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Effect of Ensiling Alfalfa at Low and High Dry Matter on Production of Milk Used to Make Grana Cheese

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ABSTRACT

The effect of alfalfa ensiled in bunker silos at high moisture [HM, 34% dry matter (DM)] and low moisture (LM, 56% DM) content on milk production and Grana Padano cheese quality was studied. Forty Italian Friesian lactating cows were allotted to two groups and fed, in a crossover design experiment, two corn silage-based diets containing 27% of the total DM as HM or LM. Each of the two periods included 10 d of adaptation and 3 experimental weeks. Forage was cut in the mid-vegetative stage with, on average, 34% neutral detergent fiber and 19% crude protein (DM basis). The two alfalfa silages showed a different fermentation pattern with 4.04 and 1.25% of lactic acid, 1.95 and 0.42% of acetic acid, 9.1 and 4.8% of total N ammonia-N for HM and LM, respectively. No butyric acid was found. Clostridial spores and yeast showed no growth in both silages except in the first 2 wk of the experiment where slight aerobic deterioration occurred. The HM treatment resulted in slightly lower DM intake (19.3 vs. 19.9 kg/d) and milk protein content (3.33 vs. 3.38%), higher milk fat content (3.56 vs. 3.37%), and 4% fat-corrected milk (25.7 vs. 24.4 kg/d). Totally, 38 cheeses obtained from over 19 tons of milk with an average yield efficiency of 6.8%, were produced. The milk renneting and microbiological properties and the cheese quality were not significantly different between treatments. However, both treatments had on average 40% of low quality (butyric fermentation) cheeses observed mainly in the first 2 wk of the experiment, when the number of clostridial spores found in alfalfa silages was significantly higher than in the subsequent weeks. The data obtained suggest that the microbial quality of milk depends more on careful management and monitoring all of the steps in milk production, from silage harvest through to cheese making, than on the moisture level of alfalfa silage, provided that the latter is in a range of 35 to 55% DM.
(**Key words:** alfalfa silage, milk quality, clostridial spores, cheese quality)

Abbreviation key: FM = fresh matter, **HM** = high moisture, **LM** = low moisture, **MPN** = most probable number, **SBC** = standard bacteria counts, **WSC** = water-soluble carbohydrates.

INTRODUCTION

Recent restrictions on the use of animal protein in ruminant feeding, increasing costs of protein supplements, and the need for more sustainable agriculture increase the need for homegrown protein crops (as feeds) for milk production. Moreover, increased awareness of the nutritional value of high quality alfalfa in terms of potential energy savings and protein supplements for ruminants, has caused many dairy farmers to reevaluate current harvest strategies and conservation methods as alternatives to traditional haymaking. Although alfalfa is traditionally made into hay, haymaking is not recommended for first cuts in areas of high spring rainfall such as the Po Valley of Italy. Good hay curing weather usually occurs too late in this region to produce high quality forage. Successful ensiling provides an opportunity to maintain the high feeding value of the young herbage, since the latter rapidly declines with morphological stage (Kalu and Fick, 1981). The DM content at which alfalfa is harvested for ensiling varies from unwilted up to 70%, depending on management practices, type of storage and environmental conditions during wilting (Luchini et al., 1997). Increasing DM content usually reduces the extent of fermentation, resulting in a higher pH, higher concentrations of water-soluble carbohydrates (**WSC**), and decreased proteolysis with a consequent decrease in the NPN content of the ensiled forage (Muck, 1987). Forages, particularly alfalfa silage, are characterized by an increased proportion of rumen degradable nitrogen, with NPN representing from 50 to 70% of total N (Luchini et al., 1997). Since an excessive amount of rumen degradable nitrogen can induce alkalosis and depresses N utilization,

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it seems advisable to encourage conditions that reduce the extent of proteolysis in the silage (Makoni et al., 1994). Proteolysis rate is negatively correlated with the DM content of the herbage, and little proteolysis occurs above 55% DM (Cavallarin et al., 2000). Furthermore, ensiling at a DM content near 50% maximizes the DMI of dairy cows (Van Vuuren et al., 1995), but increases the risks of aerobic deterioration due to higher porosity of the silage (Williams et al., 1994).

Ensiling alfalfa forage that is not sufficiently wilted (<35% DM) will result in effluent losses from the silo and may allow clostridial fermentation (Kung et al., 1985; Muck, 1990). Growth of clostridia in silage can take place during the acidification phase, the storage phase, and upon exposure of the material to air, as demonstrated by Jonsson (1991). When silage is exposed to air, the oxygen penetrates deep into it, and aciduric aerobic organisms (e.g., yeast) consume oxygen and, at the same time, the substances that inhibit the clostridia growth, i.e., organic acids produced by anaerobic fermentation. The consumption of both acids and oxygen leads to the development of micro-niches with less inhibitory activity, which might allow the growth of clostridia (Jonsson, 1989). Growth of clostridia in silage can have two quite different undesirable effects. They are involved in the anaerobic spoilage of silages and the deterioration of hard cheeses. Clostridial spores from highly contaminated silages may pass into the milk (Stadhouders and Spoelstra, 1990) via dung contamination, even if good hygienic milking conditions are practiced (Stadhouders and Jørgensen, 1990). Spores of clostridia survive milk pasteurization and pass unaffected into cheese. By their ability to ferment lactate, *Clostridium tyrobutyricum* and *Clostridium butyricum* can multiply in many types of hard and semihard cheeses (Grana, Tilsit, Parmigiano-Reggiano, Emmenthal, Gruyère, Gouda, Edam, etc.) resulting in a defect called 'late blowing,' because of abnormal gas production (H_2 and CO_2) that accompanies butyric acid production (Colombari and Fantuzzi, 1991; Gouet and Bergère, 1973).

The objective of this experiment was to compare the effect of ensiling alfalfa at two different moisture contents on silage quality and the effects of feeding this silage in corn silage-based diets on performance of lactating cows and the quality of the Grana cheese produced.

MATERIALS AND METHODS

Crop and Silages

The experiment was conducted in 1997 at the experimental farm of the Istituto Superiore Lattiero Caseario of Mantova (25 m above sea level, 45°09′N, 10°48′E, mean annual rainfall 632 mm, mean annual temperature 13°C) in the Po Valley. Pure stands of about 7.6 ha of alfalfa cultivar "Delta" were used.

A primary regrowth alfalfa at midvegetative stage was cut on 15 April (4 cm stubble height) using a mower conditioner. Tedding was applied about 2 h after mowing. The weather was favorable for field drying and no rain fell during the drying period. Forage was harvested from alternate windrows at two levels of wilting, using a self-loading forage wagon (Kemper Cargo L 9000, Stadtloon, Germany) set to a chop length of 40 mm. The levels expected were high moisture (**HM**) and low moisture (**LM**) with a DM content of 35 and 55%, respectively, obtained after 1 and 2 d of field drying. These two levels of moisture were chosen as the extremes normally compatible with obtaining good bunker silage under normal farm conditions.

Forage was ensiled in two low, narrow bunker silos made of transferable, prefabricated walls, 0.9 m high, spaced 4.0 m apart. The silage was carefully compacted with a tractor, covered with two films of polyethylene and weighted down with 150 kg/m^2 of gravel. Silos were opened after 170 d. The feed-out rate of alfalfa silage was about 20 cm/d.

The rations included whole plant corn silage produced from a corn crop (*Zea mays* L., FAO class 600) 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. The feed-out rate of corn silage was 10 cm/d.

Chemical and Microbiological Analyses of Forages

Samples of alfalfa obtained at cutting were analyzed for: DM content by oven drying at 90°C until a constant weight was achieved, CP (Kjeldahl N × 6.25), ash by ignition to 550°C, and NDF and ADF by the sequential analysis of Goering and Van Soest (1970) measured on an ash-free basis.

Silage was sampled weekly during feeding by coring (diameter 5 cm) 30 cm deep inside the mass of the silage immediately after the feeding out. Aqueous extracts (with water for 24 h at 4°C) were analyzed to determine pH, total N, and ammonia-N concentration by the method of Byrne and Power (1974). Soluble N was determined with a phosphoborate buffer at pH 6.8 and 39°C for 1 h (Krishnamoorthy, 1982). Lactate, acetate, and butyrate were determined by HPLC as described by Canale et al. (1984). Silage density was calculated by measuring the corer volume and weights of the samples.

Microbiological analyses of clostridial spores were made following the most probable number (**MPN**) technique with lactate-acetate agar (Spoelstra, 1984) after

Table 1. Composition and nutrient analysis of diets fed.

	Diet[1] HM	LM
Component (% of dietary DM)		
Alfalfa silage	27.2	27.5
Corn silage	23.2	23.0
Italian ryegrass hay	18.9	18.8
Concentrate[2,3]	30.7	30.7
Total diet (TMR)		
DM, %	50.5	59.0
Ash, % of DM	8.8	8.3
CP, % of DM	14.4	14.0
Soluble-N, % of TN	51.0	46.0
NDF, % of DM	37.8	38.2
ADF, % of DM	22.8	23.0
NE_L, Mcal/kg DM	1.54	1.54
pH	5.2	5.5
Clostridial spores, log MPN/g	2.74	2.92
Yeasts, log cfu/g	6.33	6.30
Molds, log cfu/g	5.08	4.81

[1]HM = High moisture alfalfa; LM = low moisture alfalfa; TN = total nitrogen; MPN = most probable number.

[2]Composition (%): corn grain, 17.0; sugar beet pulp, 17.0; wheat bran, 16.3; soybean meal, 14.0; barley grain, 8.5; wheat middling, 8.0; corn gluten feed, 7.0; linseed meal solv., 4.0; sugarcane molasses, 2.0; sodium bicarbonate, 1.5; dicalcium phosphate, 1.3; limestone, 1.2; mineral-vitamin supplement, 1.0; magnesium oxide, 0.7; and salt, 0.5.

[3]Supplied per kilogram of concentrate: 50,000 IU of vitamin A, 2,000 IU of vitamin D_3, 50 IU of vitamin E, 100 mg of Zn, 30 mg of Mn, 15 mg of Fe, 15 mg of Cu, 3 mg of I, 0.20 mg of Se, 0.20 mg of Co.

incubation at 37°C for 7 d. Colony-forming units (cfu) of yeasts and molds were counted using the pour plate technique with 40.0 g/L of yeast extract glucose chloramphenicol agar (YGC agar, DIFCO, West Molesey, Surrey, UK) after incubation at 25°C for 3 d.

Animals and Management

Forty lactating Italian Friesian cows in stanchion-type housing were allotted to two groups of 20 animals each. Groups were balanced for parity (2.5), stage of lactation (143 DIM), and individual daily milk yield (28.1 kg).

A crossover design was used, with 10 d of adaptation followed by 3 experimental weeks for each of the two phases. Diets included alfalfa silage, corn silage, Italian ryegrass hay, and concentrate and were fed ad libitum as TMR. Composition and analyses of the two diets are reported in Table 1. On average, the two TMR diets contained (on DM), 27% alfalfa silage, 23% corn silage, 19% ryegrass hay (57% NDF on DM), and 31% concentrate. The beginning of the experiment was synchronized with the opening of the alfalfa silos.

At the beginning of each adaptation period, rations were fed to meet predicted maintenance and production requirements, but adjustments in the quantity of TMR offered to each group were made according to appetite. Drinking water was available ad libitum. The feeding experiment was conducted in October and November.

Intake and Ration Analyses

The DMI was recorded daily for each group of cows. Each morning before feeding, refusals were collected, weighed, and analyzed for DM content. During the experimental periods, the TMR fed to each group of cows was sampled three times each week and analyzed for CP, ash, NDF, clostridial spores and yeasts. Net energy for lactation (NE_L) was predicted following the French system (Andrieu and Demarquilly, 1987).

Fecal Analyses

Samples of feces were collected three times during each experimental week directly from six cows in each group (three cows of high and three cows of low production level) after the morning milking. On these samples, the concentration of clostridial spores was determined to study the possible influence of a presumed different rate of feed turnover in the gastrointestinal tract on the spore content of the feces.

Milk Production and Cheese Analyses

Individual daily milk yields were recorded twice weekly from both morning and afternoon milkings. Individual daily milk was sampled proportionately to milk yield from the p.m. and a.m. milkings and analyzed for fat, protein, and lactose contents (Milk-O-Scan 605, Foss Technology, Hillerød, Denmark), and for the SCC (Fossomatic 360, Foss Technology, Hillerød, Denmark). The daily (sum of a.m. and p.m. milkings) bulk milk of each group of cows was sampled three times per week and analyzed for microbiological characteristics [standard bacteria count (**SBC**), proteolytic bacteria, coliforms, propionic acid and lactic acid bacteria, and clostridial spores], nitrogenous fractions (casein N, NPN, whey protein N, and urea N), and acidity according the methods of International Dairy Federation (Bull. FIL/IDF). Renneting properties (r = rennet clotting time, time required to start clotting (min); k_{20} = rate of curd firming, time (min) to reach a standard clot consistency (20 mm at the trombelastograph tracing); a_{30} = curd firmess after 30 min from the beginning of clotting (mm of the trombelastograph tracing) (Tarodo de la Fuente et al., 1969).

The milk obtained in the experiment was used for the production of Grana Padano cheese (weighing 35 kg, on average) following the traditional steps and procedures for production of this typical Italian cheese.

Cheese production started with the first experimental week (after the adaptation phase). In total, 38 Grana Padano cheeses were produced from over 19 tons of milk, with an average yield efficiency of 6.8% after 12 mo. The bulk milks of the morning milking were stored at 8 to 10°C for 12 h and mixed with the respective bulk milks of the afternoon milking. The milk was then taken to the experimental dairy plant at the Istituto Superiore Lattiero Caseario of Mantova. During the night, the milk was held in basins (12 h) to induce fat to float to the surface (temperature 14.5°C). The milk was cooled by dipping stainless steel tubes in the basins with chilled water circulating inside. The whey culture, characterized by a medium acidity (28 to 29°SH/50 ml), was added to milk at a level of 3.7% to induce clotting and to enrich the clot with lactic acid bacteria. This addition resulted in an increase of milk acidity to about 0.85°SH/50 ml. Lysozyme (16 ppm) was also added to the milk to prevent late blowing of the cheese. The common range of inclusion of lysozyme in milk destined for Grana Padano cheese is from 16 to 20 ppm (Bottazzi and Battistotti, 1999). Heating lasted 5 min; the temperature at clotting was fixed at 34.4°C; 3.6 ppm of rennet was added during the cheese-making process. Cooking temperature of the clot was constant at 54.6°C. Working time from the addition of the rennet until the end of cooking was 16.5 min. After removing the heat source, the clot sank to the bottom of the boiler and was left there for 63 min to permit a satisfactory aggregation of the clots. At the end of the process the cheese was put in salted water for 14 d.

The quality of the Grana Padano cheese produced from the milk in the experiment was checked after 12 mo of maturation to evaluate the influence of treatment. The evaluation of cheese quality was carried out by an expert from the Grana Padano Consortium (Zapparoli, 2000, personal communication). Cheese that presented fermentation damage was analyzed by gas chromatography (Contarini et al., 1989) to identify the microbiological source of the damage.

Statistical Analysis

Chemical and microbiological data of forages and silages were analyzed by ANOVA (SAS, 1994). For yeast, molds and spores of silages, time (d) computed from the beginning of the trial was used as covariate in the following model:

$$Y_{ij} = \mu + T_i + \beta(X_{ij} - \bar{x}) + e_{ij},$$

where Y_{ijk} = dependent variable; μ = general mean; T_i = type of alfalfa silage (i = 1–2); β = covariate effect (j = 1, 2, . . ., 15); e_{ij} = error.

Treatment differences for milk yield and milk fat, protein, lactose contents and yields, and SCC were examined using the following crossover model with a GLM procedure (SAS, 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 = 1–20); T_k = type of alfalfa silage (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.

Dry matter intake and chemical-microbiological data of the bulk milk (acidity, r, k_{20}, a_{30}, SBC, proteolytic, coliforms, propionic and lactic acid bacteria, clostridial spores), the nitrogenous fractions and the data on spore content of the feces were analyzed 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 alfalfa silage (j = 1–2); e_{ijk} = error.

SCC data were first converted to linear scores (LS = $\log_2$[cells/12500]) because of their nonnormal distribution. Similarly, the microbiological data on the feeds, feces and milk were first transformed to $\log_{10}$.

RESULTS AND DISCUSSION

Alfalfa Herbage and Silage Quality

For a satisfactory compromise between quality and quantity, alfalfa was cut at the midvegetative stage (mean stage by count = 1.9 following Kalu and Fick (1981)) with a high leaf to stem ratio (0.73) and a field yield of 2.5 t DM/ha. The main qualitative characteristics of the herbage at cutting and wilted prior to ensiling are reported in Table 2. The DM contents of the forages at ensiling were 36 and 58% for HM and LM, respectively. The early cutting and the good wilting conditions permitted very low NDF and ADF contents (less than 36 and 30%, respectively). The CP contents were lower than the 21 to 23% expected for this stage of growth and showed a slight decrease following wilting due to mechanical leaf losses. Soluble-N remained almost constant during wilting. The mean nitrate content was about 294 mg/kg of fresh matter (**FM**), as expected for a legume. This low nitrate content would not be expected to inhibit clostridial activity during silage fermentation, because higher values (around 1000 mg/kg of FM) are needed (Spoelstra, 1985). The ash content did not increase significantly during wilting, indicating

Table 2. Nutrient composition of alfalfa at cutting and wilted herbage before ensiling.

Item	Fresh herbage	Wilted forage[1]			
		HM	LM	SE	Effect[2]
DM, %	19.0	36.1	58.2	4.5	**
NDF, % of DM	31.6	33.0	35.2	0.6	*
ADF, % of DM	26.2	28.2	29.6	0.4	*
CP, % of DM	19.9	19.0	18.9	0.2	NS
Ash, % of DM	11.2	11.1	12.8	0.5	NS
Soluble-N, % of TN	37.9	40.9	40.9	0.5	NS
Nitrate, mg/kg FM	294	226	294	69	NS

[1]HM = High moisture alfalfa; LM = low moisture alfalfa.

[2]NS $P > 0.05$; *$P < 0.05$; **$P < 0.01$; for differences between HM and LM treatments.

low soil contamination during the mechanical treatments from cutting to harvesting. The main chemical and microbial characteristics of the silage during feedout for the two treatments are reported in Table 3.

The two alfalfa silages made at different DM contents (34.3 and 56.2%) varied for wet bulk density and fermentation patterns. Increased DM content reduced the extent of fermentation, resulting in a higher pH and lower lactic and acetic acid concentrations. Longer wilting also reduced N breakdown, giving lower value for soluble and ammonia nitrogen. Due to the lower bulk density, the drier silage had higher aerobic microbial activity with higher yeast and mold counts. Furthermore, we observed that the microbiological and conservation quality of both alfalfa silages improved with time during the experiment, as indicated by the yeast counts reported in Figure 1. The experiment started just after the opening of the silos. Therefore, in the first 2 experimental weeks the silages showed a higher yeast and mold counts associated with the higher ambient temperature than in the subsequent weeks. This microbial activity during preservation of the silages fed in the first 2 wk slightly influenced clostridial growth as shown by the higher level of butyric acid and clostridial spores, compared with the subsequent weeks of the experiment. Butyric acid (% of DM) was 0.041 in the first 2 wk versus 0.001 in other weeks for HM and 0.018 vs. 0.002 for LM, and spores (log MPN/g of FM) were 2.54 vs. 1.47 and 2.65 vs. 1.78 for HM and LM, respectively. The values of 1.47 and 1.78 were not significantly different from that found in fresh forage at ensiling. The growth of clostridia due to microinfiltration of air during ensiling has been reported by many authors and discussed by Jonsson (1991).

Corn Silage Quality

The composition of the corn silage is reported in Table 3. The DM content of the silage was typical of corn

Table 3. Chemical and microbial composition of alfalfa and corn silages sampled during feeding.

Item	Alfalfa silage[1]				Corn silage	
	HM	LM	SE	Effect[2]		SE
DM, %	34.3	56.2	3.5	**	35.2	0.8
Wet bulk density, kg/m^3	586	381	36	**	711	37
DM density, kg/m^3	207	214	10	NS	246	16
pH	4.7	5.6	0.2	**	3.9	0.1
Ash, % of DM	13.0	12.1	0.2	**	4.1	0.03
Total N, % of DM	3.06	2.94	0.04	**	1.26	0.01
Ammonia-N, % of TN	9.10	4.83	0.69	**	6.3	0.7
Soluble-N, % of TN	77.1	64.6	2.1	**	53.4	0.5
Nitrate, mg/kg FM	80	286	47	**	. . .	. . .
Lactic acid, % of DM	4.04	1.25	0.46	**	3.8	0.6
Acetic acid, % of DM	1.95	0.42	0.25	**	1.4	0.2
Butyric acid, % of DM	0.001	0.002	0.004	NS	0.000	. . .
Clostridial spores, log MPN/g FM	1.94	2.11	0.08	NS[3]	2.34	0.35
Yeast, log cfu/g FM	2.94	3.33	0.26	*[3]	6.65	0.16
Molds, log cfu/g FM	1.97	2.50	0.25	*[3]	4.75	0.34

[1]HM = High moisture alfalfa; LM = low moisture alfalfa; FM = fresh matter; TN = total nitrogen; MPN = most probable number.

[2]NS $P > 0.05$; *$P < 0.05$; **$P < 0.01$; for differences between HM and LM silages.

[3]ANOVA with 'day of the experiment' as covariate.

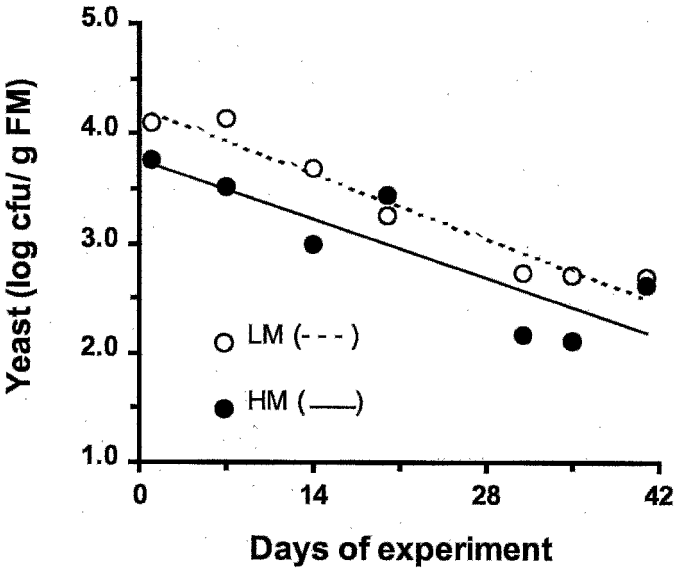

Figure 1. Yeast count evolution in alfalfa silages during experimental period. HM = High moisture alfalfa; LM = low moisture alfalfa.

harvested at the dough stage (60% kernel milk line; 42% NDF; 23% ADF; 34% starch; 7.9% CP, on DM) of maturity in the Po Valley. The fermentation characteristics were good and clostridial spores were higher than in alfalfa, but no growth occurred because the pH was always lower than 4.0, and no butyric acid was found. Yeast counts were higher than those observed in alfalfa. This was probably due to the low feeding-out rates of 10 versus 20 cm/d and the higher susceptibility of corn silage to aerobic deterioration (O'Kiely and Muck, 1992).

Feed Intake

Both HM and LM fed cows registered satisfactory feed intakes despite the high NDF content of the diets. This indicates that the quality and the palatability of the two rations were good. Moreover, DMI was slightly but significantly higher for the LM treatment (Table 4). The trend for higher feed intake by cows fed silage with a lower moisture content was reported earlier (Wright et al., 2000). In our experiment, the difference in moisture content between the two diets was less than 10 percentage points (50 vs. 59% DM) and this could explain the slight effect on DMI.

Feces

The spore contamination of the feces was very limited and not significantly different between treatments: on average 2.32 and 2.36 log MPN/g for HM and LM, respectively. No significant differences were also observed for spore content between high and low yielding cows (2.29 vs. 2.39 log MPN/g). As already seen for the alfalfa silages, a higher count of spores in the feces was detected during the first 2 experimental weeks than in the subsequent period (cf. Table 5). However, all the values found were very low in comparison with other studies (Gouet and Contrepois, 1971; Colombari and Fantuzzi, 1991) where concentrations of 10^5 to 10^6 spores per gram were observed when the rations included silages with a high clostridial spore count.

Milk Yield and Composition

The data reported in Table 4 reveal a similar milk yield for the two treatments. However, because of the higher milk fat content ($P < 0.01$), HM-fed cows registered significantly higher ($P < 0.01$) 4% FCM and fat yields. On the other hand, LM-fed cows produced a milk with a higher protein content ($P < 0.01$), but there were no differences in protein yield. The data obtained indicate a lack of relationship between DMI and animal performance. This is consistent with the results of other studies (Wright et al., 2000) which reported that, although wilting generally results in increased DMI, responses in animal performance have often been negative or small.

Lower fat and higher protein content of milk from cows fed alfalfa silage with low moisture in comparison with a high moisture content was also found by Campbell and Buchanan-Smith (1991) and Colombari et al. (1999). No significant differences between treatments were observed for lactose and SCC. The nitrogenous fractions of the milk were all within the normal range. Nitrogen urea content was significantly higher in HM treatment compared with LM ($P < 0.05$). The lower N urea content of LM milk is in agreement with the results obtained in a previous experiment (Colombari et al., 1999) and can be attributed to the slightly lower CP content and the lower level of soluble N of LM diet. The alfalfa silages contributed about one-third of total dietary nitrogen and other work (Campbell and Buchanan-Smith, 1991) found that the higher the DM content of the silage, the lower its rumen protein degradability.

Microbiological Characteristics and Renneting Properties of Milk

The overnight storage of the bulk milk in the basins resulted in a decrease in fat content of about 1 point percentage and a final casein to fat ratio near to 1. Keeping a constant low temperature (8 to 10°C) inside the milk slowed global microbial growth so much that the balance between the microorganisms generated by

Table 4. Dry matter intake, milk yields, milk composition, nitrogenous fractions, renneting properties, and microbiological characteristics of the milk.

Item	Diet[1]			Effect[2]
	HM	LM	SE	
DMI, kg/d	19.3	19.9	0.8	**
Milk yield and composition				
Milk yield, kg/d	27.8	27.3	0.3	NS
Milk fat, %	3.56	3.37	0.03	**
4% FCM, kg/d	25.7	24.4	0.3	**
Milk protein, %	3.33	3.38	0.01	**
Milk lactose, %	4.90	4.91	0.01	NS
Fat yield, g/d	970	900	12	**
Protein yield, g/d	909	906	10	NS
Lactose yield, g/d	1367	1341	16	NS
SCC, linear score	4.22	4.40	0.07	NS
Nitrogenous fractions				
Caseinic N, % of TN	77.2	77.1	0.8	NS
NPN, % of TN	5.2	4.9	0.2	NS
Whey protein N, % of TN	17.7	18.0	0.6	NS
Milk urea, mg N/100 ml	15.6	13.7	0.6	*
Renneting properties				
Milk acidity, °SH/50 ml	3.20	3.23	0.04	NS
r, min	17.5	15.6	0.5	**
k_{20}, min	11.6	11.4	0.04	NS
a_{30}, mm	19.7	23.6	1.4	NS
Microbiological characteristics				
SBC, log/ml	4.95	4.68	0.09	NS
Proteolytic bacteria, log/ml	2.58	2.80	0.58	NS
Coliforms, log/ml	1.91	2.25	0.11	NS
Propionic bacteria, log/ml	2.94	2.97	0.25	NS
Lactic acid bacteria, log/ml	3.49	3.35	0.04	NS
Clostridial spores, log MPN/L	2.27	2.08	0.10	NS

[1]HM = High moisture alfalfa; LM = low moisture alfalfa; SBC = standard bacteria count; TN = total nitrogen; MPN = most probable number.

[2]NS $P > 0.05$; *$P < 0.05$; **$P < 0.01$.

microbial multiplication and those removed by fat floating was negative (data not shown). In Table 5 the effect of fat floating on reduction of clostridial spores is reported. In fact, in the morning the SBC of milk in boiler was, on average, 3 to 4 times lower than that of the preceding evening (25 versus 83 thousand/ml). As a consequence, milk acidity in the boiler, instead of increasing, decreased on average from 3.22 to 3.13°SH/

Table 5. Clostridial spore contents of the different factors involved in the production of the Grana Padano cheese during the first 2 wk and the following 4 wk of the experiment.

Item	Period				Comparisons[2]		
	1st–2nd wk		3rd–6th wk				
	HM[1]	LM	HM	LM	HM vs. LM	Period	T × P
Clostridial spores							
Alfalfa silage, log MPN/g	2.54	2.65	1.47	1.78	NS	*	NS
TMR, log MPN/g	2.68	3.23	1.85	2.78	NS	**	NS
Feces, log MPN/g	3.04	2.82	2.25	2.49	NS	*	NS
Milk, log MPN/L	2.27	2.20	2.44	2.33	NS	NS	NS
Milk after fat floating, log MPN/L	1.77	1.72	1.70	1.72	NS	NS	NS
Cheese quality							
Bad quality batches/total batches	6/7	6/7	3/12	1/12			
Type of fermentative defect	butyric/ heterolactic	butyric/ heterolactic	heterolactic/ propionic	herterolactic			

[1]HM = High moisture alfalfa; LM = low moisture alfalfa; MPN = most probable number.

[2]NS $P > 0.05$; *$P < 0.05$; **$P < 0.01$.

50 ml. The content of proteolytic, coliforms, lactic acid, and propionic acid bacteria and clostridial spores was also quite low for both treatments (Table 4).

When we examined the renneting properties of the milk, LM treatment showed a significantly lower renneting clotting time. This result is positive because it means a shorter time to start clotting and can be related to the lower N urea content of the LM milk. The k_{20} and a_{30} means were not statistically different between the two treatments.

The reactions of HM and LM milks to the addition of rennet were almost identical and constant in terms of time of clotting (497 s, on average), hardening (63 s) and breaking of the clot (210 s). Cheese yield after 24 h was 8.0% on average, with no difference between treatments.

Cheese Results

No differences were found between HM and LM treatments in terms of commercial cheese quality during the 12 mo of maturation. From Table 5 it can be calculated that, despite the low spore contamination of the milk, HM and LM treatments yielded overall 47 and 37% of bad quality cheeses, respectively.

Most of the low quality cheeses were produced in the first 2 wk of the experiment and were attributable mainly to butyric acid and, to a minor extent, heterolactic fermentations (Table 5). Since other conditions did not change during the experiment, including the microbial profile of the corn silage, it can be concluded that the high incidence of low quality cheese was due to the poor microbial quality of the alfalfa silages at the beginning of the silo. In the first 2 wk there was clostridial growth in the alfalfa silages (Table 5). However, this phenomenon did not affect the spore content of the milk, which was constant throughout the experiment. However, despite the quantitative similarity, the qualitative profile of the clostridial count in the milk may have been quite different in the two periods. In fact, clostridia growing in silage can select strains of bacteria able to utilize, as substrate, lactic acid at low pH. When these selected clostridia pass out in the feces and, through dung contamination, appear in cheese, the late blowing defect can occur (Stadhousers and Spoelstra, 1990). This happened despite the presence of lysozyme, but it must be emphasized that lysozyme is mainly active against *C. tyrobutyricum* (Wasserfall and Teuber, 1979), while blowing in cheese can also be due to *C. butyricum* which also grows in silage.

CONCLUSIONS

Different levels of wilting of alfalfa silage (34 vs. 56% DM) did not greatly affect milk production and quality. The feeding of LM silage tended to increase milk protein content and to decrease content of milk butterfat and urea nitrogen.

The utilization of alfalfa silage in dairy cows whose milk is used for making Grana cheese needs to be done with great care, because cheese making is very sensitive to all the microbiological changes occurring in silages. If silages are well managed a successful cheese making can be achieved, but even moderate microbial growth due to air penetration in the silage can result in great fermentation damage to Grana cheese.

These data suggest that a high microbial quality milk depends more on careful management and monitoring of all the steps in milk production, from silage harvest through to cheese making, than on the moisture level of the alfalfa silage, provided that the latter is in a range of 35 to 55% DM.

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