Comparison between two hay based diets in buffalo nutrition: microbiological characteristics of milk, curd and mozzarella cheese

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ABSTRACT: Two groups of twenty milking Mediterranean buffalo cows were fed two diets, with the same energy and protein content, based on Italian ryegrass hay (diet G1) and Italian ryegrass hay silage (diet G2). Presumptive lactobacilli and lactococci were determined on milk, curd and mozzarella cheese, collected at 30, 60, 90 days from the beginning of the trial. Milk and mozzarella cheese at 30, 60, 90 days showed significantly higher microbial counts in diet G2 with respect to diet G1, apart from lactococci in milk at 90 days. For curd at 30, 60, 90 days the numbers of lactobacilli and lactococci in diet G1 were significantly higher with respect to diet G2, apart from lactococci at 30 days. Genetic analysis by 16S rDNA characterization of colonies, randomly isolated, showed in diet G2 also the presence of non lactic species. Probably Italian ryegrass hay silage, present in diet G2, could bring bacteria in the environment and consequently in the milk that are detrimental for the proper growing of lactic microflora in the curd and that are resistant to the high temperature used in the mozzarella cheese making process.

Key words: Buffalo milk, Mozzarella cheese, Microbiology, Mediterranean forages.

INTRODUCTION - This work is part of a major project concerning a comparison between the use of typical Mediterranean forages and maize silage in buffalo nutrition, to sustain the agricultural system of the southern Italy and to allow a better quality of buffalo products. The mozzarella cheese is produced with raw milk and natural whey culture used as starter (Addeo and Coppola 1983; Coppola et al. 1988; Coppola et al. 1990). The milk, whey and consequently curd contain a large number of microorganisms, their quantity and biodiversity is responsible for the taste of the final product. Many studies point out the influence of milk, curd and natural whey microflora on the production and quality of mozzarella cheese (Coppola et al. 1988; Coppola et al. 1990, Mauriello et al 2003, Ercolini et al 2004). The object of the present work is to screen the ecology of the natural bacterial communities present in milk, curd and mozzarella cheese, in relation to two hay based diets. In particular, it has been assessed the feed microflora as factor influencing the quality of the mozzarella cheese In present trial Italian ryegrass hay and Italian ryegrass hay silage are tested, further studies on other typical Mediterranean forages are in progress.
MATERIAL AND METHODS - Forty milking Mediterranean buffalo cows divided in two groups were fed two different diets based on Italian ryegrass hay (diet G1) and Italian ryegrass hay silage (diet G2). The diets had the same protein (CP:172.5 g/kg DM) and energy content (0.92 Milk FU/kg dry matter). The composition of the administered diets is reported in Table 1.

Samples of milk, curd before stretching process and mozzarella cheese (salted and stored for 24h at 4°C) were collected at 30, 60 and 90 days from the beginning of the trial. The mozzarella cheese was made by raw whole milk of the morning mixed with that of the evening milking, inoculated with natural whey, obtained from the previous day mozzarella production. The natural whey used was the same for mozzarella cheese manufacture of both diets.

The samples were immediately frozen and stored at -80°C. Then were thawed, the mozzarella and curd samples were homogenized in a stomacher with 2% sodium citrate solution and decimal dilutions of milk, curd and mozzarella cheese homogenates were opportune plated on Petri dishes in order to count presumptive lactobacilli (MRS agar Oxoid, incubated 72h at 37°C, under anaerobic conditions) and presumptive lactococci (M17 agar Oxoid, incubated 24-48h at 30°C).

The data obtained were analyzed according to the t Student test.

For the genetic analysis, from each media and collection step, 20 colonies were randomly isolated and grown in MRS or M17 liquid medium, DNA extraction was performed by GenElute Bacterial Genomic DNA Kit (Sigma). The rDNA 16S region was amplified by PCR technique (Frothingham et al. 1991), the purified amplicons were sequenced using BigDye v1.1 and analyzed on ABI prism 310 (Applied Biosystems).

RESULTS AND CONCLUSIONS - The figure 2 shows the number of presumptive lactobacilli and presumptive lactococci for the two diets along the trial. In diet G2 the milk at 30, 60, 90 days showed significantly higher microbial counts in comparison to diet G1, apart from lactococci at 90 days that showed not significant differences.

The mozzarella cheese at 30, 60 and 90 days followed the same trend of the milk as the diet G2 had higher microflora counts in respect to diet G1. Opposite results were obtained from curd at 30, 60, 90 days, as the numbers of lactobacilli and lactococci for diet G1 were significantly higher with respect to diet G2, apart from lactococci at 30 days, that showed a significant higher level (P≤0.01) for diet G2 with respect to diet G1. In general for both diets, the curd showed, along the trial, a high diversity in shape and color of colonies with

| Table 1. Composition of the two diets administered in the trial, expressed as % DM. |
|---------------------------------|----------------|----------------|
| Diet G1                         | Diet G2        |
| Italian ryegrass hay 20.3%      | 4.1%           |
| Italian ryegrass hay silage     | -              | 41.1%          |
| straw 10.1%                     | 12.0%          |
| dehydrated alpha-alpha 20.3%    | 9.6%           |
| concentrate 44.2%               | 30.9%          |
| flaked soybean 5.1%             | 1.7%           |
| fat 0.5%                        | 0.5%           |
| CaCO₃ 0.1%                      | -              | 0.1%           |
Table 2. Number of microorganisms of milk, curd and mozzarella cheese for the two diets expressed as cfu/ml of milk or cfu/g of curd and cheese.

|                | Lactobacilli | Lactococci |
|----------------|--------------|------------|
|                | Diet G1      | Diet G2    | Diet G1 | Diet G2 |
| 30 days        |              |            |
| Milk           | 1.13x10^4 b  | 4.02x10^4 a| 3.07x10^4 B | 3.84x10^5 A |
| Curd           | 7.45x10^6 B  | 1.07x10^6 A| 9.15x10^7 A | 2.68x10^7 B |
| Cheese         | 8.50x10^3 b  | 1.17x10^4 a| 9.55x10^3 B | 2.61x10^4 A |
| 60 days        |              |            |
| Milk           | 1.97x10^4 B  | 4.40x10^4 A| 1.21x10^5 b | 1.27x10^6 a |
| Curd           | 1.88x10^6 a  | 1.30x10^6 b| 1.15x10^8 A | 4.55x10^6 B |
| Cheese         | 9.56x10^3 b  | 1.13x10^4 a| 7.97x10^3 b | 1.34x10^4 a |
| 90 days        |              |            |
| Milk           | 3.87x10^4 B  | 8.20x10^4 A| 2.30x10^5 n.s. | 2.00x10^5 n.s. |
| Curd           | 2.08x10^6 A  | 1.10x10^5 B| 2.30x10^8 A | 1.51x10^8 B |
| Cheese         | 3.16x10^3 B  | 1.31x10^4 A| 2.77x10^2 B | 7.73x10^3 A |

For the same product and same bacteria group, within rows and between diets: A,B indicate differences at P≤0.01; a,b indicate differences at P≤0.05.

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