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Quality of milk and of Canestrato Pugliese cheese from ewes exposed to different ventilation regimens

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Effects of ventilation regimen on the quality of ewes' milk and on proteolysis in Canestrato Pugliese cheese during ripening were studied. Cheeses were manufactured from the bulk milk of Comisana ewes subjected to three different ventilation regimens, which were designated low (LOV, 23 m^3/h per ewe), moderate (MOV, 47 m^3/h per ewe) and programmed ventilation regimen (PROV, 73 m^3/h per ewe; fan set to maintain 70% relative humidity). Bulk milk was analysed for chemical and microbial composition, renneting parameters and plasmin-plasminogen activities. At 1, 15, 30 and 45 d of ripening, the cheeses were analysed for gross chemical composition, nitrogen fractions, and plasmin and plasminogen activities. The pH 4·6-insoluble nitrogen fractions were analysed by urea-PAGE. Free amino acid content was determined at the end of ripening. Lower concentrations of bulk milk somatic cell count (BMSCC) and of mesophilic bacteria were found in the MOV group than in the LOV and the PROV groups. A lower plasminogen (PG) to plasmin (PL) ratio (PG/PL) was observed in the MOV and PROV than in the LOV cheeses. Irrespective of treatment, PL activity in cheeses was higher at 15 d of ripening, while a sudden decrease of PL and PG activities was observed at 30 d, which was associated with a marked increase in non-protein nitrogen. The peptide profile characterized in the urea-PAGE showed a greater intensity of α- and β-CN hydrolysis in the MOV than in the PROV and LOV cheeses. The results provide evidence that a proper ventilation regimen is critical for optimizing the hygienic quality of milk and the proteolysis of Canestrato Pugliese cheese during ripening.

Keywords: Cheese ripening, proteolysis, plasmin, ewes' milk.

The increasing use of intensive production systems in dairy flocks emphasizes the need for specifications for ventilation regimens and focuses attention on the design and construction of sheep houses. A number of recent studies have addressed the control of micro-climate and of air pollution in animal houses as well as the specifications of good management practices aimed at optimizing milk production and animal welfare without minimizing the impact of animal husbandry on the environment (Wathes, 1994; Barkema et al. 1999; Frank & Swensson, 2002). Sevi et al. (2002) showed that poor ventilation can adversely affect udder health and the yield and quality of ewe milk. These authors suggested a minimum ventilation rate of 66 m^3/h per ewe to sustain the welfare and performance of lactating ewes during the summer season. The importance of ventilation during the winter season is often underestimated. Inadequate ventilation can adversely affect both productivity and animal health in intensive systems, owing to high levels of gaseous pollutants. In addition, in winter the increased moisture content of the air in the animal house and the condensation on internal surfaces enhance the growth and multiplication of micro-organisms in the air and in the litter (Sevi et al. 2001).

Ewes' milk is used almost totally for dairy products. Canestrato Pugliese cheese is manufactured from raw milk to preserve its nutritional features and to preserve the indigenous microflora and sensory quality during ripening (Albenzio et al. 2001). EU directive 46/92 details the requirements for hygienic quality of ewes' milk destined for cheese production without heat treatment. Moreover, cheese quality can be affected by several factors associated with udder efficiency, such as somatic cell count (SCC) and plasmin (PL) activity, which affect physical and

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chemical characteristics of cheese (Zachos et al. 1992; Auldist et al. 1996; Bastian & Brown, 1996; Park, 2001).

There is a lack of information on effects of sheep housing conditions on the quality of ovine cheese made from raw milk. A minimum ventilation rate of 30 m^3/h per ewe is recommended in sheep housing during the winter season (Chiumenti, 1987). However, little is known about the effects of ventilation regimen on milk quality and on subsequent processing. Previously, we (Sevi et al. 2003) assessed 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 m^3/h per ewe) and a moderate ventilation rate (47 m^3/h per ewe). In the present study, we investigated the effect of ventilation rate in the sheep house on the quality of ewes' milk and of Canestrato Pugliese cheese during ripening, with particular respect to patterns of proteolysis.

Materials and Methods

Experimental design and animal management

The experiment was conducted during winter (February and March) 2002 at Segezia research station of the Italian Istituto Sperimentale per la Zootecnia (latitude: 41° 27′ 6″ and longitude: 15° 33′ 5″). The climate is Mediterranean, with a winter rainfall of about 130 mm, and mean winter temperature of 8·3 °C over the last 20 years.

Thirty-six mid-lactation Comisana ewes (106 ± 1·87 d of lactation, mean ± SE) were divided into three groups of 12 each, which were balanced for age, parity, time of lambing and number of lambs suckled. Groups were separately housed on straw litter in 8 m × 3 m and 3·5 m-high rooms in the same building. 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. Each room was provided with a negative-pressure mechanical system of ventilation, in which a 0·28 m^2 suction fan (Vortice, Tribiano – Milan, Italy) was placed 2·5 m from the floor and two 0·36 m^2 air inlets were placed at ground level on the opposite wall. The three groups were low (LOV), moderate (MOV) and programmed ventilation regimen (PROV). 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 7.00 to 18.00 and five during night-time from 21.00 to 05.00. 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. The ventilation system was programmed to operate at 70% relative humidity with a fan speed of 1 m/s. 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 mean ventilation rates of 23, 47 and 73 m^3/h per ewe, respectively. Air temperature and relative humidity inside each room were continuously monitored throughout the trial. Thermo-hygrographs, TIG2-TH (LSI, Milan, Italy), were used, placed at a height of 1·5 m from the floor. In each pen, a layer of straw (about 0·4 kg/m^2) was strewn on litter daily. Air sampling was performed twice weekly, in the morning, starting from 09.00, and in the afternoon, starting from 16.30. Air was sampled on the same day in each room at 0·6 m height above the floor. Air concentrations of microorganisms and dust and of gaseous pollutants were measured as described by Sevi et al. (2003). The ewes were fed a diet containing a pelleted concentrate, oat grains and ryegrass hay (32%, 6% and 62% of total diet respectively), which was offered as a total mixed ration twice daily.

Sampling and analysis of milk, whey and cheese

After the ewes had been exposed to the three different ventilation regimens for 6 weeks, three milk samples were collected from the batches containing the bulk milk of the morning and evening machine milkings of two consecutive days from each experimental group. Samples of whey were collected after extraction of the curd. Cheeses from each batch were manufactured using a traditional protocol, as previously described (Albenzio et al. 2001). Briefly, raw milk was heated to 39 °C and 20 ml/100 l of standard calf rennet (CHR Hansen Spa, Italy) containing 77% chymosin was added. Cheesemaking procedures included curdling, cutting of coagulum (size of the curd after cutting was 0·5–1·0 cm), draining off the whey, heating the curd in hot whey (about 80 °C for 30 min) and manual pressing of the curd into reed containers. The curd was held at about 20 °C for 14 h; subsequently cheeses were dry-salted after 27 ± 1 h for 3–4 d and ripened at 10–15 °C and 95% relative humidity. One cheese, for each experimental group, was taken to our laboratory under refrigeration (at 4 °C) and analysed in triplicate at 1, 15, 30 and 45 d of ripening.

Bulk milk and whey samples were analysed for fat, total protein and lactose (Milko Scan 133B; Foss Electric, DK-3400 Hillerød, Denmark); total nitrogen (TN), non-casein nitrogen (NCN) and non-protein nitrogen (NPN) were determined by standard procedures using the Kjeldahl method (IDF, 1993). Casein nitrogen was calculated as the difference between TN and NCN (multiplied by a conversion factor of 6·38); whey protein was calculated as the difference between NCN and NPN (multiplied by a conversion factor of 6·38). SCC in milk was determined by a Fossomatic 90 (Foss Electric) using the standard of the International Dairy Federation (IDF, 1995); renneting characteristics (clotting time, rate of clot formation and clot firmness after 30 min) were measured using a Foss Electric Formagraph apparatus and the method of Zannoni & Annibaldi (1981). Total mesophilic bacteria were detected on Plate Count Agar (PCA, Difco Laboratories, Detroit, USA) at 30 °C for 48 h. Calcium content and pH

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of milk and cheese, and moisture and NaCl contents in cheese were determined by standard procedures (IDF, 1992, 1989, 1986, 1988, respectively). TN, pH 4·6 soluble-N, and NPN were determined as described by Gripon et al. (1975), and water soluble nitrogen (WSN) was measured as proposed by Stadhouders (1960). TN minus the WSN gave casein nitrogen; WSN minus NPN gave proteoso-peptone nitrogen (PPN) (Prieto et al. 2002). Fat was determined by the Soxhlet method using diethyl ether. The pH 4·6-insoluble fractions were analysed by urea-PAGE electrophoresis using a Protean II xi vertical slab gel unit (Bio-Rad, Watford, UK) according to the stacking and separating system described by Andrews (1983). Samples diluted with sample buffer were heated to 50 °C for 3 min and then loaded on the gels. Gels were stained with Coomassie Brillant Blue G250 by the method of Blakesley & Boezi (1977).

PL plus plasminogen (PG) activities in milk were determined according to Baldi et al. (1996); PL and PG were dissociated from casein micelles by incubation of skim milk with 50 mM-ε-amino caproic acid (EACA) for 2 h as described by Korycka-Dahl et al. (1983). The reaction mixture was 250 µl of 0·1 M-Tris–HCl buffer, pH 7·4, 0·6 mM-Val-Leu-Lys-p-nitroanilide (V7127; Sigma Chemical Co.), 30 plough units (2·5 µl) of urokinase (U0633; Sigma Chemical Co.) and 30 µl of milk serum. PL activity was measured in the same reaction mixture without added urokinase. PG-derived activity was the difference. A similar reaction mixture without sample was used as control. The reaction mixture was incubated at 37 °C for 3 h and A_{405} was measured at 30-min intervals with a microtitre plate reader (Anthos 2020 version 1.0; Diessechem, Milan, Italy). One unit of PL or PG activity was defined as the amount of enzyme that produces a change in absorbance at 405 nm of 0·1 in 60 min. PL and PG activities in cheese were determined using a modification of the method of Richardson & Pearce (1981). Grated cheese (5 g) was dispersed in 20 ml 0·4 M-sodium citrate, pH 8·5 and, after equilibration at 38 °C for 15 min, the mixture was homogenized in a Stomacher Lab-Blender 400 (PBI International, Milan, Italy) for 5 min. Assay of PL and PG activities was as described above for milk samples.

Total and individual amino acids were analysed in freeze-dried water soluble extracts of the cheeses by a single injection onto a Beckman model 63000 amino acid analyser (Beckman Instruments Ltd, High Wycombe, UK) using a Beckman P-N-338052 Na cation exchange column (12 × 0·4 cm, i.d.). A standard amino acid mixture (Beckman) was used to calibrate the column and norleucine (Sigma) was added to all samples before injection as an internal standard. Each freeze-dried sample (2·5–5·0 mg) was dissolved in sample buffer (0·2 M-sodium citrate, pH 2·2), filtered through Whatman 0·22-µm filters and 50 µl of filtrate loaded onto the column. Amino acids were post-column derivatized with ninhydrin and detected by absorbance at 440 nm (proline and hydroxyproline) or 570 nm (all other amino acids). Results were analysed using a VG Minichrom computer system with a chromatography data handling software package (Lynch et al. 1996).

Statistical analysis

All variables were tested for normal distribution using the Shapiro-Wilk test (Shapiro & Wilk, 1965). Bulk milk SCC (BMSCC) and total mesophilic bacteria were transformed into logarithmic form to normalize their frequency distributions before performing statistical analysis. Cheese variables were processed using the GLM procedure for repeated measures (SAS, 1999). Variation due to treatment, time of ripening and their interaction was tested. Replication within treatment was used as the error term. Milk variables were analysed with ANOVA (SAS, 1999) with one factor (treatment). When significant effects were found (at $P<0·05$, unless otherwise stated), Student's *t*-test was used to locate significant differences between means.

Results

Microenvironment

Microenvironmental parameters recorded during the 2 weeks before the collection of milk for cheesemaking are reported in Table 1. Averages of air temperatures were strictly similar among treatments, while relative humidity increased from 73·5 to 75·5 and 76·5% passing from the PROV to the MOV and the LOV rooms. Significant differences were observed for total dust, which was higher ($P<0·01$) for PROV and LOV than for MOV, and for respirable dust, which was higher ($P<0·01$) for PROV than for the two other treatments. Conversely, higher concentrations of ammonia ($P<0·001$) and of carbon dioxide ($P<0·01$) were recorded for LOV than for MOV and PROV. Concentrations of mesophilic bacteria were lower in the air of the MOV room than in that of PROV ($P<0·01$) and LOV ($P<0·001$) rooms.

Milk quality

Significant differences ($P<0·001$) were found for BMSCC, which was higher for LOV than for PROV ($P<0·01$) and greater for PROV than for MOV ($P<0·001$; Table 2). Higher concentrations of mesophilic bacteria were also found in the LOV and PROV milk than in the milk from MOV ($P<0·001$). MOV milk had lower PL activity and a higher PG/PL ratio than PROV ($P<0·001$) and LOV milk ($P<0·05$). No differences were observed for the other milk components, pH, or milk coagulating properties.

Cheese yield and quality

About 35 l of bulk milk was used in cheesemaking, which gave four cheeses of 1·5 kg for each experimental group. Cheese moisture was 17·62%, 17·92% and 18·45%

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Table 1. Averages of air temperature, relative humidity, air dust, and gaseous pollutants in rooms provided with a low (LOV), moderate (MOV) and programmed ventilation regimen (PROV) during the 2 weeks before the collection of milk for cheese-making

Values are means ± SEM for $n=8$

	LOV 23 m^3/h per ewe	MOV 47 m^3/h per ewe	PROV 73 m^3/h per ewe	SEM	Statistical significance of effects of (P†)		
					Treatment	Time	Treatment × time
Ambient temperature, °C	12·7	12·9	12·6	0·43	—	—	—
Relative humidity, %	76·5	75·5	73·5	1·55	—	—	—
Total dust, mg/m^3	0·83^b	0·63^a	0·93^b	0·04	***	*	NS
Respirable dust, mg/m^3	0·18^a	0·21^a	0·30^b	0·02	***	*	***
NH_3, ppm	19·0^b	9·5^a	8·3^a	0·9	***	*	***
CO_2, ppm	1062^b	712^a	700^a	65	**	*	NS
Mesophilic bacteria	2·08^b	1·68^a	1·94^b	0·06	**	**	*

† NS, not significant; * $P<0·05$; ** $P<0·01$; *** $P<0·001$. Means within a row followed by different superscript letters are significantly different ($P<0·05$)

Table 2. Chemical composition and mesophilic cell load of ewes' bulk milk for Canestrato Pugliese cheesemaking

Values are means ± SEM for $n=3$ batches

	Bulk milk†			SEM	Statistical significance (P††) of treatment effect
	LOV	MOV	PROV		
pH	6·74	6·75	6·73	0·01	NS
SCC, log_{10} cells/ml	6·09^c	5·85^a	6·01^b	0·02	***
Total mesophilic bacteria, log_{10}cfu/ml	5·83^c	5·47^a	5·77^b	0·05	**
Fat, %	6·11	6·06	6·11	0·27	NS
Protein, %	6·06	5·82	5·64	0·17	NS
Casein, %	4·72	4·55	4·64	0·07	NS
Whey proteins, %	1·12^c	1·07^b	1·02^a	0·06	***
Lactose, %	4·66	4·84	4·76	0·05	NS
Calcium, %	0·19	0·19	0·18	0·18	NS
Clotting time, min	17·4	17·6	17·3	0·66	NS
Rate of firming, min	3	3	3	0·13	NS
Curd firmness, mm	62	58	59	2·16	NS
Plasmin (PL), Units/ml	9·76^b	7·64^a	10·51^c	0·57	*
Plasminogen (PG), Units/ml	17·29	16·76	16·34	0·58	NS
PG/PL	1·79^a	2·21^b	1·56^a	0·11	*

† LOV, bulk milk obtained from ewes subjected to low ventilation regimen; MOV, moderate ventilation regimen; PROV, programmed ventilation regimen (see text for details)

†† NS, not significant; * $P<0·05$; ** $P<0·01$; *** $P<0·001$

[a,b,c] Means within a row followed by different superscript letters are significantly different at $P<0·05$

respectively from the LOV, MOV and PROV milk (data not shown). Cheese pH was lower ($P<0·05$) for LOV than for MOV and PROV samples at 1 and 15 d but, at 30 and 45 d, MOV cheeses had lower pH values ($P<0·001$) than LOV and PROV cheeses (Table 3). As expected, the moisture content decreased during ripening for all cheeses; the lowest values ($P<0·001$) were found in MOV cheeses at 30 and 45 d of ripening. Changes in Ca content were observed only at the end of ripening (45 d), the LOV cheeses showing the highest Ca concentrations ($P<0·05$) at this time. Conversely, NaCl content increased in all cheeses during ripening and was similar among treatments (about 40·4 g/kg) at the end of the study period. No differences were found for the fat content in cheeses (mean value about 379 g/kg of dry matter (DM) at 45 d of ripening). Protein content at 45 d was significantly lower for LOV (374 g/kg of DM) than for PROV (427 g/kg of DM), an intermediate value was observed for MOV (404 g/kg of DM).

Proteolysis in cheeses

Distribution of nitrogen fractions was significantly affected by experimental treatment (Table 4). WSN increased during ripening in all cheeses; at 45 d the PROV samples showed higher values than MOV and LOV ($P<0·001$). The pH 4·6-soluble N showed the same trend as WSN, being higher for PROV than for MOV and LOV (6·3 g/kg *v.* 4·6 g/kg and 5·2 g/kg; $P<0·001$). A higher casein content was found in LOV cheeses, both when fresh and at 15 and 30 d

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Table 3. Least square means ± SEM ($n=3$) of the gross composition of Canestrato Pugliese cheese during ripening

	Days of ripening (d)	Cheeses†				Statistical significance of effects (P††)		
		LOV	MOV	PROV	SEM	Treatment	Time	Treatment × time
pH	1	5·53^{a}	5·61^{b}	5·63^{b}				
	15	4·95^{a}	5·01^{b}	5·06^{b}				
	30	5·13^{b}	5·01^{a}	5·07^{a}				
	45	5·09^{b}	4·92^{a}	5·11^{b}	0·03	***	***	***
Moisture, g/kg	1	510·8^{c}	498·1^{b}	496·6^{a}				
	15	411·3^{a}	430·9^{c}	417·0^{b}				
	30	390·4^{b}	364·7^{a}	414·2^{c}				
	45	355·4^{b}	332·9^{a}	341·5^{c}	0·31	***	***	***
Ash, g/kg	1	28·8	29·1	29·7				
	15	43·9^{a}	63·2^{b}	72·0^{b}				
	30	64·2^{a}	74·5^{b}	81·1^{c}				
	45	72·5^{a}	77·2^{b}	81·5^{c}	0·90	***	***	***
Calcium, g/kg dry matter	1	11·2	11·4	10·5				
	15	12·1	14·2	14·1				
	30	15·4	15·3	16·7				
	45	19·2^{b}	17·5^{a}	17·1^{a}	0·32	NS	***	*
Salt, g/kg	1	2·7	1·5	1·5				
	15	19·1^{a}	29·2^{b}	36·1^{c}				
	30	38·2^{a}	39·8^{b}	38·9^{a}				
	45	44·1^{ab}	42·4^{a}	45·5^{b}	0·90	***	***	***

† Cheeses obtained by ewes subjected to different ventilation regimen: LOV, low ventilation regimen; MOV, moderate ventilation regimen; PROV, programmed ventilation regimen (see text for details)
†† NS, not significant; * $P<0·05$; ** $P<0·01$; *** $P<0·001$
^{a,b,c} Means within a row without a common superscript letter are significantly different at $P<0·05$

of ripening ($P<0·001$). MOV cheeses had the lowest casein concentrations ($P<0·01$) at the end of ripening.

PPN (peptides of small size) increased through out ripening in all groups, being always higher ($P<0·001$) in the MOV and PROV than in LOV cheeses. As might be expected, NPN increased during ripening and was higher ($P<0·001$) in LOV than in MOV and PROV cheeses when fresh and at 15 and 45 d of ripening. Ripening index (water soluble-N/TN) was significantly higher for MOV and PROV than for LOV at 15 and 30 d ($P<0·001$) and higher for MOV than for LOV and PROV cheeses ($P<0·05$) at 45 d of ripening.

The highest PL activity ($P<0·001$) was observed in PROV cheeses at 1 and 15 d and in MOV cheeses at 30 and 45 d of ripening, LOV cheeses having the lowest PL activity throughout the ripening period (Fig. 1). PG activity was higher ($P<0·001$) in LOV and MOV than in PROV fresh cheeses (Fig. 2), but at 15 d of ripening the highest PG activities were found in PROV cheeses. PG activity abruptly decreased at 30 d in all experimental cheeses and then peaked at the end of the ripening period, being higher ($P<0·001$) in PROV than in LOV cheeses and greater in LOV than in MOV. No differences were found for PG/PL ratio during the first 2 weeks of ripening, while at 30 and 45 d the PG/PL ratio was significantly higher ($P<0·001$) for LOV cheese than for the other two groups (Table 4).

Profiles of individual free amino acids at the end of ripening are shown in Table 5. PROV cheeses had the lowest values of serine, isoleucine, and tyrosine, while MOV cheeses had the highest concentrations of threonine, serine, alanine, as well as the lowest levels of histidine. No differences were found for the other amino acids.

Urea-PAGE patterns of pH 4·6-insoluble N (Fig. 3) showed moderate hydrolysis of α-CN in all fresh cheeses. Extensive degradation of α-CN and β-CN was observed in all cheeses at 45 d of ripening; in particular, the degradative patterns of caseins were more intense for peptides of low (γ-CN) and high (products of α-CN breakdown) electrophoretic mobility in the MOV cheeses.

In whey samples, fat content was higher ($P<0·01$) in LOV (26·5 g/kg) than in MOV (22·4 g/kg) and PROV (23·2 g/kg).

Discussion

We showed previously that inadequate ventilation regimens in sheep houses can result in poor air quality and reduced efficiency of animal production (Sevi et al. 2003). In particular, low ventilation rates can lead to increased relative humidity and higher air concentrations of ammonia and carbon dioxide, which may be ascribed to failure to remove efficiently the moisture and gases

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Table 4. Least squares means ± SEM (n=3) of changes in nitrogen fractions and plasminogen to plasmin ratio of Canestrato Pugliese cheese during ripening

Item	Days of ripening (d)	Cheeses†			SEM	Statistical significance of effects (P††)		
		LOV	MOV	PROV		Treatment	Time	Treatment × Time
Water soluble nitrogen, g/kg	1	5·81	6·15	5·95				
	15	6·60^{a}	7·61^{b}	7·51^{b}				
	30	7·61^{a}	8·85^{b}	8·75^{b}				
	45	9·35^{a}	10·20^{b}	11·30^{b}	2·31	***	***	*
Casein N/TN	1	0·84^{b}	0·83^{a}	0·83^{a}				
	15	0·83^{b}	0·79^{a}	0·78^{a}				
	30	0·81^{b}	0·77^{a}	0·78^{a}				
	45	0·75^{b}	0·74^{a}	0·75^{b}	0·01	***	***	***
Proteoso peptone N/TN	1	0·08^{a}	0·11^{b}	0·11^{b}				
	15	0·09^{a}	0·15^{b}	0·16^{b}				
	30	0·11^{a}	0·15^{b}	0·14^{b}				
	45	0·14^{a}	0·19^{b}	0·18^{b}	0·01	***	***	NS
WSN/TN	1	15·11	16·32	16·78				
	15	16·84^{a}	20·57^{b}	20·93^{b}				
	30	18·81^{a}	22·92^{c}	21·81^{b}				
	45	24·66^{a}	25·01^{b}	24·18^{a}	0·63	***	***	***
Non Protein Nitrogen, g/kg	1	2·6^{b}	1·7^{a}	1·8^{a}				
	15	2·8^{b}	1·8^{a}	1·7^{a}				
	30	3·0	3·0	2·9				
	45	3·7^{b}	2·5^{a}	2·4^{a}	1·11	***	***	***
PG/PL	1	1·21	0·72	0·32				
	15	0·52	0·52	0·58				
	30	6·35^{b}	0·22^{a}	0·21^{a}				
	45	8·99^{b}	0·51^{a}	3·83^{a}	1·52	**	*	NS

†Cheeses obtained by ewes subjected to different ventilation regimen: LOV, low ventilation regimen; MOV, moderate ventilation regimen; PROV, programmed ventilation regimen (see text for details)
††NS, not significant; * P<0·05; ** P<0·01; *** P<0·001
[a,b,c] Means within a row followed by different superscript letters are significantly different (P<0·05)

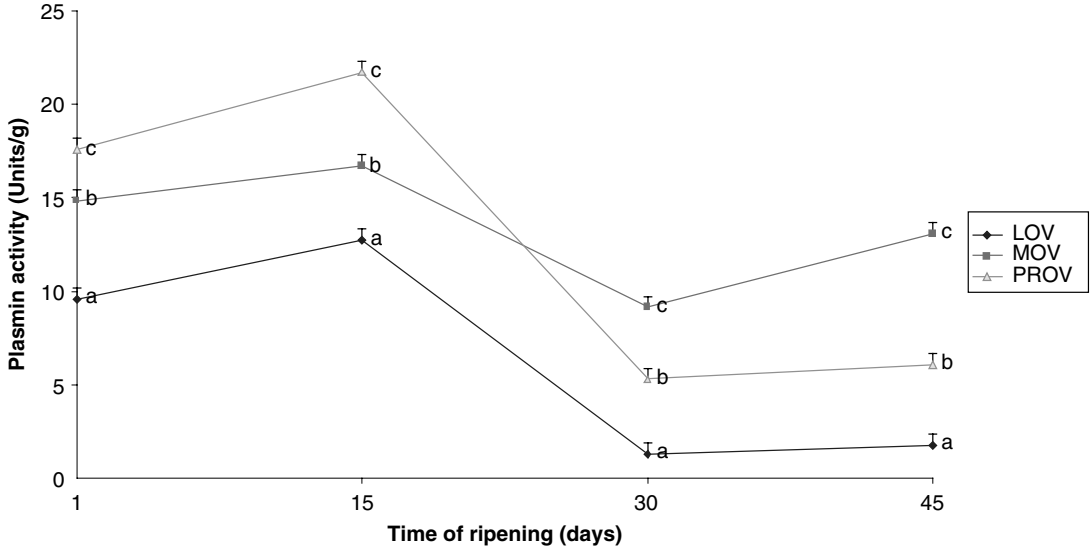

Fig. 1. Changes in Plasmin (PL) activity of Canestrato Pugliese cheese manufactured from ewes subjected to different ventilation regimens: LOV, low ventilation regimen (23 m^3/h per ewe); MOV, moderate ventilation regimen (47 m^3/h per ewe) and PROV, programmed ventilation regimen (73 m^3/h per ewe), during ripening.

Values within a ripening time accompanied by different letters (a, b, c) are significantly different (P<0·05)

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Table 5. Concentration of free amino acids (mg/100 g dry matter) in 45-d ripened Canestrato Pugliese cheese

Values are means ± SEM (n = 3)

	Cheeses†				
	LOV	MOV	PROV	SEM	Statistical significance of treatment effects (P††)
Asp	7·79	7·10	7·28	0·59	NS
Thr	6·60[a]	7·30[b]	6·43[a]	0·20	*
Ser	16·12[b]	16·45[c]	15·31[a]	0·28	***
Glu	36·41	35·82	34·70	1·11	NS
Pro	16·89	16·36	15·98	0·49	NS
Gly	5·04	4·97	4·94	0·20	NS
Ala	6·38[a]	6·84[b]	6·09[a]	0·13	*
Cys	3·79	3·88	3·41	0·38	NS
Val	15·16	14·72	14·38	1·08	NS
Met	3·79	3·76	3·61	0·14	NS
Ile	14·11[b]	14·27[b]	13·41[a]	0·33	***
Leu	13·15	13·65	13·07	0·69	NS
Tyr	4·25[b]	4·32[b]	3·70[a]	0·19	**
Phe	10·60	9·82	9·93	0·39	NS
His	6·02[b]	5·63[a]	6·01[b]	0·13	*
Lys	19·24	18·18	18·68	1·10	NS
Arg	11·19	10·33	10·54	1·09	NS
Trp	1·64	1·57	1·81	0·18	NS
Total free amino acids	198·17	194·97	189·28	8·3	NS

† Cheeses obtained by ewes subjected to different ventilation regimen: LOV, low ventilation regimen; MOV, moderate ventilation regimen; PROV, programmed ventilation regimen (see text for details)
†† NS, not significant; * P<0·05; ** P<0·01; *** P<0·001
[a,b,c] Means within a row followed by different superscript letters are significantly different (P<0·05)

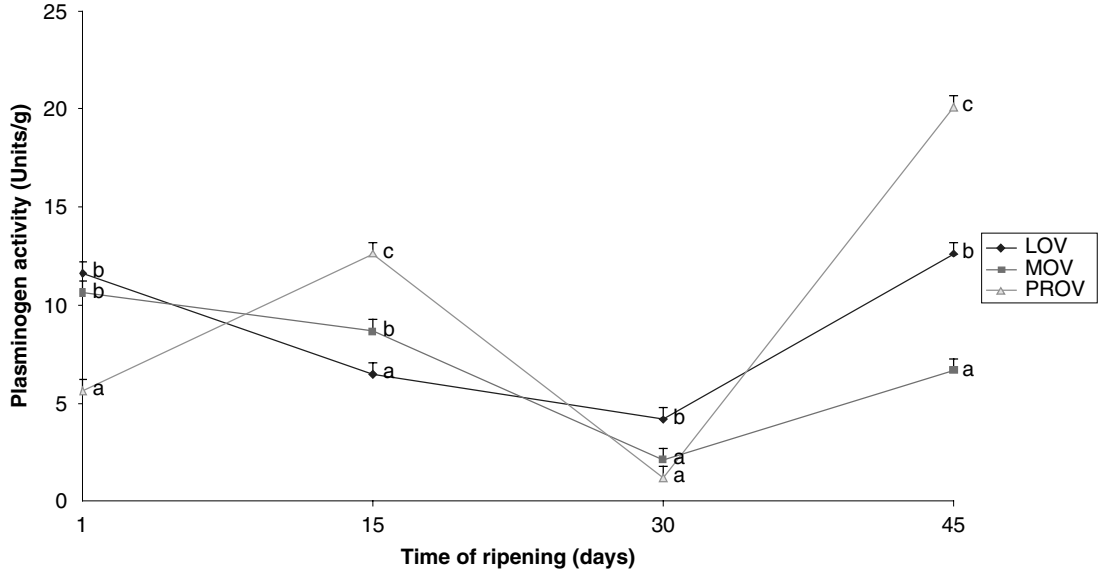

Fig. 2. Changes in Plasminogen (PG) activity of Canestrato Pugliese cheese manufactured from ewes subjected to different ventilation regimens: LOV, low ventilation regimen (23 m^3/h per ewe); MOV, moderate ventilation regimen (47 m^3/h per ewe) and PROV, programmed ventilation regimen (73 m^3/h per ewe), during ripening.

Values within a ripening time accompanied by different letters (a, b, c) are significantly different (P<0·05)

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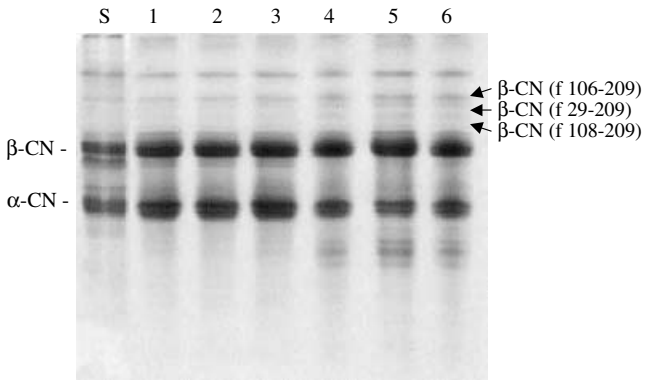

Fig. 3. Urea-PAGE of pH 4·6-insoluble fractions of Canestrato Pugliese cheese manufactured from ewes subjected to different ventilation regimens: LOV, low ventilation regimen (23 m^3/h per ewe); MOV, moderate ventilation regimen (47 m^3/h per ewe) and PROV, programmed ventilation regimen (73 m^3/h per ewe). Lane S (Standard): Ovine Na Caseinate purified. Lanes 1 and 4: LOV cheese after 1 and 45 d of ripening. Lanes 2 and 5: MOV cheese after 1 and 45 d of ripening. Lanes 3 and 6: PROV cheese after 1 and 45 d of ripening.

originating from the animals' respiration and the decomposition and fermentation of manure. On the other hand, very high ventilation rates can instead result in higher air dust concentrations, probably owing to reduced humidity and to turbulent air currents keeping dust particles suspended in the air for longer. The present study suggested that a 6-week exposure of the ewes to the LOV and PROV treatments resulted in increased dust and airborne microorganism concentrations, which in turn led to a rise in BMSCC and mesophilic bacteria counts compared with MOV. Microorganism concentrations in air and milk are closely related (Sevi et al. 1999). Indeed, bacterial penetration into the udder is determined by the balance between the natural defence mechanisms of the teat and mammary gland and the numbers and pathogenicity of the microorganisms in contact with the entrance to the teat canal (Klastrup et al. 1987). The greater bacterial load in LOV and PROV milk accounts for increased SCC, because leucocyte infiltration of the alveoli constitutes one of the main defence mechanisms of the animal against invading bacteria (Burvenich et al. 2000). Enzymes produced by the bacterial flora may act as PG activators (Fajardo-Lira & Nielsen, 1998). Elevated PL activity is also found in milk with high SCC, which has been ascribed to the release of PG activators (Grufferty & Fox, 1988) or to proteolytic enzymes occurring in somatic cells (Verdi & Barbano, 1991). These considerations might explain why the bulk milk from LOV and PROV ewes, which had higher SSC and higher microbial counts than the bulk milk collected from MOV, also had a greater PL activity and a higher PG/PL ratio, which is regarded as an index of the conversion of PG to PL (Zachos et al. 1992). Husbandry practices, such as stocking density and concentrate supplementation, can affect PL activity in milk (Kelly & McSweeney, 2002). In milk the primary PL cleavage sites in β-casein lead to the formation of the polypeptides (γ_1-CN, γ_2-CN, γ_3-CN, and proteose peptone) which are associated with deterioration of milk coagulating properties. In the present trial, the higher PL activity in LOV and PROV bulk milk probably impaired the ability of the casein matrix to incorporate the fat globules, thus allowing a greater release of fat into the whey during cheesemaking.

The contribution of PL to proteolysis is relevant to cheese quality through hydrolysis of casein (Kelly & McSweeney, 2002), which is probably the most important biochemical event during the ripening of most cheese varieties (Fox, 1989). The increase in the PL activity in cheeses, as a result of either PG activation or exogenous PL addition, ameliorates the flavour and the overall quality of cheese (Farkye & Landkammer, 1992). Apart from PL activity, proteolysis in cheese depends on the rennet enzymes, microbial proteinases, moisture and pH of curd, salt content, and temperature, humidity and time of ripening (Park, 2001).

In the present trial, MOV resulted in the lowest PL activity in milk and in the highest activity of PL in cheese. This suggests that the better hygienic quality of the MOV milk led to a slower conversion of PG to PL prior to manufacture and, consequently, to a higher release of PL during cheese ripening, which resulted in an accelerated proteolysis. The lower PG/PL ratio in MOV, and to a lesser degree in PROV cheeses, shows that these cheeses underwent a better ripening process than LOV cheeses, owing to more intense proteolysis. This is confirmed by the fact that the WSN was significantly higher for MOV and PROV than for LOV cheeses. In fact, WSN contains numerous small- and medium-sized peptides, free amino acids and their degradation products, organic acids and their salts, which are widely used as reliable indexes of cheese ripening (McSweeney & Fox, 1997). These data match with the urea-PAGE of the pH 4·6-insoluble N fractions. Indeed, at 45 d of ripening, the MOV peptide profiles showed a more intense proteolysis of the α-CN and β-CN, which was highlighted by the higher expression of degradation products with high and low electrophoretic mobility, respectively. The higher degradation of α-CN in MOV than in LOV and PROV cheeses might relate to the lower pH values of MOV cheeses (Lawrence et al. 1987). During ripening, the decrease of PL and PG activities coincided with an increase in NPN, which suggests that NPN molecules derived from CN-hydrolysis may inhibit the PG and PL enzyme system.

Moisture content is one of the main indicators of cheese quality, given that mature cheeses with high moisture levels are generally of poor quality (Pearce & Gilles, 1979). In previous experiments, Albenzio et al. (2001) and Corbo et al. (2001) found a moisture content of 36·5–39·5% in Canestrato Pugliese cheeses after 60 d of ripening. Hence moisture content ranging from 33% for MOV to 35·5% for LOV cheeses after 45 d of ripening suggests that the Canestrato Pugliese cheeses manufactured under our

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experimental conditions were of good quality. This is true particularly for the MOV cheeses, which seemed to have even better quality and accelerated ripening.

Salt concentration in cheese influences many of the chemical, enzymic and microbiological processes occurring during ripening so giving the typical flavour and texture (Melilli et al. 2003). Grufferty & Fox (1988) stated that, as the pH of the curd decreases and salt is added, PL is transferred from the casein matrix into the aqueous phase of the cheese. The presence of salt in the aqueous phase of cheese promotes release of intact casein from the casein matrix into solution in the cheese water and makes this portion of casein available for proteolysis (Guo et al. 1997). This may explain why MOV and PROV cheeses had lower casein, on average, and higher PPN contents than the LOV cheeses during ripening.

Inhibition of β-casein breakdown is desirable since the peptide fragments contribute to bitter flavour. Hydrolysis of β-CN is strongly inhibited by a NaCl content in the cheese of 5% (Fox, 2003). In the present experiment, the reduced levels of small peptides (γ_1-CN, γ_2-CN, γ_3-CN) derived from the cleavage of β-CN, as shown in urea-PAGE, support the hypothesis that β-CN hydrolysis may be inhibited by a salt concentration of about 4%.

Free amino acids from proteolysis are the major source of aroma compounds in cheese, the aromatic amino acids (phenylalanine, tyrosine and tryptophan) and the branched-chain amino acids (leucine, isoleucine and valine) being the precursors of these aromatic compounds (Banks, 2002). On the whole, the amino acid profile obtained at the end of ripening indicated higher concentration of free amino acids in the present study than in other investigations (Albenzio et al. 2001; Corbo et al. 2001; Gobbetti & Di Cagno, 2002).

In conclusion, the present results provide evidence that the choice of a proper ventilation regimen in dairy sheep housing is critical for optimizing the hygienic quality and cheesemaking efficiency of ovine milk but further investigations are needed to verify these results. Our results indicate that, during the winter season, a mean ventilation rate of about 50 m^3/h per ewe is required to control hygiene risks for cheese manufactured from raw milk, and to ensure a more accelerated proteolysis in Canestrato Pugliese cheese during ripening.

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