ROLE OF INDIGENOUS PROTEOLYTIC ENZYMES AND OF MACROPHAGES AND NEUTROPHILS IN CHEESE-MAKING ABILITY OF SHEEP MILK

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Overview

Indigenous proteolytic enzymes are able to cleave caseins prior to cheese-making impairing milk coagulation properties.

Plasmin system is the main native proteolytic enzyme and play a major role in milk casein breakdown. Other indigenous proteolytic enzymes are Elastase, Cathepsin, and Collagenase.

These enzymes are associated to somatic cell types (Lymphocytes, Polymorphonuclear neutrophilic leucocytes, Macrophages).

Changes in enzyme activity could be predictive of milk quality in terms of milk coagulation properties

Aim of the study

The aim of the present study was to determine the role of milk indigenous proteolytic enzymes in sheep milk cheesemaking ability during lactation.

Given that leukocyte populations in milk affect the amount and type of indigenous proteolytic enzymes, changes in macrophage and neutrophil levels in milk were also investigated

Materials and Methods

- ✓ The experiment was conducted in an intensively managed flocks of Comisana ewes on bulk milk samples during lactation.

- ✓ Ewes were healthy at the beginning of the experiment and were monitored for health by veterinarian throughout the study.

- ✓ Milk analyses: pH, fat, total protein, lactose, SCC, nitrogen fractions, CN, renneting characteristics.

Determination of Enzymatic content in milk

- Plasmin, plasminogen activities, and plasminogen activators activity (Baldi et al., 1996);
- Cathepsin D activity (Perlmann and Lorand (1970; Smith and Turk, 1974);
- Elastase activity (Bieth, 1974).

Leukocyte Differential Count

Milk lymphocytes, macrophages, and PMNL were detected by flow cytometry.

Statistical analyses

Data were processed by ANOVA, using the GLM procedures of SAS Insitute (1999).

The variation due to stage of lactation was tested. Bulk milk SCC and isolated macrophage and PMNL counts were transformed into logarithms to normalize their frequency distributions before performing statistical analysis. Percentage composition of the casein and casein degradation products were tested for stage of lactation, time of incubation and their interaction.

When significant effects were found (at $P < 0.05$), the Student t-test was used to locate significant differences between means.

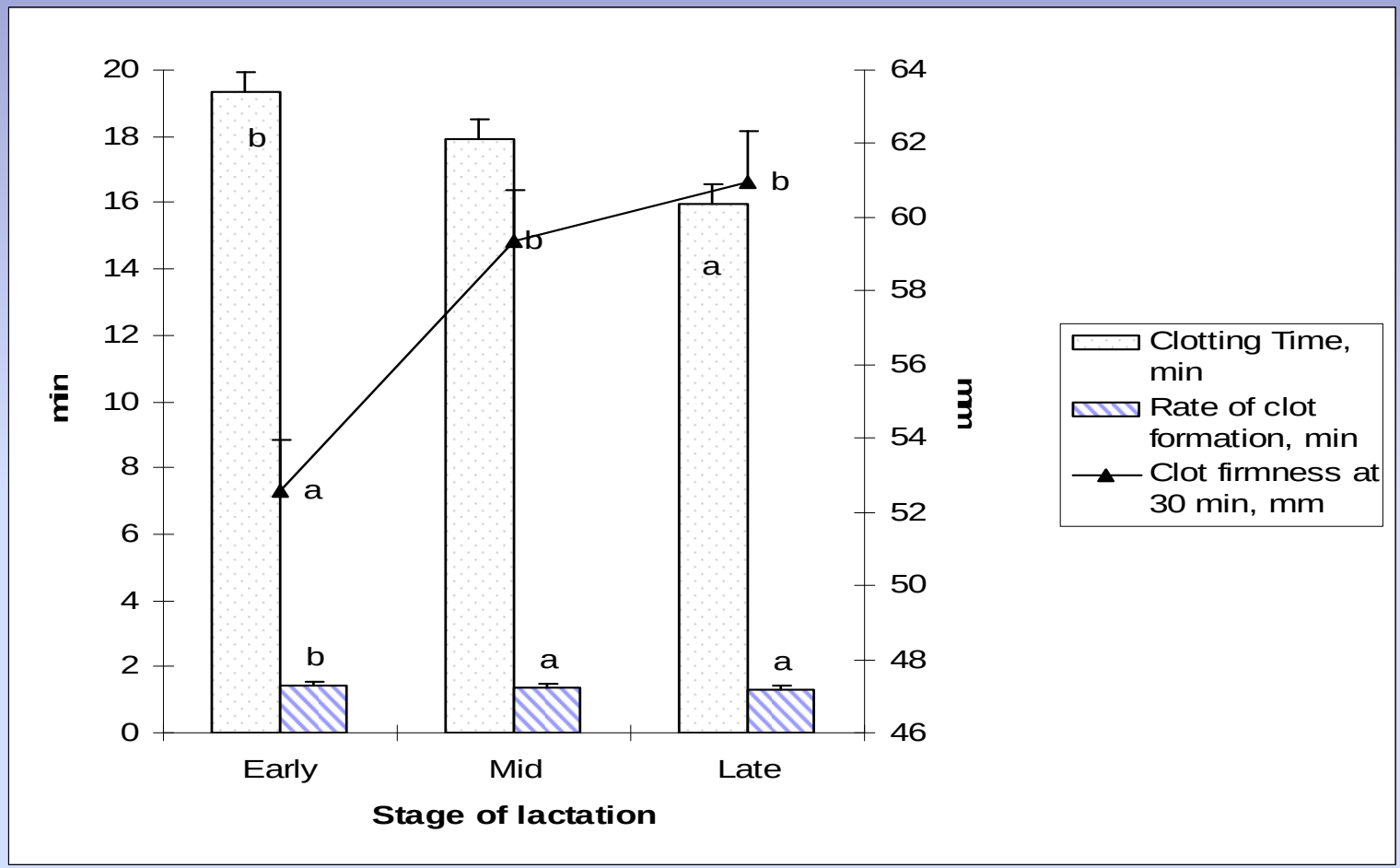
Percentage of differential cell populations in sheep milk during lactation

Item	Stage of Lactation			SEM	Effect
	Early	Mid	Late		Stage of lactation
Lymphocytes, %	43.02a	40.57a	26.15b	3.52	***
PMNL, %	49.39b	57.07b	70.58a	3.68	***
Macrophages, %	7.59b	2.36a	3.27a	0.74	***

Least squares means ± SEM of Plasmin, Plasminogen, Elastase, and cathepsin D in sheep milk during lactation

	Stage of Lactation			SEM	Effect
Item	Early	Mid	Late		Stage of lactation
Plasmin, mg/L	0.84	1.32	1.48	0.18	NS
Plasminogen, mg/L	1.34	1.24	1.87	0.18	NS
Elastase, mg/L	0.10a	0.19b	0.95c	0.09	**
Cathepsin D, mg/L	2.41b	3.24c	2.04a	0.06	***

Changes in milk coagulation properties during lactation



Correlation coefficients between casein, cathepsin D and milk coagulation properties.

	Clotting time	Rate of clot formation	Curd firmness
Casein	-0.54**	-0.60***	0.71***
Cathepsin D	0.46*	0.22^{NS}	-0.03^{NS}

CONCLUSIONS

Differential cell count showed that PMNL cells increased physiologically throughout lactation, being largely predominant in late lactation milk.

Changes in elastase levels in milk followed closely those found in PMNL suggesting that elastase concentration could be a reliable indicator of mammary gland involution in healthy udders.

- The positive correlation between clotting time and Cathepsin D levels suggests that casein hydrolysis carried out by this enzyme can impair the coagulating behaviour of sheep milk.

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