

# Special Issue

of the International Dairy Federation

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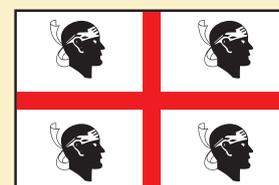


## Scientific Posters Presented at the IDF 5<sup>th</sup> International Symposium on the Challenge to Sheep and Goats Milk Sectors

18-20 April 2007, Alghero, Italy



ITALIA



Istituto Zootecnico e  
Caseario per la Sardegna

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## Special Issue of the International Dairy Federation 0801/Part 1

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# Special Issue of the International Dairy Federation

0801/ Part 1

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# The Challenge to Sheep and Goats Milk Sectors

## Foreword

This issue of the Bulletin contains the Scientific Posters presented at the IDF 5<sup>th</sup> International Symposium on the Challenge to Sheep and Goats Milk Sectors, which took place from 18 to 20 April 2007, in Alghero, Italy.

About 300 delegates, including a significant number of postgraduate students from 30 countries, attended the event. 24 main lectures (which are being published in the peer reviewed journal "Small Ruminant Research") were presented, and 163 posters exhibited.

The Symposium, held under the auspices of the IDF, was the fifth in a series, presenting latest findings in the research area related to the sheep and goats dairy sector. The overall objective of the Symposium was to provide comprehensive insight into the most recent knowledge including latest research findings on husbandry and milk production, technology, chemistry, physics, microbiology, nutrition etc, without losing sight of the significance of markets and appropriate policies. The core themes were related to the sheep and goat milk, processing and product, characteristics of the product and market and perspectives.

Several aspects were presented and discussed over a three-day programme. In the field of raw milk, genetic, analytical and quality aspects were approached, particularly to enhance the nutritional and beneficial effects for human health. New process treatments for this sector were presented while particular interest was given to functional products. Nutritional and health components together with the analytical and sensorial aspects were presented in the session dedicated to the characteristics of the products. Finally market aspects focused on the valorisation of the traditional and "artisanal" cheeses.

The high number of delegates that attended the Symposium demonstrate that the sheep and goat sector is one of the most dynamic and increasingly stimulates interest in the world.

The IDF is most grateful to the Istituto Zootecnico e Caseario per la Sardegna and the Italian National Committee of the IDF for organizing and hosting the event, and more particularly to Dr A. Pirisi and Dr G. Piredda, as well as to the members of the Programme and Organizing Committees and to all authors for their valuable contribution to the work of IDF.

Christian Robert

June 2008

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## Posters Session I. Raw Milk

### I-P001: Livestock System and Sheep Milk Quality in the PDO Sardinian Cheeses Production

M. Addis<sup>1</sup>, S. Gattu<sup>1</sup>, G. Riu<sup>1</sup>, A. Pirisi<sup>1</sup>, M. Fiori<sup>1</sup>, S. Spada<sup>1</sup>, G. Galistu<sup>2</sup> and G. Piredda<sup>1</sup>

#### Summary

In the last twenty years the sheep livestock system has evolved in different ways from farm to farm, without a common protocol aimed at protecting the characteristics of milk, in particular that destined to the PDO traditional cheese production.

This work studied how some factors such as geographical farm position, animal feeding and milk refrigeration can affect the characteristics of sheep milk produced in Sardinia and used mainly to produce PDO Pecorino Romano cheese.

Thirty-two farms heterogeneous in livestock system and facilities, all located in four different Sardinian areas and all of them lending their milk to four different dairies, were monitored from February to July, and milk samples were collected monthly. Chemical analysis were carried out on milk samples.

Milk collection area strongly affects milk features, particularly pH, DM, proteins, fatty acids composition and lipolysis.

#### 1. Introduction

In the last years the value of PDO cheeses has greatly increased, not only in economic terms but also as a traditional cultural value. To assure all that, PDO cheese making process requires milk with chemical, nutritional and sensorial traits linked to the milk origin (animal specie, breed, geographical area, feedstuffs and feeding system), and with a good cheese making aptitude. This work studied how some factors such as geographical farm position, animal feeding, milk refrigeration etc, can affect the characteristics of sheep milk produced in Sardinia and used mainly to produce PDO Pecorino Romano cheese.

#### 2. Material and methods

Thirty-two farms heterogeneous in livestock system and facilities, all located in four different Sardinian areas (Table 1) and all of them lending their milk to four different dairies (named A, B, C and D), were monitored from February to July, and milk samples were collected monthly.

**Table 1:** Features of monitored farms (means  $\pm$  sd)

	A	B	C	D
Geographical position	Nord-East	Nord-West	Sud-West	Centre-East
Altimetry (m)	606 $\pm$ 12	406 $\pm$ 245	68 $\pm$ 10	77,5 $\pm$ 23,1
Total productive area (Ha)	69,8 $\pm$ 30,9	44,9 $\pm$ 47,8	82,9 $\pm$ 42	45,9 $\pm$ 23,7
Heads/Ha	6,6 $\pm$ 2	7,9 $\pm$ 4,5	8,2 $\pm$ 4,1	5,0 $\pm$ 1,9
Irrigated area (Ha)	0 $\pm$ 0	0 $\pm$ 0	4,9 $\pm$ 7,5	8,3 $\pm$ 11,7
Grain production area (Ha)	1,0 $\pm$ 2,8	3,8 $\pm$ 6,7	14,1 $\pm$ 13,5	4,0 $\pm$ 7,0
Forage crop area (Ha)	18,4 $\pm$ 12	24,9 $\pm$ 17,7	39,9 $\pm$ 34,9	16,5 $\pm$ 7,6
Milk cooling before delivery	62,5%	25%	100%	50%

Chemical analysis, including, dry matter (G.U.C.E.; 1992), protein, (FIL-IDF,1993: 20 B, part I), fat (Rose-Gottlieb method, A.O.A.C. 989.05), pH (potentiometric method), fatty acids

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<sup>2</sup> Consorzio per la Tutela del formaggio Pecorino Romano

composition (Murphy et al., 1990; Chin et al., 1992), lipolysis (De Jong et al., 1990) were carried out on milk samples. Statistical treatment of the data was performed using the Minitab statistical package release 12 (Minitab Inc., USA). The results of the milk chemical composition were examined using a bifactorial ANOVA model with dairy type and period as fixed effects.

### 3. Results and discussion

Milk collection area strongly affects milk features, particularly its chemical composition (pH, DM and proteins,  $P < 0.01$ ). The lowest pH value of milk from farms B and D (fig. 1), which confer the milk daily, was probably due to the fact that, in most of these farms, usually, the farmers do not refrigerate the milk before its delivery, causing a high bacterial acidification. The higher level of protein content in milk from farms A (fig. 2), respect to the other milks, could be due to the higher animal supplementation level adopted in most of the farms of this area. The altitude and the geographical position of this area caused, in fact, a prolonged vegetative stasis of the grazing lands.

As showed in figures 3 and 4, milk from displaced farms (farms C) shows higher levels of free fatty acids (short chain fatty acids and long chain fatty acids), because of very long refrigeration times were adopted before milk delivery to dairy (24, 36 hours). Particularly this can negatively affect cheese characteristics.

A strong interaction exists between livestock system and milk fatty acid composition. Feeding supplementation in animal diet involves a deficit in the milk synthesis of short chain fatty acids (milk from farms A, data not shown). Grazing sheep milk (farms D) is characterised by highest levels of fatty acids with high nutritional value such as polyunsaturated fatty acids (PUFA) and conjugated linoleic acid (CLA), as already observed by other authors (Cabiddu et al., 2005). Similar differences in milk fatty acid composition were mirrored even in corresponding produced cheeses (data not shown). The monitored parameters in milk evolved during the season like to that reported by several authors.

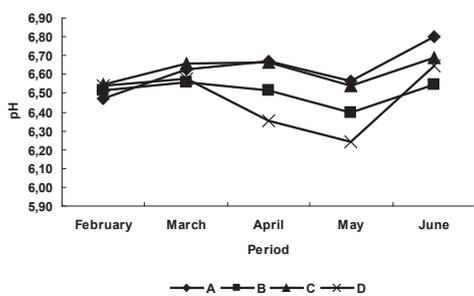


Figure 1. pH in milk samples

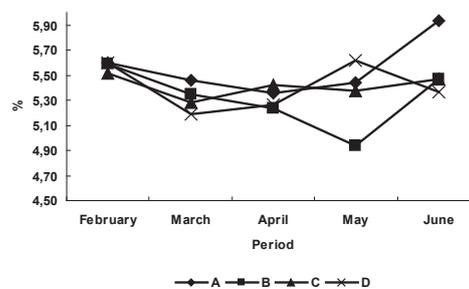


Figure 2. protein content in milk samples

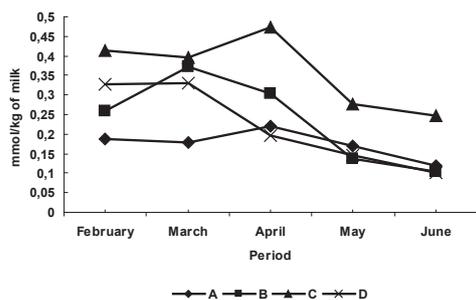


Figure 3. short chain fatty acids (C4:0-C10:0) in milk samples

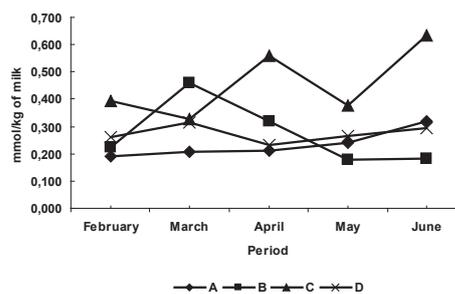
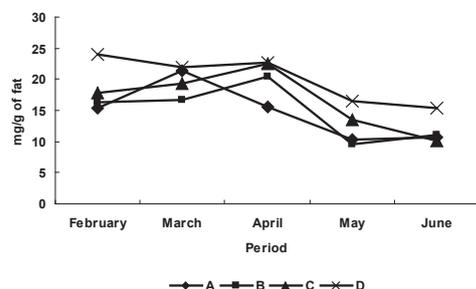
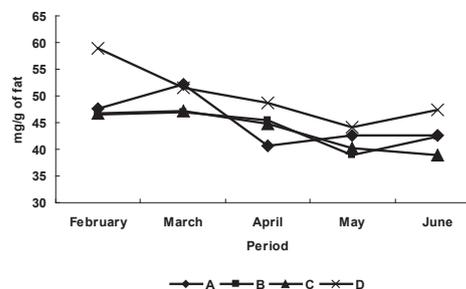


Figure 4. long chain free fatty acids in milk samples



**Figure 5.** CLA in milk samples



**Figure 6.** PUFA in milk samples

## 4. Conclusion

The milk collection area strongly affected milk characteristics. The observed differences are due particularly to the relationships network among farms, their local areas and the collecting dairies. The milk lipid quality was highly linked to the animal feeding system. In particular has been observed, on the whole, that grazing system can contribute to improve nutritional milk quality.

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## I-P002: Determination of Fat, Protein, Casein, Total Solids and Fatty Acids in Ovine Milk by Near-infrared Reflectance Spectroscopy

E. Albanell<sup>1</sup>, Y. Moussaoui<sup>1</sup>, G. Caja<sup>1</sup>, R. Casals<sup>1</sup>, AAK Salama<sup>1</sup>, X. Such<sup>1</sup>

### Summary

A NIRS Foss 5000 (scanning range 1,100 to 2,500 nm) was used for predicting composition in ovine milk. Calibration equations were developed using 122 crude milk samples from individual Manchega and Lacaune dairy ewes at different stages of lactation. Prediction equations for milk components showed satisfactory  $R^2$  values: fat (0.98), crude protein (0.99), true protein (0.99), casein (0.96) and total solids (0.95). Nevertheless the results were moderate for milk fatty acids: caproic (0.82), caprylic (0.84), capric (0.75), myristic (0.68), palmitic (0.74) palmitoleic (0.78) and oleic (0.80).

### 1. Introduction

In Europe and the Mediterranean region, large numbers of ewes are milked to produce milk for the manufacture of cheese and other dairy products. The determination of the principal milk constituents is a key issue for the different products in the dairy industry.

Near-infrared spectroscopy (NIRS) is widely used, especially by the food industry, because it presents several advantages such as rapidity, precision, no need of sample preparation, and non-destructive aspects. The development of these automated infrared instruments has made possible the rapid analysis of milk components and the routine analysis of samples in the dairy herd improvement schemes and the dairy industry. Nevertheless, few applications of NIRS techniques for the analysis of ovine milk components have been reported (Fernandez et al. 1995; Albanell et al. 1999).

The aim of this study was to investigate the potential of NIR spectroscopy for measurement of the contents of fat, protein, casein, total solids and fatty acids in unhomogenized ovine milk.

### 2. Material and methods

#### **Sample Collection and Preparation**

Milk samples were taken from individual ewes of Manchega and Lacaune breeds from the herd of the Universitat Autònoma de Barcelona. A total of 122 samples were collected at different stages of lactation, and preserved with Bronopol (BroadSpectrum Micro-tabs II, D & F Control Systems Inc., USA) as a conserving product, stored at 4°C, and then analyzed optically and chemically the day after collection.

#### **Chemical Analysis**

Milk protein and casein contents were analyzed using the Kjeldahl method as a reference (N x 6.38). The Gerber method was used as the reference for milk fat content, and total solids were determined by oven drying. All these methods adhere to the International Dairy Federation Standard and all measurements were made in duplicate.

The fatty acid (FA) profile was determined in 61 samples. For this analysis additional milk sub-samples were taken and immediately cooled, the fat fraction was separated by centrifugation and stored at -80°C. Milk FA was extracted and methylated using the procedure of Palmquist and Jenkins (2003). The methyl esters from milk samples were determined using a gas chromatograph (HP 6890; Agilent Technologies Inc., Palo Alto, CA) equipped with flame ionization detector and capillary column (CP-Sil-88; Varian Inc., Palo Alto, CA). Individual FA

<sup>1</sup> Grup de Recerca en Remugants. Facultat de Veterinària. Universitat Autònoma de Barcelona. 08193 Bellaterra, Barcelona, Spain.

were identified by checking the retention times using those of pure standards (Sigma-Aldrich Química, Spain) and expressed as percentages of the total FA detected as FA methyl esters.

### **NIRS Analysis**

NIRS data were recorded from 1,100 to 2,500 nm using a Foss NIRSystems 5000 scanning monochromator. Calibration equations were developed using 122 milk samples stored at -4 °C until analysis, heated to 40 °C and scanned in duplicate using a gold reflectant cell (path length, 0.5 mm). Reflectance (R) data were collected every 2 nm, transformed to  $\log 1/R$  and, subsequently, first and second derivatives were calculated. Mathematical treatment of the spectral data was performed using WinISI III (v. 1.6) software and calibrations were developed by the modified partial least squares regression technique. Cross validation was undertaken using the standard methodology in the NIRS software program.

## **3. Results and discussion**

Table 1 shows the characteristics of the sample sets used in the study. Chemical analysis indicated a wide variation of composition between milk samples. The range of variation of each component was a consequence of different breed and lactation stage.

Table 2 summarizes the results obtained in the development of calibration equations for milk composition and for the main fatty acids: caproic (C6:0), caprylic (C8:0), capric (C10:0), myristic (C14:0), palmitic (C16:0), palmitoleic (C16:1) and oleic (C18:1) acid.

Prediction equations for milk fat, protein (crude and true), casein and total solids content were satisfactory in all cases. The coefficients of determination ( $R^2$ ) were 0.98; 0.99; 0.99; 0.96 and 0.95, respectively, and the coefficients of determination for cross validation (1-VR) ranged from 0.85 to 0.99. These results are similar or greater than those obtained by Albanell et al. (1999) who used an instrument supplied with 19 filters. The NIRS fatty acid equations had moderate  $R^2$  and ranged from 0.68 for C14:0 to 0.84 for C8:0. The 1-VR values ranged from 0.42 for C10:0 to 0.64 for C18:1. Soyeurt et al. (2006) estimated fatty acid concentrations in cow milk using Mid-Infrared spectrometry and obtained similar results ( $R^2$  from 0.41 to 0.89; 1-VR from 0.37 to 0.67). To estimate the efficiency of the calibration, the SD/SECv was calculated and the best results were obtained in C6:0, C8:0, C16:1 and C18:1 calibrations and the lowest value was obtained in C14:0. In contrast with our results, Soyeurt et al. obtained the highest value for C14:0 (1.71) and the lowest value for C16:1 (1.24).

**Table 1:** Composition of ovine milk samples used for NIRS measurements

Parameter, % <sup>a</sup>	Range	Mean	SD
Fat	2.90-10.50	6.792	1.483
Crude Protein	4.19-16.84	6.004	1.920
True Protein	3.85-14.21	5.559	1.628
Casein	1.86-10.39	4.168	0.845
Total Solids	11.78-23.74	17.507	1.683
C6:0	1.31-2.12	1.615	0.180
C8:0	1.69-2.93	2.329	0.247
C10:0	5.09-10.25	7.833	0.917
C14:0	7.96-12.77	10.210	0.931
C16:0	25.56-34.59	29.219	1.905
C16:1	0.61-1.33	0.8854	0.148
C18:1	19.07-25.77	22.332	1.706

<sup>a</sup>Fatty acids are expressed as percentage of the total fatty acid

**Table 2:** Calibration and cross validation statistics for ovine milk samples

Parameter	Math <sup>a</sup> treatment	Scatter <sup>b</sup> correction	R <sup>2</sup>	1-VR	SEC	SECV	SD/SEC	SD/SECV
Fat	1,4,4,1	SNV & DT	0.977	0.952	0.211	0.297	7.0	5.0
Crude Protein	1,4,4,1	SNV & DT	0.994	0.991	0.087	0.106	22.1	18.1
True Protein	2,4,4,1	SNV & DT	0.994	0.991	0.107	0.129	15.2	12.6
Casein	2,4,4,1	DT	0.957	0.850	0.120	0.225	7.0	3.8
Total Solids	1,4,4,1	SNV	0.953	0.925	0.350	0.423	4.8	4.0
C6:0	2,4,4,1	MSC	0.816	0.563	0.071	0.108	2.5	1.7
C8:0	2,4,4,1	MSC	0.837	0.516	0.081	0.144	3.0	1.7
C10:0	2,4,4,1	MSC	0.750	0.424	0.395	0.660	2.3	1.6
C14:0	2,4,4,1	None	0.675	0.465	0.628	0.724	1.5	1.3
C16:0	2,4,4,1	None	0.735	0.516	0.954	1.273	2.0	1.5
C16:1	2,4,4,1	SNV & DT	0.784	0.510	0.059	0.085	2.5	1.7
C18:1	2,4,4,1	MSC	0.798	0.637	0.750	0.995	2.3	1.7

<sup>a</sup>Designations: derivate order, gap, first smoothing, and second smoothing. <sup>b</sup>Standard Normal Variance, Detrend and Multiplicative Scattering Correction transformations. R<sup>2</sup> = coefficient of determination for calibration. 1-VR = coefficient of determination for cross validation. SEC = standard error of calibration. SECV = standard error of cross validation. SD = standard deviation of reference data.

#### 4. Conclusion

In conclusion, this study showed that NIRS is a useful technique for the rapid prediction of the main milk components and could have the potential to predict fatty acids in ovine milk.

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## I-P003: Crystallization Behavior of Anhydrous Goat Milkfat Using Coupled Time-resolved Synchrotron X-ray Diffraction and DSC

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### Summary

The aim of this study is to characterize the physical properties of anhydrous goat milkfat (AGMF). Crystallization and melting properties of triacylglycerols (TG) in AGMF are investigated by X-ray diffraction as a function of temperature (XRDT) coupled with differential scanning calorimetry (DSC), using synchrotron radiation and MICROCALIX. The polymorphic behavior of AGMF was monitored by varying the cooling rates between 5 and 0.1°C/min from 45 to -20°C with their subsequent melting at 1°C/min. Quenching of AGMF at -20°C was also examined. At intermediate cooling rates, TG's AGMF crystallize, from about 18°C, in 2 different lamellar structures with triple-chain length (3L) stacking characterized by lines at 72, 36, 24 and 18 Å and a double-chain length (2L) stacking of 48 Å, which are correlated to two overlapped exothermic peaks recorded by DSC. Subsequent heating at 1°C/min, shows numerous structural rearrangements before final melting at about 38°C. Three unstable crystalline varieties are observed after quenching with a complex XRDT pattern evolution. This complex thermal behavior of AGMF might have consequences on textural, rheological and organoleptic properties.

### 1. Introduction

Over the past 20 years, a new and increased interest for goat's milk and its dairy products has occurred everywhere in the world. From an industrial point of view, goat's milk is directed mainly to cheese manufacture, and in a very small proportion to other dairy products such as yogurt, butter and milk powder. Goat's milkfat (GMF) plays an important role in dairy products, especially by contributing the typical and unique flavours, tastes and aromas [1]. Indeed, milkfat crystallization is important for technological applications of high-fat content products [2]. The diversity of TG and their polymorphism are responsible for the complex crystallisation and melting properties of GMF. The aim of this study is to examine the crystalline structures formed by TG of AGMF and their polymorphism, during cooling at slow [3], intermediate and fast cooling rates [4] as well as during quenching.

### 2. Material and methods

**Samples.** Goat milk was obtained from a goat herd (Alpine race) belonging to the Arid Region Institute (ARI, Medenine, Tunisia). Anhydrous Goat Milkfat (AGMF) was extracted from cream using the following procedure: first, after manual churning of cream (at T = 12-15°C), 10 g of butter are melted to 60°C and centrifuged for 2 min at 3000g. The upper organic phase AGMF was separated and filtered at 50°C in presence of sulfate anhydrous sodium (Na<sub>2</sub>SO<sub>4</sub>) on a filter of glass wool.

**DSC measurements.** Thermal analysis of AGMF were conducted with a DSC-7 Perkin Elmer (St Quentin en Yvelines, France) and running under Pyris Software (version 3.52). AGMF samples were loaded in aluminium pans of 40µl that were hermetically sealed. AGMF samples were heated at 70°C during 5 min in order to melt all crystals and nuclei. Crystallization curves were recorded from 60 to -40°C at different cooling rates: 0.1, 0.3, 1, 3 and 5°C/min. Then, following cooling, all the melting curves were recorded from -40 to 60°C at 1°C/min.

**XRDT/DSC measurements.** Experiments were performed using a technique that allows

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simultaneous synchrotron radiation X-ray diffraction as a function of temperature (XRDT) and high-sensitivity differential scanning calorimetry (DSC): Microcalix, to be carried out in the same apparatus [5, 6]. The experiments were conducted on D<sub>22</sub> bench of the synchrotron beam at LURE (Laboratoire pour l'Utilisation du Rayonnement Electromagnétique, Orsay, France), using setup described previously [4]. Briefly, two linear detectors allow the recording of simultaneous small- ( $q = 0-0.45 \text{ \AA}^{-1}$ ) and wide- ( $q = 1.1-2.1 \text{ \AA}^{-1}$ ) angle XRD patterns. Both XRDT data and DSC signals are simultaneously collected from the same sample with a single computer in order to avoid any time or temperature shift in the data collection. The crystallization behavior of AGMF was conducted on cooling at 5 and 1°C/min from 45 to -20°C at  $dT/dt = 1, 5^\circ\text{C/min}$  using capillary-contained samples. The melting behavior was monitored by heating of the samples at  $dT/dt = 1^\circ\text{C/min}$ . Quenching of AGMF at -20°C was also examined. Each XRD pattern recorded as a function of temperature simultaneously at small and wide angles was analyzed using IGOR PRO 4.0 software (Wavemetrics, USA).

### 3. Results and discussion

The structural and thermal characteristics observed for AGMF by XRDT and DSC, after cooling at different rates and during the subsequent heating at 1 °C/min, are summarized in Table 1.

Indeed, slow cooling of AGMF favors the formation of stable species whereas fast cooling and quenching induce the crystallization under unstable varieties.

**Table 1:** Summary of the structural and thermal characteristics observed for anhydrous goat milk fat by XRDT and DSC, after cooling at different rates indicated and during melting at 1 °C/min.

Cooling rate	0.1 °C/min	1 °C/min	5 °C/min	Quenching
<b>Thermal events</b>				
*Number of crystallization exotherms	3 (or 4+1 exotherm)	2	2	–
*Initial temperature of crystallization (°C)	26	18	12	–
*Final melting temperature (°C)	40	37	37	38
<b>Varieties observed on cooling</b>				
*Chain packing	$\alpha$ then $\beta'$ $\beta$ (trace)	$\alpha \leftrightarrow \text{sub } \alpha$	$\alpha \leftrightarrow \text{sub } \alpha$	sub $\alpha$
*Molecule stacking	$2L_1$ (41.5 Å)  $3L_1$ (72, 35.3 Å) $3L_2$ (64.7, 32.3 Å) $2L_2$ (38.2 Å)	$3L$ (72.4, 36.4, 24.2, 18 Å) $2L$ (48 Å)	$3L_{001}$ (71.5, 36.4, 24 Å) $2L1$ (46.7, 24 Å) $2L2$ (38 Å)	$2L_1$ (48.3, 24 Å) $2L_2$ (44 Å) $2L_3$ (37 Å)
<b>Thermal behavior on heating at 1 °C/min</b>				
*Chain packing	$\beta'$ and traces of $\beta$	sub $\alpha \leftrightarrow \alpha$ or $\alpha \leftrightarrow \beta'$	sub $\alpha \leftrightarrow \alpha \rightarrow \beta'$	sub $\alpha \leftrightarrow \alpha \rightarrow \beta'$
*Longitudinal transition	No polymorphic transition	polymorphic transition	polymorphic transition	polymorphic transition
*DSC melting events	4 Endotherms (LMF, MMF, 1 or 2 HMF)	4 Endotherms (LMF, MMF, HMF) and 1 exotherm	4 Endotherms (LMF, MMF, HMF) and 1 exotherm	5 Endotherms (LMF, MMF, HMF) +2 exotherms

### 4. Conclusion

To the author's knowledge, this is the first dynamic study performed as a function of time and temperature, to characterize the crystallization properties of GMF, at a molecular level. The use of the synchrotron radiation allowed studying the polymorphism and phase transitions displayed by complex TG mixtures, as a function of temperature and comparing these data with DSC recording. These tools revealed the existence of 2 different forms ( $3L \alpha$  and  $\beta'$ ) and numerous  $2L$  ( $\alpha$  and  $\beta'$ ) in AGMF depending on crystallization conditions. Although the dominant stable form

is  $\beta'$ , four different polymorphic subcell types: sub  $\alpha$ ,  $\alpha$ ,  $\beta'$  and  $\beta$  were observed. Such information increases the knowledge of the structure's complex fat in dairy products, that is of tremendous importance to better understand, control and improve the physical properties (rheological, technological, functional, nutritional and sensory) of AGMF's products.

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## I-P004: Separation, Identification and Quantification of Casein Fractions. Differences Between Portuguese Sheep and Goat Breeds

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### Summary

The purpose of this preliminary study was to separate, identify and quantify the relative proportions (%) of casein fractions ( $\alpha$ ,  $\beta$  and  $\kappa$ ) in the milk of the most representative Portuguese dairy sheep and goat breeds.

The study was based on an analysis of a total of 129 raw bulk milk samples (84 from ewe and 45 from goat) collected all over the country during three years from eight sheep breeds and four goat breeds. Protein separation and quantification were performed by a RP-HPLC method.

Casein fractions were characterized by comparing elution order and retention times with a reference standard. The average concentrations of the casein fractions and the range of variation of each fraction for each breed (%) for each of the two species were determined. The chromatographic profiles for each sheep and goat breed were also recorded. No significant differences were observed among the several sheep and goat breeds but there was a significant difference between sheep and goat casein fractions  $\alpha$  and  $\beta$  average results (%).

### 1. Introduction

Casein, a phospho-protein, is the main milk protein in all species. Casein consists of individual components ( $\alpha_{s1}$ -,  $\alpha_{s2}$ -,  $\beta$ - and  $\kappa$ -casein) with different properties, due to the variations in their amino acid composition. Total casein content, as well as the composition of fractions are important factors, which can influence micelle formation and stability and, consequently, the processing properties of the milk, and consequently its behaviour in cheese making. Over the last few years, several electrophoretic and chromatographic techniques have been developed and used for separation and quantification of casein. The aim of this work was to conduct a preliminary characterisation of the casein fractions of the milk of the main national dairy sheep and goat breeds.

### 2. Material and methods

Samples of raw sheep (84) and goat (45) milk of the most predominant Portuguese breeds were collected in three different periods of lactation and from all over the country, during three years (1998 to 2001). Each sample collected comprised 750 mL of raw, fresh, mixed herd milk. Four breeds of goat [Serrana (CS), Charnequeira (CC), Serpentina (CSP) and Algarvia (CA)] and eight breeds of sheep [Saloia (OS), Merino da Beira Baixa (MBB), Mondegueira (OM), Bordaleira Serra da Estrela (BSE), Churra Badana (CB), Churra da Terra Quente (CTQ), Campaniça (OC) and Merino, Branco e Preto (MBP)] were chosen as the most representative for milk production. Casein samples of skim milk were prepared by acid precipitation at pH 4.6 and centrifuged. The precipitate was washed and precipitated two further times. Finally, the casein was lyophilised. The HPLC equipment used was a Merck- Hitachi D-7000 interface and pump L-7100. The casein separation was performed by reverse-phase HPLC, using a C<sub>18</sub> Nucleosil column (300 Å, 250 x 4,6 mm i.d., 5 µm particle size). Gradient elution was effected using a mixture of solvent A [0.1% (v/v) trifluoroacetic acid in water] and solvent B [0.1% trifluoroacetic acid in acetonitrile-water (95:5, v/v)]. The elution was performed for 37 min.

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### 3. Results and discussion

Whilst the chromatographic profiles of goat and sheep milks share certain similarities (figures 1 and 2), they also reveal some differences, namely in the % of the different casein fractions (tables 1 and 2). For  $\kappa$ -CN in sheep milk there were no significant differences between breeds ( $p>0.05$ ) with the exception of breed CB ( $p=0.050$ ). A similar pattern was revealed for  $\alpha$ -CN ( $p>0.05$ ). For  $\beta$ -CN, the OC breed was significantly different ( $p<0.05$ ) and the value for the CTQ breed was also significant ( $p=0.054$ ). As regards goat milk, no statistically significant differences were revealed between any of the fractions ( $p>0.05$ ).

**Table 1:** Quantification of sheep milk casein fractions (relative %)

Breed	n	$\kappa$ -CN			$\alpha$ -CN			$\beta$ -CN		
		Mean	SD	Min/Max	Mean	SD	Min/Max	Mean	SD	Min/Max
OC	6	16.3	2.1	13.0/18.6	53.1	2.0	50.7/56.3	30.5	1.1	28.4/31.5
CB	12	16.5	2.4	11.9/19.8	51.0	3.8	45.6/58.6	32.5	3.5	27.7/39.2
CTQ	12	15.3	1.7	11.9/17.6	47.1	2.7	42.5/52.6	37.6	1.8	34.1/40.7
MBB	12	15.3	2.1	12.5/20.0	50.3	3.1	45.8/55.0	34.4	3.5	30.3/40.6
MBP	12	16.0	1.9	12.9/19.2	47.9	3.3	42.5/53.8	36.1	3.1	30.0/39.5
OM	12	16.2	2.0	13.6/19.7	46.8	3.4	40.9/54.5	37.0	2.2	31.8/39.6
OS	6	16.1	1.7	14.5/19.6	46.7	2.2	43.6/49.5	37.2	1.7	35.2/39.8
BSE	12	17.1	2.0	13.6/19.8	48.6	2.9	44.4/55.7	34.3	2.3	30.7/38.7

**Table 2:** Quantification of goat milk casein fractions (relative %)

Breed	n	$\kappa$ -CN			$\alpha$ -CN			$\beta$ -CN		
		Mean	SD	Min/Max	Mean	SD	Min/Max	Mean	SD	Min/Max
CC	12	17.9	2.8	12.2/21.2	32.6	3.2	28.8/38.4	49.4	3.1	44.6/55.1
CSP	9	16.9	2.4	12.6/19.7	32.5	2.5	27.0/35.4	50.6	4.1	45.0/57.5
CA	12	16.0	2.5	10.6/19.6	31.5	3.0	27.1/37.1	52.4	2.8	48.0/57.5
CS	12	18.2	1.8	12.9/19.7	28.9	1.8	26.5/33.6	52.9	2.1	47.8/57.1

### 4. Conclusion

The mean values obtained for the different casein components in Portuguese sheep and goat milk are consistent with those previously reported. Notwithstanding, we found some differences among the range of variation for the different fractions amongst the different breeds. For sheep milk we found some significant differences in the relative means of the casein fractions among the different breeds. For goat milk we did not observe statistically significant differences among the breeds. Comparing the means of the casein fractions of the different breeds in both, ovine and caprine milk, we did not uncover significant differences for the  $\kappa$ -CN fraction, but we did find significant differences in  $\alpha$ -CN and  $\beta$ -CN, for both species.

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## I-P006: Effect of Rumen-protected Choline and Vitamin E Supplementation on Milk Production Responses of Dairy Goats

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### Summary

We investigated the effects of rumen protected choline (RPC) administration on milk production during the periparturient period in dairy goats. Forty-eight Saanen multiparous goats were selected and assigned to one of the 4 experimental groups: CTR, control group; RPC, supplementation of 4 g/day choline chloride in rumen-protected form, VITE, supplementation of 200 IU/day  $\alpha$ -tocopherol in a rumen-protected form, RPCE supplementation of 4 g/day RPC and 200 IU/day  $\alpha$ -tocopherol. Supplements were administered individually starting 30 days before expected kidding and continuing for 35 days after parturition. During the first 42 days of lactation, milk yield was 9% and 7.9% higher ( $P < 0.05$ ) in the RPC and RPCE groups, respectively, compared to the CTR group. Milk yield in the VITE group was unaffected by  $\alpha$ -tocopherol supplementation. The RPCE group supplementation was associated with greater ( $P < 0.05$ ) milk fat content than in CRT group. Fat-corrected milk (FCM) yield was greater ( $P < 0.05$ ) in the RPC and RPCE groups than the CTR group. Milk protein content did not differ between groups. Compared to CTR, fat and protein yields were higher in the RPC and RPCE groups. Furthermore RPC administration increased milk free choline content by 20 and 30%, respectively in RPC and RPCE groups compared to both CTR and VITE ones. In conclusion results of this experiment indicate that RPC supplementation increases milk yield and fat content in transition dairy goats.

### 1. Introduction

In dairy ruminants, the dietary availability of choline is low, but the output of methylated compounds in milk is high, and precursors from the tetrahydrofolate pathway are often limiting, especially at the onset of lactation (Pinotti et al., 2002; Girard and Matte, 2004). Results in transition and early lactating dairy cows suggest that greater choline availability can improve not only milk production, lipid and methyl group metabolism, but also in lesser extent the vitamin E status (Baldi and Pinotti, 2006). However data on choline supplementation in transition dairy goats are lacking. Thus the aim of this study was to investigate the effect of rumen-protected choline and vitamin E administration on milk production during the periparturient period of dairy goats.

### 2. Material and methods

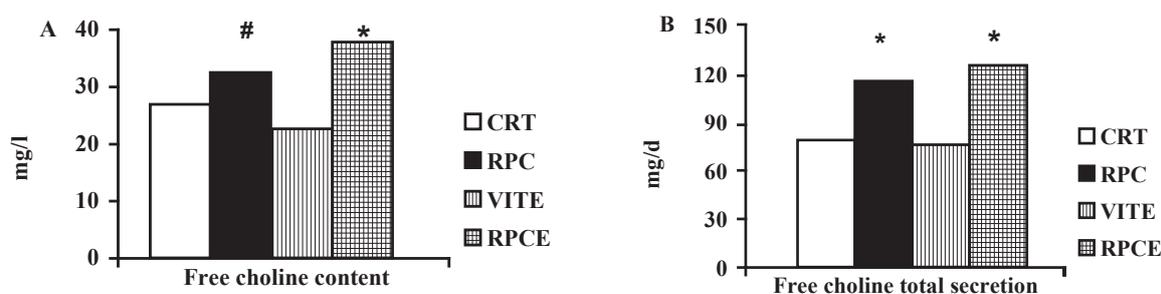
Forty-eight Saanen multiparous goats were selected and assigned to one of the 4 experimental groups: CTR, control group; RPC, supplementation of 4 g/day choline chloride in rumen-protected form, VITE, supplementation of 200 IU/day  $\alpha$ -tocopherol in a rumen-protected form, RPCE supplementation of 4 g/day RPC and 200 IU/day  $\alpha$ -tocopherol. Supplements were administered individually before the morning feed to ensure complete consumption, starting 30 days before expected kidding and continuing for 35 days after parturition. During this period the goats were fed with a basal diet formulated to provide ME 2.00 Mcal/kg DM, 2.40 Mcal/kg DM, CP 11.50% kg DM, 14.30% kg DM, for pre-kidding and lactation phase, respectively. Lactation diet contained 1.54% methionine of metabolisable protein. Through the first 30 days of lactation milk yield and composition were measured weekly. On sampling days morning and evening milk samples from each animal were collected and composited in proportion to milk yield. Milk samples of each animal were treated with preservative (sodium azide) and stored at 5°C pending analysis for milk fat, milk protein (Milkoscan, Foss Technology, Denmark), and SCC

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(Fossmatic Somatic Cell Counter, Foss Technology, Denmark). On day 21 of lactation (middle of lactation trial) two milk samples from each animal were obtained. One was treated with sodium azide and stored at 5°C pending analysis as reported. The other was analyzed for free choline by the method of Takayama et al. (1977) with the phospholipase-D step skipped, as described in Pinotti et. (2003). Total free choline daily secretion was obtained multiply its concentration by milk yield recorded on day 21 of lactation for each goat. Milk yield and milk composition measurements were analysed using the PROC MIXED procedure of SAS (1999). The statistical model included the fixed effects of treatment, sampling time (day relative to kidding) and their interactions, random effect of cows nested within treatment, and residual error. Differences were considered significance for  $P < 0.05$ , and indicating a tendency for  $P < 0.15$ .

### 3. Results and discussion

During the first 42 days of lactation, milk yield was 9% and 7.9% higher in the RPC and RPCE groups, respectively, compared to the CTR group ( $P < 0.05$ ; 3141 and 3107 vs. 2880g/d). Milk yield in the VITE group (3063 g/d) was unaffected by  $\alpha$ -tocopherol supplementation. The RPCE group supplementation was associated with greater ( $P < 0.05$ ) milk fat content than in CRT group. Fat-corrected milk (FCM) yield was greater ( $P < 0.05$ ) in the RPC and RPCE groups (3100 and 3147g/d, respectively) than the CTR group (2717 g/d); FCM milk yield in VITE goats was 2941g/d ( $P = 0.10$ ). Milk protein content did not differ between groups. Compared to CTR, fat and protein yields were higher in the RPC and RPCE groups. In the present experiment, a greater choline availability (by feeding rumen-protected choline) increased milk production in goats, as also reported in dairy cows (Baldi and Pinotti, 2006). The link between choline supplementation and milk response has been mainly attributed to the metabolic interchangeability of choline and methionine, in the sense that both can furnish labile methyl groups. Furthermore as expected, free choline and total free choline secretion in milk were affected by choline supplementation: when measured at 21 DIM, free choline and total free choline secretion in milk, respectively, were higher in the RPC and RPCE groups than CTR and VITE groups (figure 1).



**Figure 1.** Milk free choline content (A) and total secretion (B) on day 21 in milk according to treatment group. #  $P = 0.08$ ; \*  $P < 0.05$

These findings show that choline supplemented in rumen-protected form is available for absorption in goats, and that milk choline is sensitive to postruminal choline supply and bioavailability in goats, as is also the case dairy cows (Deuchler et al., 1998; Baldi and Pinotti, 2006). The free choline levels found in the milk of the animals not given choline were similar to those recently reported for transition dairy cows (Newbold et al., 2005).

### 4. Conclusion

In conclusion results of this experiment indicate that RPC supplementation not only increases milk yield and fat content, but also choline and total choline secretion in transition dairy goats. The magnitude of the production response is likely to be affected by basal diet composition, the dose and mode of administration of the rumen-protected choline, and the stage of lactation, as discussed in dairy cows. With regard to vitamin E, its administration alone did not affect milk yield.

## Acknowledgement

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## I-P007: Selection of Udder Functional Traits in Dairy Sheep

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### Summary

The emphasis for udder functional traits has resulted from the knowledge established during the last ten years that exclusive selection on milk traits would lead in the long term to udders more difficult to milk by machine and probably also more susceptible to mastitis. In a first part, we present a review of the genetic variability of traits related to udder health and machine milking ability and their relationships with milk production. In a second part, current selection in the French Lacaune and Italian Sardinian dairy sheep breeds is described. Evolutions in progress regarding the breeding objectives of these two breeds are presented.

### 1. Introduction

Functional traits are defined as those traits of an animal that increase its biological and economic efficiency not by higher outputs of products but by reduced costs of production. Functional traits have become important in dairy sheep, due to increased costs of production relative to milk prices and consumers demand for quality and safety products (as PDO cheese) with respect to animal welfare. Therefore, given the increasing interest of the breeders for improving machine milking ability of dairy sheep and the fact that udder health or udder morphology are two main causes of involuntary culling, research has focused for the last ten years on functional traits related to the udder. We will first present the genetic knowledge established during the last decade on udder functional traits and their relationships with milk production traits. Then we will describe the current selection in Lacaune and Sardinian dairy sheep breeds.

### 2. Polygenic variability for udder functional traits in dairy sheep

Milking is the main labour cost in dairy sheep production and influences milk production and udder health. On the other hand, investments in machine milking and parlours have increased noticeably in countries as France, Italy and Spain for the last 20 years, explaining the present breeder's interest for machine milking ability and udder health (mastitis resistance).

Machine milking ability includes two components, milk flow kinetics (or milking speed) and milking ease depending on udder morphology traits, such as teat placement or udder depth. Since there are not yet electronic meters approved by ICAR for dairy sheep, genetic variability of milking speed has been investigated presently only in INRA and IZCS experimental flocks: important genetic variation exist for milk flow kinetics traits, with high heritabilities between 0.50 to 0.65 according to the milking speed trait (Marie-Etancelin et al., 2006), while the genetic correlation with milk yield is clearly positive (0.30 to 0.46). Regarding udder morphology traits, an appraisal method based on 9 point linear scales was proposed first in Churra breed (de la Fuente et al., 1996) and has been now adapted to other dairy sheep breeds, as reviewed by Barillet et al., (2006). Heritability estimates were moderate to high and ranged between 0.16 and 0.42 according to the scored trait and the breed. The genetic correlations between milk yield and teat placement or udder depth are always slighty (-0.10) to clearly antagonistic (- 0.55). An efficient selection on milk production over several decades will result in an indirect genetic gain in milking speed, but also in an undesirable genetic change in udder conformation with teats placed more horizontally and more pendulous udders more difficult to milk by machine.

The second cause of unvoluntary culling is related to udder health, i.e. mastitis resistance. In practice improving udder health in dairy sheep could be done by selection based on milk somatic cell count (SCC). Estimates of heritability of the lactation somatic cell score (LSCS) ranged for sheep (as for cattle) between 0.10 to 0.20 as reviewed by Barillet et al., (2006). Relationships

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between milk yield and LSCS were low, ranging from from favourable (-0.29) to slightly antagonistic (0.20), according to the breed. Estimates in Lacaune and Sarda breeds showed this moderate antagonism, in agreement with the well established dairy cattle literature (Rupp and Boichard, 2003). Finally the genetic correlations between LSCS and several linear-udder traits as teat placement, degree of udder suspension or udder depth were moderately favourable (Legarra et al., 2005, Barillet. et al., 2006, Sechi et al., 2007) as already shown in cattle. Thus selection for udder linear traits will improve both milking ease and udder health (SCS).

### 3. Detection and location of quantitative trait LOCI (QTL)

Regarding QTL, results are promising since numerous QTL have now been detected on milking speed and udder morphology traits or SCS (Carta et al., 2002, Sara Casu, 2004, Barillet et al., 2005) based on 2 resource populations managed by IZCS (backcross Sarda x Lacaune families) and INRA (French Lacaune or Manech granddaughter families).

### 4. Current selection in the French Lacaune and Italian Sarda dairy sheep breeds

Lacaune and Sardinian breeds benefit from well structured breeding schemes based on open nuclei with a wide use of AI in Lacaune and the combination of AI and controlled natural mating in the Sardinian breed. Lacaune and Sardinian schemes totalize 184000 and 200000 dairy ewes in 400 and 900 registered flocks respectively, genetically connected via 136000 and 18000 AI per year. It allow on-farm progeny-testing of about 420 AI rams and 500 young rams per year respectively. In addition to the selection on milk yield and now on scrapie resistance, the challenge is presently to tackle the negative genetic trend on udder functional traits and especially udder morphology. Selection on udder morphology will allow improving milking ease and mastitis resistance. Of course, performances recording must be done at the nucleus flocks level, which is the only relevant level for genetically improving a whole large population on several partly antagonistic traits (Barillet, 1997). This is the genetic strategy implemented in the Sardinian breed where udder scoring has been extended to all the primiparous ewes of the registered population since 1999 (Sara Casu et al., 2006). Another challenge is implementing a qualitative milk recording (using a simplified design as promoted by ICAR) to select both for milk composition (fat and protein content) and SCS, in addition to milk yield and udder morphology. This corresponds to the new breeding objectives chosen in 2005 by the Lacaune breeders, who have been giving now the same relative weights to production (milk yield and composition) and udder functional traits (udder scoring and SCS). The genetic gain for milk yield is expected to be 39 l in 10 years, i.e. 62 % of the gain when selecting milk traits only, but the SCC is expected to decrease by 54 %, with a clear favourable change in udder morphology (Barillet et al., 2006).

### 5. Conclusion

Genetic improvement of udder functional traits has become a challenge in dairy sheep, since there is now evidence that selection on milk traits only will lead in the long term to udders more difficult to milk by machine and more susceptible to mastitis. Thus including udder scoring and SCS in the breeding goals, at the moment on a quantitative genetic basis at the population level, is a new challenge in progress for the Lacaune and Sardinian breeds. Moreover modern technology will give new opportunities: when electronic meters, approved by ICAR for dairy sheep, are available, direct selection for milking speed could be considered. Finally windows opened by molecular genetics will be profitable for udder functional traits, as it is already the case of the major PrP gene for scrapie resistance. As a whole, it is crucial to benefit from a registred population of an optimum size, between 10 to 20 % of the whole population to be improved (Barillet, 1997), in order to assure a number of selection candidates sufficient to permit an efficient selection on several breeding goals.

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## I-P008: Evolution of Ewe Milk Production at the Spooner Agricultural Research Station from 1996 through 2006

Y. M. Berger<sup>1</sup>, D. L. Thomas<sup>2</sup>

### Summary

Ewe milking started at the Spooner Agricultural Research Station of the University of Wisconsin-Madison in 1996, and the evolution of the productivity of the flock through 2006 is a reflection of the changes that have taken place in the dairy sheep industry in North America. Milk production per ewe increased from 80 kg in 1996 to 336 kg in 2006. Much of this increase can be attributed to breed substitution with more milking ewes of a higher dairy percentage (East Friesian and/or Lacaune). However, the change in breed composition is confounded with some important management changes such as milking shortly after lambing and lambing earlier in the year. The management changes led to a greater persistency of lactation which is certainly the main reason for the large increase in milk yield in a lactation over the past 10 years.

### 1. Introduction

The University of Wisconsin-Madison operates the only dairy sheep research farm in North America (NA) at the Spooner Agricultural Research Station in northwestern Wisconsin, USA (latitude: 45° 49'; longitude: 91° 53'). The station is at an elevation of 332 m with a northern temperate climate (average temperature: January = -12°C, July = 21°C; average annual precipitation: 764 mm). Ewe milking started at the Spooner Station in 1996 with mostly Dorset-cross ewes. The average milk production per ewe has increased by over 4 times in the past 10 years. This article is a reflection on the possible causes of such an increase by genetic and non genetic factors.

### 2. Discussion

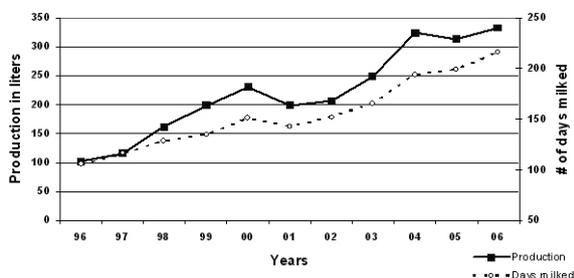
Milk production per ewe increased from 80 kg in 1996 to 336 kg in 2006 (Figure 1). Much of this increase can be attributed to breed substitution. In 1996, 30% of the ewes contained no dairy breeding and the remaining 70% of the ewes contained 50% or less of East Friesian breeding. By 2006, all ewes contained at least 50% dairy breeding (East Friesian and/or Lacaune), and over 85% of ewes contained over 75% dairy breeding (Figure 2). The non-dairy ewes averaged 67 kg of milk, and ewes of 75% and over dairy breeding averaged 274 kg of milk (Figure 3). However, the change in breed composition is confounded with some important management changes. In the first three years, 60 to 100% of the ewes were not milked until 30 days post-partum after their lambs were weaned, but during the last four years, an increasing proportion of ewes have been milked from shortly after parturition; reaching 100% in 2006. (Figure 4). Lambing has gradually moved earlier in the year, from March in 1996 to January in 2004, but ewes have dried-off by mid-September, regardless of the date of lambing. Persistency of lactation probably has improved as a result of a greater percentage of dairy breeding, but greater milk production in recent years due to reduced prepartum photoperiod also may be an explanation (Mikolayunas et al., 2007).

### 3. Conclusion

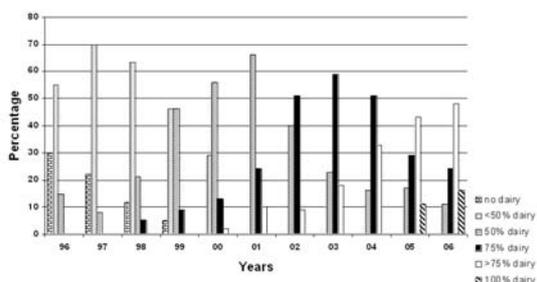
Both genetic and non-genetic factors have a large influence on milk production per ewe per lactation. Countries or regions with infant dairy sheep industries can make very rapid improvements in milk production per ewe by importing improved dairy genetics and applying sensible management practices.

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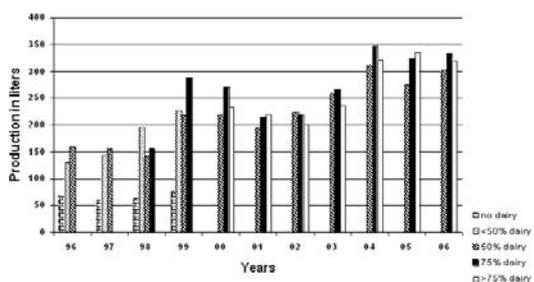
<sup>2</sup> Department of Animal Sciences, University of Wisconsin-Madison, 1675 Observatory Dr., Madison, Wisconsin 53706, USA



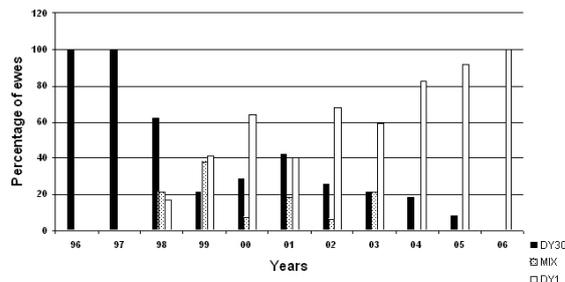
**Figure 1.** Milk Production per ewe per year



**Figure 2.** Dairy composition of the flock



**Figure 3.** Milk production per ewe in litres according to dairy percentage (adjusted for age of ewes)



**Figure 4.** Evolution of the type of lamb management (weaning systems)

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## I-P009: Use of DeLaval Cell Counter (DCC) in Goats

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### Summary

Milk quality is measured by a variety of parameters and one of these is the number of somatic cells in the milk. Maximum levels on bulk milk cell counts are being introduced into the caprine dairy production area. The reference method for cell counting in goats is the direct microscope method using the Pyronin Y-methyl green stain, which stains cell DNA. The use of the DeLaval cell counter (DCC) on goats' milk was compared to this reference method for samples from 102 udder halves. The DeLaval cell counter (DCC) showed large co-efficients of regression (1.04) and correlation (0.95) when compared with the microscope cell count ( $F_{1,199} = 1080.0$   $P < 0.001$ ). Goat, udder half and reader type were significant factors.

### 1. Introduction

There is more focus on milk quality issues for goats, with a maximum bulk milk cell count level in place in the USA at 1,000,000 cells per ml and levels under discussion in Europe. The production of milk in goats is largely apocrine in nature and cytoplasmic particles are normal constituents of goat milk. Procedures which are specific for DNA are necessary to obtain accurate cell counts for goat milk. It is accepted that cell count increases with infection, lactation length and parity and that cell counts of goats may be higher than those seen in cows. A cell count of greater than 700,000 to 1,000,000 cells per ml is taken as indicative of an infection.

This study seeks to show how the DCC method compares with the reference method and what factors may affect this, but not repeatability of the DCC for caprine milk.

### 2. Materials and methods

Foremilk samples were taken from functioning halves of 52 goats (102 samples in total). For each sample – one value was obtained using the DCC and two values were obtained using the microscope reference method (ISO13366) using the Pyronin Y-methyl green stain. Each smear was read by two of four possible readers. Statistical analysis was performed on  $\log_{10}$  transformed data using Genstat v8. Least square linear regression was used to compare the arithmetic mean of the two microscope readings with the DCC cell count. A general linear model was used to examine the goat, udder half nested within goat, and the method of counting the cells counts. A factor "Method" was created, with five levels: DCC, microscope reader 1, microscope reader 2, microscope reader 3 and microscope reader 4.

### 3. Results and discussion

The cell count values ( $\pm$  s.e.) were:- arithmetic mean: 674 ( $\pm$  53)  $\times 10^3$ /ml and logarithmic mean: 5.66 ( $\pm$  0.03) (microscope method) and arithmetic mean: 799 ( $\pm$  59)  $\times 10^3$ /ml and logarithmic mean: 5.56 ( $\pm$  0.03) (DCC method). The cell count values obtained with the DCC were significantly positively correlated with the cell count values obtained using the microscope method, explaining 95 % of the variation in the latter. ( $F_{1,199} = 1080.0$   $P < 0.001$ ). Taking into account variation due to goat number and goat half, the DCC values were significantly higher than all four readers ( $p < 0.001$ ) (Table 1). However the mean differences were small (de-transformed 118,000 cells between all readers). Somatic cell count was not significantly different between microscope readers.

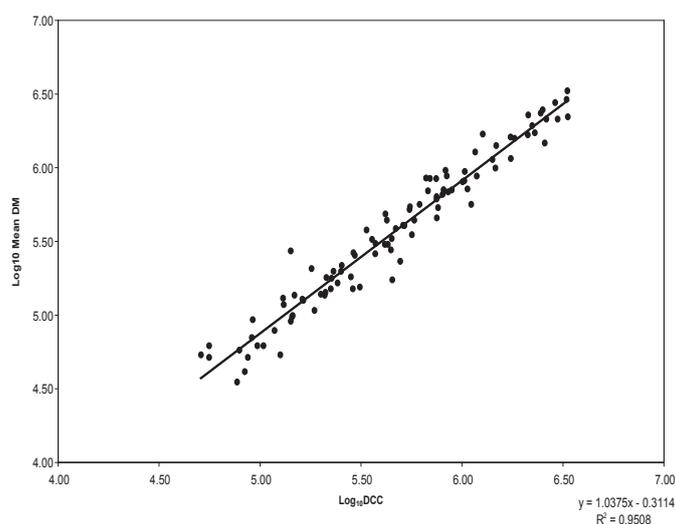
### 4. Conclusion

The reference method for cell counting in goat milk is the microscope method which is a time consuming and laboratory based method. The DeLaval cell counter (DCC) offers a field based

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system to determine the cell count of milk. This study demonstrates that the results generated by the DCC correlate with the microscope (95%) for undiluted milk. The DCC uses a single use cassette and this may offer some advantage over other mechanised cell counting systems



**Figure 1.** Relationship between the DCC cell counts and the direct microscope (DM) cell counts:  $\text{Log}_{10} \text{ mean DM} = -0.31 + 1.04 \log_{10} \text{ DCC}$   $R^2$  value =95%

**Table 1:** General Linear Model 1: The influence of method; 4 different microscope readers and the DeLaval Cell Counter (DCC) on cell count.  $R\text{-Sq}(\text{adj}) = 88.7 \%$

Source	DF	Adjusted sum of squares	Adjusted Mean squares	F value	P value
GoatID	51	49.53	0.97	33.66	<0.001
GoatID(Half)	46	20.25	0.44	15.26	<0.001
Reader	4	0.71	0.18	6.14	<0.001
Residual	196	5.65	0.03		
Total	297	76.14	0.26		

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## I-P011: Dietary Factors Associated with the Concentration of Milk Urea Nitrogen in Grazing Goats

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### Summary

This investigation aimed to individuate the dietary factors affecting the milk urea nitrogen (MUN) in lactating goats grazing herbaceous pasture, using 205 observations of dietary and milk variables from 37 Girgentana goats. The MUN concentration was closely correlated with most of variables considered. The stepwise selection showed dietary CP percentage to be the single variable explaining the most variation in MUN ( $R^2=0.56$ ;  $P=0.0001$ ). The other variables entering into the model were diet NDF, fat-corrected milk, DM intake and NDF intake (total  $R^2=0.66$ ). A linear regression, fitting mean data ( $n=28$ ) of MUN and CP concentrations ( $R^2=0.79$ ), indicates MUN could be used to estimate the dietary CP content.

### 1. Introduction

The major determinants of urea levels in plasma and milk of ruminants are known to be the amount of daily crude protein (CP) intake and the dietary ratio of CP to energy intake (Cannas et al., 1998; Nousiainen et al., 2004). In fact, a nitrogen excess of diet, not balanced with available energy, causes a surplus of N used for microbial growth in the rumen, and then enhances the urea concentration. This unbalance, resulting in high urea level, increases the risk of mastitis and has a negative impact on reproductive performance of animals (Mellado et al., 2004). Both plasma and milk urea nitrogen (MUN) are considered to be good indicators of protein feeding; since milk is easier to collect than blood, and MUN can be accurately determined, it is suggested to use MUN determination to evaluate the on farm efficiency of dietary N utilization, the adequacy and balancing of diets (Cannas et al., 1998; Shepers and Meijer, 1998). In the Mediterranean areas the breeding system of dairy sheep and goats is mainly based on grazing pasture. In these conditions, a prediction of pasture quality and an easy indicator for monitoring the feeding ration should permit the development of appropriate feeding strategies, providing the adequate supplementary feed aimed at balancing grazed forage. In this way, MUN might represent a fundamental nutritional tool also for the grazing small ruminants. This investigation aimed to individuate the dietary factors affecting MUN concentration in goats grazing herbaceous pasture, and verify the adequacy of MUN in estimating dietary CP content.

### 2. Material and methods

In Spring, over a 45-d period, 37 goats of Girgentana breed, initially averaging  $154 \pm 14$  days in milk and  $38.1 \pm 5.4$  kg of live weight, were allowed to daily graze ryegrass as a monoculture (16 goats) or a mixture with berseem clover (21 goats), and fed with 500 g/d of barley meal. From weekly measurements and sampling, regarding sward biomass, selected forage, dry matter (DM) intake and milk yield of goats, a total of 205 observations were collected. DM intake of goats at pasture and diet DM digestibility were assessed by the *n*-alkane technique (Dove & Mayes, 1991). Analyses for DM, CP, fat, ash and structural carbohydrates were carried out on selected herbage and barley. The estimation of net energy for lactation ( $NE_L$ ) of diet was based on estimated digestibility and equations of Van Soest & Fox (1992). Milk samples were analysed for fat, protein ( $N \times 6.38$ ) and casein; MUN content was determined by enzymatic method using difference in pH. Milk yield was corrected at 3.5% fat (FCM). The simple Pearson correlations were calculated among MUN and all variables. To investigate the relationships of MUN with dietary variables and milk yield, a multiple stepwise regression model was run setting the level of significance at 0.15. With the aim of predicting the diet CP content based on MUN values,

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simple regression models were fitted using individual ( $n=205$ ) or mean treatment *per* time data ( $n=28$ ). Statistical analysis was performed using CORR and REG procedures of SAS (9.1.2).

### 3. Results and discussion

MUN concentration (9.7-35.4 mg/dl) resulted closely correlated ( $P<0.001$ ) with most of dietary and milk variables. The highest correlation of MUN was observed with the diet CP percentage (13.7-26.0% DM;  $r=0.75$ ); MUN was also directly correlated with pasture allowance (39-151 kg DM/goat;  $r=0.42$ ), diet  $NE_L$  (1.5-1.9 Mcal/kg DM;  $r=0.37$ ), FCM (376-2115 g/d;  $r=0.23$ ) and milk protein (3.6-5.9 %;  $r=0.25$ ), whereas negative correlations resulted with diet NDF (18.7-37.4% of DM;  $r=-0.69$ ) and diet digestibility (72.6-92.4%;  $r=-0.33$ ). These relationships evidences how a higher grass availability at pasture allowed the goats to select forage with high nitrogen and lower NDF content, thus with a higher energy concentration, linked to an increase in milk yield and MUN. The stepwise selection using dietary variables and milk yield (Table 1) showed diet CP percentage to be the single variable explaining the most variation in MUN ( $R^2=0.56$ ), similarly to dairy cows (Nousiainen et al., 2004) and sheep (Cannas et al., 1998). The other variables entering into the model, until reaching a total  $R^2$  of 0.66, were diet NDF, with 6,8% as partial  $R^2$ , FCM, DM intake and NDF intake, which supplied very low partial contributions. Including the CP/ $NE_L$  (82-166 g/Mcal;  $r=0.70$ ,  $P<0.001$ ) and CP/NDF (0.49-1.31 g/g;  $r=0.77$ ,  $P<0.001$ ) ratios of diet in the stepwise regression model, the CP/NDF ratio alone explained 60.1% of MUN variability, with a slope of +5.28; therefore, the dietary ratio of CP to NDF was the most important nutritional factor affecting MUN, underlining the importance of balancing dietary protein to NDF for optimizing fermentation activity and nitrogen utilization in the rumen and controlling the MUN concentration. In the subsequent steps of selection, diet CP content, barley proportion in the diet and FCM entered into the regression model, absorbing an extra 4.6% of MUN variability, thus determining only a slight increase in the total  $R^2$  for MUN ( $R^2=0.65$ ). The close relationship of MUN with diet CP suggested the possibility of using MUN for predicting the CP concentration of the diet at pasture. Since the linear regression fitted between diet CP and MUN concentration with individual data of goats ( $n=205$ ) [ $CP$  (% DM)=10.231 ( $\pm 0.67$ ) + 0.46 \* MUN (mg/dl) ( $\pm 0.03$ ) ( $R^2=0.56$ )] was less accurate, mean treatment *per* time data were used, as suggested by Cannas et al. (1998) for reducing the large individual variation in MUN of animals fed the same ration. In this case, the proportion of variation explained by the relationship between CP and MUN greatly increased [ $CP$  (% DM)=6.91 ( $\pm 1.42$ ) + 0.61 \* MUN (mg/dl) ( $\pm 0.06$ );  $R^2=0.79$ ].

**Table 1:** Individuation of dietary and milk yield variables which provide a significant contribution in regressions with milk urea N using multiple stepwise regression models ( $n=205$ ).

Variable step entered	Intercept	Slopes	P-value	$R^2$
	37.20		0.0001	
Crude protein, % DM		1.00	0.0001	0.5554
NDF, % DM		-1.16	0.0001	0.0684
Fat-corrected milk g/d		0.002	0.0191	0.0126
DM intake, g/d		-0.027	0.0001	0.0053
NDF intake, g/d		0.079	0.0013	0.0050
Total $R^2$				0.6564

### 4. Conclusion

The CP percentage and CP/NDF ratio of diet resulted the best variables explaining the MUN variability. The linear relation found between mean values of dietary CP concentration and MUN

suggested the possibility of using MUN to estimate the CP content of diet, as a useful tool for developing appropriate feeding strategies aimed at balancing the rations of goats grazing at pasture. Further experiments are required in order to extend available data regarding grazing goats and to define the relationship between MUN and reproductive performance. On the other hand, further investigations could be used to put on MUN reference values for grazing goat.

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## I-P012: Effects of Feeding Soybean Oil to Dairy Goats on Milk Yield and Composition and CLA Content

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### Summary

A total of 24 Murciano-Granadina dairy goats were used to study the effects of feeding soybean oil (SBO) on lactational performance and milk fatty acids (FA) content, particularly conjugated linoleic acid (CLA) and *trans*-vaccenic acid (TVA). The experiment consisted of a two period (28 d each) crossover design with two dietary treatments: C (control) and SBO (6% as fed in the concentrate). No effect of SBO was observed on DMI, milk yield nor energy corrected milk. SBO increased (10%) the milkfat content and yield as well as total solids content, but had no effect on milk crude protein and true protein contents. Short and medium chain FA were reduced by SBO, while long chain FA increased. SBO also reduced the saturated to unsaturated FA ratio and the atherogenicity index. Compared with the control, milk contents of *cis*-9, *trans*-11 CLA and TVA in the SBO treatment were almost triplicated.

### 1. Introduction

Research data on dietary factors affecting milk CLA in dairy goats is less available than in dairy cows and has been described mainly by French researches (Chilliard et al., 2006). According to these studies, when fat sources such as oleic or linoleic sunflower oil, or linseed oil were tested, the main factor of variation was the nature of oil, the higher CLA levels being obtained when sunflower oil (C18:2-rich) was fed. In contrast, raw, extruded or formaldehyde treated oilseeds gave poorer results (Bernard et al. 2005, Chilliard et al. 2006), the milk CLA content depending also on the nature of forage.

Data on lactational effects of feeding other C18:2-rich fat sources, like soybean oil (SBO), to dairy goats is limited, and there is no information on their influence on the CLA and TVA contents in milk. Therefore, the aim of this study was to investigate the effects of including SBO in the concentrate of dairy goats on milking performance and milk fatty acids profile, with special attention in CLA and TVA contents in milk.

### 2. Material and methods

In this experiment, a total of 24 Murciano-Granadina dairy goats milked once daily throughout lactation were used. Three weeks after parturition, goats were allocated to two balanced groups according to number of lactation, BW and daily milk yield, and kept in two separate pens. The experiment consisted of a two period (28 days each, 14 for adaptation and 14 for sampling) crossover design with two dietary treatments: C (control) and SBO (6% as fed in the concentrate). Goats were fed dehydrated fescue (*ad libitum*), alfalfa pellets (0.5 kg/d), and experimental concentrate (1 kg/d) to which the SBO was or was not added. Forage was offered in the pens, and the concentrate was individually fed in two equal portions. Final SBO content in the consumed SBO diet was 2.5% (DM basis). Diets were isonitrogenous (17.4%), but their total FA content varied from 2.2% (Control) to 4.6% (SBO).

Milk yield was registered for 5 days during each sampling period, and individual milk samples were collected at each milk yield registration. Milk samples were preserved in 100 ml pots containing 2 tablets of Bronopol (Broad Spectrum Micro-tabs II, D&F Control Systems Inc., USA) as a conserving product, and refrigerated at 4° C before being analyzed for fat, CP (N x 6.38), true protein, casein and TS. The analysis was performed using a near-infrared spectroscopy analyzer (NIRS Systems 5000, Foss Electric A/S, Hillerød, Denmark). Calibration was checked using the AOAC (1990) reference methods.

To analyze milk FA profile, additional milk sub-samples were taken without preservative. Milk FA were analyzed after extraction of milkfat samples and methylation, as described by Casals

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et al. (2006). Separation and quantification of the methyl esters from milk samples was carried out using a gas chromatograph (HP 6890, N. Agilent Technologies, Palo Alto, CA) equipped with flame ionization detector and capillary column (CP-Sil-88; 100 m x 0.25 mm i.d. with 0.20- $\mu$ m of capillary thickness; Varian Inc., Palo Alto, CA, USA).

### 3. Results and discussion

There was no effect of SBO on dry matter intake (2.1 kg/d), milk yield (1.87 kg/d), energy corrected milk (2.09 kg/d), energy corrected milk conversion rate (0.98 kg/kg DM), BW (40.5 kg), and BCS (2.7). Feeding SBO increased ( $P < 0.05$ ) the milkfat content (+10%) and yield as well as total solids content. SBO had no effect on milk crude protein (average of 3.4%) and true protein (average of 2.9%) contents, but it reduced ( $P < 0.001$ ) milk casein content (2.5 vs 2.3 %). Short and medium chain FA were reduced by SBO, while long chain FA were increased ( $P < 0.01$ ). Feeding preformed linoleic acid through SBO increased milk concentrations of linoleic, oleic and stearic FA, but reduced levels of linolenic and palmitic FA ( $P < 0.001$ ). As a consequence, feeding SBO reduced ( $P < 0.001$ ) the saturated to unsaturated FA ratio (3.01 vs. 2.29) and the atherogenicity index (3.29 vs. 2.2).

The level of *cis*-9, *trans*-11 CLA (rumenic acid) in the milk of goats fed SBO was almost 3 times superior to the control (0.68 vs. 2.03% of total FA). In contrast, the *trans*-10, *cis*-12 CLA was not detected in the milkfat samples. Similarly, Chilliard et al. (2006) indicated that this CLA isomer always remained at trace levels in goat milk. Rumenic acid is synthesized in the rumen during the biohydrogenation of linoleic acid, but it can also be obtained in the mammary gland by the desaturation action of the  $\Delta$ -9 desaturase on TVA, another intermediate product of that process.

The milk TVA content was increased by the SBO supplementation (2.04 vs. 6.41%;  $P < 0.001$ ), and the ratio TVA/CLA remained unchanged, showing that TVA and CLA evolved in the same way. This would indicate that the  $\Delta$ -9 desaturase way was relevant in the milk CLA biosynthesis. There is generally a wide linear correlation between rumenic acid and TVA levels in milk under a large variety of diets, either in dairy goats (Nudda et al., 2005) or in cows (Griinari and Bauman, 2003).

In this study, the level of CLA in milk from control goats (0.68%) was in the range of normal values observed for goats receiving non fat supplemented diets (Chilliard et al., 2006). In contrast, the CLA content (2.03%) in milk from goats fed SBO was very close to the level described by Chilliard et al. (2006) using sunflower oil. The same authors (Chilliard et al., 2006) indicated a considerable increase of RA content (from 0.3 to 5.1%) in goat milk with combinations of five different forages and with or without some oil addition. Similar levels of CLA to those obtained in the present study with SBO have been observed in dairy ewes (Bouattour et al., 2005) after SBO supplementation, and in dairy cows fed either fresh pasture (Dhiman et al., 1999) or calcium soaps of SBO (Chouinard et al., 2001).

### 4. Conclusion

Feeding a reasonable dose of soybean oil to dairy goats allowed the production of enriched CLA and TVA milk, without negative effects on DMI, milk yield, and milk protein content, but with a negative effect on milk casein content and yield. This could be a valuable tool for farmers of dairy goats under intensive feeding systems in order to produce enriched CLA milk and dairy products, more unsaturated too, which are considered healthier for human consumers.

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## I-P013: Effects of Feeding Whole Linseed and Linseed Oil to Lacaune Ewes on CLA Concentration and Milk Fatty Acids Composition

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### Summary

Thirty lactating Lacaune dairy ewes were blocked in a 3x3 latin square experiment in order to investigate the effects of feeding whole linseed (WLS) or linseed oil (LSO) to dairy ewes on lactational performance, milk and cheese fatty acids profile and conjugated linoleic acid (CLA) content. Medium chain fatty acids (FA) and saturated FA were decreased and long chain and unsaturated FA (including mono and poly-unsaturated FA) were increased by WLS and LSO. Feeding WLS was more useful on increasing milk  $\alpha$ -linolenic acid content, while feeding LSO allowed a higher increase of CLA (rumenic acid). Similarly, *trans*-11 C18:1 (*trans* vaccenic acid or TVA), precursor of CLA, was only increased by LSO. Except for short chain FA, the FA profile of 60-d-old cheeses made from milk of the ewes receiving the experimental treatments was similar to the FA profile of the milk.

### 1. Introduction

Several studies showed the ability of vegetal fat to modify the FA profile of the milk and to increase CLA and TVA contents. CLA and TVA are considered functional foods because of their beneficial effects on consumer's health. Available information on studying the use of whole linseed (WLS) and linseed oil (LSO) in dairy ewes is very limited. Linseed supplements are rich in  $\alpha$ -linolenic acid and could help to increase not only the CLA but also the n-3 FA content of the milk. Therefore, the aim of this work was to investigate the effects of feeding WLS or LSO to dairy ewes on their milk FA profile and dairy performance, when compared with a traditional control diet containing calcium soaps of FA.

### 2. Material and methods

Thirty Lacaune dairy ewes were blocked in 3 pens of 10 animals and used in a 3 × 3 Latin square (20 d periods: 14+6). Ewes were fed a diet with 53% forage (alfalfa and fescue dehydrated mixture, 1:1), 43.6% of the corresponding experimental concentrate, and 3.4% of barley grain offered daily in the milking parlor. Experimental treatments were: 1) Control (6% calcium soaps of palm oil in the concentrate); 2) WLS (15.0%); and, 3) LSO (5.0%). Diets were isonitrogenous (18.6% CP), and had similar levels of fat (5.1% EE).

Milk yield was registered for 3 days during each sampling period, and individual milk samples were collected at each milk yield registration. Milk samples were preserved in 100 ml pots containing 2 tablets of Bronopol (Broad Spectrum Micro-tabs II, D&F Control Systems Inc., USA) as a conserving product, and refrigerated at 4° C before being analyzed for fat, CP (N × 6.38), true protein, casein and TS. The analysis was performed using a near-infrared spectroscopy analyzer (NIRS Systems 5000, Foss Electric A/S, Hillerød, Denmark). Calibration was checked using the AOAC (1990) reference methods.

To analyze milk FA profile, additional milk sub-samples were taken without preservative. Milk FA were analyzed after extraction of milkfat samples and methylation (Casals et al, 2006). Separation and quantification of the methyl esters from milk samples was carried out using a gas chromatograph (HP 6890, N. Agilent Technologies, Palo Alto, CA) equipped with flame ionization detector and capillary column (CP-Sil-88; 100 m × 0.25 mm i.d. with 0.20- $\mu$ m of capillary thickness; Varian Inc., Palo Alto, CA, USA). Regarding Cheese making, the ripening took place for 60 days at 14°C temperature and 85% relative humidity. After ripening, samples from the 60-d-old cheeses were taken to be analyzed similarly than milk samples in order to determine FA content.

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Individual data for BW, BCS, milk yield and composition were analyzed using the PROC MIXED procedure with repeated measures of SAS (SAS v. 8.2; SAS Inst., Inc., Cary, NC) using Tukey's multiple comparison test.

### 3. Results and discussion

Milk yield (1.9 kg/d), ECM (1.6 kg/d) and milk protein (5.2%) and casein (4.1%) contents were unaffected by treatments. In contrast, milkfat (C: 5.70; WLS: 5.85; LSO: 6.08%) and total solids (16.3; 16.6; 16.9%) contents were increased ( $P < 0.05$ ) by LSO and tended to be increased ( $P < 0.10$ ) by WLS. Also LSO increased yields of milkfat (107; 107; 114 g/d) and total solids (312, 308, 316 g/d), but reduced true protein content (5.20; 5.11; 4.97%) of milk.

Regarding milk FA profile, both WLS and LSO supplementations decreased the total saturated and increased the total unsaturated FA ( $P < 0.001$ ). The increase of unsaturated FA concerned both MUFA (20.8; 24.17; 24.74% of total FA) and PUFA (3.35; 4.24; 4.37%). This was a direct repercussion of the fat sources fed to the ewes, with FA profile of linseed supplements highly rich in unsaturated FA (90%).

As indicated in Table 1, milk content of  $\alpha$ -linolenic acid (n-3) was much more increased by WLS than by LSO, while the CLA (*cis*-9, *trans*-11 C18:2 or ruminic acid) level was much more positively affected by LSO than by WLS. Other CLA isomer, the *trans*-10, *cis*-12 CLA, usually present in cow's milk, was not found at significant levels. As indicated by Lock et al. (2006), usually this isomer is not found in ewe's milk. The *trans*-11 vaccenic acid, precursor of CLA, was duplicated by LSO, but not modified by WLS (table 1). The TVA/CLA ratio remained constant (average of 2.0) indicating a uni-sense evolution of RA and TVA. Fatty acids composition of 60-d-old cheeses showed a similar FA profile than milk, particularly for medium and long chain FA.

According to Grinaari et al., 2000, the main part of the CLA is synthesized in the mammary gland from the TVA. The remaining CLA is produced during the rumen biohydrogenation. In our experiment this way is supported by the higher C18:1 concentration in both WLS and LSO milks, indicating a higher ruminal biohydrogenation activity in the linseed treatments than in the control. Nevertheless, the TVA, was not changed by WLS treatment, which seems to indicate that the FA biohydrogenation was more active in the rumen of ewes receiving LSO than in those receiving CSFA or WLS. If there is a high biohydrogenation activity, rumen TVA content tends to be increased and the next step of biohydrogenation becomes a limiting factor (Chilliard et al., 2006). In addition, the co-evolution of CLA and TVA (constant ratio of TVA/CLA) supports the important role of mammary desaturation of TVA in CLA biosynthesis. The higher TVA concentration observed only in the case of LSO and the constant ratio TVA/CLA of both WLS and LSO treatments agrees with the higher CLA increase observed in milk from LSO group. TVA is a precursor of RA by the mammary gland  $\Delta$ -<sup>9</sup> desaturase pathway (Griinari et al., 2000). TVA was not changed by WLS treatment, indicating a lower rate of biohydrogenation of the unsaturated FA in the whole grain.

**Table 1:** Effects of whole linseed (WLS) and linseed oil (LSO) on n-3 fatty acids (FA), trans vaccenic acid (TVA) and conjugated linoleic acid (CLA) content (% of total FA) of milk from Lacaune dairy ewes.

	Treatments			SEM	Contrast $P < ^1$		
	Control	WLS	LSO		C vs. WLS	C vs. LSO	WLS vs. LSO
C18:3 n-3	0.78	1.91	1.38	0.308	***	**	**
n-6/n-3 FA ratio	4.17	1.46	2.29	0.391	***	***	NS
TVA ( <i>trans</i> -11 C18:1)	1.31	1.70	3.13	0.337	NS	**	**
CLA ( <i>cis</i> 9- <i>trans</i> 11)	0.66	0.79	1.46	0.135	*	***	**
TVA/ CLA	1.93	1.96	2.09	0.103	NS	NS	NS

<sup>1</sup> $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.0001$

## 4. Conclusion

In Lacaune dairy ewes, feeding whole linseed grains or linseed oil in moderated doses was useful to increase milkfat content, n-3 FA and CLA. Whole linseed was better than linseed oil to increase n-3 FA content and reduce the n-6/n-3 ratio of milkfat. In contrast, only linseed oil increased milk TVA content, and the increase of milk CLA (rumenic acid) was higher with the oil than with the whole seed. Similar effects on the FA profile were observed in the cheese.

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## I-P014: Milk CLA and Fatty Acids Profile in Milk from Lacaune Ewes Fed Whole Safflower Grains

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### Summary

In the present study, a total of 24 Lacaune dairy ewes at  $49 \pm 7$  DIM were used to study the effects of adding whole safflower seeds (WSF) to the concentrate on fatty acids profile, Conjugated Linoleic Acid (CLA) in milk and dairy performance. Ewes were allocated to two balanced groups and kept in two separate pens, they were fed a mixture of 53% forage (dehydrated fescue:alfalfa hay; 1:1) and 47% concentrate. Dietary treatments were: C (control) and WSF (16.3% in the concentrate). Feeding WSF increased concentrations of long chain and unsaturated fatty acids (FA), and decreased short chain and saturated FA. Concentrations of rumenic (*cis*-9, *trans*-11 CLA) and *trans* vaccenic (TVA) acids in milk were increased in animals fed WSF. In addition, WSF reduced the saturated/unsaturated FA ratio and the atherogenicity index of the milk fat, but increased the ratio n-6/n-3 FA.

### 1. Introduction

There are few studies on the effects of whole safflower seeds (WSF), a relatively unsaturated source of FA, on dairy performance and milk FA profile, and they have been mainly done with bovine. Scholljegerdes et al. (2004) compared the fermentation pathways under supplementation with two kinds of safflower (the first rich in oleic acid and the second rich in linoleic acid) in heifers fitted with ruminal and duodenal cannulas. The authors concluded that both kinds of safflower seeds increased the duodenal flux of *trans*-11 C18:1 or TVA and that the safflower rich in linoleic acid increased it much more.

As TVA is a direct precursor of CLA, and both CLA and TVA are considered to be functional foods (Bauman et al., 2006), the aim of this study was to investigate in dairy ewes the effects of feeding rich linoleic WSF on dairy performance and milk fatty acids profile, particularly CLA and TVA, when compared with a traditional and isoenergetic control diet including calcium soaps of FA.

### 2. Material and methods

A total of 24 Lacaune dairy ewes milked twice daily were used to study the effects of adding whole safflower seeds (WSF) to the concentrate on dairy performance and Conjugated Linoleic Acid (CLA) in milk. Ewes were allocated to two balanced groups according to number of lactation, body weight and daily milk yield, and kept in two separate pens. Experimental diets contained a mixture of 53% forage (dehydrated fescue and alfalfa, 1:1) and 47% concentrate, to which the WSF was or not added. Dietary treatments were: 1) Control, and 2) WSF (16.3% as fed in the concentrate). Control concentrate contained calcium soaps of palm oil (6%) in order to have final diets with similar levels of total fat (5% EE) and energy density (1.6 Mcal/kg DM). Diets were also isonitrogenous (19% CP), but their FA profile was different, being the linoleic acid content in the WSF diet much higher than in the control (18 vs. 31% of total FA). The experiment consisted of a 2x2 crossover design (20 days period, 14 days for adaptation and 6 for sampling), during which the total mixed ration was offered *ad libitum* in the pens. Milk yield was registered for 3 days during each sampling period, and individual milk samples were collected at each milk yield registration. Milk samples were preserved in 100 ml pots containing 2 tablets of Bronopol (Broad Spectrum Micro-tabs II, D&F Control Systems Inc., USA) as a conserving product, and refrigerated at 4° C before being analyzed for fat, CP (N x 6.38), true protein, casein and TS. The analysis was performed using a near-infrared spectroscopy analyzer (NIRS Systems 5000, Foss Electric A/S, Hillerød, Denmark). Calibration was checked using the AOAC (1990) reference methods.

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To analyze milk FA profile, additional milk sub-samples were taken without preservative. Milk FA were analyzed after extraction of milkfat samples and methylation (Casals et al, 2006). Separation and quantification of the methyl esters from milk samples was carried out using a gas chromatograph (HP 6890, N. Agilent Technologies, Palo Alto, CA) equipped with flame ionization detector and capillary column (CP-Sil-88; 100 m x 0.25 mm i.d. with 0.20- $\mu$ m of capillary thickness; Varian Inc., Palo Alto, CA, USA).

### 3. Results and discussion

Ewes fed WSF showed a lower dry matter intake (2.4 vs. 2.3 kg/d) that could be related with the hull hardness of the whole seeds. Therefore, feeding WSF decreased milk (1.58 vs. 1.48 kg/d) and milkfat (111 vs 99 g/d) yields, energy corrected milk (1.47 vs. 1.34 kg/d) and milk conversion rate (0.60 vs. 0.57 kg/kg DM), but did not modify milkfat (6.73%), protein (5.30%), casein (4.19%) and total solids contents (17.86%). True protein content ( $P < 0.001$ ) was increased (5.16 vs. 5.43%) and milkfat and protein yields were decreased ( $P < 0.01$ ) by the WSF treatment, mainly due to the milk yield depression. Regarding milk FA profile, WSF increased ( $P < 0.01$ ) concentrations of long chain FA (38.09 vs. 48.75%) and unsaturated FA (29.59 vs. 33.70%), and decreased ( $P < 0.01$ ) short chain FA (13.46 vs. 12.28%) and saturated FA (70.14 vs. 65.95%). Concentrations of main FA are shown in Table 1. As a consequence of WSF supplementation, concentrations of rumenic acid (*cis*-9, *trans*-11 CLA, 0.60 vs. 0.88% of total FA) and TVA (*trans*-11 C18:1, 1.19 vs. 1.70%) in milk were increased ( $P < 0.001$ ). In addition, feeding WSF reduced ( $P < 0.01$ ) the saturated/unsaturated FA ratio (2.37 vs. 1.96%), the desaturase index (0.72 vs. 0.64) of the oleic acid and the atherogenicity index (2.69 vs. 2.08) of milkfat, but increased ( $P < 0.01$ ) the ratio n-6/n-3 FA.

Given that the C18:1 FA are not synthesized by animal tissue, we can assume that the increase of the C18:1 isomers was a direct consequence of the biohydrogenation of C18:2. Part of the CLA is synthesized during the rumen biohydrogenation, this pathway being supported by the higher C18:1 concentration in the milk of the WSF group (Table 1). However, the increase of TVA concentration, precursor of RA by the mammary gland  $\Delta 9$  desaturase pathway, in addition to the co-evolution of CLA and TVA (constant ratio of TVA/RA), supports the importance of the desaturation way in the CLA biosynthesis. In dairy cows, the main portion of CLA (from 64 to 98%) in milk fat is produced in the mammary gland by  $\Delta 9$  desaturase from TVA (Griinari et al., 2000).

Adding WSF could be a useful tool to obtain CLA concentrations similar to fresh pasture in systems with low availability of fresh pastures and forage quality. Supplementation with WSF could also be useful to avoid the decrease of CLA concentration caused by the forage maturity and season effect.

**Table 1:** Effects of feeding whole safflower seeds (WSF) on main fatty acids content (% of total FA) of milk from Lacaune dairy ewes.

	Treatments		SEM	Effect $P < ^1$
	Control	WSF		
C18:1 <i>cis</i> -9	20.72	23.41	0.594	*
C18:2 n-6c	3.17	3.50	0.133	*
C18:3 n-3	0.87	0.89	0.031	NS
n-6/n-3	3.87	4.47	0.161	**
TVA ( <i>trans</i> -11 C18:1)	1.19	1.70	0.137	***
CLA ( <i>cis</i> -9, <i>trans</i> -11 C18:2)	0.60	0.88	0.039	***
TVA/ CLA	2.03	1.93	0.123	NS
Atherogenicity index <sup>2</sup>	2.69	2.08	0.083	***

<sup>1</sup> \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.0001$ .

<sup>2</sup> Calculated as: (C12+4 C14+C16)/sum of unsaturated FA

## 4. Conclusion

Feeding whole safflower seeds to Lacaune dairy ewes enhanced the nutritional quality of the milk, increasing CLA (rumenic acid) and trans vaccenic acid levels and reducing the saturated FA concentrations as well as the atherogenicity index of the milk fat. However, the safflower seeds were detrimental for the milk, fat and yield. We suggest further investigations on processing safflower seeds in order to avoid a possible negative effect of the hull hardness on feed intake.

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## I-P015: Technological Characterization of Lactic Acid Bacteria Isolated from Goat Natural Starter

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### Summary

The study was set up to compare the lactic acid bacteria naturally present in goat's milk with the bacteria characteristic of cow's milk, both milks coming from the same production area in the Lombardy Region.

One hundred and forty strains were isolated from the raw goat's milk, and all were characterized phenotypically and technologically to assess the biodiversity within these naturally wild microbial populations.

In addition, a comparison was made of the activity of the strains common to goat's milk and cow's milk, and it was observed that some strains showed higher metabolic activity in one milk than in the other.

On comparing the strains belonging to the most frequently dominant species (*Lactococcus lactis subsp. lactis*, *Enterococcus faecium* and *Enterococcus faecalis*) isolated from goat's milk with as many strains from the same species in the cow's milk, a large biodiversity was found.

### 1. Introduction

The goat population and the production of goat's milk in Italy has increased significantly over the last decade, and is mainly linked to the production of goat cheeses. Goat rearing tends to be concentrated in mountainous regions where goat's milk plays a significant role in the economy. The majority of goat milk producers are small-scale farmers who process the milk into various types of cheese. The transformation of goat's milk is achieved through natural starters or, in recent years, with selected starters of defined strains generally of cow origin [1]. The activity of the individual starter cultures varies considerably, depending on the type of milk used [1,3]. Variations in the biochemical performance of the starters, grown in goat's and cow's milk, have been reported [4,5]. The objective of the present work was to identify a large number of strains of lactic acid bacteria predominant in natural goat starters prepared without the addition of selected cultures, and to study their technological characteristics in both cow's and goat's milk.

### 2. Material and methods

Fifteen natural goat starters traditionally made with raw milk without any addition of selected strains were collected from small producers in the Lombardy region.

Strains were identified by Api 20 Strep, API 50CHL (Api system BioMérieux France)

#### **Technological Characterization**

Cow's milk and goat's milk were prepared from reconstituted skimmed milk powder (10% w/v).

The milk was inoculated (at 1%) with fresh culture of each strain to obtain approximately  $10^6$  cfu ml<sup>-1</sup>.

**Acidification activity:** titratable acidity: (FIL-IDF 306:1995) after 2 ,4 ,6 ,8, 24 h and 15 d of incubation at 37 °C. The variation in pH was determined over 24 h during waterbath incubation at 37 °C by multi-channel pH-meter: Cinac 3 Ysabaert, France,

**Reduction activity:** the variation in redox potential was determined over 24 h during waterbath incubation at 37 °C by Eh-meter Hanna Instruments, Italy.

**Proteolysis**

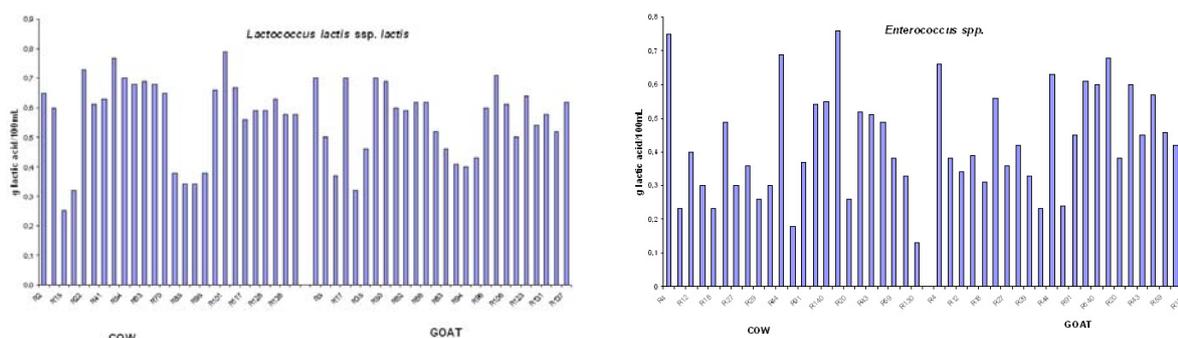
Hull's method [6]

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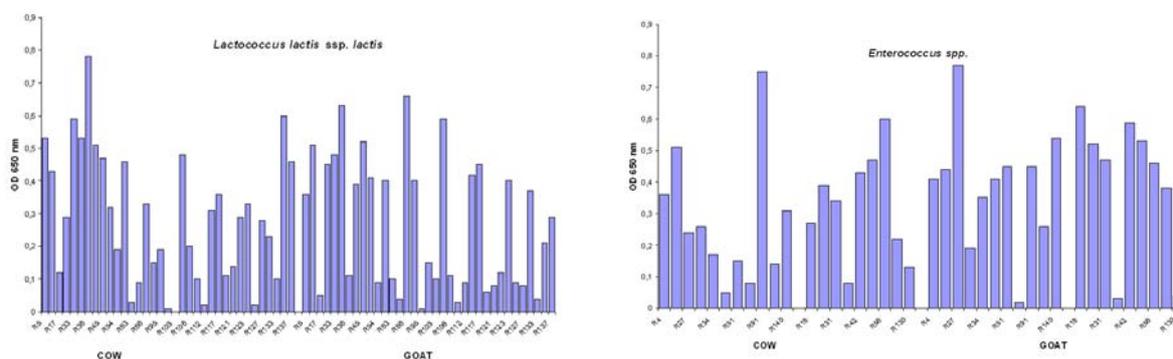
### 3. Results and discussion

One hundred and forty strains of lactic acid bacteria were isolated from natural starters.

The composition of the starters varied from area to area. Three genera were found: *Lactococcus* (62%), *Enterococcus* (32%), *Leuconostoc* (6%). The predominant species were *Lactococcus lactis*, ssp. *lactis* (72 strains), *Enterococcus faecalis* (23 strains), and *Enterococcus faecium* (16 strains). Comparing the activity of strains in goat's and cow's milk it was observed that some strains showed higher metabolic activity in one milk than in the other. Fig. 1 indicates the acid production of *Lactococcus lactis* ssp. *lactis* strains and *Enterococcus* spp. strains. A significant difference ( $p < 0.5$ ) in the level of acid production and proteolysis was observed for *Enterococcus* spp. and for proteolysis of *L. lactis* ssp. *lactis*. For 62% of the strains of *Lactococcus lactis* ssp. *lactis* acid production was higher in cow milk. Differently, lactic acid production was higher in goat's milk for 74% of the strains belonging to the *Enterococcus* spp. genus. With the exception of *Lactococcus lactis* ssp. *lactis*, the species that had similar Eh evolution in the two milks, the other species tested had reduction curves postponed for approximately one - two hours in cow's milk.



**Figure 1.** Comparison between the acidity produced in cow's and goat's milk.



**Figure 2.** Comparison between the proteolysis produced in cow's and goat's milk.

### 4. Conclusion

The acidifying, proteolytic and reducing activities of given strains were found to differ in the two milks. Our results enrich the very scarce scientific information available on the growth of lactic acid bacteria in goat's milk [7]. Furthermore, the results indicate the usefulness of testing starter cultures for technological performance in goat's milk before using such starters in cheese-making [8].

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## I-P018: Electronic Identification (EID) vs Ear Tattoo (ET) during controls on milk production of Sarda sheep

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### Summary

In agreement with EU Reg. 21/2004, the electronic identification (EID) of sheep will be compulsory in EU Countries from the 1<sup>st</sup> of January 2008. According to such Regulation, AA. compared the reliability and the efficacy of EID vs ear tattoo on 553 Sarda breed sheep, during milking.

The trial was carried out in 2 different farms of Sardinia belonging to the Genealogical Register of the Provincial Association of Farmers (APA) of Nuoro (Sardinia-Italy). In both farms, animals had been previously identified by means of endorumenal ceramic bolus (transponder HDX 134.2 kHz, according to ISO Standards 11784-11785) and by ear tattoo (APA alphanumeric 8 typeface code). In both farms, at machine milking of animals in 24 ties stall, 2 controllers for milk production worked as follows: one checked and recorded individual transponder's code by a handheld reader (Gesimpex Com. S.L., Barcelona, Spain); the other one visually checked and reported individual ear tattoo code on a handheld data store equipment (ABB Immediate Business System PLC – Radix).

Averaged times of reading and recording of EID vs ear tattoo code of animals resulted 6"/sheep vs 11"/sheep, respectively. On the whole of the check on 553 animals, reading and recording of transponder's code resulted 100% correct; ear tattoo code visual check and reporting was uncertain in 18 animals (3.2%). In particular, the technician couldn't read ear tattoo codes because totally or partly illegible, in 10 animals (1.8%). A wrong association of the same ear tattoo code to 2 different animals (double identification) concerned 8 animals (1.4%).

The field trial carried out under usual rearing conditions of Sarda breed sheep highlights that EID offers reliability and efficacy in individual identification of animals. EID shows to make easier the fundamental but hard and repetitive animal identity detection by the operator, during controls on milk production.

### 1. Introduction

The electronic identification (EID) represents an eminent topic within animal identification and anagraphical concerns, considering that starting from the 1st of January 2008 [4] it will be compulsory in the whole EU Countries. In agreement with the Reg. CE 21/2004 sheep will be identified by endorumenal boluses containing a transponder, according to ISO Standards 11784-11785 [5-6], deployed for the double identification of animals, paired to the application of an eartag as the first device for animal individual identification. The tattoo represents another device for individual animal's identification, deployed for double identification paired to eartag [4]. In this paper, Authors aimed to test the reliability, the efficiency and the accuracy offered by the electronic identification (EID) compared to the ear tattoo (ET), during milk production monitoring, as required for sheep farms of the Genealogical Register of Sarda breed [1].

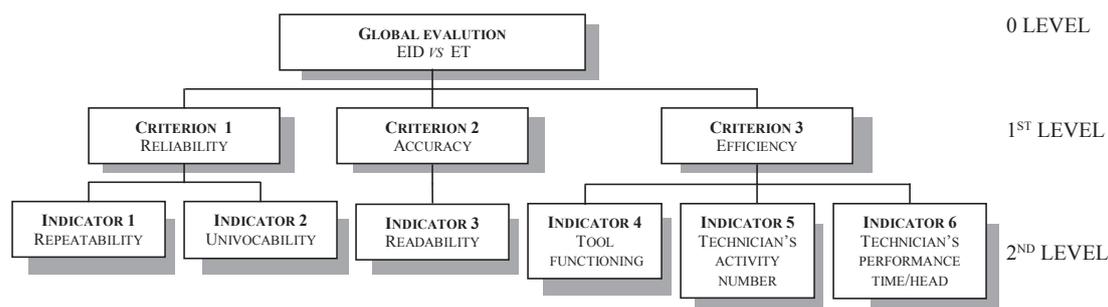
### 2. Materials and methods

The trial was carried out in 2 farms, under usual field conditions, at 2 successive monthly milk production controls. A total of 553 Sarda sheep (315 + 238 sheep per flock) of the Genealogical Register of Sarda breed kept by the Provincial Association of Farmers (APA) of Nuoro (Sardinia-Italy) were previously identified by ear tattoo (ET) consisting of APA alphanumeric 8 typeface code and by the electronic identification (EID) by the deployment of a passive HDX transponder

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(32.5×3.8 mm, ISO 11784-11785 Tiris 32 mm) administered by ceramic boluses (75 g. 70x21 mm *RUMITAG bolus*<sup>®</sup>). In both farms, sheep were milked by linear milking machine in 24 ties stall per cycle. 2 technicians for milk production controls worked as follows: one checked and recorded individual transponder's code by a handheld reader (Gesimpex Com. S.L.); the other one followed APA work activity and visually checked and reported individual ear tattoo code on a handheld data store equipment (ABB Immediate Business System PLC – Radix). Technicians activities performed in the whole process have been valued. The specific activities by 2 technicians according to the deployment of the two devices of identification have been classified as technical relationships and analysed. The Reliability of each device (EID vs ET) was valued comparing univocability (%) of individual anagraphical datum and the repeatability of reading identity codes (cross check of data) between the 2 successive monthly controls. The Accuracy of each device (EID vs ET) was valued according to readability (%) of individual anagraphical sheep datum. The Efficiency of each device (EID vs ET) was valued on the functioning of the equipments (n. turn off/control), the activities number (n. activity/technician/milking cycle) and the speed (min/technician/milking cycle) of performing. A matrix of technical items (Reliability, Efficiency and Accuracy) was analyzed by an Analytic Hierarchy Process (AHP) approach, using a matrix of technical items (Pinna et al., 2007), in agreement with the diagram exploded in Figure 1.



**Figure 1.** AHP evaluation (Pinna et al. 2007).

**Table 1:** Adjusted matrix of scored weights related to Reliability, Accuracy and Efficiency

	Investigated criteria			Averaged D score
	Reliability Y	Accuracy Y	Efficiency Y	
Reliability Y	0,546	0,571	0,50	0,539
Accuracy	0,273	0,286	0,33	0,297
Efficiency Y	0,182	0,143	0,167	0,167

A 3 score scale "alike" = 1, "favoured" = 2 "highly favoured" = 3 was used. Averaged scores 0.539, 0.297 and 0.167 related to Reliability, Accuracy and Efficiency, respectively, were calculated as reported in the matrix in Table 1.

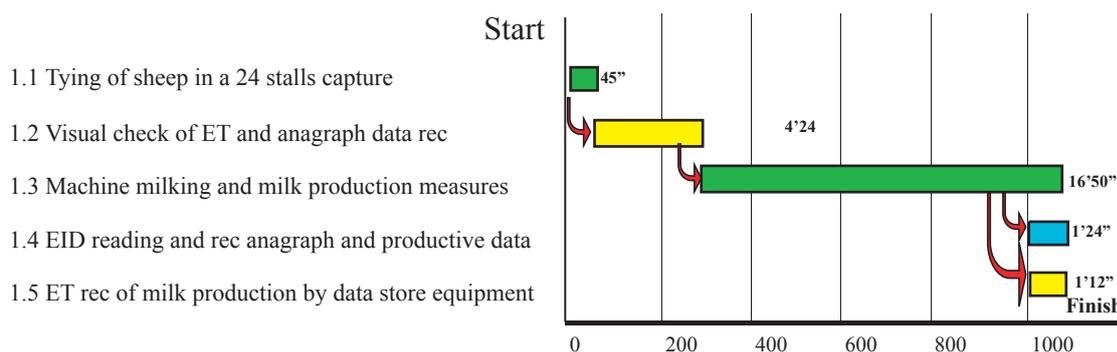
### 3. Results and discussion

Table 2 shows the whole process carried out on a 24 sheep cycle at machine milking and individual milk production controls as regards to time, links and the technical relationships: start to start (SS), start to finish (SF), finish to finish (FF), finish to start (FS).

**Table 2:** Process activities times, links and technical relationships.

CODE ID	ACTIVITIES	TIME	LINKS	TECHNICAL RELATIONSHIPS
1. Sarda sheep	<b>Start</b>			
1.1. (common)	Tying of sheep in a 24 stalls capture	45"± 8"	1	SS
1.2. (ET)	Visual check of ear tattoo and anagraphical data recording	4' 24"±15"	1.1	SS
1.3. (common)	Machine milking and measuring individual production of sheep	16'±50"	1.2	FS
1.4 EID	EID reading and recording anagraphical and productive data on handy reader	1'24"±5"	1.3	FF
1.5. (ET)	Recording of milk individual production on handy data store equipment	1'12"±15"	1.3	FF
	<b>Finish</b>			

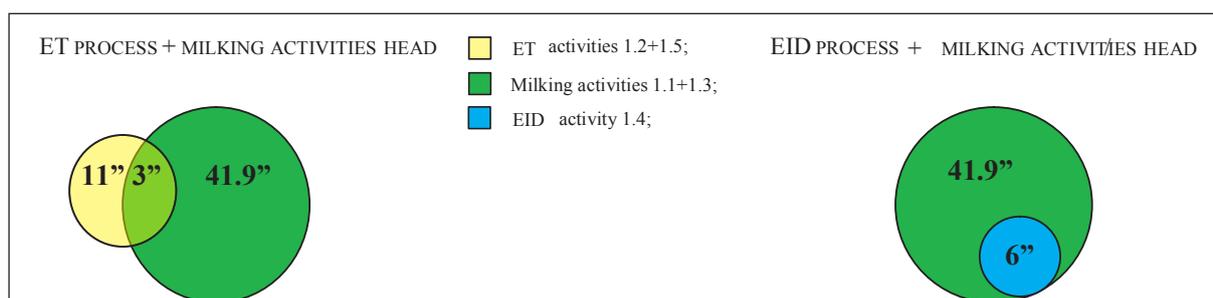
2 (1.1 and 1.3) of the 5 activities were identified like "common activities"; 2 activities (1.2 and 1.5) were specific of ET technician; 1 activity (1.4) was specific of EID technician. The timetable of activities reported in Table 2 is represented by a Gantt diagram (Fig. 2).



**Figure 2.** Gantt diagram of activities

On the whole process, milking time for a 24 sheep cycle took 16'45"± 58", on average, that's to say 41.9"± 2"/head. The time needed for the total activity by EID checks overlapped the milking time and took the only EID technician deployment for 6"/head (to read and record anagraphical + productive data). The time needed for activities by ET checks partly took an added time to milking time with the deployment of both technician and farmer for 11"/head (to read and record the anagraphical data) and partly took time for productive data recording overlapping the milking time with the only ET technician deployment for 3"/head.

EID and ET activities related to milking activities are outlined in Figure 3.

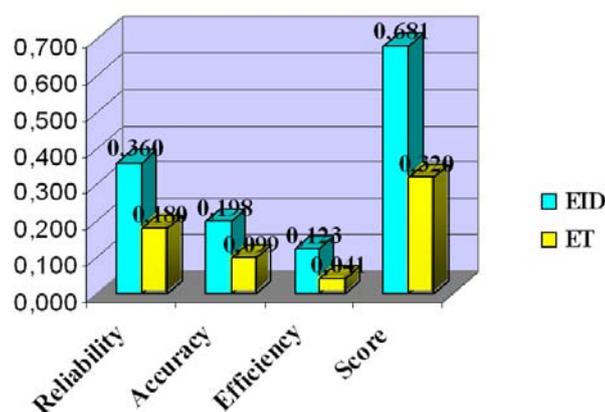


**Figure 3.** ET and EID activities related to the whole milking activities

On the whole of the 1106 checks on 553 animals, reading and recording of transponder's code resulted 100% and showed a 100% univocability of data. The repeatability of readings and cross checking of data recorded during the first and the second control by each device (EID vs ET) highlighted the correspondence between EID anagraphical data recorded and uncertain identity in 3.2% of sheep checked by ET. In particular, the ET technician was not able to read ear tattoo codes (because totally or partly illegible), in 10 animals (1.8%) with a ET readability of 98.2% (Accuracy loss). A wrong association of the same ear tattoo code to 2 different animals (double wrong identification) concerned 8 animals (1.4%) with a univocability of 98.6% (Reliability loss).

The handy reader for EID detection turned off twice (0.18%) on the total of 1106 (Efficiency loss). After turning on the handy reader, the transponder's readability and data recording were 100%.

Figure 4 shows the results of the global evaluation concerning the comparison between EID vs ET according to Reliability, Accuracy and Efficiency.



**Figure 4.** Reliability, Accuracy, Efficiency and global score EID vs ET

The global evaluation scores of EID vs ET were 0.681 vs 0.320 respectively. EID resulted more reliable (0.360 vs 0.180); more accurate (0.198 vs 0.099) and more efficient (0.123 vs 0.041) than ET during production controls.

#### 4. Conclusion

Results emerged from the comparison EID vs ET under usual field condition as regards to dairy sheep production, show a net lead of EID as far as reliability, accuracy and efficiency of the system for individual identification of heads, during milk production controls. The liking expressed by technicians for milk production controls as regards to simplification and facility of activities for anagraphical checks for sheep belonging to Genealogical Register of Sarda breed, confirm the results here reported.

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## I-P019: Occurrence of $\alpha_{s2}$ -casein (*CSN1S2*) B Variant in Sarda and Comisana Ovine Breeds

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### Summary

At the  $\alpha_{s2}$ -casein (*CSN1S2*) level, a protein polymorphism was identified in Gentile di Puglia, a fine-wooled ovine breed from southern Italy. The variant was named as *CSN1S2*\*B. In the present work we demonstrated the occurrence of *CSN1S2*\*B also in Sarda and Comisana breeds, which are important Italian dairy ovine breeds. Milk individual samples were analysed by isoelectrofocusing (IEF) carried out on ultrathin polyacrylamide gels with carrier ampholytes.

Two protein variants were found at *CSN1S2* in both breeds: *CSN1S2*\*A was predominant, but *CSN1S2*\*B was observed either in Sarda or in Comisana breed. Hardy-Weinberg equilibrium was demonstrated in both breeds. The large applicability of IEF, which is a cheap analytical test for milk analysis, could be widely exploited for typing lactating ewes at milk protein polymorphisms.

### 1. Introduction

The importance of the milk protein genetic polymorphisms is well known mainly for their effects on milk composition and technological quality. Several studies were developed in cattle and goat, while the ovine milk protein variability has been less extensively investigated. Despite the limited genetic knowledge on milk protein variability in sheep, interesting relationships between some ovine milk protein variants and traits of economical interest have been already described (Rampilli et al., 1992; Pirisi et al., 1999). These effects demonstrate the importance of a deeper knowledge on genetic casein variation in the ovine species.

At the  $\alpha_{s2}$ -casein (*CSN1S2*) level, a protein polymorphism was identified in Gentile di Puglia, a fine-wooled ovine breed from southern Italy, mainly reared for wool and meat production. The variant was named as *CSN1S2*\*B (Chessa et al., 2003). If compared with *CSN1S2*\*A variant, it is characterised by a higher isoelectric point, which could result either from a lost of negative charge (acid to neutral amino acid substitution), or from a gain of positive charge (neutral to basic amino acid substitution). A titration curve analysis suggested that *CSN1S2*\*A differs from *CSN1S2*\*B because of the replacement of a neutral amino acid by a basic one, which seems to be histidine on the basis of the comparison between the theoretical curves calculated for the ovine  $\alpha_{s2}$ -casein and obtained by adding an His to the deduced amino acid sequence of the  $\alpha_{s2}$ -cn mRNA (Di Luccia et al., 2003).

In the present work we demonstrated the occurrence of *CSN1S2*\*B also in Sarda and Comisana breeds (<http://www.assonapa.com/>), which are the most consistent dairy sheep breeds in Italy.

### 2. Material and methods

Milk individual samples from Comisana (n = 45) and Sarda (n = 39) breeds were analysed by isoelectrofocusing (IEF) carried out on ultrathin polyacrylamide gels (mm 2500\*1150\*0.3) with carrier ampholytes, by a protocol modified from bovine milk typing (Erhardt et al., 1998). The gel solution contained 0.17% (p/v) bisacrylamide, 4.9% (p/v) acrylamide, 48% (p/v) urea and a mixture of ampholines Pharmalyte at 6.5% (v/v): pH 2.5-5 (240 $\mu$ l), pH 4.2-4.9 (230 $\mu$ l), pH 5-7 (230 $\mu$ l). Both skimmed milk and isoelectric casein samples were analysed.

Genotype and allele frequencies were calculated. Moreover, Hardy Weinberg (HW) equilibrium was verified by  $X^2$  test.

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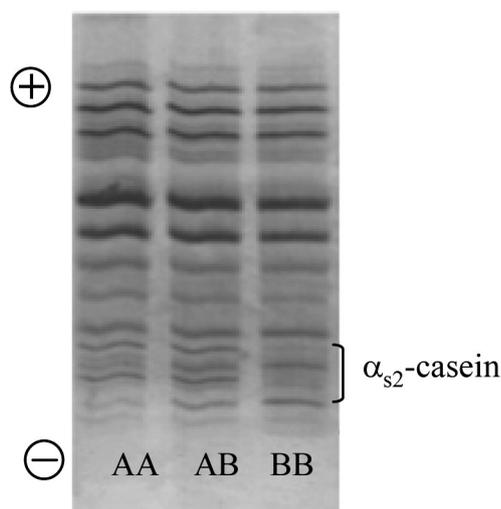
### 3. Results and discussion

Two protein variants were found at *CSN1S2* in both breeds. At IEF, the BB phenotype exhibits a more basic couple of the main  $\alpha_{s2}$ -casein bands with respect to the AA phenotype (Figure 1), as described in Gentile di Puglia breed.

The genotype frequencies, both observed and expected on the basis of HW equilibrium, are shown in Table 1. *CSN1S2*\*A allele was predominant, but *CSN1S2*\*B was observed either in Sarda or in Comisana breed (Table 2). As in Gentile di Puglia breed, Hardy-Weinberg equilibrium was demonstrated at *CSN1S2* locus.

The large applicability of IEF, which is a cheap analytical test for milk analysis, could be widely exploited for typing lactating ewes at milk protein polymorphisms.

The significance of the protein variation described at the *CSN1S2* level needs to be further investigated, due to the possible effects on milk composition, technological quality, and/or nutritional properties. In particular, the suggested replacement of a neutral amino acid by a basic one (Di Luccia et al., 2003) could strongly affect the casein micelle biochemical properties and the interactions among the four casein fractions within the micelle itself.



**Figure 1.** Isoelectrofocusing patterns of the three different genotypes observed at ovine  $\alpha_{s2}$ -casein (*CSN1S2*).

**Table 1:** Genotype observed and expected (HW equilibrium) frequencies at *CSN1S2* in the two breeds.

<i>CSN1S2</i>	Comisana (n = 45)		Sarda (n = 39)	
	Observed absolute frequency	Expected absolute frequency	Observed absolute frequency	Expected absolute frequency
AA	32	32.94	18	18.01
AB	13	11.12	17	16.99
BB	0	0.94	4	4.01

**Table 2:** Allele frequencies at *CSN1S2* and  $\chi^2$  test results (HW equilibrium) in the two breeds.

<i>CSN1S2</i>	Comisana (n = 45)		Sarda (n = 39)			
	Allele	Frequency	$\chi^2$ test	Allele	Frequency	$\chi^2$ test
A	A	0.856	$\chi^2_{[DF=1]} = 1.28$	A	0.679	$\chi^2_{[DF=1]} = 0.00$
B	B	0.144	$P > 0.257$	B	0.321	$P > 0.996$

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## I-P020: Lactational Effects of Omitting Two Milkings Weekly During Early- and Mid-lactation in Manchega and Lacaune Dairy Ewes

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### Summary

The aim of this work was to explore the effects of omitting 2 milkings weekly in early- (wk 7 to 15) and mid-lactation (wk 15 to 22) on milk yield, milk composition, and somatic cell count (SCC) of Manchega (MN, n = 42) and Lacaune (LC, n = 18) dairy sheep. Milk production was the most affected parameter by milking omission and varied between breeds. In MN breed, total milk yield only decreased significantly when milking omission was performed in early-lactation. Nevertheless, in LC ewes, the milking omission strategies did not cause losses in total milk yield. Milk composition was not affected by milking omission and SCC steadied throughout lactation in both breeds. In conclusion, omitting 2 milkings weekly could be an interesting strategy to reduce farm labor if it is performed at the suitable lactation stage according to breed characteristics.

### 1. Introduction

Mediterranean sheep milk is mainly produced in family flocks, where ewes are milked twice a day (morning and evening). This practice doesn't permit farmers to spend much time to other farming practices and/or to other activities off the farm. For this reason, any possible reduction in the number of milkings would improve the farmers' quality of life, making easier the sector durability and future growth.

For milking omission to become a practical strategy, it should have no deleterious effects on milk yield or milk quality. However, milking omission may reduce milk yield in dairy ruminants. In this sense, several studies have shown milk production decreases ranging from 10% in Assaf ewes (Hervás et al., 2006) to 69% in Tsigay (Mikus et al., 1983) when one or two milking omissions weekly were performed. In contrast, a study carried out in Manchega ewes showed no significant milk losses associated with one milking omission per week (Huidobro, 1988).

This study was conducted to evaluate the effects of omitting two milkings weekly in early- or mid-lactation on total milk yield, milk composition, and somatic cell count (SCC) in two dairy ewe breeds; a high-yielding (LC) and a medium-yielding (MN).

### 2. Material and methods

Sixty dairy ewes (MN, n = 42; and LC, n = 18) were used from the weaning of the lambs (wk 5) to wk 22. Ewes were divided into 3 groups according to the lactation stage at which milking omission was performed: **early-lactation** (wk 7 to 15), mid-lactation (wk 15 to 22), and **no milking omission** (wk 7 to 22). Ewes were milked twice daily (8:00 and 18:00 h) from the weaning of the lambs. From wk 7, the ewes submitted to milking omission treatments were milked twice daily from Monday to Friday, and once daily on Saturday and Sunday (16:00 and 14:00 h, respectively). Data were individually collected on Thursday for milk yield (weekly), milk composition (biweekly), and SCC (monthly).

### 3. Results and discussion

The milking omission treatments had different ( $P < 0.001$ ) effects according to breed (Figure 1). In MN ewes, during the experimental period (wk 7 to 22), total milk yield (control, 103 L) decreased significantly (-15%,  $P < 0.05$ ) when two milkings weekly were omitted in early-lactation. However, no significant losses (-4%,  $P = 0.47$ ) were observed when milking omission was

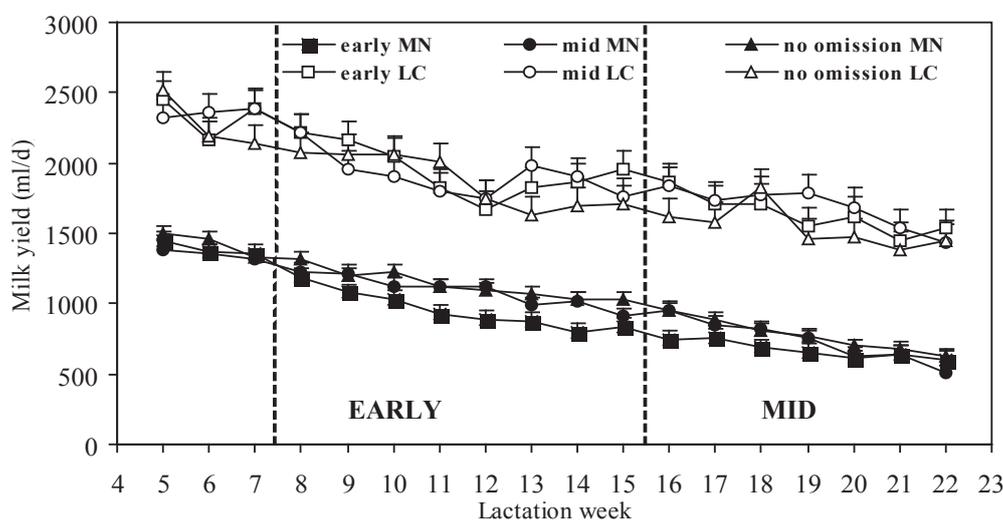
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performed in mid-lactation. In LC (control, 188 L), the milking omission strategies did not cause significant losses in total milk yield. Sheep breed, determinant of some characteristics such as the potential of production, mammary morphology or cisternal capacity of dairy ewes, may affect the response to the omission of some milkings weekly (Labussi re, 1988). Moreover, studies performed in dairy ewes (McKusick et al., 2002), dairy cows (Ayadi et al., 2003) and dairy goats (Salama et al., 2004) showed that the size of mammary cisterns, and the milk distribution within the cisternal and the alveolar compartments, are important factors in determining milk yield losses associated with extended milking intervals. Thus, the greater capacity of LC ewes to store milk in the cisternal compartment, in comparison with MN, could explain the better aptitude of this breed to tolerate extended milking intervals with few milk yield losses.

As it was previously mentioned, in medium-yielding ewes (MN), milk yield losses from ewes submitted to milking omission were significantly lower in medium- than in early-lactation. These results are in agreement with previous studies in dairy cows, where the greatest reduction in milk yield also occurred when once daily milking was performed in early-lactation (Carruthers et al., 1993; Stelwagen and Knight, 1997). We propose that the decrease of milk yield throughout lactation allows mammary cistern to become big enough to store greater proportions of milk during mid-lactation, favoring the decrease of the negative effects on milk yield, produced by local intramammary control.

Regarding milk composition, milking omission treatments did not affect fat (MN, 7.2%; LC, 6.2%), crude protein (MN, 5.9%; LC, 5.0%), casein (MN, 4.7%; LC, 3.9%), lactose (MN, 4.7%; LC, 4.5%), or total solids (MN, 19.0%; LC, 17.0%) concentrations. Moreover, the logSCC was similar between breeds (MN, 5.04; LC, 5.12) and steady throughout lactation without effects of milking omission treatments in both breeds. However, these results must be interpreted with caution because milk components, especially fat, may vary significantly the days after the milking omissions (Ayadi et al., 2003; Herv s, 2006).



**Figure 1.** Effects of omitting two milkings weekly in early- (□, ■), mid-lactation (○, ●) and no milking omission (Δ, ▲) on milk yield in MN (in black) and LC (in white) dairy ewes

#### 4. Conclusions

Our results suggested that omitting two milkings weekly in mid-lactation could be a useful management strategy to reduce farm labor without negative impact on milk yield, milk composition, or udder health in dairy sheep. High yielding dairy sheep are also capable to satisfactorily tolerate weekend milking omission in early-lactation.

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## I-P021: Relationships Between SCC and Udder Morphology Traits in Sardinian Sheep

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### Summary

The aim of this paper was studying the phenotypic and genetic relationships between udder morphology traits and udder health in Sardinian sheep. Data were collected in an experimental flock from 1999 to 2006. Primiparous ewes were scored for 4 udder traits with a linear scoring method 3 times/year on average. Somatic Cell Count (SCC) were measured once a month on milk samples from a.m. and p.m. milking. Mastitis records were collected from 2000. First, a logistic regression was performed to evaluate the risk for a ewe of having either a mastitis or at least 2 TD with  $CCS > 8 \times 10^5$  cells/ml in one of her lactations as function of udder traits scored in 1<sup>st</sup> lactation. Secondly, heritability of SCC and genetic correlations with udder traits were estimated. Test day records of SCS (log transformation of SCC) and udder traits were adjusted for the main environmental effects by using repeated measurement models which included the ewe as random effect. The sum of ewe solution and residuals was averaged by individual, in order to obtain a lactation measure of each trait to be used for genetic analyses. Relationships between animals included 3,989 individuals, 171 of which were sires of ewes with performance. Variance and covariance components were estimated by a REML method applied to four bi-trait animal models. Logistic regression indicated that the risk for a ewe to have a mastitis or high SCC values during its productive life increased as the cistern height increased and the degree of udder suspension decreased. Genetic correlations between lactation SCC and udder traits were high and favourable. Thus selection for udder morphology, recently implemented in the breeding scheme of the Sardinian breed, will lead to a favourable correlated genetic response on SCC.

### 1. Introduction

Udder health is an important issue for dairy sheep since it can affect the milk production and the functional longevity of animals [1]. Somatic cell count (SCC) is considered an indicator of mammary gland health state [2]. Indeed, threshold values of SCC have been proposed to detect subclinical infections [3]. Generally, favourable phenotypic and genetic relationships have been reported between some udder conformation traits and SCC in cattle [4]. Recently, a linear scoring method to appraise 4 udder traits has been set up and implemented in the registered flocks of the Sardinian breed [5]. The overall objective of this study was to investigate relationships between udder health and udder conformation traits. Specific objectives were 1) investigating phenotypic relationships between primiparous udder morphology and the risk of occurrence of a mammary inflammatory status during the ewes' productive life 2) estimating the genetic correlations between lactation somatic cell score (LSCS) and udder traits in primiparous ewes.

### 2. Material and methods

Udder traits, SCC and mastitis records were collected in an experimental flock of Sardinian ewes from 1999 to 2006. Teat placement (TP), udder depth (UD), degree of separation of the 2 halves (DS), and degree of suspension of the udder (SU) were scored 3 times a year on average on primiparous ewes using the 9-point linear scoring method of the Sardinian breed [5]. SCC were measured by a Fossomatic cell counter from milk samples monthly collected at a.m. and p.m. milking. Daily SCC were computed as the weighted mean of evening and morning values. Test-day SCC were log-transformed to Somatic cell score (SCS) [6]. From 2000 ewes showing clinical mastitis signs (modification of the colour or consistency of the milk; hot, swollen or painful

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udder) were recorded. In order to study the risk of a mammary inflammatory status during the productive life as a function of udder morphology, the binary variable MIS (mammary inflammatory status) was defined 'POSITIVE' either for a ewe with a recorded mastitis or a ewe showing at least 2 daily SCC records higher than 800,000 cell/ml in at least one of their recorded lactations. A logistic regression was performed to model the risk of MIS being POSITIVE as a function of SU, TP, UD and DS scored in first lactation. Heritabilities of SCS and genetic correlations with udder traits were estimated on records from primiparous ewes (n=2029). Test day records for SCS (n=8651) and udder traits (n=5972) were adjusted for the main environmental effects by using repeated measurement univariate models which included the ewe as random effect. The fixed effects for SCS were test date (50 levels), stage of lactation (12 levels) and number of suckled lambs (1, 2 or more). The fixed effects for udder traits were year of scoring - lambing period interaction (15 levels), classifier within date of scoring (66 levels), year of scoring-number of suckling lambs interaction (16 levels). The sum of ewe solution and residuals of the mixed models was averaged by individual, in order to obtain a lactation measure of each trait to be used for genetic analyses. Variance and covariance components were estimated by a REML method applied to four bi-trait models. Relationships between animals included 3,989 individuals, 171 of which were sires of ewes with performance.

### 3. Results and discussion

Table 1 shows basic statistics of the recorded traits.

**Table 1:** Number of records, means and standard deviation of daily Somatic Cell Score in first (SCS 1<sup>rst</sup>) and all lactations (SCS<sub>all</sub>), degree of suspension of the udder (SU), teat placement (TP), udder depth (UD), degree of separation of the 2 halves (DS)

Trait	n	Mean ± s. d.
SCS 1 <sup>rst</sup>	8651	4.22 ± 2.00
SCS <sub>all</sub>	31037	4.66 ± 2.05
SU	4515	4.72 ± 1.41
UD	5936	6.66 ± 0.89
TP	5965	7.31 ± 1.20
DS	5831	6.68 ± 1.06

MIS resulted POSITIVE for 44.99% of ewes, 18.22% of which showed clinical mastitis signs. The logistic analyses showed that the risk for a ewe of an inflammatory status of the udder significantly increased as SU and TP scores worsened. No significant relationship was found between MIS and UD or DS. Repeatability estimate of daily SCS in first lactation was 0.36.

Heritability of SCS lactation measure (table 2) was low but fell in the range of estimates found in literature [1; 2; 7]. Udder traits  $h^2$  were higher than values estimated in the Sardinian herd book [5]. Estimates of genetic correlations between SCS lactation measure and udder traits ranged from 0.15 to 0.66 (absolute values) and were favourable (Table 2).

**Table 2:** Heritability ( $h^2$ ) of lactation Somatic Cell Score (LSCS), degree of suspension of the udder (SU), teat placement (TP), udder depth (UD), degree of separation of the 2 halves (DS) and genetic correlations ( $r_g$ ) between LSCS and udder traits in primiparous Sardinian ewes

	$h^2(\pm SE)$	$r_g(\pm SE)$
LSCS <sup>1</sup>	0.15-0.17 ± 0.04	-
SU	0.56 ± 0.06	-0.66 ± 0.10
UD	0.58 ± 0.06	-0.64 ± 0.10
TP	0.58 ± 0.05	+0.45 ± 0.12
DS	0.33 ± 0.05	-0.15 ± 0.16

<sup>1</sup> range of estimates by the bi-trait analyses

## 4. Conclusion

Results show that both phenotypic and genetic relationships exist between udder morphology and udder health in dairy sheep. Udder scoring performed in first lactation can be a useful tool to detect animals with high risk of a mammary inflammatory status or showing high SCS during their productive life. Genetic correlations between LSCS and udder traits indicate that selection for udder morphology, recently implemented in the breeding scheme of the Sardinian breed, will lead to a favorable correlated genetic response on SCC.

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## I-P022: Occurrence of Genetic Polymorphism at Goat $\beta$ -CN Locus

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### Summary

In this work the occurrence of a new genetic variant of goat  $\beta$ -CN was reported. Samples taken from a population of goats in the Campania region of Italy were analysed by means of an immuno-electrophoretic technique and LC/ESI/MS analysis. Results obtained by PAGE at alkaline pH and UTLIEF analysis showed the new variant had the lowest mobility towards the anode by PAGE at alkaline pH and an highest pI value by UTLIEF respect to the common variant C. By means of LC/ESI/MS analysis the molecular weight difference between them was  $\Delta M = 20$  Da. The relatives MW are in accord to Tyr<sup>47</sup>(E)→Asp<sup>47</sup>(C) and Ala<sup>177</sup>(E)→Val<sup>177</sup>(C) substitutions as confirmed by LC/ESI/MS/MS analysis.

### 1. Introduction

$\beta$ -CN is the most abundant casein fraction in goat milk; his composition was variable among individual milks due to genetic polymorphism or post-traslational modifications such as the different phosphorylation degree (Chianese et al., 1993). At present, the genetic polymorphism of goat  $\beta$ -CN is due to the presence of 5 alleles A,B,C,D e 0 (null allele); the genetic variants  $\beta$ -CN A, C and D, whose each primary structure are known, are indistinguishable each other with the useful electrophoretic technique such as PAGE at alkaline pH or UTLIEF, due to their silent a.a. substitution Ala<sup>177</sup>(A)→Val<sup>177</sup>(C) (Neveu et al., 2002) and Val<sup>207</sup>(C)→Asn<sup>207</sup>(D) (Galiano et al., 2004). The occurrence of "full" and "null" alleles to which correspond the presence or the absence of goat  $\beta$ -CN in the milk had a very important impact on technological quality of milk. In fact, when was assessed the milk aptitude of milk containing null allele of  $\beta$ -CN, in comparison to the one of "full" alleles, a worsening of the rheological parameters ( $r$ , K20 and A30) was recorded (Chianese et al., 1993).

Moreover the immunostaining with specific polyclonal antibodies of UTLIEF profiles of individual goat casein (Chianese et al., 1993; Chianese et al., 2000) showed a variable number of bands in  $\beta$ -CN depending by multiple phosphorilations of the protein chain. Samples having 1-6 or 2-6 phosphate groups (P)/molecule were found, although the most frequent phenotype had 5-6 P/molecule.

In this study the occurrence of a new genetic variant of  $\beta$ -CN in goats autochthonous breed reared in Campania region, exhibiting the lowest negative charge at alkaline pH respect to the common variant  $\beta$ -CN C, was reported.

### 2. Material and methods

A total of 80 individual milk samples were analysed from an autochthonous goat breed, reared in southern Italy (Campania region). Individual caprine milk was skimmed by centrifugation at 4000 rpm and the isoelectric casein prepared following the procedure described by Aschaffenburg & Drewry (1959). The determination of casein genetic polymorphism was carried out according to the procedure of Chianese et al. (1992). High-Resolution ESI/MS/MS was performed with Micromass QTOF Ultima Api (Waters) as described by Ferranti et al. (1998).

### 3. Results and discussion

#### *PAGE analysis and immunoblotting of whole casein*

The most common  $\beta$ -CN phenotypes in lanes 1, 2, 3 (Fig. 1 - panel A), unlike sample in lane 5 where 3 components are showed, are consisting in the two main components  $\beta$ -CN 6P and  $\beta$ -CN 5P having the same anodic mobility towards the anode and higher than the counterparts components in lane 4. The belonging to  $\beta$ -CN casein family of  $\beta$ -CN components was confirmed

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by immunoblotting with specific polyclonal antibodies (Fig. 1 - panel B). In accordance to the official nomenclature in this field, the new  $\beta$ -CN variant was identified with the letter E. Comparing among them the most common phenotype in lane 3 with  $\beta$ -CN in lane 5, we can suppose that the latest profile is an heterozygous form of the common A or C variants with the new variant E.

#### ESI/MS analysis

To verify our hypothesis, the three samples were submitted to ESI/MS analysis to determine the relative MW before and after phosphatase alkaline action. The results are reported in following table 1.

From this data we can conclude that:

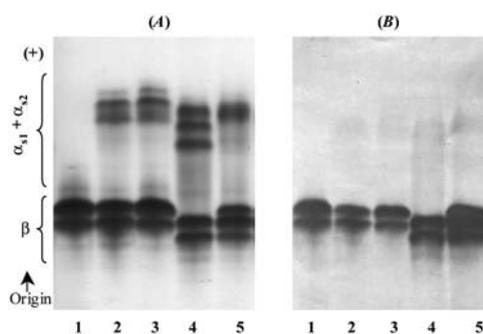
- the most common phenotype of  $\beta$ -CN in lane 3 is  $\beta$ -CN CC;
- in this flock the new  $\beta$ -CN EE variant was occurred in homozygous and heterozygous forms (lane 4 e 5); its MW was 20 Da higher than  $\beta$ -CN CC.

This different mass does not correspond to any single amino acid substitution. To this aim the tryptic digest of both  $\beta$ -CN EE and  $\beta$ -CN-CC was analysed by LC/MS to identify the modified peptide(s).

The peptide sequence determined for the E variant was identical with variant C except for two substitutions located in peptides 33-48 and 177-181 (table 2).

Peptide sequencing using MS/MS analysis demonstrated that  $\Delta M$  was consisted with substitution Tyr<sup>47</sup>(E)→Asp<sup>47</sup>(C) in 33-48 peptide and Ala<sup>177</sup>(E)→Val<sup>177</sup>(C) in 177-181 peptide.

The identification of these amino acid substitutions justified the lowest negative charge at alkaline pH respect to the common variant  $\beta$ -CN C.



**Figure 1.** Disc-PAGE at pH 8.6 of whole caprine samples containing different  $\beta$ -CN variants. Staining was carried out with Coomassie R-250 brilliant blue (A) and polyclonal antibodies against (B)  $\beta$ -casein.

**Table 1:** Proteins identified by LC/MS analysis

Sample	Molecular mass (Da)		$\Delta M^a$	Genotype
	Native protein	Dephosphorilated protein		
3	23849 (6P)	23369		CC
	23769 (5P)			
4	23869 (6P)	23389	20	EE
	23789 (5P)			
5	23849 (6P)	23369	20	CE
	23769 (5P)			
	23869 (6P)	23389		
	23789 (5P)			

<sup>a</sup> difference of MW

**Table 2:** Comparison of amino acid sequences of goat  $\beta$ -casein variants

Variable peptide	Quasi-molecular ion, m/z (Da)	Variant
33-48	1981.9	C
	2029.9	E
177-181	569.3	C
	597.4	E

## 4. Conclusion

Today most attention was done to safety of milk (either microbiological or intolerance point of view ) mainly in infant nutrition when human milk is replaced by "bovine" infant formula. In this regard in New Zealand bovine milks labelled " $\beta$ A1 free" are in the market targeted for infants can develop diabetes mellitus. The difference between bovine  $\beta$ A1 and  $\beta$ A2 consists in amino-acid substitution Pro<sup>67</sup>(A2) $\rightarrow$ His<sup>67</sup>(A1).

Even if only few data are disposable about positive effects of goat milk in infant intolerant to bovine milk (Bevilacqua et al., 2000) this kind of milk is already in the Italian market for infant feed as replacer of bovine milk.

One raison of this better tolerance could depend on the presence of Pro67 in all goat  $\beta$ -genetic variants.

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## I-P023: Primary Structure of Ovine Deleted Variant $\alpha_{s1}$ -CN E

L. Chianese<sup>1</sup>, S. Caira<sup>2</sup>, G. Garro<sup>1</sup>, S. Lilla<sup>1</sup>, F. Addeo<sup>1</sup>

### Summary

$\alpha_{s1}$ -CN E was detected in the Italian ovine Leccese breed and characterised using a proteomic approach.  $\alpha_{s1}$ -CN was specifically localised along the 2D map through polyclonal antibodies against  $\alpha_{s1}$ -CN. Of the four stained spots, the major one in the Coomassie-Blue stained gel was excised and the molecular mass measured by ESI-TOF/MS. Native  $\alpha_{s1}$ -CN E accounted for 22316Da while that of the dephosphorylated protein using alkaline phosphatase was 21918Da. The 399Da mass shift indicated that native protein contain five phosphate groups. Analysis with nanoLC-ESI-MS/MS of the tryptic digest allowed us to identify  $\alpha_{s1}$ -CN E as an internally deleted protein lacking of peptide 70-77. This deletion cause the loss of four SerP residues located at sites 64, 66, 68 and 75.

Compared to other known ovine  $\alpha_{s1}$ -CN variants A, B, C and D, the structural features of the  $\alpha_{s1}$ -CN E justify the previous electrophoretic findings indicating the latter as that *i)* moving slower towards the anode by PAGE at alkaline pH; and *ii)* having higher isoelectric point.

### 1. Introduction

“Proteomics” combining 2D gel electrophoresis with very sensitive methods of polypeptide identification provides detailed analysis at the protein level comprising the number and identity of the proteins present. Proteomics involves combination of a high resolution separation technique (e.g. 2D gel electrophoresis) and analytical processes on micro-scale (e.g. tryptic digestion and mass spectrometry) for single protein identification, and characterisation of post-translational modifications.

In combination with image analysis, proteomics becomes a powerful means for detecting changes in protein composition, and identifying the proteins affected by the changes.

The importance of the proteomic approach has been demonstrated in the recent determination of ovine casein phosphoproteome (Mamone et al., 2003). Moreover, the protein heterogeneity of ovine casein is complicated by the presence of multiple phosphorylated chains depending either from the activity of the native mammary kinases (Mercier J.-C., 1981) or from an abnormally high alkaline phosphatase activity

depending on the increased somatic cell counts. Recent application of this analytical means to individual caseins from ewes belonging to Leccese breed, reared in the Puglia region, has shown genetic diversity of  $\alpha_{s1}$ -caseins.

The use of powerful 2D technique assisted by the use of specific polyclonal antibodies was able to exactly define the profile of  $\alpha_{s1}$ -casein components (Chianese et al., 1992; Mamone et al., 2003). The aim of this present study was to establish characterisation of the mutant  $\alpha_{s1}$ -CN E differing, as previously reported by Chianese et al. (1996), for the lower negative charge and higher pI compared to that of A, C and D counterpart. Ewe's  $\alpha_{s1}$ -CN E has resulted in an internally deleted protein which adds to the expression of six alternative splice-variants each shorter than the 199-residue long mature  $\alpha_{s1}$ -CN (Ferranti et al., 1998). This study provides a basis for future experiments to examine genotypic and cheese-making interactions.

### 2. Materials and methods

Individual whole ovine casein from local breeds was prepared following the procedure described by Aschaffenburg & Drewry (1959). Vertical disc-PAGE at pH 8.6, two dimensional (2D) electrophoresis, preparation of casein samples and immunoblotting were carried out according to the procedure of Chianese et al. (1992). The in-gel tryptic digestion was performed following the procedure described by Mamone et al. (2003). High-Resolution nanoLC-ESI/TOF-MS was performed with Q-TOF Ultima Api coupled with CapLC (Waters, Milford. MA) .

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### 3. Results and discussion

#### *Immunoelectrophoretic analysis*

The most common phenotypes  $\alpha_{s1}$ -CN AA, BB, CC, CD and CE detected in single milks are grouped Figure 1; each  $\alpha_{s1}$ -CN phenotype consists of three main phosphorylated bands. With respect to C, the D variant had lost three phosphate groups e.g. Ser64, Ser66 and Ser68 (Ferranti et al., 1995) which justifies its lower mobility towards the anode. Therefore  $\alpha_{s1}$ -D is 6P and 5P. In comparison to " $\alpha_{s1}$ -C", " $\alpha_{s1}$ -D" containing milk has demonstrated the worst aptitude to cheese-making (Pirisi et al., 1999). Mobility of  $\alpha_{s1}$ -E is lower than that of the D variant. Using 2D analysis (PAGE vs-UTLIEF) and specific polyclonal antibodies raised against the  $\alpha_{s1}$ -CN (Figure 2, panel b) assisted the recognition of the spot of the new casein variant along the Blue-Coomassie stained gel (Figure 2, panel a). Indeed, four components belonging to  $\alpha_{s1}$ -CN E were detected. After recovery of each gel spot whole protein and tryptic digests were submitted to MS analysis according to the procedure previously outlined by Mamone et al. (2003).

#### *ESI/MS analysis*

The molecular mass measured by ESI/MS analysis gave a 22316Da value for the native protein under the spot 1 (Figure 2, panel c) which decreased to 21918Da after dephosphorylation by alkaline phosphatase treatment. The molecular mass difference was indicative of a 5P protein. The 840Da mass difference respect to  $\alpha_{s1}$ -CN C taken as reference variant indicated a probable peptide deletion. Tryptic digests of native and dephosphorylated spot 1 were analysed by nanoLC/ESI-MS. In the tryptic map of  $\alpha_{s1}$ -CN E the 1805Da  $\alpha_{s1}$ -CN C(f62-79) fragment was missing, replaced by a peptide having mass of 2313Da. The MS/MS analysis of this peptide show the presence of two missed cleavage sites by trypsin, and the lacking of sequence stretch between Glu69 and Gln78 of  $\alpha_{s1}$ C. By comparison with C, the E variant was internally deleted for the peptide (f70-77) accounting for 840Da matching the molecular mass difference between E and C variant.

### 4. Conclusion

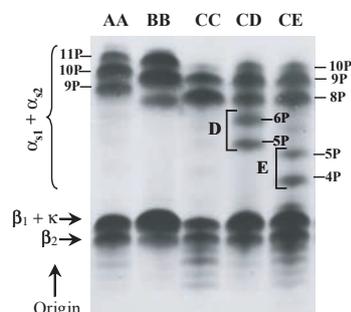
The casein fraction, both in total amount and functional composition, play an important role in determining the cheese-making aptitude of milk. Knowledge of genotype alone does not allow to control the consequences of milk quality fluctuations; on the other side a detailed analysis of protein composition can provide extensive information because of its direct relationship to phenotype. Identification of individual caseins as markers of phenotype has a great value for quality of ovine cheese. A further reason for detailed casein composition is to gather information about changes occurring by introducing genetic improvement through male introduction from abroad. Particularly important in determining the functional properties of milk are the caseins involved in micelle formation. The achievement of more detailed information about the proteins within breeds complement studies on cheeses.

Studies of ovine caseins having provided a wide range of genetic variability within  $\alpha_{s1}$ -CN fraction, affording to the characterisation of the internally deleted  $\alpha_{s1}$ -CN E variant.

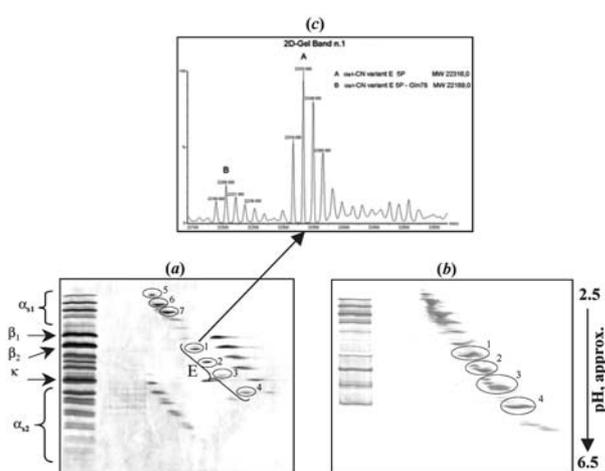
The simultaneous loss of four phosphorylated groups in the  $\alpha_{s1}$ -CN C (f70-77) peptide, including residues SerP 64, 66, 68 and 75, has detrimental effects on cheese-making aptitude of milk as it enhances the ability of casein to remain soluble in the presence of calcium, as previously suggested (Chianese et al., 1996).

Other ovine  $\alpha_{s1}$ -CN, namely the D variant, are underphosphorylated but this variation depends on the amino acid substitution within the sequence consensus for phosphorylation. Therefore,  $\alpha_{s1}$ -CN E has both the lowest number of phosphorylated residues and chain length shorter than the mature ovine counterparts. In this paper, we have highlighted the potential of proteomics to describe milk quality on molecular terms, and illustrated the quality concept with an argument of caseins diversity: the underphosphorylated  $\alpha_{s1}$ -CN E and D and fully phosphorylated  $\alpha_{s1}$ -CN variants. In addition, we emphasize that further methods are essential for analysing relative proportions of these variants in cheese-milk.

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**Figure 1.** PAGE at pH 8.6 of individual whole ovine casein samples containing different  $\alpha_{s1}$ -CN phenotypes indicated on the top of each lane. Staining was with Coomassie brilliant blue. P=phosphate group.



**Figure 2.** 2D electrophoresis (disc-PAGE in the first dimension followed by isoelectric focusing (UTLIEF) in the second dimension) of whole ovine casein sample containing  $\alpha_{s1}$ -CN E variant. Staining with Coomassie stain (a) and with antibodies preparation against (b)  $\beta$ -CN. Inset (c) shows the ESI/TOF-MS of the 2D gel, spot 1.

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## I-P024: Furosine Content and the Acid-soluble Whey Protein Composition - Possible Heat Indicators in Sheep and Goats Milk

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### Summary

The furosine contents and the acid-soluble contents of the different whey proteins were determined in representative bulk milk samples of ovine and caprine milk after holder pasteurization or HTST-heating and compared with results on bovine milk. No appreciable denaturation of whey proteins was measured in bovine, ovine and caprine milk after heat treatment. However, a significant increase of the furosine content was observed in all cases after holder pasteurization (HP), but not after HTST-heating. Besides activity determination of alkaline phosphatase (ALP), determination of the furosine content is thus an appropriate indicator for characterizing the heat treatment of sheep and goats milk and cheese.

### 1. Introduction

Heat induced reactions are well investigated and used for the characterization of heat treated bovine milk. For sheep and goats milk, however, only few data are available about suitable parameters for characterizing heat treatment. The furosine content has proved to be an appropriate indicator both for assessing the heat treatment of bovine milk and dairy products (cheese) and for monitoring milk adulterations. We have therefore examined the suitability of the chemical parameter furosine along with other possible heat indicators like the denaturation of the different whey proteins for characterizing the heat treatment of sheep and goats milk and cheese.

### 2. Material and methods

Furosine contents were determined in the acid hydrolyzate of milk samples by ion-pair reversed-phase chromatography [1-3]. The determination of the acid soluble content of whey proteins ( $\alpha$ -Lactalbumin,  $\beta$ -Lactoglobulin and Immunoglobulin) was performed by reversed-phase HPLC calibrated with bovine protein standards [4,5]. The ALP-activity was determined by the Fluorophos<sup>®</sup> method [6]. Holder pasteurization (HP I: 62°C, 30 min and HP II: 65°C, 32 min) was carried out by heating the milk in a water bath; short-time heating (HTST: 75°C, 28 s) was carried out in a plate heat exchanger (APV, Unna, Germany).

### 3. Results and discussion

For recording seasonal variations the furosine content of sheep and goats bulk milk samples was examined every 2 weeks from approx. 4 weeks *post partum* until the end of the lactation period and compared with results on bovine milk. Independently of lactation stage and period relatively constant furosine contents between 5.5 and 6.4 mg/100 g protein were measured in bovine raw milk. According to the examined first lactation period the furosine content of raw sheep and goats milk showed greater variations (8.0 – 11.4 and 6.0 – 8.8 mg/100 g protein).

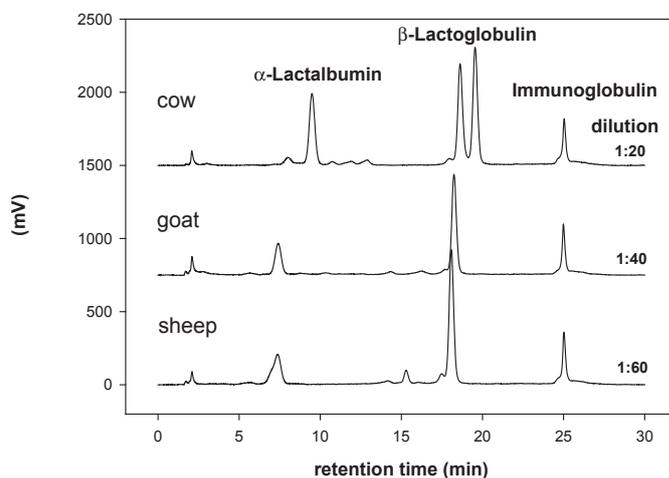
After holder pasteurization (HP I and II) a distinct increase of the furosine content in the milk of all three species by approx. 5 mg/100 g protein was recorded relative to the raw milk (Tab. 1). In HTST-heated milk only a small increase of the furosine content of approx. 1 mg/100 g protein was determined. The acid-soluble contents of the whey proteins (Fig. 1) showed large seasonal variations in sheep and goats raw milk as well as in bovine raw milk (Tab. 2). No significant denaturation of  $\alpha$ -lactalbumin and  $\beta$ -lactoglobulin and only a slight denaturation of the more heat sensitive immunoglobulin was assessed (at HTST up to 30%). Initial results showed that

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the determination of the furosine content can also be used for characterization the heat treatment of cheese from sheep and goats milk (Tab. 3).



**Figure 1.** HPLC chromatograms of the different whey proteins of bovine, caprine and ovine milk

**Table 1:** Furosine content of holder pasteurized bovine, ovine and caprine milk

holder pasteurization	raw milk	furosine (mg / 100 g protein)			
		HP I (62 °C, 30 min)		HP II (65 °C, 32 min)	
cow	4.9	10.9	± 0.08	13.5	± 0.29
sheep*	10.6	10.8	± 0.08	16.7	± 0.33
goat*	6.6	9.2	± 0.10	11.0	± 0.22

\*Raw milk samples stored before investigation at -20 °C

**Table 2:** Seasonal variation of the different whey proteins

bulk milk 14-day (May-November)	α-Lactalbumin (mg/100 ml)		β-Lactoglobulin (mg/100 ml)		Immunoglobulin (mg/100 ml)	
	mean	min-max	mean	min-max	mean	min-max
cow (n=9)	118	90 - 150	411	300 - 577	67	40 - 103
sheep* (n=9)	157	125 - 219	642	522 - 742	131	97 - 147
goat* (n=13)	104	87 - 165	292	201 - 425	79	56 - 106

\*Raw milk samples stored before investigation at -20 °C

**Tab. 3:** Furosine content and APL-activity of commercial cheese samples

Cheese samples	heat treatment	furosine (mg/100 g protein)	ALP (U/kg)
hard cheese (sheep)	pasteurized	6.4	<0.01
soft cheese (sheep)	pasteurized	27.9	<0.01
hard cheese (sheep)	pasteurized	34.5	<0.01
hard cheese (sheep)	raw milk	23.9	<0.01
fresh cheese (goat)	heat treated	6.9	<0.01
fresh cheese (goat)	pasteurized	12.8	<0.01
soft cheese (goat)	pasteurized	8.1	<0.01
soft cheese (goat)	raw milk	11.4	<0.01
soft cheese (goat)	raw milk	5.8	340.0

## 4. Conclusion

Besides activity determination of ALP, the determination of the furosine content has proved to be an appropriate indicator for characterizing the heat treatment of sheep and goats milk.

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## I-P025: Study on the Discriminating Power of Fatty Acids of Saanen Breed Goat Milk

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### Summary

The aim of this research was to investigate the influence of the stage of lactation on the fatty acid composition of goat milk fat. Fatty acid composition, together with the fat, protein and lactose content, was determined on 22 goat milk samples, three times during lactation. Univariate and multivariate statistical techniques were applied to evaluate the data set. The results of PCA showed the presence of three groups according to the sampling periods. The ability of fatty acid composition to discriminate the different lactation periods was demonstrated by applying the LDA on a reduced data set, including only variables selected on the basis of their discriminating power. Validation was performed with an external evaluation set.

### 1. Introduction

The fatty acid composition of milk fat is subjected to variations mainly due to differences in the diet of the animals, the season and the stage of lactation. The influence of these factors on the cow milk fat has been extensively investigated, while little information is available on goat milk fat, particularly as far as the stage of lactation is concerned (Kondyli et al. 2002; Chilliard et al. 2003; Soryal et al. 2004). The aim of this research was to evaluate the fatty acid composition of milk of goats, fed the same diet, and to investigate the ability of these compounds to discriminate between the different lactation stages.

### 2. Materials and methods

Twenty-two multiparous Saanen breed goats having approximately the same parturition date, were selected for the milk sampling. During all the lactation period the goats were fed hay (2.5kg/die) and concentrate (0.8 kg/die). In addition to this controlled diet, the goats received a small amount of energy from grazing in a fenced wood. Milk samples were taken three times: in May, July and September corresponding to a mean period of lactation of 71, 140 and 202 days, respectively. Milk yield was recorded and gross composition (protein, fat, lactose) was determined by mid infrared spectroscopy. The milk fat was extracted by ISO 14156 (2001) and analysed as methyl esters (ISO 15884:2002) by GC (ISO 15885:2002). Methyl nonanoate was added as internal standard, and the results, for each fatty acid, were expressed as mg/100g of fat.

All the results were statistically analysed by ANOVA. The data set consisting of 66 objects (22 milk samples collected three times) and 23 variables (23 fatty acids) was categorized on the basis of the 3 sampling periods (M, J, S), normalized by autoscaling and submitted to multivariate statistical analysis by applying Principal Component (PCA) and Linear Discriminant Analyses (LDA).

In order to evaluate the performances of the LDA classification rules, 6 other milk samples collected from 6 different goats of the same herd, at the same sampling periods (2 for each sampling), were analyzed for fatty acid composition and the results were used as evaluation set.

### 3. Results and discussion

The results obtained by ANOVA (Table 1) showed significant differences between the sampling periods for all the variables except for C15AI, C15 and C16I. The plot on the 1<sup>st</sup> and 2<sup>nd</sup> eigenvector of the scores of the 66 samples categorized on the basis of sampling time (M, J, S), obtained by the PCA performed on 23 fatty acid, showed that the 1<sup>st</sup> component did not contain discriminant information related to the lactation stage. On the contrary, in the plot on the 2<sup>nd</sup> and 3<sup>rd</sup> component (Figure 1), the samples collected at early-lactation (M) appeared well separated from those collected at end-lactation stage (S). Moreover it was possible to observe

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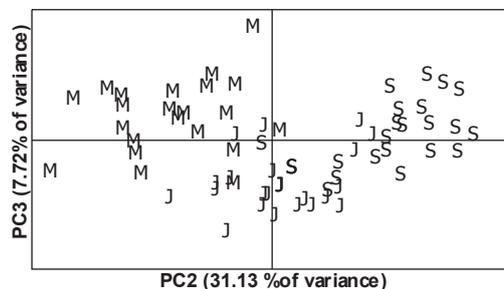
<sup>2</sup> CRA – Istituto Sperimentale per la Zootecnia, Monterotondo (RM) Italy

a discrimination, even though not completely defined, also for the mid-lactation samples (J). In order to improve the separation, the most discriminant variables, i.e. those having the highest loading on the 2<sup>nd</sup> and 3<sup>rd</sup> component, were selected and PCA was recalculated. Figure 2 reports the biplot of the scores of the objects and the loadings of the 12 most discriminant fatty acids selected. This type of plot allowed some considerations to be made; the separation of the three lactation stages occurred mainly along the axis of the 1st eigenvector; M samples presented higher values of short chain fatty acids (C4,C6,C8) and linoleic, while higher concentration of branched chain and unsaturated fatty acids (C15I, C17I, C14:1, C16:1, C17:1, C18:1 and CLA) was observed in S samples. The improved separation of samples taken at mid-lactation period (J) was also due to the variables loading on the 2<sup>nd</sup> component, particularly C17AI. The presence of an S sample (indicated by an arrow) far from the samples of its own category was detected. It was considered as an outlier and excluded from the further calculation. Figure 3 reports the plot of the discriminant scores obtained by LDA on both the 65 objects used to calculate the model (training set) and the 6 objects (Me,Je,Se) of the evaluation set. The latter set was not used for the calculation, but only for the validation of the classification rules. Dotted lines indicate the delimiter of each category. It is worth noting that the 12 variables selected were able to correctly classify all the samples in their own category, except one J sample (indicated by the arrow). Moreover all the 6 samples of the evaluation set were correctly assigned to the category corresponding to their lactation stage.

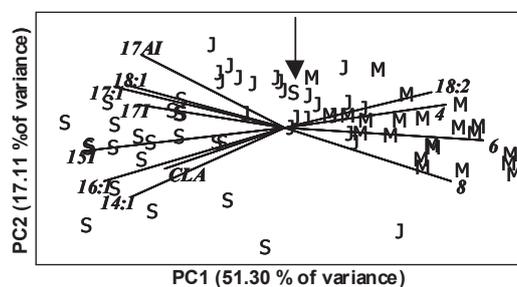
**Table 1:** Milk yield (kg/day), composition (g/day) and FA content (mg/100g fat) of goat milk samples.

Sampling period	May (n=22)		July (n=22)		September (n=22)	
Variable	Mean±SD		Mean±SD		Mean±SD	
Yield	2.11 ± 0.58	a	2.60 ± 0.69	b	1.52 ± 0.39	c
Fat	69.9 ± 20.8	a	69.9 ± 21.3	ab	58.2 ± 13.4	c
Protein	56.5 ± 13.9	a	68.3 ± 16.7	b	58.9 ± 12.7	ac
Lactose	95.5 ± 26.5	a	111.3 ± 30.1	ab	63.2 ± 15.9	c
C4	3.51 ± 0.60	a	2.91 ± 0.51	b	2.54 ± 0.40	c
C6	2.57 ± 0.26	a	2.13 ± 0.20	b	1.95 ± 0.20	c
C8	2.40 ± 0.27	a	1.98 ± 0.26	bc	1.95 ± 0.23	c
C10	7.04 ± 0.97	a	6.03 ± 1.03	bc	6.21 ± 1.02	c
C10:1	0.16 ± 0.05	a	0.12 ± 0.03	b	0.22 ± 0.06	c
C12	3.19 ± 0.46	a	2.85 ± 0.72	ab	3.36 ± 0.74	ac
C14	8.91 ± 0.91	a	8.94 ± 1.28	ab	10.23 ± 1.52	c
C14:1	0.12 ± 0.03	a	0.13 ± 0.05	ab	0.31 ± 0.12	c
C15I	0.18 ± 0.03	a	0.26 ± 0.04	b	0.36 ± 0.07	c
C15AI	0.45 ± 0.09	a	0.47 ± 0.07	a	0.48 ± 0.08	a
C15	0.94 ± 0.14	a	1.00 ± 0.10	a	0.95 ± 0.11	a
C16I	0.26 ± 0.05	a	0.26 ± 0.05	a	0.24 ± 0.06	a
C16	21.99 ± 1.72	a	23.18 ± 1.80	bc	22.82 ± 2.24	ac
C16:1	0.55 ± 0.07	a	0.62 ± 0.07	b	0.88 ± 0.14	c
C17I	0.50 ± 0.06	a	0.52 ± 0.09	ab	0.62 ± 0.13	c
C17AI	0.45 ± 0.08	a	0.60 ± 0.10	bc	0.57 ± 0.09	c
C17	0.63 ± 0.08	a	0.84 ± 0.13	b	0.67 ± 0.12	ac
C17:1	0.28 ± 0.07	a	0.36 ± 0.08	b	0.41 ± 0.07	c
C18	13.17 ± 1.58	a	14.25 ± 2.51	ab	10.07 ± 2.29	c
C18:1	25.36 ± 2.95	a	26.81 ± 2.99	ab	29.35 ± 2.84	c
C18:2	3.73 ± 0.53	a	2.78 ± 0.48	b	2.27 ± 0.33	c
C18:3	0.54 ± 0.11	a	0.52 ± 0.13	ab	0.37 ± 0.09	c
CLA	0.68 ± 0.16	a	0.56 ± 0.11	b	0.93 ± 0.19	c

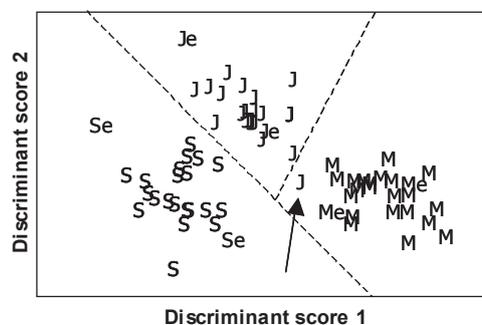
Values followed by different letters differ significantly (P=0.05)



**Figure 1.** Scores plot of PCA: 66 objects, 23 variables.



**Figure 2.** Biplot of PCA: 66 objects, 12 variables.



**Figure 1.** LDA on 12 variables and 65 (training) + 6 (evaluation) objects.

## 4. Conclusion

Results obtained in this research lead to the conclusion that lactation stage affected significantly the fatty acid composition of goat milk. Moreover, under a controlled grazing and feeding system, the fatty acid composition seems to be able to discriminate milk produced at early, mid and end lactation periods and correctly predict to which stage an unknown sample belongs.

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## I-P026: Using Goat Milk Samples to Diagnosed Caprine Arthritis Encephalitis Virus by ELISA

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### Summary

This work was designed to compare the ELISA serologic diagnosis of caprine arthritis encephalitis virus (CAEV) using milk (whey) and blood samples. Paired samples of blood and milk from 66 Dairy goats from two herds were studied and 264 serological analyses were performed using a commercial test kit of CAEV diagnosis (ELISA Kit MVV/CAEV serum monocupule, Institut Pourquier, France). The results showed a total agreement (Kappa=1) between the results positive and negative for whey and serum and the correlation coefficient of testing paired sera and whey in the ELISA was very high ( $r=0,98$ ). These results, comparing with previous studies using goat milk, shows that whey is a better substrate than milk to perform the ELISA test for CAEV determination in goat.

### 1. Introduction

Since detection of Caprine Arthritis Encephalitis Virus (CAEV) in indigenous goat breed in Spain (1998) some control programs are implemented to fight against the disease. The use of milk sample to diagnosis bacterial or viral infections is a valuable tool to control diseases, especially in dairy herds running official dairy control, where milk samples are routinely processed. In a previous study, Motha and Ralston (1994) demonstrated the good accuracy of individual milk samples to detect CAEV goat infection. The objective of this work is to study the agreement between results from blood sera and whey from individual milk samples to detect CAEV infection in Murciano-Granadina goat using a commercial ELISA kit.

### 2. Material and methods

Paired samples of blood and milk from 66 Dairy goats of Murciano-Granadina breed from two herds under official dairy control were studied. One herd was negative to the CAEV infection ( $n=21$  negative goats) and the other was positive to CAEV infection (30% prevalence - $n=45$  positive goats-). Samples carried out by duplicate and a total of 264 serological analyses were performed using a commercial test kit of CAEV diagnosis (ELISA Kit MVV/CAEV serum monocupule, Institut Pourquier, France).

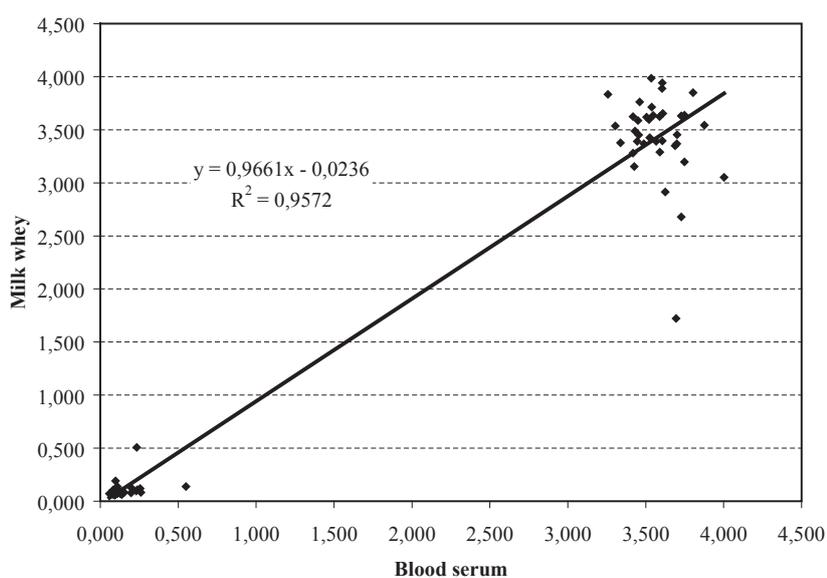
Whey was obtained from milk samples by centrifugation ( $1500g\ 10'$ ) and both, whey and sera samples were stored at  $-20^{\circ}C$  until analyses. ELISA results were obtained at 450nm. To verify the agreement between the sera and whey diagnosis results (positive or negative) the Kappa Test was used and to compare the correlation between the optical density (OD) measures of whey and sera samples the linear regression was performed.

### 3. Results and discussion

The results show a total agreement (Kappa=1) between the results positive and negative for whey and serum and the correlation coefficient of testing paired sera and whey in the ELISA was very high ( $r= 0,98$ ) (Figure 1). Previously, Motha and Ralston compared the efficacy of CAEV diagnosis in sera and milk using one ELISA method. These authors obtained also a high correlation coefficient between testing sera and milk of 0.94 but 3% of false negative diagnosis was obtained by them. They explained that the false negative results could be caused by a lack of antibodies in milk and the false positive diagnosis due to nature of milk (excess fat or any other unknown factor present in the milk). Because whey concentrated the milk antibodies and but did not contain fat or other milk components, the use of whey instead of milk could explain

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the best results obtained by us. Because of these results, whey could be a better substrate than milk to perform the ELISA test for CAEV determination in goat. The use of whey obtained from milk samples instead of blood sera will help to simplify the routine diagnosis, especially in selected herds under official dairy control and could help to avoid the presence of false diagnosis obtained by using milk samples. The accuracy of this diagnosis is necessary when culling strategies are taken to control CAEV infection.



**Figure 1.** LDA on 12 variables and 65 (training) + 6 (evaluation) objects.

#### 4. Conclusion

Whey is a suitable substrate to perform ELISA analysis to detect CAEV antibodies in goat, because no false diagnoses were obtained when comparing their results and the obtained using blood sera samples.

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## I-P028: Casein Haplotypes in Six Goat Breeds Reared in Southern Italy

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### Summary

The 250 kb region spanning over the four goat casein genes has been characterized using a set of thirty-two polymorphic SNPs. The survey was carried out on six autochthonous goat breeds reared in Southern Italy. A total of 174 unrelated heads have been genotyped by Mass Array technology. Individual genotypes have been used to predict intragenic haplotype frequencies. In the whole sample thirty-three haplotypes have been detected, whereas fifty-six haplotypes resulted from the analysis of separate breeds. For each *locus* the two most frequent haplotypes accounted for more than 50%, except for Messinese and Rossa Mediterranea at CSN1S2 *locus*. Aspromonte and Argentata showed the highest variability with 34 and 33 haplotypes respectively. Molecular data have been used to calculate genetic distances and draw phylogenetic tree.

### 1. Introduction

Casein gene polymorphism affects protein content in goat milk (Martin et al., 2002). Mutations in the promoter region as well as in the transcription units can influence the gene expression level. Casein haplotypes have been recently associated to milk production traits (Hayes et al., 2006) suggesting the use of Haplotype Assisted Selection (HAS) in the goat breeding system. SNP markers at casein genes cluster were used to assess genetic biodiversity in six goat breeds of Southern Italy.

### 2. Material and methods

DNA was extracted from blood of 174 unrelated goats: 31 Girgentana (GIR), 30 Argentata dell'Etna (ARG), 30 Maltese (MAL), 22 Rossa Mediterranea (ROS), 30 Messinese (MES), 31 Aspromonte (ASP). A set of 32 polymorphic SNPs (12 at CSN1S1, 7 at CSN2, 4 at CSN1S2 and 9 at CSN3 *locus*) were amplified in three PCR-multiplex and analysed by means of MassArray System (Sequenom). FastPHASE 1.1 software (Scheet and Stephens, 2006) was used to estimate haplotypes, within each *locus*, from SNP genotypes. Haplotypes with a frequency of less than 1% were omitted from the dataset. The average number of haplotypes, the percentage of shared and private haplotypes per breed were calculated. The linkage disequilibrium (LD) was assessed by HAPLOVIEW program (Barrett, 2005) using  $r^2$  statistic. *Da* (Nei et al., 1983) and *Ds* (Nei, 1972) genetic distances among breeds were obtained from haplotype frequencies using DISPAN program (Ota, 1993). UPGMA dendrogram and bootstrap analysis on *Da* distance were constructed by Phylip 3.66 (Felsenstein, 2005) and SplitsTree4 4.6 softwares (Huson and Briant, 2006).

### 3. Results and discussion

All SNP markers resulted polymorphic in each breed, except for GIR and ROS that highlighted two different monomorphic sites at CSN2 *locus*; at those sites the other breeds showed frequencies always lower than 8.1% for the same allele. In the whole sample 33 haplotypes have been detected (12 CSN1S1, 8 CSN2, 6 CSN1S2, 7 CSN3). A higher number of haplotypes (56) resulted from the analysis of separate breeds (25 CSN1S1, 12 CSN2, 8 CSN1S2, 11 CSN3) when 1% frequency was required for each breed (Table 1). For each *locus* the two most frequent haplotypes accounted for more than 50%, except for MES and ROS at CSN1S2 *locus*. The lowest number of haplotypes (28) was observed in GIR, MAL and ROS, the breeds with higher

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production and management level. The highest variability was detected in ASP and ARG goat accounting for 34 and 33 haplotypes respectively and 4 private haplotypes each. CSN1S2 *locus*, despite the lowest number of SNP markers, appeared to be the most informative according to the differentiation index  $G_{st}$  (0.058), the gene diversity ( $H_t=0.818$ ) and the low internal linkage evaluated by  $r^2$ . LD was not equally spread over the chromosome segment; CSN1S1 and CSN3 loci highlighted the highest  $r^2$  values. In terms of pair-wise distances, the closest value was observed between ASP and ARG both at  $D_s$  and  $D_a$ , ASP and MAL were the most divergent at  $D_s$ , ASP and GIR at  $D_a$  (Table 2). The UPGMA dendrogram showed high bootstrap values indicating two main clusters (Figure 1).

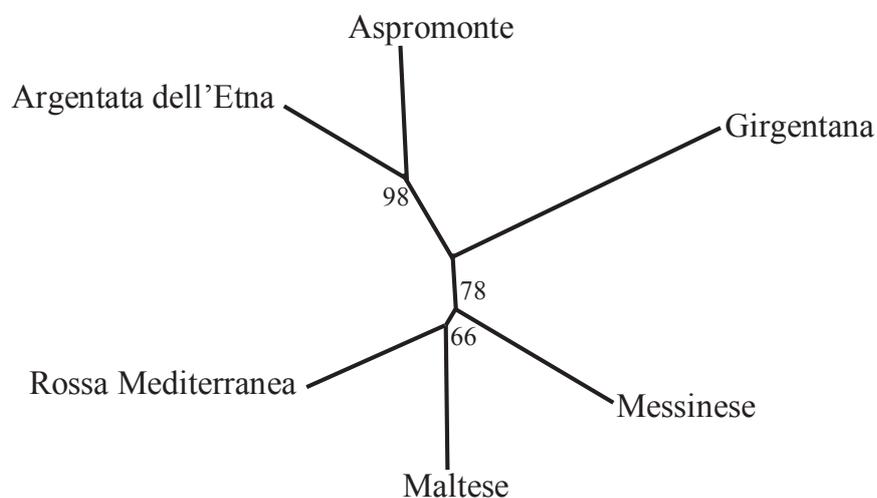
**Table 1:** Number of haplotypes with frequency >1% per locus and breed.

Locus \ Breed	MES	ARG	ASP	MAL	ROS	GIR	Haplotypes per locus	
							Separate breeds	Whole sample
CSN1S1	8 (1)	12 (3)	12 (3)	10 (2)	9 (2)	9 (1)	25 (12)	12
CSN2	8 (1)	9 (1)	8	7 (1)	6 (1)	6	12 (4)	8
CSN1S2	6	6	7 (1)	5	6	6 (1)	8 (2)	6
CSN3	7 (1)	6	7	6	7 (1)	7 (2)	11 (4)	7
Total	29 (3)	33 (4)	34 (4)	28 (3)	28 (4)	28 (4)	56 (22)	33

Private haplotypes between brackets

**Table 2:** Pair-wise genetic distances:  $D_s$  above the diagonal and  $D_a$  below the diagonal.

Breed	MES	ARG	ASP	MAL	ROS	GIR
Messinese		0.077	0.109	0.113	0.048	0.132
Argentata dell'Etna	0.118		0.014	0.139	0.035	0.138
Aspromonte	0.117	0.081		0.235	0.101	0.200
Maltese	0.113	0.139	0.176		0.032	0.181
Rossa Mediterranea	0.096	0.112	0.155	0.092		0.085
Girgentana	0.144	0.150	0.189	0.174	0.161	



**Figure 1.** UPGMA dendrogram obtained from  $D_a$  distance with 1000 bootstraps.

## 4. Conclusion

Goat breeds of Southern Italy are characterized by a high level of variability. SNP markers have proven useful in assessing biodiversity of local breeds. However the integration of SNPs data with neutral markers information may allow to better characterize their genetic structure. The use of tightly linked SNP markers allows to produce robust haplotypes useful in improving goat breeding system by mean of HAS.

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## I-P029: Fatty Acid Composition of Tsigai Sheep Milk as a Physiological Advantage

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### Summary

The fatty acid (FA) composition of sheep milk and contrasting to cow milk is controversial in the literature.

The fatty acid analysis of the Tsigai milk samples was determined with Chrompack CP 9000 gas chromatograph after the special preparation (esterification) of the samples.

C14:00 (11.30%), C16:00 (26.19%), C18:00 (9.98%), and C18:1 (29.98%) were the dominant FAs, which gave the 77.45% in all FA.

The high ratio of C18:1 (29.98%) and the assay of the different nutritional FA group (SFA, UFA, PUFA) suggest that the physiological properties of Tsigai sheep milk fat much more favourable in every aspect than cow milk.

### 1. Introduction

During the last decades the milkfat in the cow milk and its composition was thoroughly studied but regarding the sheep milk's fatty acid composition much less publication was appeared.

According to the statement of some authors the fatty acid composition of the sheep milk is similar as the cow milk's one (1.), (2.), (9.), (11.).

Others established noticeably differences regarding the fatty acids comparing to the cow milk's fatty acid composition (5.), (7.), (7.), (8.), (9.). In opinion of some authors first of all the more favourable physiological determination of the sheep milk's fat comparing to the cow milk can be explained by its higher ratio of unsaturated and 4-12C fatty acids (3.), (4.).

Our objective was to explore the fatty acid composition of milk from milking Tsigai sheep.

### 2. Material and methods

The fatty acid composition of milk from milking Tsigai sheep was determined from individual milk samples (from 8 ewes) and bulk milk samples in a whole (165 day) lactation. The forage of the flock was based on the grazing and was characteristically extensive type. We analysed separated milk fat samples from daily milk samples (morning + evening milking) were stored in -25°C until the analysis.

The determination of fatty acid composition.

*Preparation:* the samples were destroyed in hot water bath with concentrated hydrochloric acid and mixed with ethanol. Afterwards the lipids extracted by ether and petrol ether (<60°C). After combining the organic phases the solvent was removed by means of a rotating vacuum evaporator.

*Hydrolysis and esterification:* The evaporated samples were boiled with 0.5M methanol sodium hydroxide solution (appr. 5 minutes) and further the boiling was continued for 3 minutes with 14% methanol boron-trifluoride solution. We boiled for another 1 minute adding dried hexane and after cooling down mixed it with salted water solution. After separation of the phases we took 0.5 – 2 µl sample from the organic phase and injected it into Chrompack CP 9000 gas chromatograph.

### 3. Results and discussion

Tsigais' milk fat composition did not differ basically from the figures published in the literature. The reason of the existing differences can be dedicated to the different genotypes and the environmental conditions (first of all in the different forage). The fatty acids detected in the highest quantities (furthermore as the dominant fatty acids) according to the expectations were

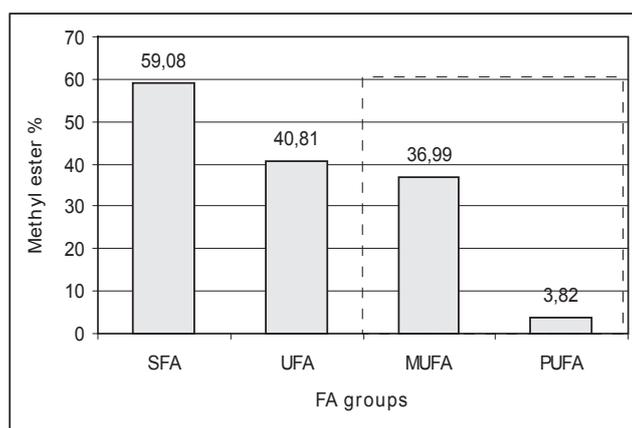
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the saturated myristic acid, the palmitic acid, the stearic acid and the unsaturated oleic acid, which formed the 77.45–78.1% of the fatty acid quantities. Summarized amount of C10:00, C14:00, C16:00, C18:00 and C18:1n9c fatty acids was 81,52% (but C4:0 and C6:0 were not investigated), and higher than published in Park et al. 2007 (>75%).

Some other fatty acids were measured in lower amount than in (10.). Caprylic (C8:0) 1.22% (2.6%), capric (C10:0) 4.07% (7.8%) and lauric acid (C12:0) were 3.67% (4.4%) in Tsigai milkfat. Contrarily the amount of C18:1 total was remarkable higher 34.85%, more than 13.0% like in (10.).

The samples contained 2,17% C18:2n6c and 0,75% C18:3n3 essential fatty acids. The ratio of other, rarely analyzed PUFAs was the follow: C20:2 0.46%, C20:3n3 0.12%, C20:4n6 0.15%, and C22:2 0.15%.

The distribution of the fatty acids was significantly affected by the individual properties and abilities of ewes as well as the progress in the lactation period. Our results derived from the lactation indicate, that the ratio of the palmitic acid slightly changes in the Tsigais' milk fat (cv: 4.23%). During the lactation the quantity of the changes of the stearic acid (cv: 33.51%) were the highest, and the change in the margaric acid (cv:2.69%) was the lowest.



**Figure 1.** The ratio of nutritional FA groups in Tsigai sheep milkfat (Mean of the whole lactation)

Evaluation of the different FA groups showed that the ratio of UFA was very high (40.20%). The ratio of C18:1n9t (29.98%) was as higher 4% than cow milk's average data. The ratio of essential C18:2n6 was 2.19%, while the ratio of the investigated total PUFA was 3.82%.

So, the ratio of the total UFA mainly C18:1n9t and C18:1n9c were notably higher than in cow milk and in Merino sheep's milk described in the literature.

#### 4. Conclusion

The ratio of the unsaturated fatty acids is 40.81%, and the polyunsaturated fatty acids is 3.82% in Tsigai milkfat. The amount of C18:1n9t (29.98%) is the highest of all FA.

Tsigai milkfat contains other PUFA like C20:2n 0.46%, C20:3n3 0.12%, C20:4n6 0.15%, and C22:2n 0.15%.

These results effect that the Tsigai milkfat is very close to the HIF (Hypothetical Ideal Fatty acid composition).

Beyond that it has optimal  $\omega$ -6/  $\omega$ 3 fatty acid ratio (2.68) in itself.

The SFA/UFA ratio also optimal in Tsigai sheep milkfat as in the goat and cow milk, but the amount of total unsaturated fatty acid is higher a bit.

The explored differences confirm that sheep milk (milkfat) has more beneficial human nutritional aspects compared mainly to cow milk

The advantage of the FA composition of sheep milk and its products can be reach for customers only if the consumption of the sheep milk products would be advanced also in Hungary.

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## I-P031: *Mycoplasma* spp. in Goats from a Stud Centre of Selected Dairy Goat

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### Summary

The aim of the present study was to assess the role of goat males, their semen and the artificial insemination to spread contagious agalactia.

### 1. Introduction

Contagious agalactia (CA) is among the most significant infectious diseases that affects dairy goat flocks in terms of economic losses. Moreover, the specific European intracommunity rules of animal's movement demand farms symptom-free for the last 6 months. The role of males, their semen and the artificial insemination to spread the infection among the animals is still unclear although it is evident that goat stud's centres must be herds free of mycoplasmas in order to minimize the risk of infection when infected and non infected animals are in close contact. Another important epidemiological factor is the high percentage of mycoplasma isolations from samples collected from the ear canals of goats (Gil et al., 1999; De la Fe et al., 2005). The peculiarity and the risk of this carrier state is that it may occur independently of any clinical expression, and the most frequent location of infection identified to date is the external ear canal. As our knowledge, no information about the presence of healthy carriers in goat stud's centres is available, and for this reason, the aim of the present study was to assess their presence in one of these centres.

### 2. Material and methods

A total of 46 males and 2 goats were checked to detect carriers of mycoplasma species involved in CA. A total of 96 auricular swabs and 2 milk samples were analyzed using standardized protocols to isolate *Mycoplasma* spp. Solid and liquid pH media were inoculated with all samples, and preliminary biochemical and serological identification was carried out on isolations from previously cloned single colonies (Poveda, 1998; Poveda and Nicholas, 1998) Final identification was undertaken using PCR. Amplification was achieved in a iCycler (Bio-Rad) The PCR amplification products were analysed by gel electrophoresis on 1% (w/v) agarose gels and visualised after staining with ethidium bromide using a Syngene UV transilluminator (Bio Rad).

### 3. Results and discussion

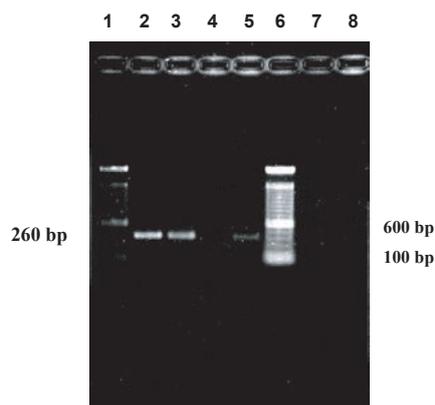
Mycoplasmas were isolated in 4 animals (3 males and 1 female). After them, these animals were conducted to the Veterinary Faculty of Murcia, where they were slaughtered. Complete necropsy was performed immediately after the four animals died, and several tissues (n=149) were collected for bacteriologic culture. All samples were removed from the carcass, inoculated onto liquid and solid PH media and incubated at 37°C. *Mycoplasma* spp. colonies were observed after 48 hours in 7 cultures inoculated with samples of auricular swabs, trachea, the joint of the knee, the mandibular lymph node and 1 culture of mammary parenchyma from the female. These isolations were identified as *Mycoplasma mycoides* subsp. *mycoides* LC type, (*Mmm* LC) (Figure 1).

Generally speaking, the mixing of animals of different origins is a major factor favouring mycoplasmosis and healthy carriers seems to be important in Spain, where the interchange of animals is a common practice (De la Fe et al., 2005), or other countries as Italy, due to in Sardinia, the disease was also introduced by carrier sheep from the Italian mainland (Sanguinetti and Chiocco, 1987). Goat males placed in a stud centre have a different geographical origin and these preliminary results seem also confirm the necessity for monitoring the presence of mycoplasma species involved in CA to decrease the risk of transmission to dairy goat flocks. The

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results also show that *Mmm* LC is the only isolated aetiological agent associated with CA on this stud centre. These findings are in contrast to previous data from the mainland of Spain, in which *M. agalactiae* was always the most frequently isolated species (Gil et al., 1999; Corrales et al., 2004). We are presently engaged in further studies designed to establish the real importance of this mycoplasma species.



**Figure 1.** PCR results: Lines 1 and 6: Molecular marker DNA 100 bp ladder (Invitrogene), Line 2. *Mmm* (LC) (Y-Goat), Line 3 and 5: Positive samples. Lines 4 and 7: Negative samples. Line 8: Negative Control.

#### 4. Conclusion

Results obtained showed that males can be healthy carriers of mycoplasma species involved in contagious agalactia. Further studies are necessary in order to establish the real epidemiological role of this health status.

#### Acknowledgements

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## I-P032: Effects of Global Warming on Milk Processing System in Traditional Goat Farming of East Mediterranean Region

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### Summary

The aim of this study was to determine effects of global warming on milk processing system in traditional goat production system in East Mediterranean region of Turkey. According to obtained results, about 87, 3 % of the farmers declared that there have been changes in climate. Most of them stated that the temperature of the atmosphere increased (82, 5 %). However, the farmers response to these questions by stating their opinions about how the changes in climates affected the milk processing. It was determined that 77,1 % of the farmers no longer use the traditional ways and 27 % of them use only caves and skins of the animals. Additionally milk products technologies and other conservation methods have developed in the region due to the climate changes.

### 1. Introduction

In Mediterranean region, to farming systems with dominant extensive grazing situations, specific technologies and conditions for slaughtering as well as for the transformation process of cheese making and its maturing (Boyazoglu and Morand-Fehr,2001). According to Ronchi and Nardone (2003), livestock systems in Mediterranean areas are far removed from an acceptable level of sustainability, considering animal health, environmental impact, quality of products and profitability. Climate change is particularly threatening agriculture in undeveloped and developing countries because sustainable water management measures are not developed together with land management policies. The aim of this study was to find out effects of global warming on milk processing system in traditional goat farming in East Mediterranean region of Turkey.

### 2. Material and methods

The public survey has been carried out in the villages of 5 districts within the borders of Adana province and in the Seyhan basin. The altitude and animal population have been taken into consideration while defining the villages and districts. Data of animal population have been obtained from The Directorates of Agriculture in districts and provinces and from the mukhtars. In this context, the animal farmers in villages of Kirazliyurt and Kayarcik in Tufanbeyli (14 Questionnaire), Himmekli in Saimbeyli (9 Questionnaire), Kökez and Dölekli in Aladag (18 Questionnaire), Gildirli, Bolacali and Güvenç in Karaisalı (37 Questionnaire), and Ataköy in Karatas (6 Questionnaire) have been interviewed. Totally 84 Questionnaire were surveyed. The public survey has been carried especially on the animal farmers out of the 10 % of the total house number in each village by Intentional Illustration Method (SPSS, 2002).

### 3. Results and discussion

During the process of getting the opinions of the farmers, it was stated that there have been some changes in milk processing and conservation methods.

Milk is processed in different ways in the business enterprises which kept milk type animals. Almost 9 % of the farmers process the milk as cheese and an important part of them sell raw milk. Some of the products are used for the family's own needs and the rest of it is sold for income. Most of the farmers stated that the cooperatives or merchants bought the milk in a very low price and even they could buy only 1 kg of feed by the income of 1 kg milk. Consequently, they stated that fresh milk selling wasn't economical. Milk is mostly used for cheese making (70, 3 %), and the rest of it is used for making yoghurt, butter and çökelek. An income is being gotten by selling the most of the cheese. White cheese and cheese encased in a goat skin is generally produced.

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The number of farmers who use commercial yeast is more than the proportion of the farmers who use natural yeast. Plug milk, sarkanak (kind of animal tissue) and dried fruit are intensively used as traditional yeasts. Questions about the changes in the technologies used in processing milk were asked to the housewives and 29.50 % of the women in farm stated that they used the same ways as they used before. 28.8 % of them stated that there has been a change and added that they used to use traditional ways but now they are used to use commercial yeasts as they are more practical. It was determined in the working area during different periods that there have been some changes in preservation methods of the processed dairy and meat products. It was seen that they are preserved in the refrigerator as they were spoiled in the past because of being embedded under snow or soil and being kept in caves. In the past, products were digged into ground or into the snow in highlands where it's impossible these days. After questions related with this topic, it was determined that 77.1 % of the farmers no longer use the traditional ways and 27 % of them use only caves and skins of the animals. When the traditional methods were asked to the farmers, most of them stated that (48.1 %) use the refrigerator, and rest of them uses the traditional methods such as caves (22.2%), skin (11.1%) for preservation of their products. Approximately, 16 % of the farmers still use the traditional methods and the rest of them used to embed their products into the soil (29.9 %), preserve them in skins (13.8 %), in caves (6.9 %), in highlands (3.4 %) but now they no longer use these methods. Most of the farmers stated that their preservation methods have been changed due to technological improvement. And 20.5 % of them thought that as the reason of this changing are climate changes. About 87.3 % of the farmers declared that there have been changes in climate in the areas on which they live. Most of them stated that the temperature of the atmosphere increased (82.5 %) and some of them stated that the temperature of the atmosphere decreased (2.9 %). It was determined that 77.1 % of the farmers no longer use the traditional ways and 27 % of them use only caves and skins of the animals. Milk products technologies and other conservation methods have developed in the region due to the climate changes. As an example, cheese is produced on daily conditions instead of traditional methods. Only, in a few regions cheese fermentation is still done by the traditional methods.

## Acknowledgements

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## I-P034: *Pseudomonas Aeruginosa* Persistence in the Milking Machine: Healing Experiences

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### Summary

The water contamination by *Pseudomonas aeruginosa* in a dairy sheep farm affects adversely the milking machine hygiene and animal health.

The aim of this work is to study a *Pseudomonas ae.* healing programme for a polluted milking machine and to verify its efficacy inside a dairy sheep farm. Sanitizing operations were verified at the end of the cleaning procedures by microbiological analysis of the milking machine rinsing water and on tampons taken from each milking machine components. This experience confirms the great difficulty in removing *Pseudomonas ae.* from the milking machine even with careful sanitization washings. Considering the expensiveness of the *Pseudomonas ae.* healing due to the great resistance and the high infectiousness, prevention represents the most useful tool.

### 1. Introduction

The water contamination by *Pseudomonas aeruginosa* in dairy sheep farm affects adversely the milking machine hygiene and animal welfare: the contamination of the milking machine pipelines, and in particular of the liners, by the microorganism, determines the mammary gland infection of the animals. The high infectiousness and the great resistance, in particular in aqueous environment, is due to the biofilm forming capacity that isolates and feeds bacteria, gives adaptation ability, antibiotic and disinfection resistance, and helps the fast and invasive growth on all surfaces, especially if moist. (Moore David M., 1997)

Aim of this work is to study a *Pseudomonas ae.* healing programme for a contaminated milking machine and to verify its efficacy.

### 2. Material and methods

The study was carried out in a dairy sheep farm located in northern Sardinia. The flock consists of about 800 dairy Sarda ewes and their milk, obtained by automatic milking, is used to make a type of raw milk cheese locally known as "Fiore Sardo", as well as a type of smoked dairy product named "Ricotta Mustia". (Dore S., 2007)

Milking is carried out by a low-line milking machine 24+24 stalls. The water used to wash the milking machine was not purified and it represented the origin of *Pseudomonas ae.* contamination; moreover, equipment maintenance was carried out not-systematically affecting the component wear and tear, in particular of the liners.

The combination of these factors determined the outbreak of *Pseudomonas ae.* udder pathologies (prevalence of 27% among the positive animals for mastitis agent) with negative consequences on quality and quantity milk production.

The milking machine cleansing was performed by two different steps.

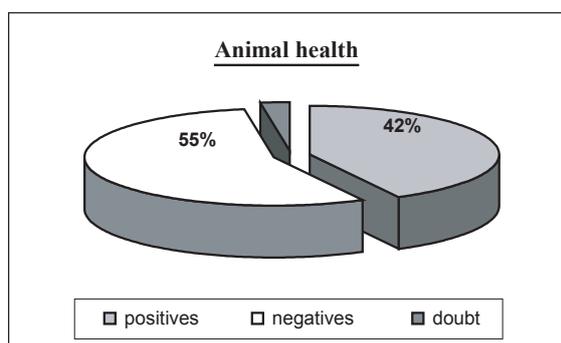
During the first one, after the water purification by UV rays and a routine acid cleaning, a disinfectant washing of the milking machine with 1:6 solution of sodium hypochlorite 5% was carried out; clusters were mechanically washed with brushes and disinfected by immersion in 1:5 solution of sodium hypochlorite 5%, for 12 hours.

The second step was carried out by the substitution of all the milking clusters and by a careful cleaning of jettors for washing and milk receiver by sodium hypochlorite 5% with brushes.

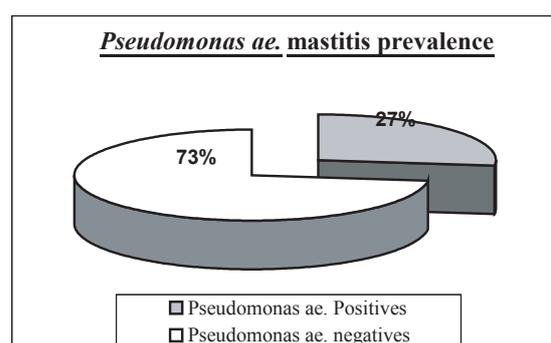
Sanitizing operations were verified at the end of the cleaning procedures by microbiological analysis of the milking machine rinsing water and on tampons taken from liners, claws, jettors for washing, short pipes and milk receiver. The tests for *Pseudomonas ae.* were based on the ability to identify the microorganism using a selective culture medium, on which it grows with particular cultural characteristics. Water (100 ml) was filtered using a membrane and then the

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membrane was sowed on CETRIMIDE-AGAR medium and finally it was incubated at 37°C for 24-48 h.; the tampons were directly sowed on CETRIMIDE-AGAR medium and incubated at 37°C for 24-48 h.. Bacterial cultures were examined by Wood's Lamp Test (Ultra Violet Light Test) and the fluorescent colonies were purified by transplantation on Nutritive/Blood – Agar. Finally, after performing Oxidase-test, biochemical identification with miniaturized system (API NE Kits) was carried out.



**Figure 1.** Results of the microbiological test for mastitis agent research carried out on individual milk of each sheep of the examined herd.



**Figure 2.** *Pseudomonas ae.* mastitis prevalence among the sheep positives to the microbiological test for mastitis agent research.

### 3. Results and discussion

The first cleansing intervention determined the absence of *Pseudomonas ae.* on the rinsing water, while all of the tampons sampled from the milking machine components were positives.

The second intervention demonstrated the absence of *Pseudomonas ae.* both on the rinsing water and on all of the tampons sampled from the milking machine components.

### 4. Conclusions

This experience confirms the great difficulty to remove *Pseudomonas ae.* from the milking machine components even with careful sanitization washings, as bibliography showed; it is essential to verify the disinfection efficacy by careful bacteriological controls of the milking machine internal surfaces, with great attention to the liners that come into contact with animal teats, and to the jettors for washing, that can represent a perfect culture medium for bacteria.

Considering the expensiveness of the *Pseudomonas ae.* healing due to the great resistance and the high infectiousness, prevention is essential, and must be carried out preventing the entrance of the microorganism inside the milking machine equipment through the surveillance of the microbiological quality of the rinsing water, the monitoring of the functioning of the purification system and an accurate mastitis control. Moreover, to avoid an upward re-contamination (from the infected teats to the liners) of the milking machine, all the positive animals for *Pseudomonas ae.* and the dubious ones (with Somatic Cell Count > 1 000 000) must be separated and milked manually.

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## I-P036: Goat $\kappa$ -Casein (CSN3) Polymorphisms in Europe, Middle East and Africa

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### Summary

A high degree of polymorphisms at the  $\kappa$ -casein (*CSN3*) locus was recently described but the number of breeds studied is still very limited. Therefore the occurrence and frequencies of the *CSN3* alleles *A*, *B/B'*, *B''*, *C*, *C'*, *D/L*, *E*, *G*, *H*, *I/K*, *J*, and *M* were analysed at the molecular level in 52 goat breeds from Europe, Asia and Africa. There was a great variation in the number (2-6) of alleles at *CSN3* between the breeds. The rare alleles *CSN3 B''*, *H*, and *I/K* could not be demonstrated. Since the casein genes are organised as a cluster, further haplotypes in the casein complex will occur which can then be used in association and phylogenetic studies.

### 1. Introduction

Mutations in the casein genes affect the level of gene expression and effects on milk production traits have thus been reported (Martin et al. 2002). A high degree of polymorphisms was recently described in the kappa-casein (*CSN3*) locus in the domesticated goat (Prinzenberg et al. 2005). The polymorphisms have been investigated both at the DNA and protein level, and a protein evolutionary model considering casein haplotypes was proposed by Caroli et al. (2006). However, analyses on the occurrence and frequencies of the *CSN3* alleles in different breeds are still limited.

### 2. Material and methods

A total of 1690 goats belonging to 52 traditional and local breeds were sampled from 16 European, Middle Eastern and African countries. For each breed, a maximum of three unrelated goats per flock from an average of 10 flocks per breed were sampled. Nine breeds are catalogued as endangered by the FAO, whereas 27 breeds were considered not at risk. For 15 breeds, there was no information about their status available.

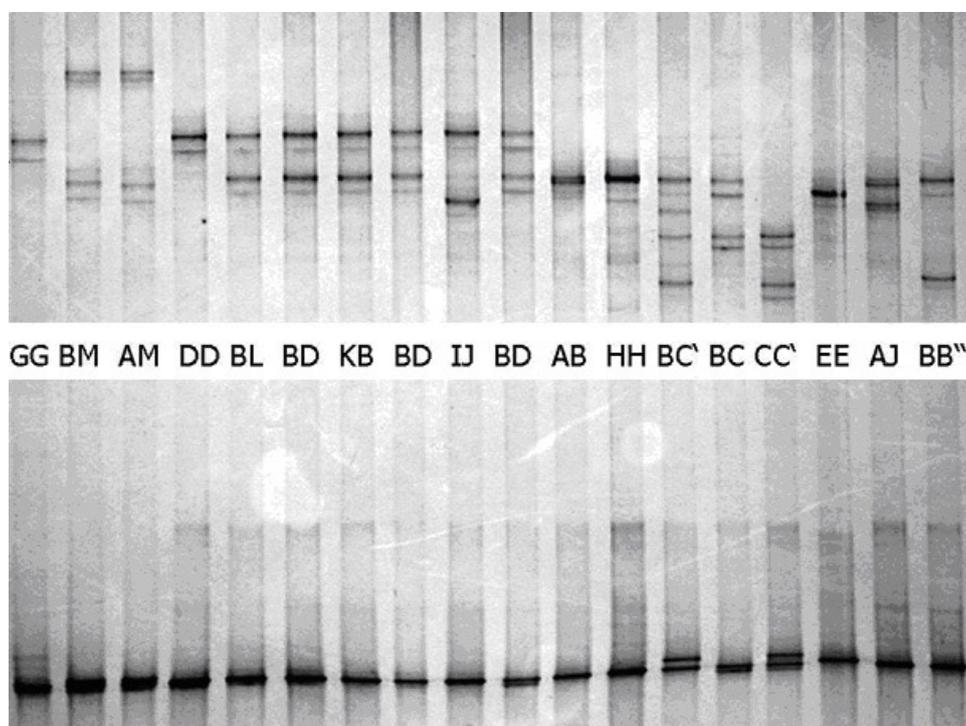
*CSN3* was analyzed by PCR-SSCP (Chessa et al. 2003) followed by 2 PCR-RFLPs to distinguish the *CSN3* alleles *A*, *B/B'*, *B''*, *C*, *C'*, *D/L*, *E*, *G*, *H*, *I/K*, *J*, and *M*.

### 3. Results and discussion

There was a great variation (Figure 1) in the number of alleles between the breeds. The alleles *CSN3 A* and *B/B'* occurred in all breeds with frequencies up to 0.523 (Alpine, Switzerland) and 0.806 (Peacock, Switzerland, Argentata dell'Etna, Italy). Only these two alleles were found in Borno (Nigeria), Guadarrama and Verata (Spain), Sarda (Italy) and Alpine (France). On the other hand, 6 of the 12 alleles analysed occurred in the breeds Capore and Dukati (Albania), in the Alpine breeds from Switzerland and Germany; in Carpathian (Romania) as well as in the goat breeds Abaza, Angora and Hair sampled in Turkey. The rare alleles *CSN3 B''*, *H*, *I/K* were not found in the breeds analyzed, while *CSN3 E* occurred in the breeds Angora (Turkey), Polish Fawn Coloured (Poland), Grigia Molisana (Italy), Mati and Capore (Albania). *CSN3 G* was identified in the breeds Abaza, Angora, and Hair goat from Turkey, and *J* in both Alpine breeds (Switzerland, Germany) and the endangered Thuringian Forest goat of Germany. The silent allele *CSN3 C'* was found in 15 of the 52 breeds with the highest frequency (0.16) in Valdostana (Italy).

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**Figure 1.** : PCR-SSCP analysis of samples with different  $\kappa$ -casein genotypes. After PCR-SSCP analysis two PCR-RFLPs can be used for further differentiation of samples with  $\kappa$ -casein D, L, I or K.

#### 4. Conclusion

As the casein genes, especially *CSN3*, affect milk production traits (Hayes et al. 2006), the demonstrated variation at *CSN3*, leading to further haplotypes, can be used in association but also in phylogeny studies in goats.

#### Acknowledgement

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## I-P037: Analysis of the Microbial Composition of Goat Milk Produced in Sardinia

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### Summary

The aim of this study was to define the microbial composition of raw goat milk sampled in different areas of Sardinia (Italy) and to determine the influence of both the sampling period and the breeding system (extensive and semi-extensive) on its structure. Goat milk was sampled from 8 farms during winter, spring and summer.

Lactococci, streptococci and enterococci were identified as the major microbial groups present in goat milk irrespective of the sampling period and the breeding system. The number of thermophilic and mesophilic lactobacilli were very low and they were not found at all in the 50% of the samples. It appeared necessary a more efficient application of the autocontrol system in order to prevent microbiological risks.

### 1. Introduction

Goat breeding is of particular interest in Sardinia even though its potentiality is currently not fully realized. This is due to a lack of local valorization of goat milk and dairy products such as yogurt and cheese. To date, the knowledge on the microbial composition of goat milk and goat dairy products manufactured in Sardinia is very limited. Indeed the aim of this study was to fill this gap and particularly to define the microbial composition of goat milk as a function of the breeding system and sampling period.

### 2. Material and methods

A total of 24 bulk tank milk samples were collected from 8 farms during winter, spring and summer. Sample preparation for microbiological analysis was carried out following the International Dairy Federation (IDF) standard protocol 122B:1992 (1992).

Total mesophilic count were determined on PCA (Oxoid) after incubation at 30°C for 2 days; lactococci were counted on M17 agar (Oxoid) after incubation at 30°C for 2 days; streptococci were counted on M17 agar after incubation at 45°C for 2 days; lactobacilli and pediococci were counted on MRS agar (Oxoid) after incubation at 30°C for 2 days; enterococci were counted on Azide agar (Oxoid) after incubation at 37°C for 2 days; staphylococci were counted in Baird Parker medium (Oxoid) after incubation at 30°C for 2 days. Presumptive colony of coagulase positive staphylococci were tested on StaphyTest (Oxoid) and Coagulase test (MICROBIOL). *Leuconostoc* spp. and micrococci were identified according to Zarate (1). *Propionibacterium* spp. strains were quantified according to Tiecco (2) while *Pseudomonas* were counted on *Pseudomonas* selective agar (MICROBIOL diagnostici, UTA, Sardegna, Italy) after incubation at 7°C for 7 day.

Total and faecal coliformes (MPN method) were quantified using BGGB (Oxoid) after incubation at 37°C and 44°C respectively for 48 h. To assess the presence of *Escherichia coli* 0,1 ml aliquots from BGGB positive tubes were incubated in tryptone water and incubated at 44°C for 48h; the indole production was highlighted using Kovac's reagent; spores of sulphite-reducing clostridia were assessed on DRCM (Merck) after pasteurization of the sample at 80°C for 20 min and incubation at 37°C for 48h. Yeasts were counted on GYEP agar pH 4.5 after incubation for 3 days at 25°C.

### 3. Results and discussion

In Table 1 we reported the average number of the different microbial groups identified in goat milk samples. These latter were collected in farms characterized by a different breeding system and during different periods of the year.

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**Table 1:** Microbial composition (mean cfu ml<sup>-1</sup>) of raw goat milk sampled from different periods of the year and in different system of goat breeding.

Microbial group	Winter	Spring	Summer	Extensive	Semi-extensive
	Mean			Mean	
TMC	1,22E+05	7,92E+06	6,24E+06	5,59E+06	3,93E+06
Lactobacilli 30°C	1,81E+03	2,37E+04	5,14E+03	1,82E+04	2,25E+03
Lactobacilli 45°C	5,08E+01	5,25E+01	1,30E+03	7,03E+01	8,65E+02
Lactococci	3,64E+04	1,01E+06	1,43E+06	9,92E+05	6,60E+05
Streptococci	2,37E+04	7,31E+04	2,99E+05	4,79E+04	2,16E+05
<i>Leuconostoc</i> spp.	7,03E+03	1,12E+05	4,22E+05	7,75E+04	2,84E+05
<i>Pediococcus</i> spp.	1,50E+02	2,60E+03	1,57E+03	1,63E+03	1,25E+03
<i>Enterococcus</i> spp.	1,80E+04	1,58E+05	1,16E+06	1,01E+05	7,88E+05
<i>Micrococcus</i> spp.	1,23E+03	1,47E+05	9,21E+04	1,56E+05	4,42E+03
<i>Propionibacterium</i> spp.	2,49E+03	5,99E+04	5,29E+03	4,14E+04	3,66E+03
CNS	8,79E+03	1,90E+05	1,12E+05	1,53E+05	5,40E+04
CPS	1,62E+02	2,00E+03	2,63E+05	2,34E+03	1,74E+05
<i>Pseudomonas</i> spp.	1,19E+04	4,65E+03	2,77E+03	7,39E+03	5,47E+03
Yeasts	1,73E+03	4,72E+03	5,73E+03	6,28E+03	1,84E+03

CNS: coagulase negative staphylococci TMC: Total mesophilic count CPS: coagulase positive staphylococci

Lactococci, streptococci and enterococci were the prevalent microbial groups, being 1-2 times higher than the other microbial groups. This is mostly in agreement with previous findings (3). The numbers of lactococci, streptococci and enterococci increased during spring and summer.

The number of thermophilic and mesophilic lactobacilli were very low and they were not found at all in 50% of the samples (not shown). This was in contrast with previous findings (1;4;5). The numbers of mesophilic lactobacilli was higher in milk samples collected from farms with an extensive breeding system compared to the semi-extensive system.

*Leuconostoc* spp., pediococci and micrococci were found as a constant component of the goat milk microflora. The numbers of *Leuconostoc* spp increased considerably during spring and summer.

Coagulase negative staphylococci were present in all the samples analyzed whilst coagulase positive staphylococci were only found in 50% of the samples (not shown). The number of coagulase negative staphylococci increased during summer and spring while the coagulase positive staphylococci were found more abundant during summer. This was particularly evident in the semi- extensive breeding system compared to extensive.

The presence of total and faecal coliformes (not shown) was very variable and, apparently, no correlation has been found between sampling period and breeding system on these microbial groups. This can be certainly related to the different hygienic and sanitary conditions of the singular farms. They were highly present in 9 samples (> 2400 MPN/ml) and in 7 of these the presumptive number of *Escherichia coli* was >2400 MPN/ml. Spores of sulphite-reducing clostridia were only found in one sample (460 MPN/ml).

## 4. Conclusions

The results from this study showed that a composite microbial population is associated to raw goat milk. In this context the mesophilic microflora can be considered as one of the main components of the total population.

Coagulase positive and negative staphylococci increased during the lactation while the breeding system seemed to influence their presence. Coagulase negative staphylococci were more abundant in samples from the extensive breeding system while coagulase positive seemed favoured in the semi-extensive system.

Above all the low presence of lactobacilli encountered in most of the milk samples seems remarkable as well as the absence of spores of sulphite-reducing clostridia. The high recovery of microbial groups indicating some poor hygienic and sanitary conditions highlights the necessity to improve the qualitative standard of goat milk. Moreover a more efficient application of the autocontrol system is required in order to prevent microbiological risks.

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## I-P038: Inhibitory Activity of Different Essential Oils on *S. Aureus* Strains Isolated from Mastitis Affected Goats and Sheeps

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### Summary

*S.aureus* is a frequent contaminant of raw milk, especially from goats and sheeps. Pasteurization is able to eliminate *S.aureus* contamination, making safe the milk itself and as ingredient of a variety of food products. The raising consumer demand of raw milk cheeses has been stimulating a lively market of these products. Depending on the cheese-manufacturing procedure, an initial *S.aureus* contamination in non-thermized milk could find favourable conditions to reach a concentration able to produce enterotoxin. Aim of the work was to evaluate the possible inhibitory activity of some EOs (extracted from edible plants) on *S.aureus*. By MIC test, cinnamon, clove, coriander, peppermint, bay, white and red thymus showed values ranging from 0.06% to 0.5%. For organoleptic reasons, red thymus was chosen to test the effect on the growth rate of *S.aureus* ATCC 29213.

### 1. Introduction

Milk products are good substrates for the growth of *S.aureus* and the production of staphylococcal enterotoxins, hence such products can be involved in food-borne diseases due to the occurrence of enterotoxigenic coagulase-positive staphylococci in raw milk and/or cross contamination during the process of manufacturing. For this, criteria on staphylococci and on staphylococcal enterotoxins have been introduced by Reg.2073/2005 [1]. Milk producing animal teats can be a weighty reservoir of *S.aureus*, hence raw milk, also from goats and sheeps, is frequently contaminated and such contamination is dramatically increased in case of mastitis. Thermization may be effective on *S.aureus* contamination but there is a huge production of goat and sheep raw milk cheeses owing to the raising consumer demand. Toxin production is conditioned by the possibility of enterotoxigenic strains to growth and multiply at concentrations of  $10^5$ - $10^6$  cfu/g and these concentrations can be reached during the first steps of manufacturing of raw milk cheeses, before the drop of pH.

The incidence of food-borne diseases stresses the need to produce safer food. Concerns over the safety of some chemical preservatives and the negative consumer perception of them has propelled a new interest towards natural alternatives. It has long been recognized that some essential oils (EOs) have antimicrobial properties. The present work is a pilot study to evaluate the inhibitory activity of 19 edible EOs on *S.aureus* strains isolated from mastitis affected goats and sheeps.

### 2. Material and methods

*microorganisms*: 65 strains of wild *S.aureus* isolated from milk of mastitis affected animals and *S.aureus* ATCC 29213 as reference strain.

*essential oils*: 19 EOs from plants or their parts (dillweed, anis, bark of cinnamon, leaf of cinnamon, coriander, tarragon, sweet fennel, leaf of clove, peppermint, east indian nutmeg, black pepper, bay, rosemary, sage, clary sage, seed of celery, white thyme, red thyme and ginger) were obtained from Sigma-Aldrich, Milan, Italy.

*determination of MICs*: the EOs were tested for the evaluation of the MICs (Minimum Inhibitory Concentration) on the bacterial strains according to NCCLS [2] with the modification of the inclusion of Tween 80 [0.5 % v/v][3] in the agar. MIC was defined as the lowest concentration of compound that completely inhibited visible growth after 18 to 24 hours of incubation at 37°C.

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*determination of growth curves at different concentrations of red thymus EO:* the effect of 1/4, 1/2, 1 MIC of red thymus EO on the growth curves of *S.aureus* ATCC 29213 was evaluated at 0, 1h, 2h, 3h and 6h. Colonies of *S.aureus* ATCC 29213 in exponential phase were suspended in Mueller Hinton broth and adjusted to the concentration of  $1.5 \times 10^8$  cfu/ml. After further dilution, 4 tubes (one as growth control and one for each concentration of thymus) containing 15 ml of MHb (plus 0.5% of Tween 80) and added with the different concentrations of thymus were inoculated at the final concentration of  $1.5 \times 10^5$  cfu/ml. The tubes were incubated at 37°C in water bath and growth curves were evaluated by colony counts at the stated intervals of time.

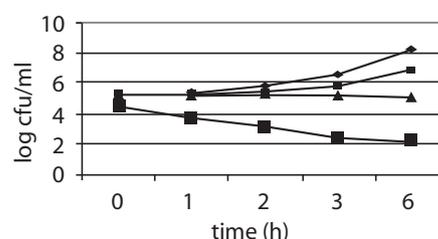
### 3. Results and discussion

Table1 reports MICs values for all the EOs tested, listed in order of activity.

Figure 1 shows the growth curves in *S.aureus* ATCC 29213 as function of the thyme concentrations included in the MHb cultures.

**Table 1:** MICs values for the EOs tested

MIC <sub>50</sub> (%)	Essential oils
0.06	bark of cinnamon
0.25	leaf of cinnamon, clove
0.5	coriander, peppermint, bay, red and white thymus
1	dillweed, sage
>1	anis, tarragon, fennel, nutmeg, blackpepper, rosemary, celery, ginger



**Figure 1.** Growth curves in *S.aureus* ATCC 29213 as function of 3 red thyme concentrations ■ 1 MIC; ▲ 1/2 MIC; ● 1/4 MIC and ◆ control.

### 4. Conclusion

*In vitro* results show that red thymus essential oil has the potential to effect the growth of *S.aureus* strains. Although further investigation is needed to explore the effect of other EOs and their activity when applied to milk, at moment we can suppose they could be applied as part of the combination of hurdles (pH, temperature, water activity, redox potential, packaging, competitive microorganisms, consortia) to satisfy the demand for "natural" cheese (from raw milk) or to contribute to prevent the possibility that highly contaminated milk is used as ingredient for a variety of food preparations, keeping high the microbiological safety level of the final product. The use of EOs could raise perplexities regarding organoleptic characteristics of the food. Appropriate EOs could be considered not only as preservatives but also as flavour components, especially in herb and spice flavoured cheeses. The major antibacterial components of thymus are terpenes such as thymol and carvacrol [4]. This suggests that some of the most active components could be considered instead of the whole oils.

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## I-P039: Effects of Dietary Supplement with Linseed at Three Different Levels on Gross Composition and Fatty Acids Content in Goat Milk

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### Summary

A study was carried out to enhance the CLA contents in goats' milk fat under field conditions by dietary means. Thus, a commercial supplement enriched in linseed (SEL) was incorporated at three different concentration levels into the goats' diet and the fatty acid profile in milk fats was thoroughly monitored. Among the SEL doses assayed, 0.7 Kg/animal/day provided the best results. Although the gross milk composition was not affected by using a SEL, significant changes were found in the lipid profile of goats' milk. A noticeable decrease in SFA levels was detected in goats fed with SEL which is highly correlated to the lower concentrations of C12:0, C14:0 and C16:0 found.

Concentrations of vaccenic acid (*trans*-11 C18:1) and C18:3n-3 in SEL diet were markedly higher than in control diet. An increase in total CLA was also observed during the period of supplementation and the principal isomer was rumenic acid, accounting for as much as 90% of the total CLA.

### 1. Introduction

Among animal feeding strategies for CLA enrichment of milk, those diets with linseed supplements rich in PUFA that provide lipid substrates for the production of rumenic acid or *trans*-vaccenic acid have proved to be effective (Luna et al., 2005; Khanal & Olson, 2004). In addition to enhancing CLA content, the dietary changes with linseed also result in milk fat containing a lower proportion of saturated FA and greater amounts of mono unsaturated FA and PUFA.

Research on this topic, could lead to the development of natural, consumer-acceptable strategies and processing systems to produce dairy foods of proven quality with enhanced healthful properties. In a previous study, Luna et al. 2005, demonstrated that dietary linseed supplementation could be a valuable means of increasing CLA in ewes' milk

The aim of this work was to study the effects of a SEL diet at three different levels on the fatty acid profile of goats' milk fat.

### 2. Material and methods

During a 10-wk period (from September to November) milk samples were collected twice a week from the three herds of goats Murciano-granadina breed located in the Castilla-La Mancha region (Spain).

For the first 3-wk the herds of goats were fed a conventional diet. Then the following 4-wk a commercial supplement (*Lactovejina Omega 3 Plus*, Cargill España S.A) enriched in linseed was incorporated at three doses (0.3; 0.5 and 0.7 Kg /animal/day) into the diet of 48 goats of each herd (herds A, B, and C, respectively). Finally, during the last 3 weeks the supplement was removed and the goats were fed the control diet.

The FA profile in milk fat was monitored by GC-FID for all the trial period. CLA isomers were determined by Ag+-HPLC. Gross milk composition was also evaluated.

### 3. Results and discussion

The effect of dietary supplement on gross milk composition was minimal and only a slight decrease in fat content was observed. Among the SEL doses assayed, 0.7 Kg/animal/day provided the best results.

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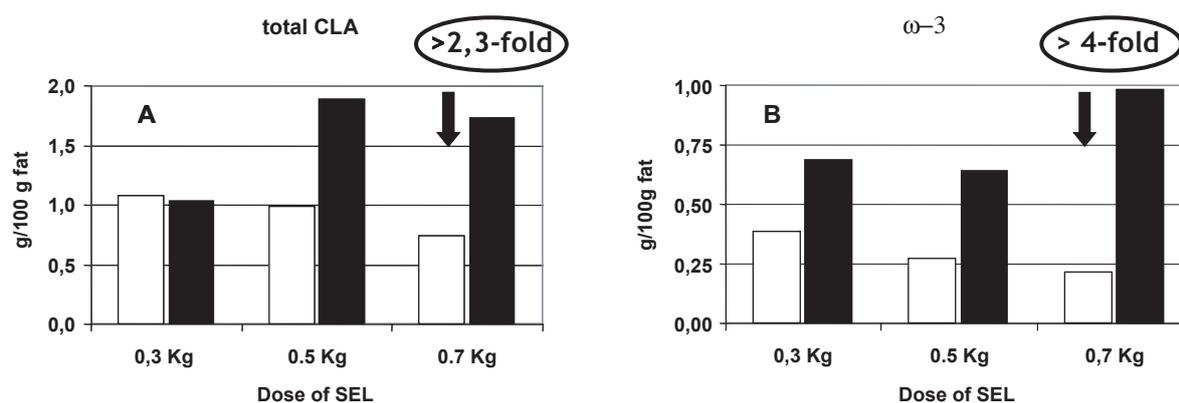
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The incorporation of 0.7 Kg of SEL to the goats' diet showed changes in milk fat composition containing a lower proportion of SFA than control. Largely the decrease in SFA level is caused by the lower concentrations of C12:0, C14:0 and C16:0 fatty acids, considered to be cholesterol-raising. Thus, proportion of these fatty acids is 6% lower (39.26%-32.87%) than in control milk fat. Meanwhile the concentration of SFA with a neutral or positive effect on human health (short fatty acids C4-C10 and estearic acid C18:0), remained constant (~32%).

No alteration in the proportion of MUFA was found. However, the increase in total content of PUFA was significant and positive (4% for the control diet and 6% for supplemented diet).

In particular, an increase in total CLA (from 0.74% to 1.74% of total FA) was observed (Figure 1A). Total CLA remained elevated only during the weeks of SEL feeding and levels returned to their original values when the supplement was removed from the diet. CLA isomer distribution obtained from Ag<sup>+</sup>-HPLC analysis revealed that C18:2 *cis9*, *trans11* (rumenic acid) represented almost 90% of total CLA.

With respect to the *trans* C18:1 fatty acids, the total content of *trans*-vaccenic acid (TVA, C18:1 *trans11*) was significantly increased during the SEL diet period, concretely its concentration became 3-fold higher than with control diet. Likewise, significant increase in omega-3 fatty acid (C18:3n-3) was also observed during the period of supplementation (Figure 1B), whereas C18:2 was not affected by adding a SEL.



**Figure 1.** Effect of 4-wk commercial supplement (SEL) diet at three different levels on total CLA (A) and C18:3n3 ( $\omega$ -3) (B) contents of goats' milk fat. Control Diet (□) and Supplemented Diet (■).

## 4. Conclusion

The SEL diet employed at 0.7 Kg/animal/day allows us to improve the nutritional quality of the goats' milk, since increases the levels of PUFA especially CLA, TVA and C18:3n-3, and reducing simultaneously SFA content.

## Acknowledgements

This study has been possible thanks to the supply of livestock feeding supplements by Cargill España S.A.

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## I-P040: Changes of Milk Yield and Composition as Affected by Subclinical Mastitis in Sheep

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### Summary

Thirty Sardinian ewes were selected and monitored from weaning to the end of lactation to investigate the intramammary infection (IMI) and its influence on milk yield and composition. Half-udder milk samples were collected and tested for bacteriological status, milk yield and composition, renneting parameters, and somatic cells count (SCC).

The main intramammary pathogens isolated were coagulase negative staphylococci (CNS); *S. aureus* was not isolated. Milk yield was significant related to IMI, with lower values ( $P < 0.01$ ) in infected halves compared with uninfected halves (0.284 vs. 0.410 kg/milking, respectively). The SCC response were significantly higher in infected halves than in the uninfected ones (5.94 vs. 5.31). Prevalence of IMI resulted greater after the second lactation. A long persistency of single-pathogen infection has been highlighted throughout lactation.

### 1. Introduction

Udder infection in dairy sheep has a major effect in reducing both yield and quality of milk, leading to greater economic losses than those reported for dairy cattle (Watson and Buswell, 1984). In dairy sheep flocks the incidence of clinical and subclinical mastitis is related to a complex of factors, including breed and management. CNS are the most common pathogens of udder infection. However, the association between CNS infectious and the increased SCC is still controversial (Menziez and Ramanoon, 2001). Although, SCC is the main factor influencing milk quality, other milk components have been found to be related to bacterial udder infection (Burriel, 1997). The aim of the present study was to identify the pathogens causing subclinical udder infections in dairy sheep, and to determine the influence of pathogens on quantity and quality of milk.

### 2. Material and methods

The trials was carried out in a flock of about 400 machine-milked Sardinian sheep located in the province of Viterbo (Italy). Thirty primiparous and multiparous healthy ewes were selected with no evidence of clinical mastitis and monitored from weaning to the end of lactation. Milk sampling and yield measurements were carried out during the morning milking at 4 weeks interval. A total of 586 udder half milk samples were collected aseptically into sterile vials for bacteriological analysis. Ewes were hand-milked and the milk yield from each udder half was determined by graduated cylinder. Milk from each udder half was collected and was analysed for fat, protein, casein (CN) and lactose content (MilkoScan FT600, Foss Electric), SCC (Fossomatic 5000, Foss Electric), pH, titratable acidity ( $^{\circ}\text{SH}$ ), and renneting characteristics (clotting time, rate of clot formation and clot firmness after 30 min; Formagraph, (Foss Electric) according to Zannoni and Annibaldi (1981).

Bacteriological analysis was done following procedures recommended by NMC (1999).

Data for all variables measured were analysed as repeated measures using the GLM procedure of SAS (SAS, 1999). Transformation Log SCC  $\text{Log}_{10}$ .

### 3. Results and discussion

The percentage of infected half-udders along the control period was of 24.2%. CNS was the main group of pathogens (77.5%) according to results from other studies (McDougall et al., 2002). During the entire lactation 67.6% of infected halves persisted for at least three consecutive months and resulted caused by a single pathogen. The 15.6% of infected halves showed

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two consecutive infections, while 16.9% exhibited only one case of infection during lactation. The prevalence of subclinical mastitis was greater in the third or further lactations than in first or second lactation ( $P < 0.01$ ). Among ewes with 66.2% had unilateral infections; the remaining showed bilateral infections. No significant differences were observed for any trait when unilateral or bilateral infections was considered. However, mammary gland with bilateral infections showed a lower milk production and higher SCC (data not shown) when compared to gland with unilateral infections.

SCC of healthy half udders were lower ( $P < 0.01$ ) than infected half udders, confirming our previous findings (Rosati et al., 2004). Milk yield was higher ( $P < 0.01$ ) in uninfected halves than infected halves (Table 1). These results agree with that reported by Leitner et al. (2003). Milk from infected halves showed lower tritric acid (P<0.01), CN/total protein ratio and lactose content, and higher ( $P < 0.01$ ) pH and percentage of samples with suboptimal renneting properties compared with uninfected halves (Table, 1).

Compared with contralateral healthy halves, infected halves had higher ( $P < 0.01$ ) SCC (5.83 vs. 5.42) and pH (6.62 vs. 6.55) and lower ( $P < 0.05$ ) tritric acid (9.1 vs. 10.4 °SH/100 ml). No significant differences were observed for milk yield and other components (data not shown). On the contrary, Leitner et al. (2004) in a previous study found a significant reduction of milk yield and fat, protein, and casein contents.

Comparing milk samples from uninfected half udder, coming from completely uninfected or from unilateral uninfected udder, a significant difference of SCC was found, with lowest values (5.31 vs. 5.94,  $P < 0.01$ ) in milk samples coming from healthy animals.

Our data confirm findings from Dulin et al. (1983), who reported a systemic effect of half udder infection on SCC of uninfected half udder in dairy goat.

Furthermore, milk yield (0.416 vs. 0.309 kg/milking), and lactose content (5.22% vs. 5.01%) were higher ( $P < 0.01$ ) in halves of completely uninfected udders. In the context of the present study, a discrimination SCC threshold between healthy and infected half udders may be fixed around 200.000 cells/ml.

**Table 1:** LSmean and SE of milk yield, SCC, milk composition and renneting parameters in infected and uninfected udder half.

	infected udder half	uninfected udder half
SCC, Log <sub>10</sub>	5.94 ± 0.45 <sup>a</sup>	5.31 ± 0.45 <sup>b</sup>
Milk, kg/milking	0.284 ± 0.14 <sup>b</sup>	0.410 ± 0.15 <sup>a</sup>
Fat, %	4.91 ± 1.64	4.87 ± 1.32
Protein, %	5.55 ± 0.46	5.38 ± 0.70
Lactose, %	4.98 ± 0.42 <sup>b</sup>	5.16 ± 0.30 <sup>a</sup>
Casein / protein ratio, %	77.32 ± 1.38 <sup>b</sup>	78.48 ± 1.44 <sup>a</sup>
pH	6.57 ± 0.17 <sup>a</sup>	6.49 ± 0.07 <sup>b</sup>
°SH/100 ml	8.55 ± 1.69 <sup>b</sup>	10.53 ± 2.62 <sup>a</sup>
Clotting time, min	21,10 ± 5,70	20.84 ± 3.27
Rate of clot formation, mm	42.00 ± 6.47	45.57 ± 11.42
Clot firmness, min	1.58 ± 0.50	1.64 ± 0.33

a, b =  $P < 0.01$

## 4. Conclusion

The long persistency of single-pathogen infection highlighted throughout lactation, together with a detrimental effect on milk yield and composition, stresses the importance of an early and effective diagnostic procedure for mastitis control in sheep flocks.

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## I-P041: Relationship Between Freezing Point and Chemical Composition of Individual Comisana Ewe Milk

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### Summary

The aim of the study was to evaluate the freezing point trend and the relationship between chemical composition and physical properties of ewe milk.

We determined, on 150 individual milk, the freezing point, fat, protein, casein, lactose, urea, pH, titratable acidity and chloride.

Significant relation coefficients ( $r$  = Pearson) have been recorded as follow showing freezing point and the following parameters: lactose ( $r$  = -0,21;  $P < 0,01$ ), protein ( $r$  = -0,36;  $P < 0,001$ ), casein ( $r$  = -0,40;  $P < 0,001$ ), urea ( $r$  = -0,39;  $P < 0,001$ ).

### 1. Introduction

Freezing point of sheep milk is still not ruled as a law limit in Italian legislation and studies related to that are insufficient. As a consequence frequent contrasts are observed between farmers and milk product manufacturers about such trait.

It is therefore important to know the range of freezing point during the lactation period on individual and bulk milk, surely safe of any adulteration, in order to determine a reference value.

### 2. Material and methods

A total of 150 individual milk samples were monthly collected in the morning milking from February to June 2006 using one flock. The samples were obtained from 30 multiparous Comisana ewes.

The ewes were pastured and housed over night; they were fed with hay and 0.4Kg of barley and pea corn per head per day.

In June ewes were fed only with straw and 0.15Kg of barley and pea corn per head per day.

Milk samples were collected by milk meter to record milk yield.

We determined the freezing point by thermistor cryoscope; fat, protein, casein, lactose and urea by Fourier Transformed Infrared analysis (MilkoScan FT600); pH; titratable acidity (Soxhlet-Henkel) and chloride by silver nitrate titration (Mettler DL50).

The statistical analysis has been performed by BMDP new system software.

### 3. Results and discussion

In the considered period ewe milk show on average freezing point  $-0.563^{\circ}\text{C} \pm 0.008$ , fat  $6.44\% \pm 1.90$ , protein  $6.03\% \pm 0.84$ , casein  $4.8\% \pm 0.72$ , lactose  $4.77\% \pm 0.34$ , pH  $6.63 \pm 0.58$ , titratable acidity  $9.89^{\circ}\text{SH} \pm 2.25$ , urea  $39.2\text{mg/dl} \pm 6.5$  and chloride  $1.00\text{g/l} \pm 0.16$ .

The average monthly freezing point trend increased from February ( $-0.567^{\circ}\text{C}$ ) to June ( $-0.556^{\circ}\text{C}$ ) (Tab.1).

The 50.4% of the samples show a freezing point ranging between  $-0.556^{\circ}\text{C}$  and  $-0.565^{\circ}\text{C}$  (Tab.2).

41% of total milk samples showed a freezing point  $> -0.560^{\circ}\text{C}$ . Amongst those samples 41% have been collected in June where we observed higher medium value of this trait and the lowest medium value of protein and lactose.

In detail the medium value of lactose decrease from 4.75% in May to 4.43% in June (Tab.1).

In the same month of June an increase of freezing point has been observed also from other Authors (Di Antonio E. et al.; Cannas A. et al.).

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Averages of fat content increased from March to June while the yield, protein, casein and lactose decreased (Tab.1).

Tritable acidity had a little variation during the considered period except in March where we recorded the minimum value (9.29°SH). The values of pH show variations with the minimum in February and the maximum in April (Tab.1).

Significant relation coefficients ( $r = \text{Pearson}$ ) have been recorded as follow showing freezing point and the following parameters: lactose ( $r = -0,21$  ( $P < 0,01$ ) observed also from Pavic V. et al.; protein ( $r = -0,36$ ;  $P < 0,001$ ), casein ( $r = -0,40$ ;  $P < 0,001$ ), urea ( $r = -0,39$ ;  $P < 0,001$ ) (Tab.3).

**Table 1:** Monthly averages and standard deviations of the traits.

	Feb.	March	April	May	June
Freezing point	-0.567±0.008	-0.564±0.006	-0.563±0.008	-0.564±0.007	-0.556±0.006
Production (g)	410±180	483±139	422±205	400±134	352±101
Fat (%)	6.27±2.09	5.00±1.41	5.86±1.48	7.25±1.60	7.84±1.49
Protein (%)	6.29±1.01	6.10±0.73	6.17±0.75	5.83±0.75	5.76±0.88
Casein (%)	5.01±0.78	4.86±0.61	4.93±0.61	4.66±0.65	4.56±0.87
Lactose (%)	4.89±0.29	4.95±0.33	4.86±0.28	4.75±0.26	4.43±0.27
Urea (mg/dl)	45.20±8.25	38.01±4.76	35.75±3.58	40.67±4.70	36.44±5.34
pH	6.49±0.16	6.79±0.08	6.77±0.09	6.60±0.23	6.63±0.25
Trit. acidity (°SH)	10.31±3.27	9.29±1.72	10.01±2.33	10.10±1.93	10.02±1.23
Chloride (Cl <sup>-</sup> ) (g/l)	0.95±0.12	1.02±0.16	1.01±0.16	1.04±0.12	1.03±0.11

**Table 2:** Frequencies (%) of freezing point samples by classes (°C).

>-0.551	-0.551 / -0.555	-0.556 / -0.560	-0.561 / -0.565	-0.566 / -0.570	-0.571 / -0.575	<-0.575
4.1%	13.1%	24.5%	25.9%	16.6%	11.0%	4.8%

**Table 3:** Coefficients of correlation ( $r_{\text{Pearson}}$ ).

	Fat	Protein	Casein	Lactose	Urea	pH	Tritable acidity	Chloride	Freezing point
Production	-0.65***	-0.29***	-0.26**	0.53***	0.059	0.25**	-0.05	-0.19*	0.15
Fat		0.29***	0.26**	-0.65***	-0.02	-0.41***	0.13	0.13	-0.029
Protein			0.96***	-0.14	0.18*	-0.19*	0.08	-0.06	-0.36***
Casein				-0.04	0.25**	-0.23**	0.09	-0.14	-0.40***
Lactose					0.25**	0.21*	-0.09	-0.48	-0.21**
Urea						-0.39***	0.16	-0.24**	-0.39***
pH							-0.16	0.03	0.11
Trit. acidity								-0.05	-0.08
Chloride									0.07

\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$

## 4. Conclusion

In this study the traits mainly related to the freezing point were protein, lactose and urea. The most evident freezing point on monthly basis variation, was observed at the end of the lactation, at the same time to a low content of lactose and protein in milk.

More studies are needed to verify the influence of season on variation of milk freezing point.

Further investigations, especially on bulk milk, are under way.

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## I-P043: Antibiotic Dry Therapy with Penethamate Hydriodide, Benethamine Penicillin and Framycetin Sulphate in Dairy Ewes: Effectiveness Against Mammary Infection and Antimicrobial Depletion in the Postpartum Period

B. Linage<sup>1</sup>, J. A. Asensio<sup>2</sup>, A. Martínez<sup>2</sup>, C. Gonzalo<sup>1</sup>

### Summary

This experiment was carried out to know the effectiveness against the intramammary infection (IMI) of an antibiotic combination containing 100 mg of penethamate hydriodide, 280 mg of benethamine penicillin and 100 mg of framycetin sulphate (Mamyzin® Secado, Boehringer Ingelheim España S.A.) used as dry therapy (1 syringe/teat) in two random lots of Assaf ewes: control lot (CL, 156 glands) and treated lot (TL, 154 glands). This formulation diminished drastically both IMI throughout dry period and SCC of milk in dairy ewes because of higher IMI cure rate and lower reinfection and new-infection rates in TL in comparison with CL. Antibiotic residues were no detected  $\geq 54$ h postpartum.

### 1. Introduction

In dairy ewes, IMI caused by mammary pathogens elicit high somatic cell counts (SCC) and important losses on milk yield (Gonzalo et al. 2002), and remain in a high percentage from one lactation to the next (Marco, 1994; Watson & Buswell, 1984). These facts make the antibiotic dry therapy necessary to control mammary infections. The objectives were: 1) To evaluate the infection dynamic throughout dry period and know the efficacy against IMI of an antibiotic combination with penethamate, penicillin and framycetin in dairy sheep, under high IMI prevalence, and 2) to study the depletion of antibiotic residues in the postpartum period.

### 2. Material and methods

**Experiment I:** This experiment was carried out in a flock with a high IMI prevalence, in 310 glands of 155 Assaf ewes which were randomly assigned to two lots: a) Treated lot (TL) with 154 glands (77 ewes), which received a complete dry therapy (1 syringe/teat) of an antibiotic combination containing 100 mg of penethamate hydriodide, 280 mg of benethamine penicillin and 100 mg of framycetin sulphate (Mamyzin® Secado, Boehringer Ingelheim España S.A.), and b) Control lot (CL) with 156 non treated glands (78 ewes). For bacteriological identification (BBL Crystal ID system), all glands were sampled twice, in different days, within 5 days previous to abrupt drying-off of the ewes, coinciding the 2nd sampling with the last milking of lactation. The average duration of dry period was 81.6 days. The glands of 155 ewes were again sampled twice within 5 days after lambing, carrying out the first sampling  $\leq 72$  h postpartum.

**Experiment II:** After a dry period of  $>60$  days (mean: 74.0 d), the presence of residual antibiotics in 50 Assaf ewes (100 glands) dry treated with the previous antibiotic formulation, using 1 syringe/teat, was measured in colostrum and milk in the first 7 milkings postpartum. Two screening tests for antibiotic detection of  $\beta$ -lactams and aminoglycosides were used (Rosa Charm® and Blue Yellow Charm®, Charm Sciences, Inc. Lawrence, Massachusetts, USA), the detection limits (by ordinal and binary logistic regression studies) being 3-4  $\mu\text{g}/\text{kg}$  for penicillin and 704-781  $\mu\text{g}/\text{kg}$  for framycetin. Maximum residue limits (MRL) established by UE in ovine milk are 4  $\mu\text{g}/\text{kg}$  and 1500  $\mu\text{g}/\text{kg}$ , respectively. Specificity of both screening test for ovine colostrum samples ( $\leq 24$  h) was 0.966

### 3. Results and discussion

Cure, reinfection, persistent infection and new infection rates are in Table 1. The SCC variation

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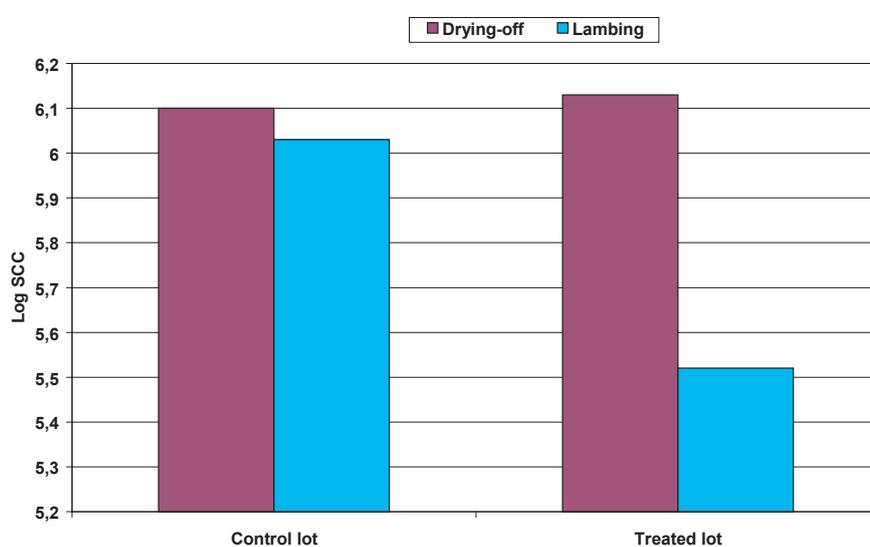
<sup>2</sup> Consorcio de Promoción del Ovino, 49630-Villalpando, Zamora, Spain.

from drying-off to lambing in both lots is in Figure 1, and the percentages of glands with antibiotic depletion in the postpartum period are in Table 2.

The IMI prevalence at drying-off and at lambing were 60.7% and 15.2% ( $P < 0.001$ ) for TL, and 53.0% and 57.1% ( $P > 0.05$ ) for CL. *Staphylococcus epidermidis* and *Streptococcus agalactiae* were the most prevalent species. The prevalence of *S. epidermidis* (42.1% at drying-off and 7.6% at lambing) and *Str. agalactiae* (9.0% at drying-off and 1.4% at lambing) significantly decreased in TL during dry period; however, CL prevalence variations for both species were not significant.

**Table 1:** IMI dynamic in both ewe lots during the dry period

Rates (gland level)	Control lot	Treated lot	$\chi^2$
Cure (%) (n/N)	13.92 (11/79)	81.82 (72/88)	76.76***
Cure-reinfection (%) (n/N)	18.99 (15/79)	5.68 (5/88)	6.99**
Persistent infect. (%) (n/N)	67.09 (53/79)	12.50 (11/88)	46.32***
New infection (%) (n/N)	24.28 (17/70)	10.53 (6/57)	4.01*



**Figure 1.** LogSCC values for ewe lots at drying-off and at lambing

**Table 2:** Percentage of dry treated glands with and without antibiotic depletion over detection limits of two screening tests used, after lambing.

Test	Result	Time postpartum						
		6h	18h	30h	42h	54h	66h	78h
Rosa Charm	Positive	5	3	2	1	0	0	0
	Negative	95	97	98	99	100	100	100
Blue Yellow Charm	Positive	3	2	1	0	0	0	0
	Doubtful	3	5	3	1	0	0	0
	Negative	94	93	96	99	100	100	100

## 4. Conclusion

The previous results show that the antibiotic formulation used as dry therapy was very effective for reducing the IMI prevalence and SCC during the dry period in dairy ewes. Antibiotic residues were not detected  $\geq 54$ h postpartum; consequently, this delay may be considered as relevant to respect MRLs as established by UE in dairy ewes

## Acknowledgements

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## THE CHALLENGE TO SHEEP AND GOAT MILK SECTORS

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*Published in four separate parts*

[Part 1: Posters I-P001 to I-P043](#)

### **ABSTRACT**

Scientific posters presented at the IDF 5th International Symposium on the Challenge to Sheep and Goats Milk Sectors, 18-20 April 2007, Alghero, Italy.  
Presented in 4 sessions related to (1) raw milk, (2) processing and product, (3) characteristics of ewe's and goat's milk products and (4) market and perspectives.

*Keywords: Acids; Artisanal; Breeding; Casein; Cheese; CLA; Ewe; Fat; Functional; Goat; Lactation; Lamb; Livestock; Market; Marketing; Milk; Non-bovine; Nutrition; Processing; Production; Protein; Quality; Raw; Rennet; Separation; Sheep; Technology; Udder; Vitamin; Yield*

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