Probiotic in rennet paste can affect lipase activity of rennet and lipolysis in ovine cheese

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ABSTRACT – Lambs were subjected to three different feeding regimes (mother suckling MS, artificial rearing AR, and artificial rearing with \(7\log_{10}\) cfu/ml \(Lactobacillus\ acidophilus\) supplementation to the milk substitute ARLb) and slaughtered at 20d and 40d of age for each feeding treatment. Lambs abomasa were processed to rennet paste and lipases activity was evaluated. Rennet paste was used for Pecorino cheese production. Free fatty acids (FFAs) and conjugated linoleic acids (CLAs) were detected in cheese at 60d of ripening. Lipase activity was found higher in ARLb than in MS and AR rennet from lambs slaughtered at an older age. A reduction of all FFAs was observed in all cheeses when passing from 20 d to 40d of slaughtering. CLAs were more abundant in ARLb cheeses at both 20 and 40d. Milk substitute with \(Lb.\ acidophilus\) improves enzymatic features of rennet, and health and nutritional characteristics of ovine cheese.

Key words: Lamb rennet paste, Probiotic, Lipolysis in cheese, Conjugated Linoleic Acids.

Introduction – Rennet pastes are artisanal products used in the manufacture of ovine and goat cheese. There is a renewed interest in promoting the use of lamb rennet paste to maintain the authenticity of traditional cheeses (Santillo et al., 2007). The enzymatic content of rennet pastes is heterogeneous and includes proteolytic, lipolytic, and milk clotting enzymes. Rennet paste contains pre-gastric esterases (PGEs) (of oral origin) and gastric lipase. Addis et al. (2005) reported that the variation in lipase activity of rennet paste could be attributed to slaughtering condition, i.e. fasting period before slaughtering, and to the balance of different lipases, i.e. PGE and gastric lipase which behave differently along with slaughtering age. Although lactic acid bacteria are weakly lipolytic, they possess esterase/lipases, which release low level of fatty acids during a long ripening period. CLAs are positional and geometric isomers of linoleic acid with conjugated double bonds: they have potential health or nutritional effects, first of all anticarcinogenic activity (Ha et al., 1990). The present aimed to assess the effects of lamb feeding regime (maternal milk, milk substitute and a milk substitute supplemented with \(Lb.\ acidophilus\)) and of lamb age at slaughtering (20d and 40d of age) on microbiological, coagulating and enzymatic features of rennet paste, and on its lipolytic activity in ovine cheese. Finally, FFAs and CLAs content have been evaluated in the analyzed cheese samples at 60d of ripening.

Material and methods – Twenty-four Comisana lambs were divided into six groups of four according to a 3x2 factorial design, with lambs being subjected to three different feeding regimes (mother suckling MS, artificial rearing AR, and artificial rearing with \(Lb.\ acidophilus\) supplementation ARLb) and slaughtered at 20d and 40d. MS lambs were kept with their dams throughout the trial. AR and ARLb lambs were fed on a commercial milk substitute. The weight of the lambs at the beginning of the trial was 4.06±0.14kg (mean ±SEM). Lyophilized culture of \(Lb.\ acidophilus\) was propagated in MRS tape...
broth under anaerobiosis at 37°C for 24h. Cells were cultured again under the same conditions, harvested by centrifugation, washed with sterile distilled water and resuspended daily in milk substitute at a concentration of 7log10 cfu/mL. The milk supplemented with Lb. acidophilus was incubated at 37°C for 1h and then given to ARLb lambs. The lipolytic activity of rennet paste was determined according to Addis et al. (2005). A lipolytic unit (L.U./g rennet paste) was defined as the amount of enzyme producing, on the sheep milk cream substrate, after 24h at 37°C, an amount of FFAs titrable with 1µeq of NaOH, until a pH of 8.5 was reached. Pecorino Foggiano cheese was made according to Santillo et al. (2007). Three cheeses were produced using each experimental rennet paste and analyzed after 60d of ripening. Volatile free fatty acids in cheese were extracted and isolated according to Ha and Lindsay (1990) and de Jong and Badings (1990), respectively. FFAs in cheese were grouped in three classes: short-chain FFA (SCFFA, C4:0-C10:0), medium-chain FFAs (MCFFA, C12:0-C16:0), and long-chain FFAs (LCFFA, C18:0-C18:3). CLA isomers were analyzed directly by HPLC according to Sehat et al. (1998). All the variables were tested for normal distribution using the Shapiro-Wilk test (Shapiro and Wilk, 1965). Data were processed by analysis of variance, using the GLM procedure of SAS (1999). The tested effects were the feeding regime, the age at slaughtering and their interaction. The Principal Component Analysis (PCA) was performed by SCAN for Windows (Minitab Inc. State College, PA, USA), in order to obtain a visual representation of CLA isomer distribution in samples cheese.

Results and conclusions – Addition of 7log10 cfu/mL Lb. acidophilus in milk substitute was carried out successfully so that a total recovery of viable cells was recorded in milk given to the lambs in the ARLb group. Lowest levels of mesophilic lactobacilli and lactococci (data not shown) in MS rennet paste could be ascribed to free access of the ewe reared lambs to the mother feeding zone so MS lambs becoming accustomed to solid feed more rapidly than artificially reared lambs.

Table 1. Effects of feeding regime and slaughter age of lambs on lipases activity in rennet paste.

| Age at slaughtering (d) | Lipases, L.U./g | Feeding regime | SEM | Effects |
|------------------------|----------------|----------------|-----|---------|
| 20                     | 210<sup>a</sup> | MS             | 2960<sup>b</sup> | 4400<sup>b</sup> | 642 *** | ns * |
| 40                     | 475<sup>a</sup> | AR             | 990<sup>a</sup> | 3240<sup>b</sup> | *** | ns |
|                        | 400<sup>b</sup> | ARLb           |                  |              |       |

<sup>1</sup>MS, mother suckling; AR, artificial rearing; ARLb, artificial rearing with Lb. acidophilus.

The lipases activities of rennet pastes (Table 1) on sheep milk cream substrate were influenced by feeding regime and the interaction of feeding regime and age at slaughtering. In ARLb and MS rennet lipases activities were not changed by slaughter age of lambs, but in ARLb rennet lipases activities were more than twenty times greater at 20d of age and about seven times higher at 40d of age than in MS rennet. Collins et al. (2003) reported that suckling stimulates the secretion of PGE at the base of the tongue, which is carried into the abomasa with the milk. In this study, rennet obtained from lambs reared with their mothers always displayed the lowest lipases activity. This could be because MS lambs had access to hay and concentrate given to their mothers so solid feed intake led to a depression in PGE synthesis. The high levels of lactococci found in ARLb rennet paste at 40d could be responsible for lipases activities remaining elevated in the abomasa from lambs supplemented with Lb. acidophilus. Indeed, both Lb. acidophilus and lactococci are able to release microbial lipases in rennet paste. The most abundant free fatty acids were: butyric acid, caproic acid, palmitic acid, oleic acid and linoleic acid, along with previous results in ovine cheese (Albenzio et al., 2001). In a previous note, Santillo et al. (2007) found that ARLb fresh curd cheese showed higher levels of lactobacilli and of lactococci than MS and AR ones. An effect of diet was found for levels of 9c,11t-18:2, and 9t,11t-18:2, which were the most abundant isomers.
of linoleic acid in cheeses. The highest values of both isomers were detected in ARLb cheese at 20d and 40 days. The PCA biplot relevant to the CLAs composition of cheese samples in function of feeding regime of lambs is shown in Figure 1. The score plot shows how cheeses from ARLb rennet paste lie in a well defined zone, being their CLA concentrations higher than the other cheese samples, as demonstrated by the loading plot of the variables. **Milk substitute supplementation with** *Lb. acidophilus* **improves enzymatic features of rennet and makes it possible to increase the slaughter age of lambs without detrimental effects on rennet characteristics. The production of CLA by *Lb. acidophilus* could be a possible way to enhance health properties of ovine cheese.**

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