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Comparison of lucerne silage and ventilated hay in maize silage-based rations for dairy cows for the production of milk destined for Grana cheese

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Abstract

Three experiments were carried out to study the effects of feeding lucerne silage (wilted to give different dry-matter (DM) contents) and ventilated hay to dairy cows on milk production, milk quality, milk-renneting properties, clostridial spore content and the quality of cheese prepared from the milk. The lucerne, cut at vegetative or early-bud stages of maturity, was harvested from alternate windrows and conserved as silage or artificially dried hay. The lucerne was wilted until it reached different DM contents of 550, 360 and 432 g kg^{-1} in the three experiments, harvested, chopped with a self-loading forage wagon and ensiled in low and narrow clamps made up of transferable prefabricated walls. The organic acid content, pH, yeast and mould counts of the lucerne silage suggested that there was no aerobic deterioration.

In each experiment, fifty Italian Friesian lactating cows were divided into two groups and fed two maize silage-based rations for 6 weeks, which only differed in the lucerne forage [silage (S) vs. ventilated hay (H)], in a cross-over experimental design. The lucerne in the rations represented 35%, 23% and 24% of the DM of the rations for the three experiments. The microbiological profiles of the ration were influenced more by the maize silage than by the lucerne silage.

Individual daily DM intakes were similar for the two treatments in Experiments 1 and 3 (on average 18·7 kg in Experiment 1 and 20·3 kg in Experiment 3) and slightly lower for S cows in comparison to H cows in Experiment 2 (18·0 vs. 19·0 kg). Milk yields of S and H cows were 21·0 and 20·8, 20·0 and 20·6 ($P < 0·01$), and 28·4 and 27·9 kg d^{-1} in Experiments 1, 2 and 3 respectively. Milk composition was similar for all the experiments for the two treatments, except that the protein content was lower and the fat content was higher in the silage treatment than in the hay. The renneting properties and microbiology of the milk were not influenced by the introduction of lucerne silage into the rations, although the season in which it was consumed had a greater effect on the microbiological content, in terms of standard bacterial counts, proteolytic, coli and lactic acid bacteria, and clostridia spores. The clostridial spore counts were always very low (< 400 per litre), thus fulfilling the requirements for top-quality milk for Grana cheese production. In the third experiment, the quality of Grana Padano cheese produced was examined, and no differences between treatments were observed after 12 months of maturation.

These results show that lucerne silage can be included in the ration of dairy cows instead of ventilated lucerne hay, which is considered to be the top-quality hay available for the production of milk destined for Grana cheese, without any negative effects on milk and cheese quality.

Introduction

To maximize the dry-matter (DM) intake of dairy cows and, consequently, milk yield, rations with a low fibre and with a rapidly digestible organic matter (OM) content are recommended (Paterson *et al.*, 1994). On the other hand, environmental nitrogen (N) pollution by intensive farming systems, as well as feeding costs, can be significantly reduced by systems where most of the feed is home grown following the use of rotations and the choice of particular forage species that guarantee a balance between N, phosphorus and potassium inputs and outputs (Tamminga, 1992; 1996). Forages

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with a high N content can therefore be successfully produced on farm, together with high energy/low protein forage (e.g. maize silage) in order to obtain rations with a high forage proportion and well balanced in terms of protein and energy supply.

Legumes can contribute to sustainable farming systems because of their capability to fix N, and their potentially high nutritional value (Sprent and 't Mannetje, 1996). Lucerne is of interest because of its low neutral-detergent fibre content (NDF), its high degradation rate and high protein content, compared with grasses (Marten *et al*., 1988; Wilman *et al*., 1996). A further improvement in the nutritive value of lucerne can be obtained by earlier cutting (Borreani *et al*., 1996). However, earlier cuts prolong the drying period (Acutis *et al*., 1994) and therefore increase the probability that the forage will be exposed to rainfall. In these situations, ensiling or artificial drying techniques are particularly advantageous because the exposure of the forage in the field is limited to a very short time. They are thus less weather dependent with drastically reduced mechanical losses compared with traditional haymaking. Ensiling requires less capital and incurs lower running costs than artificial drying techniques (Marsh, 1979), but can cause microbial stability problems during silage consumption (Woolford, 1990). Even though artificial drying results in microbiologically stable forages and hampers proteolysis, it causes serious logistic problems in harvesting.

For successful fermentation, lucerne should have a DM content higher than 350 g kg^{-1} fresh matter (FM) at ensilage, owing to its low water-soluble carbohydrate content and high buffering capacity (Ciotti *et al*., 1985). Ensiling at values of DM content around 500 g kg^{-1} FM maximizes the DM intake of dairy cows (Van Vuuren *et al*., 1995) and reduces the nitrogen transformations because of plant enzyme activity and microbial degradation (Muck, 1987) but increases the risks of aerobic deterioration as a result of the higher porosity of the silage (Williams *et al*., 1994).

In addition, the high non-protein nitrogen (NPN) content of lucerne silage, which usually ranges from 50% to 87% of total N (Broderick, 1995), produces a higher content of soluble protein and a higher rumen degradation rate of N in lucerne silage than in hay (Makoni *et al*., 1994). Degradation of digestible protein in excess of microbial needs results in elevated ruminal ammonia concentrations (most excess ammonia is absorbed and then excreted in the urine as urea) and in inefficient utilization of lucerne protein, which may lower milk and protein yields (Broderick, 1985).

High rates of DM digestion in the rumen are generally welcome in dairy ration formulation, in order to maximize rumen turn-over and feed intake, which are positively correlated with milk production: in this respect lucerne silage is superior to lucerne hay (Nelson and Satter, 1992a).

The use of silages in rations for lactating cows can be detrimental to the quality of milk destined for hard and semihard cheese production (Annibaldi, 1969; Gouet and Bergère, 1973; Colombari and Fantuzzi, 1991). Clostridia spores from highly contaminated silages may pass into the milk (Stadhouders and Spoelstra, 1990) via dung contamination, even if good hygienic milking conditions are practised (Stadhouders and Jørgensen, 1990). They survive milk pasteurization and pass unaffected into the cheese where they can cause the so-called 'late blowing' defect.

The aim of this research was to compare, at farm scale, over the whole cycle from forage to cheese, the qualitative characteristics of lucerne silage wilted at different moisture contents with ventilated lucerne hay (chosen as a top-quality hay available on the farm) and their influence on milk production, milk-renneting properties and the quality of Grana Padano cheese.

Materials and methods

Crop and silages

Three experiments were carried out at the experimental farm of the Istituto Superiore Lattiero Caseario of Mantova (45°09'N, 10°48'E) in the Po Valley on three different cuts of lucerne (*Medicago sativa* L.) cvs. Delta and Boreal, grown in a soil with a texture of 30% clay, 40% silt and 30% sand.

All the forages, mown at a stubble height of 4 cm and conditioned, were left in swaths and tedded after 3–4 h of wilting. The weather during wilting was always good and the forages were harvested from alternate windrows: half after one day of wilting with a self-loading forage wagon (Kemper Cargo L 9000, Stadtloon, Germany), set to a chop length of 40 mm, and then ensiled in low, narrow clamps made up of transferable prefabricated 0·9 m high walls that were spaced 4·0 m apart; half after 2 days of wilting at least at a DM content of 550–600 g kg^{-1} FM and then artificially dried using an on-farm drying system with drying chamber of 8 m × 10 m × 5 m dimensions and with a batch capacity of about 40 t.

The silage was carefully compacted with a tractor, covered with two films of polyethylene and weighted down with 100 kg m^{-2} of sand. The feed-out rate of lucerne silage was in the range of 25–30 cm d^{-1}. The hay was stored loose until feed-out.

In the three experiments the whole crop maize silage was produced from a maize crop (*Zea mais* L. cv. Costanza, FAO class 600) sown between 18 and 25 May, harvested at the dough-ripe stage with a precision-chop forage harvester and ensiled in bunker silos with 2·7-m-high walls, spaced 6·0 m apart.

Chemical and microbiological analyses of forages

Samples of herbage at cutting and hay were analysed for DM content by oven drying at 90°C until a constant weight was achieved, crude protein (CP = Kjeldahl N × 6·25), ash by ignition to 550°C, NDF and acid-detergent fibre (ADF) by the sequential analysis of Goering and Van Soest (1970) measured on an ash-free basis.

Silage sampling for chemical and microbiological analyses was made weekly during feeding, by coring (diameter 5 cm) at 30 cm deep inside the mass of the silage in four different parts of the front of the silo (Figure 1a–d). The decision to consider separately the most external zones of the silo derives from the fact that in these small clamps the peripheral zone (up to 20 cm depth) represents a considerable part (about 20%) of the whole volume, hence the importance of studying the fermentative and microbiological aspects of the external areas of the silage mass for a better understanding of animal performance.

Aqueous extracts (with water for 24 h at 4°C) were analysed to determine pH, total N and ammonia-N concentration determined using the method of Byrne and Power (1974). Soluble N was determined in a phosphoborate buffer at pH 6·8 and 39°C for 1 h (Krishnamoorthy *et al.*, 1982). Lactate, acetate and butyrate were determined as described by Canale *et al.* (1984).

Microbiological analyses of clostridial spores were made according to the most probable number (MPN) technique with lactate–acetate agar (Spoelstra, 1984) after 7 days of incubation at 37°C, and colony-forming units (CFU) of yeasts and moulds were enumerated using the pour plate technique with 40·0 g l^{-1} of yeast extract glucose chloramphenicol agar (YGC agar, DIFCO, West Molesey, Surrey, UK) after 3 days of incubation at 25°C.

Silage density was calculated by measuring the corer volume and the weights of the samples.

Animals and management

The three experiments were carried out using Italian Friesian lactating cows divided into two groups of twenty-five animals each. At the beginning of Experiments 1, 2 and 3 the two groups of cows were balanced, as far as possible, between the silage and the hay treatment for parity (2·6, 2·6 and 2·5 lactations), stage of lactation (166, 160 and 138 days post partum) and individual daily milk yield (21·6, 21·5 and 28·8 kg). About half of the animals present in Experiment 1 were present in Experiment 3.

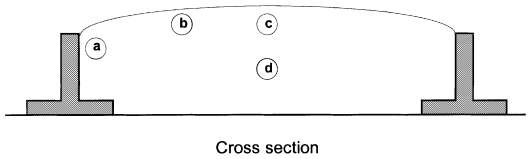

Figure 1 Sampling scheme of the lucerne silage. The shaded areas represent the silo walls.

A cross-over design was used. Each experiment lasted 12 weeks: 2 weeks of adaptation followed by 4 experimental weeks for each of the two phases. The rations, as total mixed rations (TMR), were always offered in the morning. The forage proportion of the TMR included lucerne silage or hay, maize silage and some grass hay (Table 5).

At the beginning of each adaptation period, the rations were fed to meet the predicted maintenance and production requirements, but adjustments of the quantity of the TMR offered to each group were made according to appetite. Drinking water was available *ad libitum*. Experiments 1, 2 and 3 were conducted in winter, summer and spring respectively.

Intakes and ration analyses

The DM intake was recorded daily for each group of cows. Every morning, before feeding, the refusals were collected, weighed and analysed for DM content, in order to measure the actual DM intake of each group. Three times each week, during the experimental periods, the TMR fed to each group of cows was sampled and analysed for CP, NDF, ash, clostridia spores and yeasts. Net energy for lactation (NEL) was calculated by the equations proposed by Andrieu and Demarquilly (1987).

Faecal analyses

Samples of faeces were also collected directly three times each week from two cows of each group after the afternoon milking to determine the concentration of clostridia spores.

Milk and cheese analyses

The milk yield, milk fat, protein and lactose contents, and the somatic cell count (SCC) of individual cows were recorded twice each week. Milk yield was recorded from both milkings, whereas individual milk composition was determined on the milk from the morning milking. The interval between milkings was always 12 h. The bulk milk of each group of cows was analysed three times each week for microbiological characteristics [standard bacterial count (SBC), proteolytic, coli and lactic acid bacteria, and clostridia spores],

nitrogenous fractions (urea N, whey protein N, non-protein N, caseinic N), and acidity and renneting properties [r = time required to start clotting (min); k_{20} = time (min) to reach a standard clot consistency (20 mm at the trombelastograph tracing); a_{30} = consistency of the clot after 30 min from the beginning of clotting (mm of the trombelastograph tracing; Tarodo de la Fuente *et al*., 1969)].

The quality of the Grana Padano cheese produced from the milk in Experiment 3 was checked after 12 months of maturation in order to evaluate the influence of treatment. The evaluation of cheese quality was carried out by an expert from the Grana Padano Consortium. Cheese that presented fermentation damage was analysed by gas chromatography (Contarini *et al*., 1989) to identify the microbiological source of the damage.

Statistical analysis

The significance of the differences of the treatments for milk yield, milk fat, protein, lactose and SCC contents in each experiment was examined using the following cross-over model with a GLM procedure (SAS Institute, 1994):

$$Y_{ijk} = \mu + P_i + C_j + T_k + e_{ijk}$$

where Y_{ijk} = dependent variable; μ = general mean; P_i = period effect (i = 1–2); C_j = cow effect (j = 1–25); T_k = type of conservation of the lucerne (k = 1–2); e_{ijk} = error. The interactions between period and treatment, and period and cow were not significant and therefore were not included in the model.

The data from the three experiments on the chemical–microbiological characteristics of the bulk milk (acidity, r, k_{20}, a_{30}, SBC, proteolytic and lactic acid bacteria and clostridial spores), the nitrogenous fractions and the data on the spore content of the faeces were analysed using the following model:

$$Y_{ijk} = \mu + P_i + T_j + e_{ijk}$$

where Y_{ijk} = dependent variable; μ = general mean; P_i = period effect (i = 1–2); T_j = type of conservation of the lucerne (j = 1–2); e_{ijk} = error. SCC data were first converted into linear scores (LS = $\log_2$(cells/12 500)) because of their non-normal distribution. Similarly, the microbiological data on the feeds, faeces and milk were first transformed to $\log_{10}$.

Results and discussion

Lucerne herbage quality

The date of cutting, yield, morphological stage of the crop, days of conservation and the period of consumption for the three experiments are reported in Table 1.

In all the experiments the lucerne was cut at an early stage (vegetative in the first and second, and early bud in the third). The main qualitative characteristics of the herbage at cutting, silages at feed-out and artificially dried hays are reported in Table 2. The early cutting permitted low NDF and high CP contents in the forage,

Table 1 Date of cutting, yield, stage, and days of lucerne silage conservation in the three experiments.

	Experiment		
	1	2	3
Date of cutting	7 October 1994	2 May 1995	15 September 1995
Cut	5th	1st	4th
Yield (t DM ha^{-1})	2·4	4·0	3·2
Stage	Vegetative	Vegetative	Early bud
Days of conservation	90	76	157
Period of consumption	Winter	Summer	Spring

Table 2 Main nutritional characteristics [content g kg^{-1} DM, except DM (g kg^{-1} FM)] of lucerne at cutting, silage at feed-out and hay in the three experiments.

	Herbage at cutting			Silage			Artificially dried hay		
Experiment	1	2	3	1	2	3	1	2	3
DM	220	160	208	550	360	432	850	850	890
CP	215	249	186	203	212	180	189	184	181
NDF	354	354	418	396	385	429	397	403	423
ADF	311	301	349	342	341	367	ND	ND	355
Ash	90	94	92	125	141	136	107	140	100

DM, dry matter; CP, crude protein; NDF, neutral-detergent fibre; ADF, acid-detergent fibre; ND, not determined.

Table 3 Chemical and microbial composition of lucerne silages sampled during feeding in peripheral and central zones of the clamps.

	Experiment					
	1		2		3	
Zone	Central (d)*	Peripheral (a b c)	Central (d)	Peripheral (a b c)	Central (d)	Peripheral (a b c)
DM (g kg^{-1} FM)	610	508	349	425	425	410
Wet bulk density (kg m^{-3})	439	291	834	414	480	293
DM density (kg m^{-3})	268	148	291	176	204	120
pH	6·0	6·0	5·4	4·7	4·8	5·7
Total N (g kg^{-1} DM)	32	33	34	33	29	27
Ammonia-N (g kg^{-1} TN)	41	61	162	94	80	200
Soluble-N (g kg^{-1} TN)	350	380	720	660	650	580
Lactic acid (g kg^{-1} DM)	10·0	14·3	21·2	39·2	16·0	ND
Acetic acid (g kg^{-1} DM)	4·1	9·2	41·5	19·3	14·0	ND
Butyric acid (g kg^{-1} DM)	0	0	0	0	0·3	ND
Clostridia spores (log MPN g^{-1} FM)	2·49	2·06	1·60	1·78	1·70	2·39
Yeast (log CFU g^{-1} FM)	3·79	3·46	3·00	3·15	3·30	4·55
Moulds (log CFU g^{-1} FM)	3·66	3·30	2·95	3·00	3·79	5·75

FM, fresh matter; DM, dry matter; TN, total nitrogen; MPN, most probable number; CFU, colony forming units; ND, not determined.
*See Figure 1 for description of a, b, c, and d.

and the short wilting times, owing to the favourable climatic conditions, achieved the high quality in silages and hays. The limited decrease in CP content and the increase in NDF content from herbage to silage and hay could be ascribed mainly to loss of leaf during wilting. The DM contents of the forages at ensiling were 550, 360, 432 g kg^{-1} in Experiments 1, 2, and 3 respectively. The high DM content in the first experiment was due to the high DM content at cutting and to the very good weather conditions during wilting associated with a low yield.

The increase in the ash content of the silages and hays compared with the fresh forage was due to fermentation losses and to soil contamination during mechanical treatments from cutting to harvesting.

Lucerne silage conservation

The main chemical and microbial characteristics of the silage in the three experiments, divided into central and peripheral zones of the silo, are reported in Table 3.

The DM content revealed stratification in Experiments 1 and 2, whereas in Experiment 3 the DM content was quite uniform. In Experiment 1, the upper (peripheral) part was wetter than the lower (central) one, owing to the addition of a forage with a higher moisture content to the top of the clamp to improve the consolidation of the silage characterized by a very high DM content at ensiling. In Experiment 2 the stratification was the opposite of that in Experiment 1, because of a longer drying period for the last batches of forage ensiled.

The density values in the central part of the silos were similar to those observed in forages cut with a precision-chop machine (e.g. Rees *et al.*, 1983; Darby and Jofriet, 1993), whereas in the peripheral zones they were not sufficiently high to avoid aerobic deterioration (Williams *et al.*, 1994).

The difference in DM content of the lucerne silage between the layers and experiments influenced fermentation. As found by other researchers, both with laboratory scale silos (Muck, 1987) or with large, commercial silos (Luchini *et al.*, 1997), ammonia-N concentration was inversely related to the DM content of the silage (Figure 2), with the exception of two samples of Experiment 3 taken from the peripheral area (a and b), which showed intense aerobic deterioration. In the second experiment the DM content of 360 g kg^{-1} FM reached during wilting was not sufficient to guarantee a good fermentation.

The concentration of soluble-N was high in Experiments 2 and 3 (720 and 650 g kg^{-1} TN for the central zones respectively) and, again, inversely correlated to the DM content of the silage. These results agree with the high values reported by Muck (1987) and are higher than the value (500 g kg^{-1} TN) tabulated by the Cornell Net Carbohydrate and Protein System (CNCPS) for an early bloom (350 g DM kg^{-1}) lucerne silage (Barry *et al.*, 1994). In the first experiment the soluble-N of the high DM silage was very low and similar to that of hay.

In all the experiments no clostridia development could be detected, as indicated by the low level of spores. The number of yeast and moulds was around

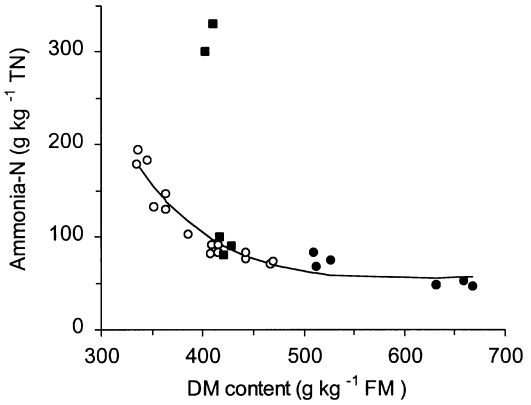

Figure 2 Relationship between ammonia-N in lucerne silages and DM content (hyperbolic regression, ammonia-N = 7·28 10^7 DM^{-2} – 2·43 10^5 DM^{-1} + 257; r^2 = 0·922; the two highest values of Experiment 3 are not included in the regression).●, Experiment 1; ○, Experiment 2; ■, Experiment 3.

3 log CFU g^{-1} FM in all the experiments, excluding the peripheral zones of the third experiment where there was an advanced aerobic deterioration shown by the higher count of yeast and moulds. Pahlow (1991) indicates the threshold of 5 log CFU g^{-1} FM as a critical yeast value for aerobic stability.

Maize silage quality

The DM content of the silages were typical of maize harvested at the dough stage of maturity in the Po Valley, being 322, 361 and 336 g kg^{-1} in Experiments 1, 2 and 3 respectively (Table 4). The chemical composition was similar with a lactic acid content ranging from 51 to 63 g kg^{-1} DM and no butyric acid.

Clostridia spores were always below 10^3 g^{-1}, indicating no butyric fermentation in the maize silages in the three experiments.

The yeast counts were higher than those observed in lucerne. This confirms, especially as far as Experiments 1 and 2 are concerned, a higher risk of aerobic deterioration spoilage for maize silage than lucerne, as found by other authors (Muck and O'Kiely, 1992; O'Kiely and Muck, 1992). The high yeast count of maize silages is probably due to the low rates of feeding-out (10, 12 and 13 cm d^{-1} in Experiments 1, 2 and 3 respectively) and to the interaction with the season of the experiments (higher in Experiment 2 in summer).

Feed intake

The composition of the rations consumed during the three experiments is reported in Table 5. In the first experiment lucerne was the main component of the ration providing 35% of the total DM, with maize silage representing 26%, whereas in the second and third experiments maize and lucerne constituted 36% and 24–25% of total DM respectively.

Table 4 Chemical and microbial composition of the maize silages sampled during feeding in the three experiments.

	Experiment		
	1	2	3
DM (g kg^{-1} FM)	322	361	336
CP (g kg^{-1} DM)	85	85	87
NDF (g kg^{-1} DM)	435	414	372
ADF (g kg^{-1} DM)	262	ND	ND
pH	3·9	4·1	3·9
Ammonia-N (g kg^{-1} TN)	86	110	ND
Soluble-N (g kg^{-1} TN)	509	569	ND
Lactic acid (g kg^{-1} DM)	62·7	60·5	51·0
Acetic acid (g kg^{-1} DM)	21·7	34·0	15·0
Butyric acid (g kg^{-1} DM)	0	0	0
Clostridia spores (log MPN g^{-1} FM)	2·66	2·31	2·85
Yeast (log CFU g^{-1} FM)	5·19	5·59	4·48
Moulds (log CFU g^{-1} FM)	3·86	4·26	4·08

Abbreviations as in Tables 2 and 3.

The chemical and microbiological analyses of the rations are reported in Table 6. As expected, the CP content was lower and the NDF content higher than international feeding standards: > 150 g CP and 280 g NDF per kg of DM for the cow size and milk yield considered (NRC, 1989). However, this reflects the need to reduce N excretion, especially in an area with a high concentration of cattle, such as that producing Grana Padano cheese. The net energy for lactation was balanced between treatments and was higher in Experiment 3 to sustain the higher milk yield.

The clostridia spore content of the rations was limited and always below 400 MPN g^{-1} (2·60 log g^{-1}), and no differences were observed between the treatments and experiments.

The yeast contents of the rations were highest in Experiment 1 and lowest in Experiment 2. As the values of the two treatments within each experiment were similar, it is likely that the rather high yeast contents of the rations did not depend primarily on the lucerne forage but mainly on the maize silage that appeared always to have a high yeast content (Table 4).

Individual daily DM intakes of the cows fed lucerne silage or hay were 18·8 and 18·5, 18·0 and 19·0, 20·5 and 20·1 kg in Experiments 1, 2 and 3 respectively. The feed intake, similar in the two treatments in Experiments 1 and 3, was slightly lower for the silage treatment in Experiment 2, probably because of the non-optimal fermentation of the lucerne silage in that experiment (see Table 3).

Table 5 Composition of the individual rations consumed (kg fresh matter d^{-1}) in the three experiments. The proportions (%) of total ration DM are shown in brackets.

	Experiment					
	1		2		3	
Treatment	**Silage**	**Hay**	**Silage**	**Hay**	**Silage**	**Hay**
Lucerne silage	12·0 (35)	–	11·3 (23)	–	11·1 (24)	–
Lucerne hay	–	7·4 (34)	–	5·5 (25)	–	5·4 (24)
Maize silage	15·0 (26)	15·0 (26)	18·5 (37)	19·0 (36)	22·2 (36)	22·6 (36)
Grass hay	2·0 (9)	2·0 (9)	1·5 (7)	1·6 (7)	2·2 (9)	2·2 (9)
Concentrate	6·5 (30)	6·5 (31)	6·8 (33)	7·0 (32)	7·2 (31)	7·0 (31)

Table 6 Main chemical and microbiological parameters of the rations fed in the three experiments.

	Experiment					
	1		2		3	
Treatment	**Silage**	**Hay**	**Silage**	**Hay**	**Silage**	**Hay**
Chemical composition						
CP (g kg^{-1} DM)	143	138	138	137	145	149
NDF (g kg^{-1} DM)	410	426	368	386	338	336
NEL (MJ kg^{-1} DM)	6·40	6·38	6·44	6·40	6·70	6·75
Microbiological composition						
Clostridia spores (log MPN g^{-1} FM)	2·24	2·20	2·09	2·60	2·35	2·21
Yeast (log CFU g^{-1} FM)	6·57	6·51	5·30	5·20	6·01	5·76

NEL, net energy for lactation; other abbreviations as in Tables 2 and 3.

Faeces

The spore contents (MPN g^{-1}) of the faeces from cows fed lucerne silage and lucerne hay were 362 and 342, 188 and 189, 558 and 76 in Experiments 1, 2, and 3 respectively. There was no difference between treatments in Experiments 1 and 2, whereas in Experiment 3 lucerne silage produced significantly higher ($P < 0·01$) spore counts in the faeces. However, all the values found were very low compared with previous studies (Gouet and Contrepois, 1971; Crovetto *et al.*, 1990; Colombari and Fantuzzi, 1991) in which concentrations of 10^5–10^6 spores per gram were found when the rations included silages with a high clostridial spore count. The low values in our experiments could be attributed to the absence of clostridia growth in the silage due to the high DM content obtained by wilting and to the technique of ensiling.

Milk yield and composition

The milk yield and composition in the three experiments are reported in Table 7. The milk yield was similar for both treatments within each experiment, independent of the production level, and the DM ratio between the maize and lucerne silage. Milk yield in Experiment 3 was higher because of the earlier stage of lactation of the animals. The hay-fed cows had a slightly but significantly higher milk yield in Experiment 2, probably owing to the higher feed intake. Similarly to our results, no significant differences in milk yield (kg d^{-1}) of cows fed early-cut lucerne silage or hay have been found by other researchers: 30·1 vs. 30·0 (Nelson and Satter, 1992b); 35·1 vs. 35·7, average of four experiments (Vagnoni and Broderick, 1997).

In Experiments 2 and 3, the protein content of milk was slightly but significantly decreased and the milk fat content significantly increased with the lucerne silage. This is consistent with the results of other studies (Nelson and Satter, 1992b; Broderick, 1995; Vagnoni and Broderick, 1997) in which 300–400 g kg^{-1} DM lucerne silage was fed to lactating cows and compared with lucerne hay. The increase in fat content of milk with silage may be attributable to a greater fibre digestion (Broderick, 1995) with consequent higher ruminal synthesis of fat precursors such as butyrate

Table 7 Milk yield, composition, nitrogenous fractions, renneting properties, and microbiological characteristics of the milk in the three experiments.

	Experiment											
	1				2				3			
Treatment	**Silage**	**Hay**	**s.e.m.**	***P***	**Silage**	**Hay**	**s.e.m.**	***P***	**Silage**	**Hay**	**s.e.m.**	***P***
Milk yield and composition												
Milk yield (kg d^{-1})	21·0	20·8	0·22	NS	20·0	20·6	0·12	**	28·4	27·9	0·18	NS
Protein (g kg^{-1})	33·3	33·1	0·15	NS	30·6	31·0	0·09	***	33·7	34·1	0·10	**
Fat (g kg^{-1})	40·7	40·5	0·35	NS	37·8	37·0	0·20	**	36·1	34·9	0·23	**
Lactose (g kg^{-1})	50·0	49·7	0·15	NS	50·4	50·1	0·80	*	50·5	50·4	0·07	NS
SCC (log ml^{-1})	5·31	5·31	4·38	NS	5·10	5·14	4·10	NS	5·30	5·33	4·12	NS
Nitrogenous fractions												
Casein N (g kg^{-1} N)	763	763	3·3	NS	766	764	2·0	NS	770	770	2·8	NS
Non-protein N (g kg^{-1} N)	57	57	2·2	NS	50	49	1·8	NS	52	55	2·3	NS
Whey protein N (g kg^{-1} N)	–	–	–	–	183	187	3·7	NS	179	175	3·4	NS
Urea (mg l^{-1})	210	210	10·4	NS	270	270	10·1	NS	264	276	12·3	NS
Renneting properties												
Acidity (°SH 50 ml^{-1})	3·46	3·41	0·032	NS	3·32	3·30	0·032	NS	3·33	3·33	0·084	NS
r (min)	15·3	15·6	0·26	NS	16·3	16·4	0·40	NS	17·7	17·2	0·31	NS
k_{20} (min)	9·1	9·2	0·28	NS	12·7	11·9	0·38	NS	8·9	8·4	0·25	NS
a_{30} (mm)	28·5	28·2	1·21	NS	19·9	20·1	0·91	NS	26·8	29·9	0·50	*
Microbiological characteristics												
SBC (log ml^{-1})	4·58	4·62	0·103	NS	5·37	5·12	0·101	NS	4·60	4·76	0·197	NS
Proteolytic bacteria (log ml^{-1})	3·13	3·11	0·109	NS	3·59	3·72	0·052	NS	2·81	3·00	0·216	NS
Coli bacteria (log ml^{-1})	1·90	1·85	0·040	NS	2·81	2·82	0·116	NS	2·41	2·49	0·237	NS
Lactic acid bacteria (log ml^{-1})	3·10	3·04	0·059	NS	3·83	3·80	0·032	NS	3·62	3·56	0·229	NS
Clostridia spores (log l^{-1})	2·16	2·06	0·082	NS	2·53	2·62	0·050	NS	2·14	1·86	0·192	NS

SCC, somatic cell count; r, time required to start clotting; k_{20}, time to reach a standard clot consistency; a_{30}, consistency of the clot after 30 min from the beginning of clotting; SBC, standard bacterial count.
Within rows and experiment, NS, not significant; $^{*}P \leq 0{\cdot}05$; $^{**}P \leq 0{\cdot}01$; $^{***}P \leq 0{\cdot}001$.

(Vagnoni and Broderick, 1997). Among volatile fatty acids, butyrate absorption through the rumen wall is mostly influenced by rumen acidity, and reaches the maximum rates at a low pH (Pitt *et al*., 1996).

The decrease in milk protein content in the silage treatment could be due to inadequate intestinal absorbed protein which, in turn, could be ascribed, on the one hand, to the very limited rumen undegradable protein (RUP) content of lucerne silage (10%, Makoni *et al*., 1991) and, on the other hand, to an excess of soluble-N with consequent excessive ammonia-N and an unbalanced ratio between the latter and amino peptide N. Microbial protein synthesis is normally lower with lucerne silage than with lucerne hay, probably because of the smaller microbial uptake of N from amino acids and peptides (Petelkova and Broderick, 1996); this finding confirms the study by Van Kessel and Russell (1996), which has shown that when the energy supply in the rumen is high the highest rates of microbial protein synthesis can be attained with a supply of amino peptide N.

Lactose content and SCC were practically constant over the treatments and experiments.

There was no significant effect of treatment on the nitrogenous fractions of milk (Table 7). The urea content in Experiment 1 was lower than that of the other two experiments. For the 'silage' rations this can be explained by the much lower N solubility of lucerne silage in Experiment 1 (35%) than in Experiments 2 (72%) and 3 (65%) (see Table 3).

However, the milk urea contents were satisfactory for Experiments 2 and 3, always being below 300 mg l^{-1} milk. It should be noted that the low urea content of milk was achieved despite the high proportion of N from lucerne silage, which is more than 50% soluble (see Table 3), and also because of the low protein level of the rations.

Milk-renneting properties and microbiology

The renneting properties and microbiological characteristics of the milk are shown in Table 7. There were no

significant treatment effects for the parameters investigated, except for the greater clot consistency (a_{30}) of the milk produced by the lucerne hay-fed cows in Experiment 3. When considering the differences between Experiment 2 conducted in the warm season and those carried out in the cold season (Experiments 1 and 3), it can be seen that the latter (characterized by higher milk protein contents) showed other positive characteristics such as a shorter time to reach the 20-mm reference clot consistency (k_{20}) and a stronger clot consistency after 30 min from the beginning of clotting (a_{30}).

The microbiological characteristics of the milk did not differ between treatments in any experiment; the levels of SBC, proteolytic, coli and lactic acid bacteria, and clostridia spores were lower in Experiments 1 and 3 than in Experiment 2. This indicates that the season (cold or warm) had a much higher effect than the conservation technique (ventilated hay vs. silage) on the microbiological content of milk.

The inclusion of lucerne silage in the ration did not increase the spore content of milk, and in each experiment the spore count was always very low (< 400 per litre) fulfilling the requirements for a top milk quality (Bottazzi and Battistotti, 1978) destined for the production of Grana Padano cheese.

Cheese quality

The trade classification of the thirty-two cheeses (sixteen per treatment) of the Grana Padano produced from the milk of Experiment 3 showed that all the cheeses were commercially good and no third-choice cheese was recorded for either group; only one cheese of the group fed on the silage treatment was evaluated as being of second choice. The second-choice cheese was analysed by gas chromatography to identify the origin of the damage; no butyric acid was detected, confirming that no clostridial development had taken place, although evidence of a heterolactic fermentation was found.

Conclusions

Lucerne forage, cut at an early stage (vegetative or early bud), harvested and chopped with a self-loading wagon, can be successfully ensiled in small clamps even with a high DM content (around 50%), provided that accurate sealing and compaction of the forage, and a correct feed-out rate are carried out in order to avoid risks of aerobic deterioration because of its high porosity.

The data obtained indicate that the substitution in the ration of lucerne silage (23–35% of ration DM) for high-quality lucerne hay did not change the milk yield and milk composition from a practical point of view. The same holds true for the milk nitrogenous fractions and the milk renneting properties.

The quality of the Grana Padano cheese after 12 months of maturation was very good and not influenced by the inclusion of lucerne silage into the ration. This is likely to be ascribed to the low spore content of the milk which, in turn, depends on the low spore contamination of the silages used.

The use of good-quality lucerne silage can increase the proportion of home-grown forage, reduce the amount of the purchased feeds and has no negative repercussions on the quantitative and qualitative milk production and subsequent cheese quality.

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