b	1·63a				
	6	2·04b	1·78ab	1·92ab	1·66a				
	7	2·07b	1·87ab	1·89ab	1·74a				
	Mean	2·08c	1·88b	1·97b	1·73a	0·04	***	**	NS‡
Mesophilic count, log_{10} of cfu/ml	2	4·59b	4·54b	3·90a	4·06a				
	4	5·08	5·05	4·87	5·14				
	6	5·48b	5·70b	5·70a	5·31ab				
	Mean	5·05c	5·10c	4·62a	4·84b	0·07	NS	***	*
Total coliforms, log_{10} of cfu/ml	2	1·33	1·50	1·06	1·10				
	4	2·27a	3·03b	2·62ab	2·35a				
	6	2·28a	2·47ab	2·20a	2·85b				
	Mean	1·96a	2·33b	1·96a	2·10ab	0·11	*	NS	NS

† In rows, means followed by different letters are significantly different at $P<0·05$
‡ NS, not significant, *$P<0·05$, ** $P<0·01$, ***$P<0·001$

257 000 cfu/ml milk and MPLV groups, and only in the MPLV group, at week 6 of the experiment, exceeded 500 000 cfu/ml milk, which is the threshold indicated by EU directive 92/46 for ewe milk intended for cheese production without heat treatment. Averages of total coliform count ranged from 340 to 1095 cfu/ml milk in the LPLV and MPLV groups, and exceeded 2000 cfu/ml milk only in the MPLV group at week 4 of the experiment.

In spite of the relatively low levels recorded, marked changes were observed in SCC and bacteria loads among the experimental groups (Table 5). Averages of SCC were affected by CP level (2·03 *v.* 1·80 log_{10} cells/ml in the LP and MP groups, respectively, $P<0·001$) and by ventilation rate (1·98 *v.* 1·85 log_{10} cells/ml in the LV and MV groups, respectively, $P<0·01$). In particular, significant differences were observed between the LP and MP groups at weeks 1 (2·04 *v.* 1·81 log_{10} cells/ml, $P<0·05$), 3 (2·04 *v.* 1·78 log_{10} cells/ml, $P<0·05$), 5 (2·18 *v.* 1·71 log_{10} cells/ml, $P<0·001$) and 6 (1·98 *v.* 1·72 log_{10} cells/ml, $P<0·05$), and between LV and MV groups at weeks 1 (2·03 *v.* 1·81 log_{10} cells/ml, $P<0·05$) and 5 (2·06 *v.* 1·83 log_{10} cells/ml, $P<0·05$). Ventilation rate ($P<0·001$) and the interaction of protein level × ventilation rate ($P<0·05$) affected the mesophilic

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concentration in milk. Indeed, averages of mesophilic count were higher in the LV than in MV groups (5·08 *v.* 4·73 cfu/ml milk, respectively) and, within the moderate ventilation rate, in the 16% CP than in the 13% CP group (4·84 *v.* 4·62 cfu/ml milk). A CP level effect was observed for total coliform count, with the MP groups having significantly higher average coliform concentrations than the LP groups (2·22 *v.* 1·96 cfu/ml, respectively, $P<0·05$). A time effect was found for bacteria loads ($P<0·001$) due to a progressive increase in milk mesophilic counts when passing from week 2 to week 4 and to week 6, and to a significant rise of coliform count from the first to the second and the third milk sampling.

Changes in the bacteriological characteristics of milk were more marked than those observed in the air. This was expected, because litter is not only the main source but also the deposition site of microorganisms arising from bedding, from the animals' skin and coat, from feed and from stockpersons' skin and clothes. Moreover, ewe udders are closer to the ground and may be more affected by the degree of bedding pollution than those of other species. Hence, the higher coliform contamination observed in the MP groups might be expected as an outcome of the greater amount of faeces excreted because it is known that the major source of coliforms is an environment contaminated by normal gut flora. In addition, the high moisture content of faeces in the MP groups could have enhanced the soiling of the bedding and, in turn, of ewe udders. Moreover, the higher efficiency of the moderate ventilation rate in removing airborne microorganisms might explain the differences observed between the MV and LV milk. In addition, results suggest that the lower faeces release recorded in the LPMV than in the MPMV group acted to reduce further the mesophilic concentration in the milk of the ewes fed the 13% CP diet and exposed to the moderate ventilation rate.

Differences in milk microbism may explain the rise in SCC observed in the less-ventilated groups, while the protein level effect on milk leucocyte concentration is not easy to interpret. In previous experiments, a positive correlation was found between dietary CP level and SCC (Cuccuru et al. 1994) but this occurred in grazing ewes consuming high amounts of very degradable protein. Nevertheless, in light of the very low milk SCC (which tends to exclude even subclinical mastitis episodes) a relationship between volumes of milk yielded and SCC was not excluded, at least for LPLV, which produced the lowest volume of milk with the highest leucocyte concentration.

In conclusion, under the conditions of the present study the ewes receiving the 13% CP ration displayed an enhanced efficiency of dietary N utilization, which resulted in milk yields comparable to that of the ewes fed the 16% CP ration. Reduction of ventilation rate from 47 to 23·5 m^3/h per ewe led to higher air NH_3 concentrations. Low ventilation also led to a moderate lowering of yield and hygienic quality of milk, assessed by SCC and mesophilic count, in the low dietary CP groups, and to a marked reduction in the efficiency of dietary N utilization in the groups receiving the 16% CP diet. Therefore, these results suggest that the choice of a proper ventilation rate is critical for high efficiency of production in the lactating ewe, especially in intensively managed flocks receiving diets high in CP.

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