

Special Issue

of the International Dairy Federation

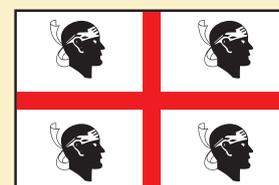


Scientific Posters Presented at the IDF 5th International Symposium on the Challenge to Sheep and Goats Milk Sectors

18-20 April 2007, Alghero, Italy



ITALIA



Istituto Zootecnico e
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International Dairy Federation (I.N.P.A.)
Diamant Building
Boulevard Auguste Reyers 80
1030 Brussels
Belgium
Phone: + 32 2 733 98 88
Fax: + 32 2 733 04 13
E-mail: info@fil-idf.org
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Special Issue of the International Dairy Federation

0801/ Part 2

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Disclaimer:

The posters in this issue of the Bulletin are presented, for information purposes, as they were received from the authors and have not been edited in any form. The views and opinions expressed in them are those of the authors and do not commit IDF in any way.



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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 5th 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-P044: Assessment of Sheep Welfare: First Results on Sarda Breed Reared in Tuscany

L. Giuliotti¹, G. Bondi², J. Goracci¹, N. Benvenuti¹, M. Mari³, G. Perfetti³

Summary

The aim of this study was to monitor the characteristics of Sarda sheep farms in order to evaluate the welfare of animals reared in semi-extensive conditions. We observed 88 flocks of Sarda sheep, located in the province of Siena. A questionnaire was drawn up to gather information about farm structures, flock management, pasture, milking systems and health conditions and filled out together with the farmer. Data showed a wide variety of farm types especially regarding size, consistency and structures.

1. Introduction

European sheep production is an important economic, environmental and sociological issue for Mediterranean countries and particularly for Italy, which along with Greece is a leader in milk sheep farming (De Rancourt et al., 2006). In Italy 70% of the ovine population are dairy ewes and Tuscany (especially the province of Siena) is the fourth-ranked region for ovine milk production. In contrast with other species, "on farm" evaluation of sheep welfare has not yet been well-defined. This assessment can play an important role in the valorization of sheep products and the underlying importance of animal welfare in public perception (Goddart et al., 2006). Generally animals bred in extensive systems show satisfactory natural behaviors although they must still be kept under strict control. Moreover, attention to animal welfare is directly linked to both high-level or good quality products and to animal health (AA.VV., 2003). Our study attempted to monitor welfare conditions in sheep farming; this could represent a first step towards the assessment of welfare levels in farms, available for certification of the quality of animal life (i.e. "Animal-friendly" or "Stress-free farm").

2. Material and methods

The research involved 88 Sarda sheep farms located in the province of Siena (Tuscany). Flock inspections were arranged with the farmer to fill out the relevant questionnaire. This report gathered information about farm structures, flock and pasture management, milking systems and animal health and every other aspect linked to sheep welfare. Data underwent statistical analysis to describe farm situations (JMP, 2002).

3. Results and discussion

Data showed a wide variety of farm types, especially regarding the number of animals (540 ± 404.2 animals) and structures. On average, flock size was medium-large; only 7% of farms reared fewer than 100 animals and the same percentage, more than 900 (Table 1). Replacement rate was 17% on average.

The interaction between human and animals in extensive system could represent a crucial interface in animal management (Goddart et al., 2006). In our study the shepherd took care of nearly 247 animals; he played an important role in controlling grazing and animal movements (Warren, 2007). Moreover, a stockperson can anticipate and prevent situations in which the animals' welfare may be at risk, by recognizing early signs of distress or ill-health (Bureau of Animal Welfare, 2001).

¹ Department of Animal Production, University of Pisa, Viale delle Piagge 2, 56124, Pisa, Italy.

² A.U.S.L. 7, Via Mascagni, 53, 53100, Siena, Italy.

³ IZS Lazio e Toscana, sez. Siena, Via Toselli, 12, 53100, Siena, Italy.

Table 1: Distribution of flock size on farms

Number of animals	%
≤100	7
101-500	45
501-900	41
900	7

In most cases, structures for animal housing were in good condition with 82% of farms built or reorganized after 1970. In 14% of cases, a place for confinement of lambing ewes was lacking; in the other cases shepherds could prevent loss of lambs due to cold weather or predation and perform adequate surveillance to ensure that ewes are not having difficulties.

Pasture was 96 ± 264.1 ha on average with mean pasture availability for animals 9 ± 0.6 ha, a good rate that assures a correct exploitation of grazing areas. Covered areas were always present with 1.4 ± 1.5 m² availability per animal. These proportions could be sufficient, taking into account the official regulation that recommends a resting area with a minimum of 0.75 m² per ewe (Council Regulation n. 1804, 1999). Reduced space could result in more rigid dominance relationships (Bøe et al., 2005). A high female/male ratio was observed (56 ± 25).

Litter always consisted of straw; cleaning frequency was twice a year, often with daily addition of material. We observed a common care for bedding cleanliness confirmed by the 93% of farm scoring good litter condition.

Pasture and hay were always provided; feed supplementation was furnished on 74% of farms and especially consisted in row feed (oat, barley, maize, sweet lupine) with 660g/day/sheep supplied on average.

Milk production displayed a fluctuation connected with milking techniques ($p \leq 0.05$); higher milk production was observed on farms where a milking machine was present (Tab. 2), most likely other factors (genetic, nutrition, management) were also involved.

Table 2: Milk production related to milking practice

Milking practice	Mean	S.D.
Manual	120.2 ^a	40.02
Mobile machine	150.6 ^b	44.17
Fixed machine	154.7 ^b	43.25

a, b: $p \leq 0.05$

Milking machines were used on 84% of farms comprising 34% of mobile types. In some cases medium-large flocks did not use milking machines, perhaps due to bonds linked with tradition. Pre-milking parlors were not provided on 64% of farms. The presence of a pre-milking parlor could help maintain a certain order of entry into the milking area; this is important because alteration of this habit could be correlated with processes that indicate stressful situations or dysfunctions in milking procedure (Villagrà et al., 2006)

The following table (Tab. 3) summarized several parameters concerning reproductive activity and health. The fertility rate of Sarda sheep was in agreement with Sanna (1992).

Table 3: Percentage incidence of some demographic parameters

	Mean	S.D.
Mastitis	1.3	2.6
Abortions	2.1	4.9
Fertility	91.6	6.7
Mortality	3.1	2.9

Parasite burden occurred on most farms and scouring in young was the pathology occurred with greater frequency.

About 60% of shepherds practiced animal mutilation, especially on tails and ears, following traditional customs. A total of 62% of farmers complained about predators, and relative damage occurred in 24% of flocks. A safe fence against wolves was provided by 38% of farmers. A number of procedures (shearing, castration, tail docking) necessary for sheep production are stressful but beneficial to the animal overall; thus proper techniques must be employed and performed quickly and competently.

Artificial insemination was never practiced but in some cases (11%) estrus synchronization was performed.

4. Conclusion

These initial results highlighted the need to improve certain husbandry practices in order to have direct positive effects on animal welfare. Concerning milking procedures, we suggest the adoption of a pre-milking parlor and the introduction of milking machines on farms with a large number of animals, where shepherds cannot ensure the same care for all the animals. Regarding flock management we noticed a high female/male ratio that could have repercussions either on animal welfare or flock fertility. On the whole, the monitoring we carried out did not show severe deficiencies regarding farm structure and management. Closer attention to the various aspects examined could prove to be an interesting focus from the perspective of a future certification regarding "on farm" welfare.

Acknowledgements

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I-P048: Conjugated Linoleic Acid in Milk from Goats Fed Supplements Enriched with Linoleic and α -Linolenic Acids

P. Luna¹, A. Bach², M.A. de la Fuente¹, J. Fontecha¹, M. Juárez¹

Summary

The aim of this research was to study milk composition and fatty acid profile, specifically conjugated linoleic acid (CLA) in modified goat milk by feeding supplements with both linseed and sunflower oil. Goats milk was monitored for a period of three months. Gas chromatography (GC) was used to analyse total CLA content and fatty acid profile. Milk fats from goats supplemented with lipid showed a healthier fatty acid profile, with lower amounts of saturated fatty acids and enhanced levels of CLA together with no substantial modification in animal performance.

1. Introduction

Total content of CLA in cow's milk has been studied and variations with different diets are relatively well known. However, less information is available on the influence of diet composition on the CLA content in goat's milk (1-2). Increasing the CLA content and changing the fatty acid profile in goat's milk by feeding a diet rich in polyunsaturated fatty acids (PUFA) may provide a value-added food. The aim of the present research was to study milk composition, animal performance and fatty acid profile, specifically CLA and vaccenic acid (VA, *trans*-11 C18:1), its precursor, in modified goat's milk fat by feeding a dietary supplement abundant in linoleic acid α -linolenic acids containing sunflower oil and linseed.

2. Material and methods

Bulk milk from a flock fed with supplemented diet in sunflower oil and linseed (Table 1) were monitored for a period of three months. Time 0 corresponded to control diet without lipid supplementation. Lactose, fat, protein, and total solids in milk were measured with a MilkoScan and a GC equipment with a capillary column was used to analyse fatty acid profile and CLA content.

Table 1: Nutrient composition of the experimental rations

Ingredients (% of dry matter)	Control	Enriched
Crude protein	18.5	18.8
Neutral detergent fibre	36.4	34.3
Nonfibre carbohydrates	35.8	34.3
Ether extract	3.62	5.1
Linoleic acid	0.51	1.11
α -Linolenic acid	0.09	0.38
Metabolisable energy (Mcal/kg)	2.81	2.98

3. Results and discussion

Milk production, milk fat, lactose, protein and total solids were not drastically affected by dietary treatments (Table 2). Milk fat from goats supplemented with PUFA showed a healthier fatty acid profile, with lower amounts of saturated fatty acids (Figure 1). *Cis*-9 *trans*-11 C18:2 or ruminic acid (RA) content, the most relevant CLA isomer, was increased in goats milk when fed the enriched diet (Figure 1) The same pattern was observed for VA in control and supplemented goats after three months (Figure 1). However, increases in other *trans* C18:1 isomers, as *trans*-10, were less remarkable.

¹ Instituto del Frío (CSIC), José Antonio Novais 10, 28040 Madrid, Spain.

² Grup de Recerca en Nutrició, Maneig i Benestar Animal, Unitat de Remugants (IRTA) 08193 Bellaterra, Spain.

Table 2: Performance of lactating goat fed a control or an enriched ration for 90 days

	Control	15 days	30 days	60 days	90 days
Milk yield (Kg/d)	2.92	2.93	2.85	2.78	2.74
Composition (%)					
Fat	4.21	4.01	3.84	3.63	4.28
Protein	3.06	3.16	3.10	3.21	3.25
Lactose	4.61	4.44	4.43	4.29	4.31
Total solids	12.75	12.60	12.48	12.04	13.01

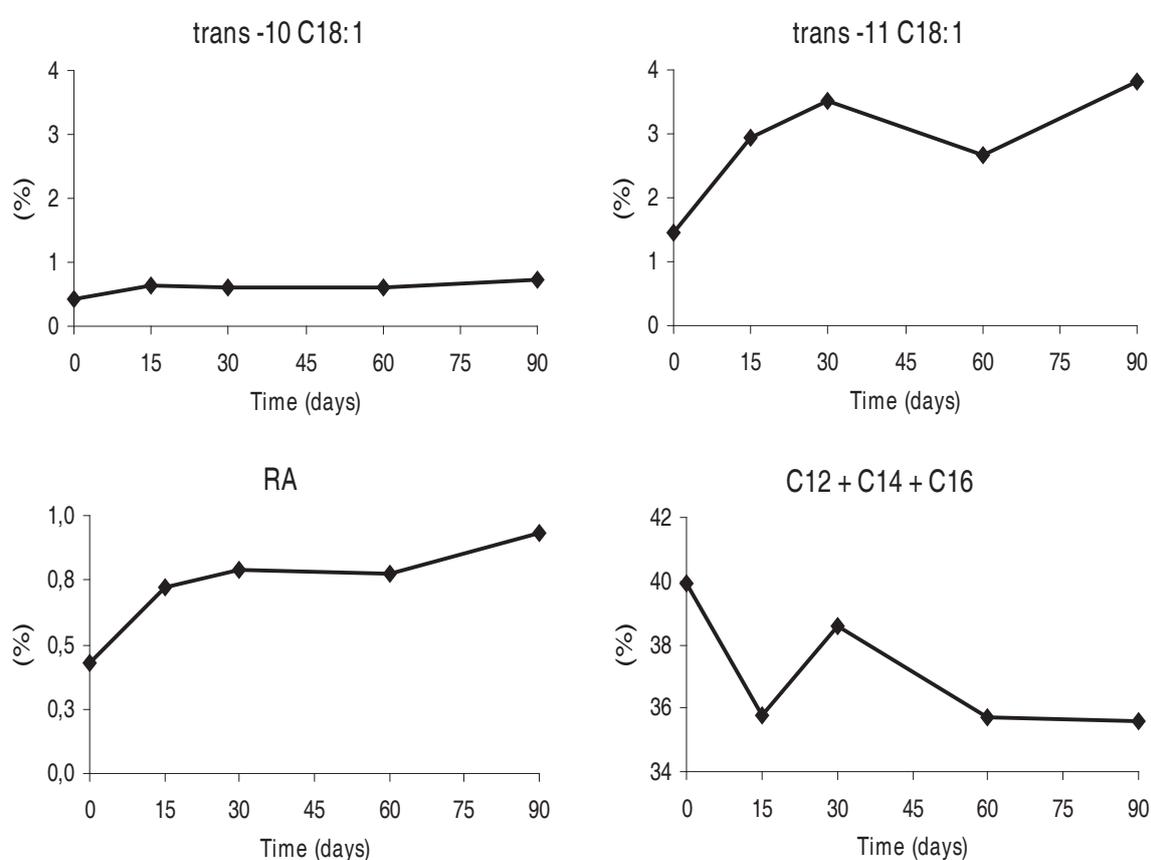


Figure 1. Evolution of *trans*-10 C18:1, *trans*-11 C18:1 (VA), *cis*-9 *trans*-11 C18:2 (RA) and the sum of C12, C14 and C16 (% of total fatty acids) in milk fat from goats fed a diet supplemented with sunflower oil and linseed for 90 days. Time 0 corresponded to control diet without lipid supplementation.

4. Conclusion

The results of this study indicate that the combined dietary supplementation of linseed and sunflower oil could be a valuable means of increasing CLA and VA and improve the fatty acid profile towards a healthier profile without important effects on animal performance.

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I-P049: The Concentration of Urea in the Milk of East Friesian and Pag Island Sheep

N. Antunac¹, B. Mioc², S. Kalit¹, D. Samarzija¹, J. Havranek¹, N. Mikulec¹, V. Pavic², Z. Prpic², Z. Barac³

Summary

The aim of the research was to determine the effect of the breed, herd, stage (0-60th day, 61st-10th day and 151th-drying off) and number of lactation I, II, III, IV and more) on the concentration of urea in sheep milk. The research was conducted between the year 2002 and 2005 on a sample of 78 East Friesian sheep (herds A and B) and 213 Pag island sheep (herds C, D and E). In total, there were 1278 individual milk samples of the morning milking. The concentration of urea in milk was determined by the spectrophotometric method. The average concentration of urea in the milk of the East-Friesian sheep was 30.18 mg/100 ml and in the milk of the Pag island sheep 36.56 mg/100 ml respectively. The influence of the breed on the concentration of urea in milk was significant ($P < 0.001$) as was also the influence of the herd ($P < 0.001$). During lactation, the concentration of urea in milk of the East-Friesian sheep was balanced, unlike the one in the Pag island sheep, where a significant increase was determined from the beginning (31.28 mg/100 ml) towards the end of lactation (36.67 mg/100 ml). Based on the research results, it can be concluded that the breed, herd and the stage of lactation have a significant influence on the concentration of urea in sheep milk.

1. Introduction

Urea is a normal constituent of milk and comprises part of the non-protein nitrogen fraction. The variation in milk urea concentration is due to the influence of factors which can be result of individual variations, and the other factors which can be result of environmental variations. Individual variations may be due to breed, milk yield, the composition of milk, stage and number of lactation. Environmental variations may be to a herd effect or seasonal variations. Urea in milk has proven to be an easy measurable indicator for the protein metabolism in dairy cattle. Their determinations in milk may serve to indicate unbalance in the ration between protein and energy and sub-optimal utilisation of feed nitrogen. The aim of the research was to determine the effect of the breed, herd, age and the stage of lactation on the concentration of urea in sheep milk.

2. Material and methods

The urea concentration was determined in the milk samples of original Pag island (213) and East-Friesian sheep (100). Milk samples (2321) were taken in month intervals from evening milking. Three herds of Pag and two herds of East-Friesian sheep were chosen. Lambs were separated from sheep at the age of 60 days. The beginning of lactation included first 60 days, the middle from 61-150 days and the end since 151 days till drying off. The winter meal of Pag sheep consisted of meadow hay and corn grits in the quantity of 200 gram/head while summer meal was based exclusively on pasture. The winter meal of East-Friesian sheep consisted of hay and mixture for lactating sheep, while summer meal consisted of green mass and ship mixture. The urea concentration in milk was determined by spectrophotometric method using Boehringer-Mannheim test. Collected data were statistically processed by using PROC ANOVA, SAS programme.

3. Results and discussion

The average concentration of urea in the milk of the Pag island sheep was 36.56 mg/100 ml and

¹ Dairy Science Department, University of Zagreb, Faculty of Agriculture, Svetošimunska 25, 10000 Zagreb, Croatia.

² Department of Animal Production, University of Zagreb, Faculty of Agriculture, Svetošimunska 25, 10000 Zagreb, Croatia.

³ Croatian Livestock Center, Ilica 101, 10000 Zagreb, Croatia.

in the milk of the East-Friesian sheep 30.18 mg/100 ml, respectively (Table 1). The influence of the breed on the concentration of urea in milk was significant ($P < 0.001$) as was also the influence of the herd ($P < 0.001$) of the analysed breeds. The effect of year was not significant for the concentration of urea in milk in Pag island sheep (37.17 mg/100 ml in 2002 and 35.52 mg/100 ml in 2003) and in milk of East-Friesian sheep (29.84 mg/100 ml in 2004 and 30.82 mg/100 ml in 2005). During lactation, the concentration of urea in milk of the East-Friesian sheep was balanced, unlike the one in the Pag island sheep, where a significant increase was determined from the beginning (31.28 mg/100 ml) towards the end of lactation (36.67 mg/100 ml).

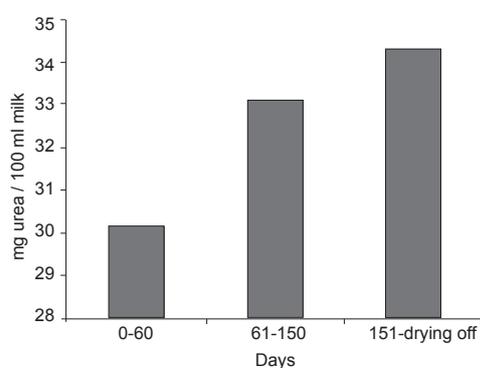


Figure 1. Effect of stage of lactation on the urea concentration in milk for both breed.

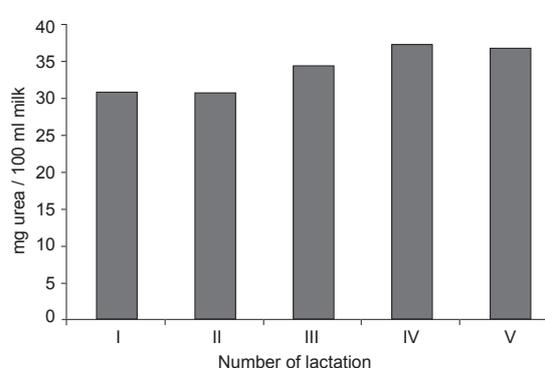


Figure 2. Effect of number of lactation on the urea concentration in milk for both breed

Table 1: Daily milk yield (DMY) and concentration of urea and protein content in milk of two different breed

Parameter	East-Friesian sheep	Paska sheep	P-value
DMY* (ml)	1034	644	$P < 0.001$
Urea (mg/100 ml)	30.18	36.56	$P < 0.001$
Protein (%)	5.04	5.75	$P < 0.001$

*Daily Milk Yield

Table 2: Influence of number of lactation on daily milk yield, concentration of urea and protein content in milk of both breed

Parameter	Number of lactation				
	I.	II.	III.	IV.	V.
DMY* (ml)	752 ^a	1026 ^b	885 ^c	661 ^a	663 ^a
Urea (mg/100 ml)	30.79 ^a	30.74 ^a	34.40 ^b	37.25 ^b	36.58 ^b
Protein (%)	5.02 ^a	5.10 ^a	5.51 ^b	5.79 ^c	5.84 ^c

*Daily Milk Yield

a,b,c Means within the same row and not sharing the same superscript letter are significantly different ($P < 0.01$).

4. Conclusions

On the urea concentration in milk, significant influence had breed, herd, stage and number of lactation, while the influence of the year was not significant. Significant correlations were

determined between the urea concentration in milk and daily milk yield i.e. protein content in milk ($0.12 \text{ i } -0.09$).

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I-P050: Influence of Some Non-genetic Parameters on Production and Quality of Milk of East-Friesian Sheep in Croatia

N. Antunac¹, S. Kalit¹, D. Samarzija¹, B. Mioc², M. Pecina³, N. Mikulec¹, J. Havranek¹, V. Pavic²

Summary

The aim of this paper is to find influences of non-genetic parameters (herd, season, stage and number of lactation) on milk production, chemical composition, physical properties and hygienic quality of East-Friesian sheep. There were no significant differences between observed parameters considering herd and season influences. But significantly lower milk production, lactose content, pH value, and higher total solids, milkfat, protein and solids non fat content were found at the end of lactation. No significant differences between beginning and middle of lactation were found considering all analyzed parameters except number of bacteria in milk. The highest milk production and freezing points were found in second and third lactation. The highest protein content was found in third lactation and the lowest lactose and solids non fat content were found in fourth and more lactation. Finally, the highest somatic cell counts and bacteria counts were found in third or higher lactation.

1. Introduction

Sheep milk production and processing has become very important in last decade in Croatia. In Croatian sheep breed structure, traditional breeds are predominant which are characterised by low milk production, but high total solids content in comparison to high milk productive breeds. Five years ago East-Friesian breed was introduced in Croatian farms. Therefore the aim of this paper is to find influences of non-genetic parameters (herd, season, stage and number of lactation) on milk production, chemical composition, physical properties and hygienic quality of East-Friesian sheep.

2. Material and methods

From 100 sheep of East-Friesian breed, the individual milk samples were taken in first, second, third, fourth and more lactation in monthly intervals. Sheep were distributed in two herds (A and B) in different part of Croatia. Analyses were done for chemical, physical and hygienic quality. During two years 1354 individual milk samples were collected. Lambs were separated from sheep at the age of 60 days. The beginning of lactation included first 60 days, the middle from 61-150 days and the end since 151 days till drying off. Sheep milk samples were taken from evening milking. The following methods were used for determining the chemical composition of milk: infrared spectrometric method (HRN ISO 9622:2001). Freezing point of milk was determined by cryoscopic method (HRN EN ISO 5764:2003) and ionometric acidity by pH meter. The somatic cell count was determined by fluoro-opto-electronic method (HRN EN ISO 13366-3:1999) and the total bacterial count by flow cytometric method (HRN ISO 4833:2003). Collected data were statistically processed by using PROC ANOVA SAS (1996) programme.

3. Results and discussion

Stage and number of lactation had significant influence on most analysed parameters (Table 1 and 2), but there were no significant differences between observed parameters considering herd and season influences.

¹ Dairy Science Department, University of Zagreb, Faculty of Agriculture, Svetosimunska 25, 10000 Zagreb, Croatia.

² Department of Animal Production, University of Zagreb, Faculty of Agriculture, Svetosimunska 25, 10000 Zagreb, Croatia.

³ Department of plant breeding, Genetic, Biometrics and Experimentation, University of Zagreb, Faculty of Agriculture, Svetosimunska 25, 10000 Zagreb, Croatia.

Table 1: Influence of stage of lactation on milk production and quality of East-Friesian sheep in Croatia

	Stage of lactation		
	0-60 days	61 – 150 days	151 – drying off
Daily milk production (ml)	1292 ^a	1226 ^a	756 ^b
Total solids (%)	16.13 ^a	15.96 ^a	17.71 ^b
Milkfat (%)	5.71 ^a	5.42 ^a	6.72 ^b
Protein (%)	4.64 ^a	4.74 ^a	5.47 ^b
Lactose (%)	4.93 ^a	4.89 ^a	4.6 ^b
Solids non fat (%)	10.38 ^a	10.54 ^a	11.00 ^b
pH value	6.66 ^a	6.64 ^a	6.59 ^b
Freezing point (°C)	-0.5672	-0.5727	-0.5734
log SCC*/ml	5.31	5.21	5.42
log CFU*/ml	4.49 ^a	5.05 ^b	4.52 ^a

^{a, b} = means in the raw with no common superscript letters are different (P<0.01).

SCC*=somatic cell count.

CFU*=colony forming unit.

Table 2: Influence of number of lactation on milk production and quality of East-Friesian sheep in Croatia

	Number of lactation				P-value
	I.	II.	III.	IV. and more	
Daily milk production (ml)	914 ^a	1247 ^b	1300 ^b	905 ^a	P<0.001
Total solids (%)	16.82	16.59	16.65	16.32	NS
Milkfat (%)	6.16	5.98	5.89	5.77	NS
Protein (%)	4.92 ^a	5.01 ^a	5.19 ^b	5.13 ^{ab}	P<0.01
Lactose (%)	4.86 ^{ab}	4.81 ^{cd}	4.83 ^{bc}	4.73 ^d	P<0.01
Solids non fat (%)	10.69 ^a	10.65 ^a	10.82 ^a	10.57 ^b	P<0.001
pH value	6.62 ^a	6.62 ^a	6.63	6.67 ^b	P<0.05
Freezing point (°C)	-0.5741 ^a	-0.5728 ^{ab}	-0.5696 ^b	-0.5775 ^a	P<0.05
log SCC/ml	5.19 ^a	5.11 ^a	5.36 ^b	5.38 ^b	P0.001
log CFU/ml	4.48 ^a	4.42 ^a	4.89 ^b	4.95 ^b	P<0.05

^{a, b} = means in the raw with no common superscript letters are different (P<0.01).

4. Conclusion

Significantly lower milk production, lactose content, pH value, and higher total solids, milkfat, protein and solids non fat content were found in last part of lactation. No significant differences between beginning and middle part of lactation were found considering all analyzed parameters except number of bacteria in milk. The highest milk production and freezing points were found in second and third lactation. The highest protein content was found in third lactation and the lowest lactose and solids non fat content were found in fourth and more lactation. Finally, the highest somatic cell counts and bacteria counts were found in third or higher lactation.

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I-P051: Amino Acid Composition and Nutritional Value of Goat Milk from the Indigenous Greek Breed

E. Kondyli¹, M.C. Katsiari¹, L.P. Voutsinas¹

Summary

Raw goat milk samples from the indigenous Greek breed in the area of Ioannina, Northwestern Greece, were collected during one lactation and analyzed for their amino acids content. The major amino acids were proline, glutamic acid, alanine and leucine with mean contents 831.9, 720.7, 273.7 and 237.6 mg/ 100ml milk, representing 23.1, 20.0, 7.6 and 6.6% of the total amino acid content, respectively. Seasonal variations were observed for almost all of the amino acids studied except serine, alanine and leucine. The observed variations of the amino acids contents might be possibly attributed to the differences in total protein content of goat milk during lactation.

1. Introduction

In the last decades an increasingly important role in the human diet has been attached to goat milk, since it possesses unique properties which distinguish it from cow milk and make it a valuable alternative not just for infants but also for adults and especially nursing mothers (Baldo, 1984). Greece is the first among the European countries in goat population (6,000,000 animals) and produces about 450,000 tones of goat milk per year. Most of the animals (85%) belong to the Greek native breed and they are frugal in feeding, durable and well adapted to the mountainous environmental conditions of Greece. Goat milk in Greece is mainly used for cheesemaking and recently there is an increasing demand for consumption as fluid.

There are many studies concerning the main constituents and some physicochemical properties of goat milk but no information at all on the amino acid profile of goat milk of the native Greek breed. Thus, the objective of the present study was to obtain data on the amino acid composition of the raw goat milk samples.

2. Materials and methods

Four milk samples were taken every month during the period from December to July and analyzed for their amino acids content. For the amino acid analysis a small volume (5 μ l) of skim goat milk was put into glass tubes and concentrated to dryness. Hydrolysis of the samples was performed in the gas phase with 6N HCL at 150° C for 1.5 h. Free amino acids were derivatized with phenyl-isothiocyanate (PITC) according to Bidlingmeyer et al., 1985. Separation of the PTC amino acids was achieved by reverse phase HPLC on a Pico Tag amino acids analysis column (Waters Milford, MA, USA).

3. Results and discussion

The major amino acids in the goat milk were praline(Pro), glutamic acid, alanine and leucine with mean contents 831.9, 720.7, 273.7 and 237.6 mg/ 100ml milk, representing 23.1, 20.0, 7.6 and 6.6% of the total amino acid content, respectively. Especially the concentration of Pro found in this study was higher than those reported in the literature for goat milk of other breeds (Haenlein, 1996) and cow milk (Lindmark-Mansson et al., 2003). The lowest mean contents were observed for methionine (Met) and cysteine (Cys) (45.6 and 9.0mg/ 100ml milk), representing 1.3 and 0.3% of the total amino acid content, respectively. Higher values of Met and Cys were reported in the literature for goat (Haenlein, 1996) and cow milk (Lindmark-Mansson et al., 2003)) than those found in this study. Higher concentrations were observed for the most of the amino acids in December and January than in the other months. No significant ($P>0.05$)

¹ National Agricultural Research Foundation, Dairy Research Institute, 452 21 Katsikas, Ioannina, Greece, email: efikon.ig@nagref.gr

differences in the concentrations of serine, alanine and leucine were observed during the whole period.

Table 1: Amino acid composition (mg/ 100ml milk) of goat milk from the indigenous Greek breed during lactation

Amino acid	December	January	February	March	April	May	June	July	Mean \pm se
Aspartic acid	244.8 ^b	235.5 ^b	199.3 ^{ab}	171.7 ^a	167.7 ^a	203.6 ^{ab}	169.1 ^a	174.3 ^a	195.8 \pm 5.1
Glutamic acid	828.2 ^{ab}	885.5 ^b	666.3 ^a	707.5 ^{ab}	642.8 ^a	699.5 ^{ab}	698.7 ^{ab}	636.9 ^a	720.7 \pm 21.1
Serine	154.6	157.6	125.1	132.4	118.7	147.2	138.2	114.5	136.1 \pm 4.9
Glycine	85.8 ^b	63.8 ^a	57.1 ^a	58.6 ^a	51.8 ^a	62.3 ^a	65.3 ^a	57.1 ^a	62.7 \pm 2.1
Histidine	96.6 ^c	88.4 ^c	65.2 ^a	74.5 ^b	63.7 ^a	75.3 ^b	74.5 ^b	62.1 ^a	75.0 \pm 2.7
Arginine	176.6 ^c	142.8 ^{bc}	111.5 ^a	123.7 ^{ab}	102.8 ^a	126.3 ^{ab}	102.1 ^a	92.3 ^a	122.3 \pm 5.3
Threonine	223.9 ^b	196.5 ^{ab}	157.2 ^a	167.9 ^{ab}	148.9 ^a	157.2 ^a	157.2 ^a	153.6 ^a	170.3 \pm 6.9
Alanine	303.6	317.2	252.1	274.4	226.3	287.7	288.6	239.6	273.7 \pm 10.4
Proline	941.6 ^b	839.1 ^{ab}	839.5 ^{ab}	864.5 ^b	836.3 ^{ab}	720.2 ^a	790.8 ^{ab}	858.3 ^b	831.9 \pm 14.5
Tyrosine	100.6 ^b	66.9 ^a	69.0 ^a	60.3 ^a	62.5 ^a	77.5 ^{ab}	71.1 ^a	75.4 ^a	72.9 \pm 2.4
Valine	249.0 ^b	226.0 ^{ab}	181.5 ^a	190.8 ^{ab}	169.8 ^a	194.9 ^{ab}	207.2 ^{ab}	166.3 ^a	198.2 \pm 6.5
Methionine	56.6 ^c	38.8 ^a	43.2 ^b	65.6 ^c	35.8 ^a	47.0 ^b	46.2 ^b	31.3 ^a	45.6 \pm 2.1
Cysteine	12.1 ^b	12.1 ^b	10.9 ^b	8.5 ^a	6.1 ^a	6.1 ^a	8.5 ^a	8.5 ^a	9.0 \pm 0.5
Isoleucine	156.6 ^b	156.1 ^b	118.1 ^{ab}	114.1 ^{ab}	101.0 ^a	131.2 ^{ab}	150.8 ^b	120.7 ^{ab}	131.0 \pm 4.7
Leucine	234.5	266.3	242.0	245.3	203.3	242.1	252.3	213.8	237.6 \pm 8.0
Phenylalanine	161.8 ^b	140.4 ^{ab}	118.9 ^a	125.5 ^{ab}	105.7 ^a	133.8 ^{ab}	145.3 ^{ab}	118.9 ^a	131.3 \pm 4.5
Lysine	197.6	188.6	190.0	178.4	176.9	187.6	181.0	194.4	186.8 \pm 5.9

^{a, b, c}: Means within a row without a superscript or bearing the same superscript do not differ significantly ($P > 0.05$). Mean: average of 32 samples. Se: standard error.

Table 2: Daily adult requirements for essential amino acids and quantities of goat milk necessary to supply them

Essential amino acid	Amino acid content (mg/ 100ml) of goat milk *	Minimum daily requirement (mg)	Goat milk necessary for supply (ml)
Phe + Tyr	204.2	1100	538
Leucine	237.6	1100	463
Isoleucine	131.0	700	534
Threonine	170.3	500	293
Met + Cys	54.6	1100	2014
Valine	198.2	800	403
Lysine	186.8	800	428

* Mean values taken from Table 1

4. Conclusions

The results of this study showed that the goat milk of the native Greek breed in the region of Ioannina, generally, contained sufficient quantity of the essential amino acids a fact which has a great impact on its nutritional quality. The daily requirements of almost all essential aminoacids could be covered by the consumption of about 500 ml (two glasses) of goat milk. This is only deficient in Met and Cys, which can be supplied by more milk or by the addition of some cheese in the diet (Renner, 1983).

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I-P052: Effect of the Type and Level of Concentrate to Grazing Dairy Goats on Milk Production and Quality of Cheese

Y. Lefrileux¹, A. Pommaret¹, N. Cirier², J. Le Scouarnec²

Summary

Because of Protected Designation of Origin regulation, farmers often use very low level of concentrates for dairy goats. It seemed useful to evaluate the interest of maintaining a complete feed or working directly with raw material.

Two levels (0.5 or 1 kg) and two kinds (pelleted feed or corn grain) were compared on a grazing flock of high genetic level. Milk yield and fat increased with pelleted feed and with the amount of concentrate. The needs of goats were covered without increasing much the concentrate quantity.

1. Introduction

Due to PDO regulation, the authorities advise producers to use mainly feedstuffs grown locally. However a maximum of 20% of feedstuffs can originate from outside the designated area. Usually, those are the pelleted concentrates used to adjust the protein and fat supply. Thus, the aim of this study was to specify the impacts of different type of feeding i.e. pelleted concentrate v.s. whole corn grain), onto grazing dairy goats milk production and cheese quality.

2. Material and methods

The experimental plan consisted in a block with two factors: Factor 1, type of feed (pelleted feed or whole corn grain) Factor 2, quantity of feed (0,5 or 1,0 kg /goat/day). The main characteristics of the experimental concentrate are a high crude protein level (26%), PDIA (115 g/kg), celluloses(8%), protected fat (9%). Four groups of 27 goats each were given different feedstuffs for 125 days (table 1), starting at the grazing season.

Each week, the following data were collected about each goat : milk yield, protein, fat, cells count; urea and non protein nitrogen of mixture milk. All along the trial, those milks have been processed into lactic cheese. Technological and tasting tests were conducted.

Table 1: Daily supply per goat (through concentrate)

Group	0,5 kg corn	1kg corn	0,5 kg granulate	1kg granulate
UF	0,55	1,09	0,50	1,01
PDIA(g)	26	52	57	115
PDIN(g)	35	70	95	190
PDIE(g)	52	103	79	158
Fat(g)	21	42	43	87

3. Results and discussion

Supplying 1 kg of concentrate increased by 13.5% the milk production and by 8% fat percentage, without modifying the protein percentage (table 2). Consequently, cheese yield has increased. Urea level in milk has been modified with the using of concentrate without affecting lactic technology ($p < 0,01$), respectively for the 4 groups, 569^a 508^a 687^b et 734^b mg/l. Non protein nitrogen analysis in milks gives very similar results. Fat increase in milk is explained by protected fat addition in concentrate ($p < 0,01$). Regarding cheese processing, acid quantities produced

¹ Station expérimentale régionale caprine, domaine Olivier de Serres 07170 Mirabel, France

² Evalis, Service Recherche, BP235 56006 Vannes Cedex, France

and acidification kinetic were not modified by the process. Triangular tasting tests showed an impact of fat level in milk as a perceptible element by tasters when there were substantial differences (> 3 points) and when maturing time was longer (14 days).

Table 2: Effects of treatments on performances of dairy goats

Group	0,5 kg corn	1kg corn	0,5 kg granulate	1kg granulate
Milk (kg) S D	4,04 ^b 0,96	4,14 ^b 0,93	4,44 ^b 0,97	4,80 ^a 0,97
Fat (g/kg) S D	36,69 ^b 3,97	38,70 ^{ab} 4,58	40,38 ^a 5,93	41,10 ^a 5,30
Protein (g/kg) S D	32,23 2,90	31,74 3,41	33,47 2,68	32,62 2,13

^{a,b} Values with different superscripts within the same line are different ($p < 0.05$)

4. Conclusion

This trial validates the positive effects of a feeding program including quality protein and protected fat so as to drive the production in terms of quantity and quality, on a high genetic level flock. The needs of the goats are covered without increasing much the amount of concentrates or cereals.

I-P053: Effect of Vitamin and Trace Elements Supplementation on the Control of Ewe Milk Somatic Cell Counts and Subclinical Mastitis

D. Bergonier¹, J.P. Guitard², X. Berthelot¹, J. Le Scouarnec³

Summary

The aim of this study was to assess the effect of a reinforced supplementation with vitamin E, A and four trace elements on the prevention of subclinical mastitis in dairy ewe. A controlled trial was performed in a Lacaune recorded flock by comparing a control (basic supplementation) and an assay (reinforced supplementation) groups during and after a differential supplementation of 3 months after weaning (n=2x50). The mean somatic cell counts and number of chronically affected udders (resp. milk yield) were significantly lower (resp. higher) in the assay than in the control group. Bacteriologically, there was no significant difference between the groups in the number, the duration, the associated bacteria and the mean titre of shedded bacteria. Most likely reinforced supplementation may have improved the neutrophil functionality (and possibly other udder defence mechanisms), and thus enhanced the bacteria phagocytosis. These results need confirmation and adaptation to the various field situations according to the different possible (sub)-deficiency status.

1. Introduction

For the dairy ewe production, one of the main challenges is the management of the hygienic quality of raw milk. To improve its bacteriological and cytological quality, prevention of mastitis is a critical point. Moreover, the inclusion of somatic cell counts (SCC) in the differential milk payment in certain areas imposes the development of large scale mastitis control programmes. One of the proposed preventive measure is the supplementation with vitamins and trace elements (TE). In dairy cattle, an extensive literature is available regarding their effect on the udder immuno-stimulation [1]. The goal of this study was to assess the effect of vitamin E, A and four TE supplementation on the prevention of subclinical mastitis in the field conditions.

2. Material and methods

A controlled trial was performed in a commercial Lacaune dairy ewe flock. Two groups of 50 lactating ewes were compared. Ewes were assigned to the groups by stratified-random sampling. The stratification was performed at the end of the suckling-milking period on the following criteria: SCC and udder clinical scores of the previous lactation and of the beginning of the current one, glutathione peroxydase (GSHpx) activity (beginning of lactation) and parity. After stratification, ewes were randomly assigned to a control and assay groups. Both of them received a ration including silage, hay and concentrates. The control group received a vitamin and TE supplement close to the usual recommendations (as for the 100 ewes during the previous lactation). The assay group supplement was reinforced for vitamin E (x6), vitamin A, selenium, zinc and cobalt (x3), and copper (x1.5). Differential supplementation lasted 3 months from the weaning to the beginning of the grazing period (fig. 1, cel1 to cel8), with a total trial length of 7 months. Ewes of the 2 groups were kept under the same husbandry conditions. They were physically separated and machine-milked after the rest of the flock. At mid-experiment, the milking order of the two groups was reversed. The induced effects were analysed by a series of 12 milk recordings (yield, protein and fat contents, SCC on composite samples performed by the opto-fluoro-electronic method), udder clinical scoring and bacteriological examinations of half-udder milk samples (5 times). Bacteria were isolated, identified by the API system (BioMerieux, France) and enumerated. Enumeration was performed by duplicate plating of

¹ INRA-National Veterinary School UMR 1225, BP 87614, 31076 Toulouse Cedex, France.

² Agronomic College, Saint-Affrique (Aveyron), France.

³ Evalis, Research Department, BP 234, 56006 Vannes Cedex, France.

several milk decimal dilutions onto tryptic soy and counting the colonies. Plasmatic vitamin and TE concentrations were also determined 3 times (data not shown).

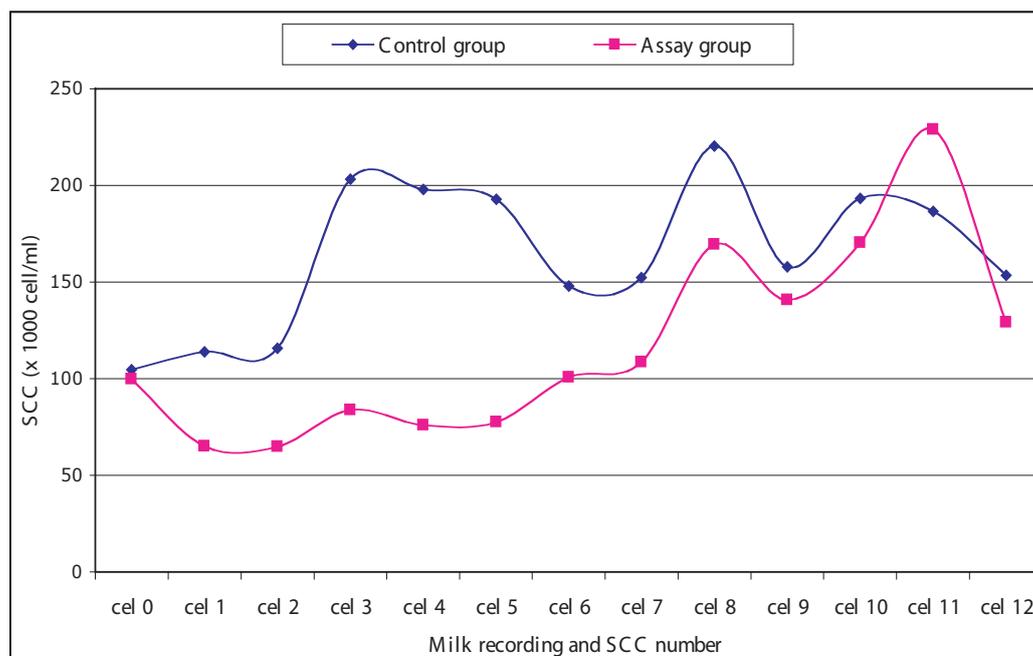


Figure 1. Somatic cell counts (geometrical mean) from the beginning of the differential supplementation (cel 1) to the end of the experimentation. Cel x: xth milk recording and composite milk somatic cell count (SCC).

3. Results and discussion

Milk yield of the assay group was higher than the control group (+7%, not significant [NS]). Geometric mean SCC of the assay group was also significantly ($p < 0.05$) lower. Before the beginning of the differential supplementation (fig. 1, cel0), the two groups had equivalent SCC geometric mean, at around 100 000 cell/ml, which corresponds to low values [2]. The main goal of this study was indeed to assess the preventive effect of the supplementation. The difference between the two groups quickly appeared and increased from cel3; this difference peaked at 125 000 cell/ml (geometrical mean). After the 8th record (cel8), too many ewes had been culled for insufficient milk production to allow a valuable interpretation of the curves (10 ewes had been culled at cel12). Reversion of the milking order of the two groups after cel6 was not associated to a reversion of the SCC kinetics. After 2 parenteral administrations of vitamin E and selenium during the dry period, a significant difference was also obtained for the SCC but not for the clinical mastitis rate in Sardinian dairy ewes [3]. In our study also, only one clinical acute mastitis occurred during the whole experiment.

The number of chronic mastitis throughout the lactation, assessed by clinical scoring, was lower in the assay group (NS). Chronic mastitis symptoms mainly appeared as 'unbalanced' udders on and after mid-lactation (after cel7). These chronic unilateral lesions of the mammary parenchyma are presumably associated with the drop in milk production particularly observed in ewes of the control group.

No difference between the 2 groups were obtained regarding the isolated bacterial species. Among the identified intra-mammary infections (IMI), 79% were due to coagulase negative staphylococci (particularly *S. xylosus* and *S. epidermidis*), 5.1% to *Streptococcus* spp., 5.1% to *Corynebacterium* spp. and 10.2% to Gram negative bacteria. This bacteriological profile is

representative of the aetiology of subclinical mastitis in dairy ewe [2]. There was no significant difference between the 2 groups in the number of new infections : 23 and 25% of udder-halves became infected during the experimentation. No effect was observed regarding the duration of the IMI, the majority (66%) being short infections (only 1 positive bacteriological result). Finally, the mean titre of shedded bacteria was lower for the assay group, but the difference was not significant. Regardless the group, the magnitude of the shedded titers was significantly higher during the *post partum* period (during suckling) and after the beginning of grass feeding ($p < 0.05$).

In dairy cattle, Vitamin E supplementation is associated with a reduction in SCC by a factor of 0.7 and a 30% decrease in the risk of clinical mastitis [1]. The reduction of IMI risk is lower (14%). Our preliminary results need to be confirmed, and will be completed by the inclusion of more individuals in the statistical analysis, and by integrating the plasmatic vitamin and TE concentrations study. However, in cattle as probably in ewes, vitamin E and selenium, in particular, appear to enhance phagocytic cell function by playing an antioxydant role which may have beneficial consequences on the intracellular killing of ingested bacteria [1].

4. Conclusion

Basically, the classical mastitis control plans remain fundamental, with preventive (milking) and curative (treatment and culling) measures. However, vitamin and TE supplementation may be added especially in case of deficiency.

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I-P054: Interactions Between Bacteria Type and Physico-chemical Properties of Goat's Milk

G. Leitner¹, O. Krifucks¹, S. Shapiro¹, N. Silanikove², U. Merin³

Summary

Coagulase negative staphylococci (CNS) are the major causative bacteria of subclinical udder infection in sheep and goats. Infections with common CNS species were studied to assess their effect on milk quality. Milk coming from glands infected by different bacteria species affected differently milk composition and curd yield. Infection with *S. xsilosus* and *S. chromogenes* elicited the strongest negative response. Our data suggest that the interaction between bacteria and the host innate immune system is specie-specific and governs the response to the infection.

1. Introduction

Udder health affects milk yield and quality and consequently cheese production. High somatic cell count (SCC) in milk of cow, sheep and goats causes longer coagulation time and weaker coagulum and leads to increased moisture content in the cheese and lower cheese yield (Auld and Hubble, 1998; Leitner et al., 2004a,b). However, it is doubtful if SCC can be used as a single criterion to correlate between intramammary infection (IMI) and cheese yield and quality, due to the complicated interrelationship between bacteria type, inflammatory response of the host immune system and proteolytic activity (caseinolysis) (Le Roux et al., 2003; Leitner et al., 2006).

It was shown by our group that the effect of four major udder pathogens: *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus dysgalactiae* and *Streptococcus chromogenes*, that are commonly associated with subclinical mastitis in dairy cow, increased SCC, modified leukocyte cell distribution, decreased lactose concentration and increased caseinolysis. These changes were associated with increased clotting time and decreased curd firmness. Although the general pattern of bacterial invasion was similar, each type of bacteria elicited the above described responses in a specific manner with no single correlation to any of the parameters tested (Leitner et al., 2006).

In dairy sheep and goats, the proportion of udder halves with subclinical IMI differ among countries and husbandries and could reach up to 70% of the glands in a given herd. The main pathogen group in infected glands comprises various species of CNS, mainly *Staphylococcus epidermidis*, *Staphylococcus symulans* and in goats also *Staphylococcus caprae*. In our previous studies, we did not differentiate between the effects of CNS sub-types on milk quality. This study focused on the interaction between intramammary infection by a specific CNS specie and milk composition and quality for cheese production in goats.

2. Materials and methods

Data from 520 Alpine, Saanen and Shami crossbreed goats (1040 udder halves) at different lactation number and stage of lactation were evaluated. Milk was sampled during the morning milking and was analyzed as described before (Leitner et al., 2004a,b; 2006).

3. Results and discussion

The interaction between SCC and milk composition or curd yield was not significant, though SCC was significantly higher ($P < 0.05$) in milk from infected glands as compared to milk from bacteria free glands, regardless of bacteria type. Infection with *S. xsilosus* and *S. chromogenes* elicited the highest increase in SCC and the greatest decrease in lactose concentration and

¹ National Mastitis Reference Center, Kimron Veterinary Institute, P.O. Box 12, Bet Dagan 50250, Israel.

² Institute of Animal Science, Agricultural Research Organization, The Volcani Center, P.O. Box 6, Bet Dagan 50250, Israel.

³ Department of Food Science, Agricultural Research Organization, The Volcani Center, P.O. Box 6, Bet Dagan 50250, Israel.

increase in fat content (Table 1). Protein was higher in the infected halves in comparison to uninfected ones. This could be attributed to increase in whey proteins, because casein content did not differ between infected and uninfected glands. The concentration of proteose peptone was 1.5 times higher in the infected glands compared to the uninfected ones. Plasmin activity was significantly higher in the infected glands, whereas plasminogen activity was undetectable. Plasmin activator was higher in the infected glands compared to the uninfected ones. Clotting time was similar in all the samples irrespective to infection status. However, curd yield was significantly lower in milk coming from glands infected with *S. caprae* and *S. chromogenes*, whereas infection with other bacteria did not affect this measure.

Table 1: Somatic cell count, milk composition, plasmin activity, milk clotting time and curd yield in goat's milk from uninfected glands and glands infected by various CNS bacteria

	Bacteria-free	<i>S. caprae</i>	<i>S. epidermidis</i>	<i>S. symulans</i>	<i>S. xsilosus</i>	<i>S. chromogenes</i>
Number*	624	155	102	98	48	12
SCC ($\times 1000$)	551 \pm 40 ^d	1186 \pm 119 ^c	1939 \pm 228 ^b	2049 \pm 179 ^b	2721 \pm 207 ^a	3294 \pm 784 ^a
Fat (%)	3.06 \pm 0.06 ^d	3.29 \pm 0.11 ^{ab}	3.43 \pm 0.01 ^{ab}	3.17 \pm 0.07 ^d	3.67 \pm 0.11 ^a	3.76 \pm 0.24 ^a
Protein (%)	3.61 \pm 0.03 ^b	3.76 \pm 0.05 ^{ab}	3.88 \pm 0.09 ^{ab}	3.86 \pm 0.08 ^{ab}	3.96 \pm 0.01 ^b	4.11 \pm 0.06 ^a
Casein (%)	2.94 \pm 0.03	3.02 \pm 0.05	3.03 \pm 0.09	3.00 \pm 0.08	3.09 \pm 0.01	3.08 \pm 0.06
Lactose (%)	4.81 \pm 0.01 ^a	4.70 \pm 0.03 ^d	4.68 \pm 0.01 ^d	4.68 \pm 0.06 ^d	4.47 \pm 0.04 ^c	4.51 \pm 0.04 ^c
Whey proteins (%)	0.67 \pm 0.02 ^c	0.74 \pm 0.06 ^c	0.85 \pm 0.04 ^b	0.86 \pm 0.04 ^b	0.87 \pm 0.04 ^b	0.102 \pm 0.04 ^a
p-p (mg/mL)	0.34 \pm 0.05 ^b	0.55 \pm 0.02 ^a	0.47 \pm 0.04 ^a	0.44 \pm 0.08 ^a	0.47 \pm 0.04 ^a	0.42 \pm 0.04 ^a
Plasmin (U/mL)**	13.5 \pm 0.05 ^c	20.3 \pm 0.10 ^b	23.5 \pm 0.19 ^b	27.2 \pm 0.12 ^a	27.7 \pm 0.07 ^a	28.1 \pm 0.08 ^a
PA (U/mL)**	609 \pm 35 ^b	722 \pm 14 ^b	883 \pm 22 ^a	613 \pm 12 ^b	704 \pm 15 ^a	975 \pm 11 ^a
Clotting time (sec)	168 \pm 10	161 \pm 9	191 \pm 4	178 \pm 6	176 \pm 3	162 \pm 7
Curd yield (g/L)	69.17 \pm 0.1 ^a	60.56 \pm 0.2 ^b	68.18 \pm 0.1 ^a	69.17 \pm 0.3 ^a	70.17 \pm 0.1 ^a	63.54 \pm 0.2 ^b

* Number of udder halves.

** U = activity unit; 1 unit is the amount of PL that produces a change in absorbance of 0.1 at 405nm in 60 min.

^{a,b,c,d} Means sharing common superscripts were not significantly different ($P < 0.05$).

4. Conclusions

Udder infection with *S. xsilosus* and *S. chromogenes*, common CNS pathogens in goats, elicited a dramatic increase in SCC in the infected glands, caused major changes in milk composition and altered milk quality as reflected by the decrease in curd yield. However, these differences did not correspond to the number of somatic cells but were rather related to the pathogen specie present in the gland. Thus, it may be concluded that different bacteria elicit different changes in the milk properties, despite a similar increase in SCC in certain cases. It is suggested that a different pattern in the response of the innate immune system, which is not necessarily reflected by SCC governs the specific interaction between bacteria specie and milk composition and quality.

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I-P055: Milk Components, SOD Activity and Oxidative Stability of Milk in Two Breeds of Dairy Goats

T.S. Marenjak¹, J. Piršljin¹, N. Poljičak-Milas¹, S. Milinković Tur¹, B. Beer-Ljubić¹, M. Benić²

Summary

The aim of the study was to evaluate factors affecting the quality of raw goat milk. The milk components were analyzed in seven each of Saanen and Alpine goats, in the late stage of lactation. Low average milkfat, SNF and lactose content were determined in all goats with no statistically differences between breeds. The significantly higher SCC was noticed in Saanen goats. The oxidative stability of milk was not different between two breeds, but was changed during the storage times. The negative correlation between TBARS and milk urea concentration was observed in Alpine goats, whereas positive correlation between TBARS and milk protein, and SCC was detected in Saanen goats. Primarily, nutritional imbalance might be a factor of lower milkfat and lactose production in both breeds of goats, influencing integrity of mammary gland tissue and SCC.

1. Introduction

The milk components greatly influence the technology properties of goat milk and are depended on the parity, stage of lactation, breed, climate conditions and moreover nutrition of dairy goats. The fodder ingredients are often a cause of off-flavours in animal products (Lacasse; 2002). For example, unsaturated fatty acids are particularly prone to oxidative changes and oxidative reactions reduce the nutritive quality of raw milk. In goat and other ruminants' milk some indigenous enzymes are considered very important for the oxidative stability of raw milk and milk products (Fox and Kelly, 2006). The superoxide dismutase (SOD) has antioxidant properties and thiobarbituric acid reactive substances (TBARS) are very often used as a simple measure of secondary oxidative reactions. Higher oxidative stability in animal products may be expected under certain nutritional conditions such as natural or synthetic antioxidant agents, enzymes or minerals (Smet et al., 2005). The main objective in our research was to compare and evaluate the milk composition and oxidative stability of milk in two breeds of dairy goats in the late stage of lactation that are reared in the same farm conditions. The relationship between milk components, somatic cell count and oxidative stability of raw milk was also studied.

2. Material and methods

The research was conducted on 14 dairy goats of Saanen and Alpine breed. The animals were kept and managed in the same indoor/outdoor farming system. The feeding procedure was identical for both breeds and daily ration consisted of mid-mature meadow hay, pasture and concentrate mixture. Hay and water was offered ad libitum, and concentrate mixture two times daily, at the milking time. The concentrate mixture consisted of: 15% wheat bran, 10% barley grains, 40% corn grains, 15% soybean meal, 15% sunflower meal, 3% Ovisan (Sano, GmbH) 1% Multisan Nektar (Sano, GmbH), 1% Camisan (Sano, GmbH). For the purpose of milk components analysis, 40 ml of individual milk samples were collected during morning milking period and transported at 4°C to the laboratory. The milk component analyses was performed 2 h after the milk sample collection and measurement of SOD activity and TBARS concentration were performed, 2 and 14 h after the collection and cold storage of samples. The milk components and somatic cell count were measured by automatic analyzer (MilcoScan 4000; Fossomatic 5000 Denmark). The milk urea concentration was measured by colorimetric method and total SOD activity was measured using the commercial kit (RANSOD, "Randox", UK). Determination of thiobarbituric acid reactive substances (TBARS) was performed according to the method of Trotta et al. (1982). The data were analyzed using one-way analyses of variance (ANOVA,

¹ Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, 10000 Zagreb, Croatia.

² Croatian veterinary institute, Savska cesta 143, 10000 Zagreb, Croatia.

Statistica, 7.1, Stat soft, USA, 2004). The multivariate test of significance was performed using the Wilks lambda test. The significance was declared at $p < 0,05$.

3. Results and discussion

The milk components were not significantly different between two breeds of dairy goats in the present study (Table 1.). The average milk protein content was higher than 2,6%. On the other hand, comparing to Kozačinski et al. (2004) study, milkfat and lactose content was rather low in both breeds of dairy goats. At the same time, lower SNF content was also determined (Table 1.). The relationship of urea and protein in milk of experimental goats revealed the nutritional imbalance and possible energy shortage and protein surplus in the ration (Figure 1.). The regularly usage of milk urea for evaluating the energy and protein supply of the diet (Marenjak et al. 2004) could also efficiently predict the nitrogen supply in dairy goats. In our research, milk urea concentration ranged over 3,3 – 3,9 mmol/L (Table 1.) and was higher than reported by Arieli et al. (2005). Furthermore, the lower energy supply might cause a decrease in milk lactose content due to the lower digestible carbohydrates supply in the ration that are important source for the lactose synthesis in mammary gland. The SCC in Alpine goats was lower and in Saanen goats higher than allowable maximum level in most European countries (Table 1.). The breed, level of milk production and stage of lactation, as well as reproductive cycle, may influence the SCC in dairy goats (Haenlein, 2002; McDougall and Voermans, 2002). The activity of milk SOD and concentration of TBARS were not different between breeds, although, to some extent, higher SOD activity and TBARS concentration was detected in Saanen milk (Table 1.). After 14 h of storage at 4°C a significant increase of secondary oxidative products and decrease of SOD activity in both breeds of dairy goats was detected, with rather lower formation of TBARS in Saanen goats.

Table 1: The composition, somatic cell count and oxidative stability in milk of two breeds of dairy goats

Milk components	Alpine	Saanen
Fat (%)	1,85 ± 0,5	2,10 ± 0,23
Protein (%)	2,62 ± 0,34	2,78 ± 0,49
Lactose (%)	4,02 ± 0,18	4,11 ± 0,29
SNF* (%)	7,42 ± 0,45	7,66 ± 0,65
Urea mmol/L	3,61 ± 0,73	3,15 ± 0,86
SCC** (x 1000)	180,28 ± 194,13	1122,43 ± 1048,57
SOD (m) U/L	3028,86 ± 690,41 ^A	3199,14 ± 2010,37 ^A
SOD (e) U/L	2155,29 ± 431,36	2486,27 ± 1301,44
TBARS (m) mmol/L	2,25 ± 0,86 ^A	2,73 ± 1,46 ^A
TBARS (e) mmol/L	3,39 ± 0,41	3,12 ± 0,76

Data are expressed as mean ± standard deviation

*SNF = solids-non fat

**SCC = somatic cell count

m- milk samples analysed two hours after the collection

e – milk samples analysed 14 hours after the collection

^A – difference between (m) and (e); $p < 0,05$

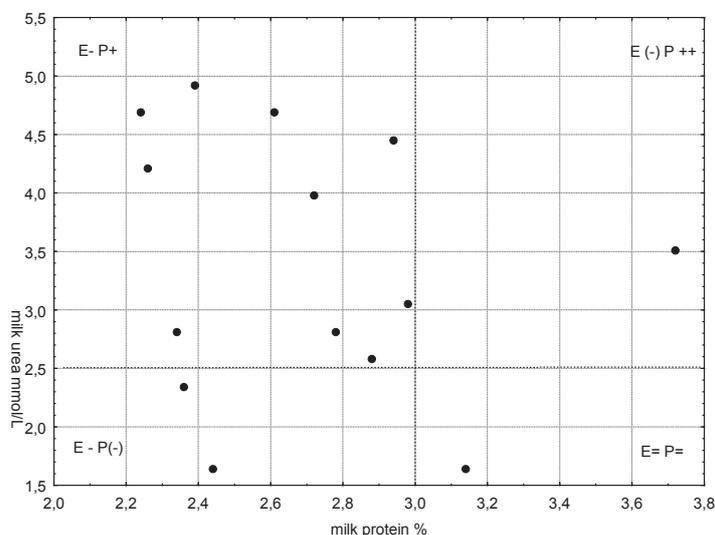


Figure 1. The relationship of protein and urea in milk of experimental goats.

4. Conclusion

According to milk components analyses, improper feeding may have been a major cause of lower production in dairy goats. The high grain diet may influence the increase in SCC in Saanen goats, and lower production of milk fat and lactose content in both breeds of dairy goats. The initiation of estrus could possibly influence the food intake and SCC. In present study, the oxidative stability of raw milk was possibly compromised by higher SCC and relevant enzyme activity.

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I-P056: Antibiotic Therapy in Dry Period: Cloxacillin Residues in Ewes' Milk

G. Marogna¹, C. Testa², N. Rubattu², G. Calaresu², G.S. Leori¹

Summary

The aim of this work was to study cloxacillin residues in ewes' milk following intramammary antibiotic dry-off therapy. The study was performed on a group of Sardinian sheep with subclinical mastitis, at the end of their milking period. Milk from ewes subjected to antibiotic therapy at drying off was tested for drug residue analysis for nine days after days lambing at daily intervals.

Liquid chromatography-mass spectrometry analytical determination showed that 5 out of 22 ewes shed antibiotic in milk, although at a level below the Maximum Residue Limit (MRL) established by the EU legislation for the drug in question.

1. Introduction

Dry-off therapy to control mastitis, sub clinical in particular, defined sometimes as Intra Mammary Infection (IMI) in dairy sheep has been introduced last few years. It was found to significantly reduce the incidence of IMI and to point up chronic infections in the next lactation. Because of the high incidence in Italy of IMI, in particular due to CNS (Coagulase Negative Staphylococci) the use of dry-off therapy has been growing. Cephazolin and Cloxacillin only have been registered in Italy for this aim. No data were found regarding antibiotic residues in milk after treatment with this antibiotics. In particular, antibiotic residues could be sucked by lambs that will be slaughtered one month after birth. This study is a part of a project directed to define drugs treatment for mastitis in traditional and organic sheep farms, and we refer data regarding antibiotic residues in sheep milk, after dry-off treatment, at the next lactation.

2. Materials and methods

27 sheep whose milk had been tested with positive results to bacteriological tests and which were closed to the dry period were used for this study: 5 of them were *Staphylococcus aureus* positive and 22 were Coagulase Negative Staphylococci (CNS) positive. Positive bacteriological tests, however, had not been associated with symptoms of mastitis and all animals were healthy and in good nutritional status (BCS evaluation). 22 sheep (4 *S. aureus* and 18 CNS positive) in the early dry period were treated by intramammary infusion with 0.3 g of cloxacillin benzatin intramammary for udder (Orbenin Extra® -Pfizer), while the remaining 5 sheep(4 CNS and 1 *S. aureus* positive) were used as control group. In accordance to the company recommendation, the drug was to be used only when the dry period is longer than 34 days; in our study, the time interval between therapy and lambing ranged between 35 and 41 days. After lambing, milk samples were aseptically daily taken for 9 days. The suckling lambs were not separated from their mothers during the sampling period.

The presence of cloxacillin in milk samples was determined using a liquid chromatographic method tandem mass spectrometry (LC/MS-MS). After extraction with acetonitrile, milk samples were submitted to a solid phase extraction on Oasis HLB cartridge. For confirmatory purpose multiple reaction acquisition mode (MRM) with two fragmentation reaction was adopted. All the determinations, microbiological and chemical, were performed in accredited laboratories of the Istituto Zooprofilattico following European standard UNI CEI EN ISO/IEC 17025/2005.

3. Results and discussion

A week after lambing bacteriological tests showed positive results in 2 ewes out of 22 in the treated and in 4 out 5 ewes in the control group.

¹ Centro di Referenza Nazionale per le Mastopatie degli Ovini e dei Caprini - Istituto Zooprofilattico Sperimentale della Sardegna, Via Duca degli Abruzzi 8, 07100 Sassari, Italy.

² Centro di Referenza Nazionale per la Zootecnia Biologica - Istituto Zooprofilattico Sperimentale della Sardegna, Via Duca degli Abruzzi 8, 07100 Sassari, Italy.

5 ewes out of the 22 treated showed cloxacillin residue in milk after lambing. However, no milk sample contained a drug concentration over the MRL, established at 30 ng/g by Council Regulation (EEC) No 2377/90. Only one of the 5 ewes excreted the drug up for a period longer than one day, while three days after lambing the other 4 positive ewes had a residue concentration below the Limit of Quantitation of the analytical method, which is estimated at 1 ng/g. In accordance to the company recommendation, the milk from ewes under dry therapy could only be used for human consumption at least 8 days after lambing. However, the results of this study show that in all sheep treated the residues of cloxacillin were below the Limit of Detection already at the fifth day after lambing.

4. Conclusion

The results of the bacteriological tests demonstrate the effectiveness of this drug. The risk of using contaminated milk for human consumption is reduced if after lambing the milk withholding period is properly observed, while this risk is not sufficiently reduced if other antibiotics are used in a similar manner.

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I-P057: Effect of Milk Yield and Lactation Stage on Body Condition and Metabolic Profiles in Assaf Ewes

M. Marques-Almeida¹, L.T. Gama², A.P.L. Martins³, R.M. Caldeira¹

Summary

The aim of this study was to evaluate body condition and metabolic profiles throughout lactation in Assaf ewes of two different milk production levels. Ewes were assigned to two groups, according to their previous milk yield. Body condition score and serum concentrations of different metabolites were assessed during lactation. The higher milk production group confirmed its superiority with a 55% higher total milk yield. Although some small differences in all analyzed variables were observed, they were not significantly relevant to differentiate the two production groups. However, as expected, significant differences among production levels were observed in body condition and some blood indicators along lactation. These results suggest that a balanced metabolic status was achieved in both groups during this trial, probably resulting from a good feeding management.

1. Introduction

The Assaf breed was developed in Israel in the 1950's from crosses between the local improved Awassi breed and the German East Friesian breed [1], with the objective of obtaining a high milk producer to be the genetic support of an intensive sheep milk production system. In Israel, Assaf ewes proved to be an excellent milk breed, producing an average of 340 kg milk in 173 lactation days [2]. This breed was later spread from Israel to different countries such as Portugal, where it has been used for more than 20 years, with the purpose of improving milk production and, consequently, the profitability of milk sheep farmers. Nevertheless, information on adaptation and production parameters for this breed is very scarce. Therefore, this study aims to: 1) contribute to a better knowledge of the performances of Assaf ewes under a semi-intensive production system and 2) investigate how milk yield is related to different indicators of metabolic status.

2. Material and methods

Information was obtained in 36 Assaf ewes, divided into two groups, with 18 ewes considered of high milk yield (H) and 18 of low milk yield (L), depending on their previous milk production being above or below the flock's average (250 kg in 150 days), respectively. All animals had free access to the same diet (commercial concentrates, alfalfa hay and pasture). Between days 30 and 220 after lambing, individual milk yield was recorded every 15 days. At days 5, 21, 45, 81 and 130 after lambing, body condition score (BCS) was assessed and blood samples were collected from 10 ewes of each production level, and serum concentrations of glucose, non-esterified fatty acids (NEFA), β -hidroxybutirate (β -HBA) and urea were determined, to evaluate the metabolic status of lactating ewes. All data were analyzed with PROC GLM of SAS [3].

3. Results and discussion

The average lactation length was 220 days. Means and standard errors for levels H and L were, respectively, 441.0 ± 77.8 and 284.5 ± 41.5 kg for total milk yield and 338.0 ± 53.6 and 214.0 ± 36.1 kg for milk yield at 150 days. Group H produced about 55% more total milk yield than group L.

¹ Centro Interdisciplinar de Investigação em Sanidade Animal – Faculdade Medicina Veterinária. Av. Universidade Técnica, Alto da Ajuda, 1300-477 Lisboa, Portugal, mplma@hotmail.com, ruicaldeira@fmv.utl.pt

² Estação Zootécnica Nacional, Instituto Nacional de Investigação Agrária, 2000-775 Vale de Santarém, Portugal, ltgama@fmv.utl.pt

³ Núcleo de Tecnologia Leite e Derivados. Departamento de Tecnologia dos Produtos Agrários, Estação Agronómica Nacional, Instituto Nacional de Investigação Agrária. Instituto Superior de Agronomia, Tapada da Ajuda, 1349-017 Lisboa, Portugal, pedrolouro@isa.utl.pt

The mean values for BCS and blood indicators are shown in Table 1. Ewes of both groups lambed with an average BCS of 2.25 but, after day 45, those in level L recovered a greater amount of body reserves until 130 days of lactation (BCS of 2.79 in H and 3.09 in L). Serum concentrations of metabolites were not affected by production level, except for NEFA, which indicates a higher mobilization in group H, especially in the beginning of lactation. As expected, lactation stage influenced all variables, except β -HBA, which can be an indication of a balanced diet. Nevertheless, serum urea concentrations are above those observed by other authors during lactation period [4,5,6], which probably indicates an excess or unbalance of nitrogen in the diet.

Table 1: Least squares means and standard errors (SE) for body condition score (BCS), glucose, non-esterified fatty acids (NEFA), β -hidroxybutirate (β -HBA) and urea serum concentrations throughout lactation in Assaf ewes of high (H) and low (L) production level*

	Production Level	Lactation stage (days)					SE
		5	21	45	81	130	
BCS	H	2.20	2.27	2.55 ^a	2.67 ^a	2.79 ^a	0.07
	L	2.25	2.25	2.85 ^b	2.90 ^b	3.09 ^b	
NEFA (μ mol/L)	H	^A 550 ^a	^A 463 ^b	^A 315 ^c	^A 208 ^c	^A 129 ^c	39
	L	^B 380 ^a	^B 301 ^b	^A 227 ^b	^A 129 ^b	^A 154 ^b	
β -HBA (mmol/L)	H	0.495	0.452	0.594	0.579	0.730	0.03
	L	0.447	0.416	0.505	0.555	0.642	
Glucose (mmol/L)	H	3.107 ^a	3.298 ^{ab}	3.599 ^{bc}	4.602 ^c	4.552 ^c	0.143
	L	4.358 ^a	3.461 ^a	3.632 ^{ab}	4.879 ^b	4.740 ^b	
Urea (mmol/L)	H	8.499 ^a	12.163 ^c	12.544 ^c	8.394 ^a	10.171 ^b	0.467
	L	8.982 ^a	12.924 ^c	11.232 ^c	8.866 ^a	10.238 ^b	

*For a given variable, means within a column with different superscripts (A-B) differ significantly ($P < 0.05$), and means within a row with different superscripts (a-d) differ significantly ($P < 0.05$).

4. Conclusions

Total milk production observed is within the normal range reported for this breed [2,7]. The evolution of BCS and serum concentrations of blood metabolites showed a balanced metabolic status, probably resulting from an adequate plane of nutrition and a good management of body reserves. However, some aspects can be improved, such as the quantity and quality of diet nitrogen. This breed has shown high production levels, without any particular management requirements.

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I-P058: Effect of Milk Yield and Lactation Stage on the Composition and Milk Clotting Properties in Assaf Ewes

M. Marques-Almeida¹, L.T. Gama², R.M. Caldeira¹, A.P.L. Martins³

Summary

The aim of this study was to evaluate the effects of milk yield on the composition and clotting properties of milk throughout lactation in Assaf sheep. Ewes were assigned to two groups, according to their previous milk yield. The most productive group presented a significantly higher protein and protein+fat yield, even though with a lower protein content. The analysis of milk properties indicates that, in spite of the lower protein content, the milk from the most productive group presented slightly better clotting properties, probably influenced by other factors, such as milk pH, which showed a more relevant effect on milk clotting properties than protein content.

1. Introduction

The Assaf breed was introduced in Portugal more than 20 years ago, with the intention of improving milk production and profitability of milk sheep farms, and today has partially replaced local breeds in some PDO cheeses areas. It is known that this breed tends to have lower protein and fat content due to its high milk production, parameters usually considered directly related to cheese yield. In order to support cheese producers in handling these new milk features, this study was conducted to evaluate the consequences of this loss in protein and fat content on cheese-making properties, by assessing the evolution of milk coagulation, a major phase for the definition of cheese characteristics.

2. Material and methods

This trial was conducted with 36 Assaf ewes, divided into two groups, with 18 ewes considered of high milk yield (H) and 18 of low milk yield (L), depending on their previous milk production being above or below the flock's average (250 kg in 150 days), respectively. Between days 30 and 220 after lambing, individual milk yield was recorded every 15 days and individual milk samples were collected and kept at 2-4°C until further analyses. The milk production variables were total milk yield (PTOT) and milk production at 150 days (P150d), protein yield (PY), fat yield (FY) and protein+fat yield (PFY). Milk samples were analysed for fat (FC) and crude protein (PC) contents using a Milkoscan, and pH was evaluated with a Metrohm 713 pHmeter (Metrohm S.A., Switzerland). The milk clotting properties were determined using an Optigraph equipment (Ysebaert, France). After setting the milk clotting temperature to 32 °C (at least 1h to equilibrate), 1 ml of a 0,08% solution in distilled water (m/v) of rennet powder containing $96 \pm 2\%$ of chymosin (Extracto de cuajo en polvo Granday® 6000) was added to each 10 ml of milk. All tests were run for 50 minutes and the studied variables were clotting time (R), rate of firming (OK20) and clot firmness (A20, A40, AR and A2R) [1]. All data were analyzed with PROC GLM of SAS [2].

3. Results and discussion

Average lactation length was 220 days. Means and standard errors for the variables analyzed are shown in Table 1. Group H produced about 55% more milk than group L, but with a lower PC. However, group H had a much higher PY and PFY, and these are two variables of major

¹ Centro Interdisciplinar de Investigação em Sanidade Animal – Faculdade Medicina Veterinária. Av. Universidade Técnica, Alto da Ajuda, 1300-477 Lisboa, Portugal, mplma@hotmail.com, ruicaldeira@fmv.utl.pt

² Estação Zootécnica Nacional, Instituto Nacional de Investigação Agrária, 2000-775 Vale de Santarém, Portugal. ltgama@fmv.utl.pt

³ Núcleo de Tecnologia Leite e Derivados. Departamento de Tecnologia dos Produtos Agrários, Estação Agronómica Nacional, Instituto Nacional de Investigação Agrária. Instituto Superior de Agronomia, Tapada da Ajuda, 1349-017 Lisboa, Portugal, pedrolouro@isa.utl.pt

importance when milk and cheese yield are considered together. Milk clotting properties were significantly different in the two studied groups, with group H having a lower clotting time (R) and better clot firmness (AR, A2R, A20, A40), which was unexpected, given its lower PC. In general, group H presented better milk clotting properties than group L. This can be due to the difference in pH, which was significantly lower in the H group. As expected, milk clotting properties along the lactation period were significantly influenced by FC, PC and pH [3] (Table 2). On the other hand, an effect of production level and stage of lactation was observed in all variables studied, with the exception of OK20, even though the interaction between level*day was not significant ($P>0.05$). The multiple regression analysis of the factors affecting clotting properties demonstrated a major effect of pH, when compared with milk fat or protein, indicating the importance of milk pH on its aptitude for coagulation and, thus, on subsequent phases of cheese-making.

Table 1: Least squares means \pm standard errors for variables analyzed in milk samples from high (H) and low (L) production level groups*

	N	H	L
Milk production:			
Total milk yield (kg)	18	441.0 \pm 77.8 ^a	284.5 \pm 41.5 ^b
Milk yield at 150 days	18	338.0 \pm 53.6 ^a	214.0 \pm 36.1 ^b
Fat Content (%)	18	6.89 \pm 0.66	6.99 \pm 0.76
Protein Content (%)	18	5.12 \pm 0.22 ^a	5.32 \pm 0.26 ^b
Fat Yield (kg)	18	30.3 \pm 5.7 ^a	19.2 \pm 3.12 ^b
Protein Yield (kg)	18	22.6 \pm 4.2 ^a	15.1 \pm 2.0 ^b
Protein + Fat Yield (kg)	18	53.0 \pm 9.5 ^a	35.0 \pm 4.9 ^b
pH	475	6.56 \pm 0.0006 ^a	6.61 \pm 0.0006 ^b
Milk clotting properties:			
R (s)	475	732 \pm 8 ^a	807 \pm 8 ^b
AR (V)	473	7.55 \pm 0.12 ^a	7.18 \pm 0.12 ^b
A2R (V)	453	10.88 \pm 0.17 ^a	10.44 \pm 0.17 ^b
A20 (V)	475	5.51 \pm 0.11 ^a	4.37 \pm 0.11 ^b
A40 (V)	475	11.51 \pm 0.17 ^a	10.35 \pm 0.17 ^b
Ok20 (s)	475	531 \pm 25 ^a	562 \pm 25 ^b

*Clotting time (R), Clot firmness (AR, A2R, A20, A40), Clot firmness rate (OK20) and sample size (n). For a given variable, means with different superscript differ significantly ($P < 0.05$).

Table 2: Results of the Analysis of Variance of milk clotting properties as a function of production level, stage of lactation, FC, PC and pH

	R	AR	A2R	A20	A40	Ok20
N	475	473	453	475	475	475
Level	**	**	**	**	**	NS
Day	**	**	**	**	**	+
Level*Day	NS	NS	NS	NS	NS	NS
FC	NS	**	**	**	**	**
PC	**	**	**	NS	**	**
pH	**	**	**	**	**	**
r ²	0.48	0.57	0.51	0.50	0.44	0.22

Clotting time (R), Clot firmness (AR, A2R, A20, A40), Clot firmness rate (OK20), Fat content (FC), Protein content (PC), Coefficient of determination (r²).

4. Conclusion

Milk production is within the normal range reported for this breed [4,5]. Also, clotting properties were the expected considering Assaf's milk composition. Although milk from group H had lower PC, it showed similar or even better clotting properties than that from group L. These results can be due to the strong influence of pH in clotting properties observed.

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I-P059: Electronic Nose Evaluation of Repeated Milk Samples in Saanen Goats and Relationships with Not-VOC Milk Traits and Fame

G. Sala¹, G. Masoero¹, G. Contarini², A. Avalli², B. Moioli¹

1. Introduction

The aroma of fresh milk is influenced by specific genetic aptitudes, achieving *maxima* expressions in pheromones, by ontogenetic factors linked to mammal evolution and by feeding conditions, animal health, microclimate and management (Ampuero and Bosset, 2003). Fat content is highly reputed to be a primary source of typical flavor of milk; nevertheless other not-Volatile Organic Compounds (VOCs) world-wide monitored in dairy production could be invoked as co-causalities into aroma patterns. This trial was carried out to ascertain if the aroma of milk should be considered a feature trait of the animal. By second the study aims to investigate the relationships between the Electronic Nose evaluation and the characteristics of the raw milk, with special emphasis to its Fatty Acid Composition. In a recent study (Masoero et al., 2007) the aroma of fresh Goat milk as appreciated by Electronic Nose was markedly distinguished from milk of Jersey ($R^2=0.57$), of Piemontese (0.76) and of Valdostana (0.76) Cattle breeds.

2. Materials and methods

In this study the aroma of fresh milk was assessed by a Electronic Nose (EN, PEN 2-AIRSENSE) produced from twenty-seventh Saanen goats belonging to a single herd. 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. The animals were controlled twice during the lactation period, in May and September. The samples of raw milk were acquired during the morning milking and immediately taken to the lab for testing. The chemical analysis of the milk and the electrical conductivity were carried out as described in Masoero et. al. (2007) The aromatic profiles were kept under flow of filtered air, as duplicate or triplicate of two grams by each fresh milk sample (N=122). Measurements were essayed in a 20 ml vial, the headspace was recorded for 50 seconds followed by 110 seconds of flush time consisting in lab air filtered by an active charcoal filter. Chamberflow was set to 340 ml/minute (12 turnover/minute). The EN used to perform the analysis is a ten metal oxide sensor (MOS) device. Each MOS detects a specific class of compounds as a change of its own resistance. The change occurs when the volatile organic compounds released from the milk get in contact with the sensor surface. The redox are recorded as digit with display of a ten-trace profile. Three-hundred digits, corresponding to the first 30 seconds for each of the ten sensors, were submitted to Variance Component Analysis (SAS System) to estimate effects due to: the Animal, the Milk records, the Replicate of Nose measurements. The repeatability of chemical were calculated as above. In the multivariate approach the olfactometric digits were elaborated as contiguous arrays, in a set of 300 elements as described in Masoero et al. (2007). The WinISI II software (Infrasoft International, PA, USA) was chosen to perform the chemometrics, using PLS method, coupled with a cross-validation system expressed as R^2 . The calibrations of chemical compounds and of the Nose replicate effects were performed separately for the two Milk records and overall, the fixed effects of the Animals only overall. Repeatability of EN profile were calculated as correlation between PC1 scores of the two Nose Replicates at the two Milk records.

3. Results and discussion

The Figure 1 reports the plot of the whole set of measurements. The MOS sensor w2 appeared to be the most sensible in milk aroma enhancing the widest amplitudes.

¹ CRA-Istituto Sperimentale per la Zootecnia, Monterotondo (RM) – Italy.

² CRA – Istituto Sperimentale Lattiero Caseario, Lodi - Italy.

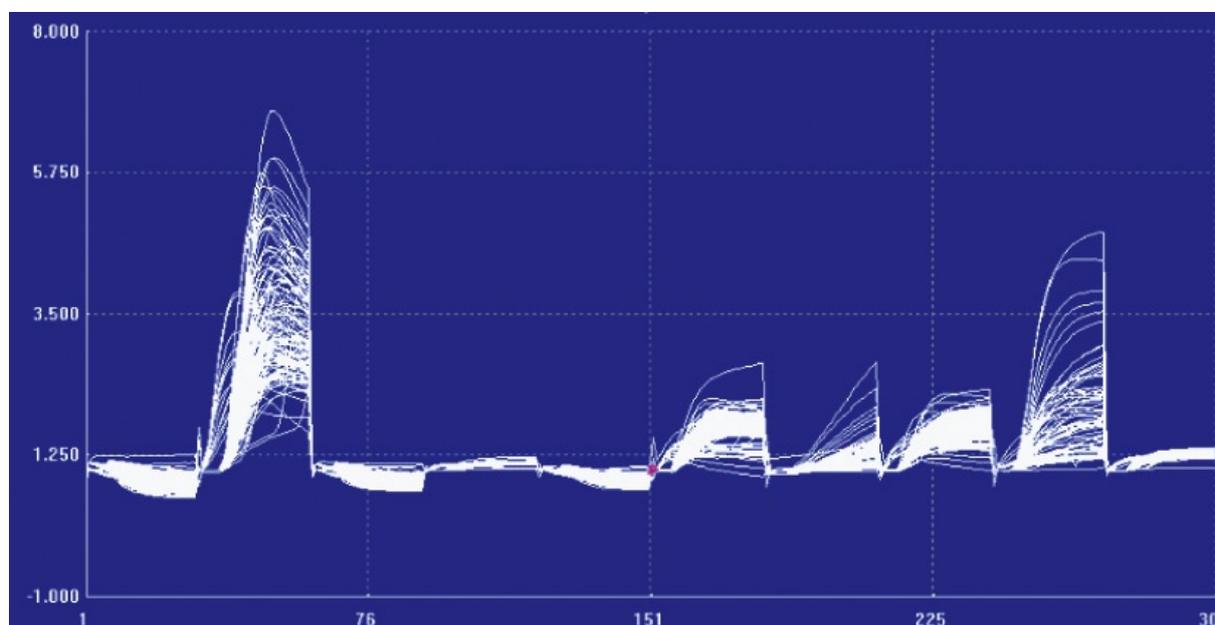


Figure 1. The plot of all the MOS sensors #1-10 of goat raw milk sample.

Table 1: Result of the Varcomp analysis of the 300 digits according to ten MOS sensors

Sensor	Sources of variance			
	Milk record	Nose Rep (Milk record)	Animal	Error
w1	54%	12%	1%	33%
w2	12%	8%	17%	63%
w3	25%	23%	4%	48%
w4	31%	5%	0%	64%
w5	34%	15%	5%	46%
w6	-2%	16%	8%	77%
w7	34%	-2%	7%	61%
w8	20%	9%	3%	68%
w9	60%	-1%	3%	38%
w10	24%	8%	2%	66%
Av.ge	29%	9%	5%	56%

In the univariate analysis of 300 digits the incidence of the Animal effect (Table 1) resulted generally low, on the average $5\% \pm 5\%$ with the exception for MOS sensors w2, w6 and w7 which results were respectively 17%; 8% and 7%. According to the chemometric PLS discrimination the Animal multivariate fit was also poor, resulting on the average 0.14. This low level of distinction for the animal effects within the same lactation appeared to be critical when it was compared to the 0.60 values for the repeatability of Fat%, and Electrical conductivity and also when compared to the values around 0.40 for the Milk Yield and the Somatic cells. Nevertheless the within-lactation repeatability for the Protein % and for many FAMES fallen below the 0.20 level, when their average repeatability was 0.28 ± 0.15 (Table 2). In partial conclusion the aromatic profile of the individual milk, as intended through the EN evaluation, is comparable to a trait with low repeatability, as, for example the monounsaturated C14:1, C16:1 and C18:1 fatty acids, which are involved in the $\Delta 9$ -desaturase activity (Soyeurt et al., 2006). A more sensible part of the monovariance (Table 1) was accounted for by the Milk records ($29\% \pm 18\%$) and this is in agreement with the perfect PLS Discrimination of the two Milk records ($R^2=0.97$, Table 2). This last finding agrees with Barker and Rayens (2003) who demonstrates superiority

of PLS discrimination to PCA analysis. The Replicate of Nose measurements accounted for $9\% \pm 8\%$ of the total monovariance (Table 1); the chemometric PLS fittings (Table 2) of the differences between replications gave more consistent figure as R^2 0.36 for the second Milk record and non for the 1st. But the instrument was repeatable at a level of 0.68 between nose replications of the same samples, a medium effective value but highly efficient when compared to GC determinations. The vectors of milk's aroma were then calibrated to Not-VOC by Partial Least Squares chemometric analysis. Results were quite surprising as the best relationships occurred with protein contents (R^2 0.65). Some FAME were significantly correlated to EN profiles, as C18:2 and C16:1 (0.65). It must be appointed however that these results outcome from a main strong effect, linked to the stage of lactation, because within each Milk record the true relationships were markedly lowered (Table 2), and no constant relationships was observed.

Table 2: Result of repeatability and PLS Calibration of effects, of milk traits and of FAME content

Constituent	Mean	S.D.	Reptb MR1 MR2	R^2 - Cross-Validation		
				M.R. 1&2	M.R. 1	M.R. 2
MilkRecord	-		-	0.97	-	-
Animal				0.14		
Nose Rep.	-		0.68	0.26	0.01	0.36
Elect.Cond	7.04	0.42	0.60	0.24	0.28	0.08
Fat %	3.48	0.69	0.62	0.32	0	0
Protein %	3.22	0.65	0.16	0.65	0	0
Lactose %	4.30	0.24	0.20	0.52	0.07	0.08
S.C.C	1730	1705	0.42	0.04	0.33	0.00
N.F.Res.%	8.24	0.57	0.28	0.50	0	0
Product kg	1.81	0.64	0.45	0.45	0.18	0.00
C4_0 %	2.98	0.62	0.24	0.55	0.08	0
C6_0 %	2.22	0.35	0.14	0.62	0.25	0
C8_0 %	2.11	0.32	0.16	0.53	0.04	0
C10_0 %	6.45	1.04	0.30	0.21	0.06	0.06
C10_1 %	0.19	0.06	0.34	0.24	0	0
C12_0 %	3.19	0.59	0.58	0.06	0	0
C13_0 %	0.14	0.09	0.06	0.92	0.17	0
C14_0 %	9.64	1.32	0.39	0.18	0	0
C14_1 %	0.22	0.13	0.20	0.47	0	0
C15I %	0.28	0.10	0.09	0.72	0.29	0
C15AI %	0.48	0.08	0.49	0.12	0.31	0
C15_0 %	0.98	0.10	0.36	0.09	0.25	0
C16I %	0.26	0.05	0.52	0.07	0	0.20
C16 %	22.93	1.86	0.49	0.04	0.17	0
C16_1 %	0.75	0.19	0.12	0.65	0	0
C17I %	0.59	0.10	0.31	0.49	0.26	0
C17AI %	0.54	0.11	0.25	0.51	0.20	0
C17_0 %	0.69	0.10	0.47	0.32	0	0
C17_1 %	0.37	0.09	0.16	0.47	0	0
C18 %	11.69	2.50	0.27	0.41	0	0
C18_1 %	28.28	2.79	0.20	0.36	0	0
C18_2 %	2.88	0.74	0.08	0.65	0.06	0
C18_3 %	0.45	0.13	0.27	0.50	0.08	0
C18_2co.%	0.81	0.21	0.32	0.33	0.06	0

4. Conclusions

These preliminary results of EN evaluation carried out with individual milk samples suggest that the aroma of milk should be considered a trait only faintly constant during the animal's lactation provided that lactation advancement and available feeds were important factor of variation. The period effects appear to be strong, so the lack of standard for EN utilization may be critical. Further studies are welcome in this field.

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I-P060: Influence of Somatic Cell Count on Ewe's Milk Composition with Particular Reference to Casein Fraction

R. Mauriello¹, S. Caira², S. De Pascale¹, A. Pirisi³, G. Piredda³, F. Addeo¹, L. Chianese¹

Summary

The objective of this work was to investigate the effect of somatic cell counts on half udder milk caseins from the same ewe, throughout three months of lactation. Casein composition was determined by using of immunoelectrophoretic techniques and LC- ESI/MS analysis

By electrophoresis at pH 8.6, comparing half udders, the left one with initial low SCC $67 \times 10^3/\text{mL}$ (Left Low, LL), and right one with initial high SCC $204 \times 10^3/\text{mL}$ (High Right, HR) throughout three months of lactation, a high heterogeneity at level of α_s1 - and β -CN casein fractions was observed in HR electrophoretic pattern (Fig. 1A). At the end of three months, in HR profile the native α_s1 - and β -CN components disappeared with a concomitant presence of new peptides immunostained with specific polyclonal antibodies and identified as γ -CN. By LC/ESI/MS, in HR casein in the last milk sample, no native casein fractions, but only γ -CN and their fragments f(124-207) were occurred. The casein modification throughout three months of lactation was due to a synergic action of two phenomena: proteolysis by plasmin action on β -casein, primarily and alterations of phosphorylation degree, likely related to an increased alkaline phosphatase activity, with a concomitant loss of phosphate groups.

1. Introduction

A significant increase in somatic cell count (SCC) is the primary characteristic change that occurs in milk during mastitis disease, therefore the SCC of milk is a parameter widely used to predict the mammary health status. Mastitis causes injury to milk secretory cells in mammary gland which interferes with synthesis of lactose, fat and protein (Scallibaum, 2001).

In ovine milk, even lacking of infective process, SCC can irregularly vary throughout lactation period, owing to other factors such as stress, season, flock management ecc. (Konig, 1985). It is known somatic cells are associated with plasminogen activators (Poltis 1989); Zachos 1992) and contain a range of proteolytic enzymes including acid proteinase cathepsin D (Verdi & Barbano, 1991), which may cause an altered composition of milk, resulting in poor quality dairy products.

The aim of this research was to evaluate the effect of somatic cell counts on half udder caseins, from milk samples of the same ewe, throughout three months of lactation. Casein composition was determined by using immunoelectrophoretic techniques and LC- ESI/MS analysis.

2. Material and methods

Half udder caseins, left with initial low SCC $67 \times 10^3/\text{mL}$ (LL), and right with initial high SCC $204 \times 10^3/\text{mL}$ (HR), from milk samples, of the same ewe, throughout three months of lactation were prepared according to Achaffenburg & Drewery (1959), SCC determined with IDF procedure (1987) using Fossomatic 90 (Foss Electric, Hillerod, Denmark). Urea-PAGE at pH 8.6 and immunoblotting analysis were carried out according to Chianese et al. (1992). High-Resolution LC/ESI/MS of the pH 4.6 insoluble nitrogen fraction of each milk sample was performed as described by Addeo et al. (1992).

3. Results and discussion

PAGE at pH 8.6 and immunoblotting

Blue Coomassie staining of PAGE analysis at pH 8.6 of LL and HR from 5 milk samples throughout three months of lactation, was shown in Fig. 1(A). In the sample 1, although both the half

¹ Dipartimento di Scienza degli Alimenti, Università degli Studi di Napoli Federico II, I-80055 Portici, Italy.

² Istituto di Scienze dell'Alimentazione, I-83100 Avellino, Italy.

³ Istituto Zootecnico e Caseario per la Sardegna, I-07040 Olmedo, Italy.

udders contained $SCC < 500 \times 10^3/mL$, in HR, in addition to native α_s1 and β casein fractions, two new formed peptides at the highest anodic mobility (asterisk in Figure) were already observed, whose electrophoretic mobility was similar to α_s1I -CN, generating by rennet action in cheese. These new products were immunospecific stained with polyclonal antibodies against α_s1 -CN (Fig. 1B). Their formation was likely due to an enzymatic action of acid proteinase cathepsin D (Verdi & Barbano, 1991). In Fig. 1B the intensity of native α_s1 -CN bands decreased throughout the lactation period, until they disappeared in HR of sample 5, owing to the detrimental effect of SCC increase, close related to lactation stage. The new formed peptides (bracket in Figure 1A) were identified as γ -CN, in Fig. 1C; the native β -CN bands also disappeared in HR of sample 5, with a concomitant presence of plasmin-generated γ -CN in addition to other new products having the highest anodic mobility.

LC/ESI/MS analysis The HPLC elution profiles of LL and HR casein in milk sample 1 were qualitatively similar, but quantitatively different (Fig. 2). In HR α_s1 -CN f(1-23) was found. A detrimental effect of SCC increasing in milk sample 5, was evident in HPLC elution profiles of HR casein in which no native casein fractions, but γ -CN and their fragments f(124-207) were occurred. α_s1 -casein peptides likely consisted of components with different phosphorylation degree (work in progress).

4. Conclusion

The SCC increase has a detrimental effect on milk quality, since it regulates quali-quantitative modifications of casein composition also in milk with initial $SCC < 500 \times 10^3/mL$. The increased heterogeneity of casein fractions α_s1 , and β -CN is due to: proteolytic phenomenon by plasmin action mainly, and likely alterations of phosphorylation degree. A more extent of casein breakdown is related to cheese yield milk while low phosphorylation level is negatively related to cheesemaking milk aptitude.

Since ovine milk is destined to cheesemaking, a monitoring of SCC in the flock needs, because high SCC in milk causes a hard casein degradation at level of α_s1 -CN and β -CN primarily, with a consequent poor quality of cheese.

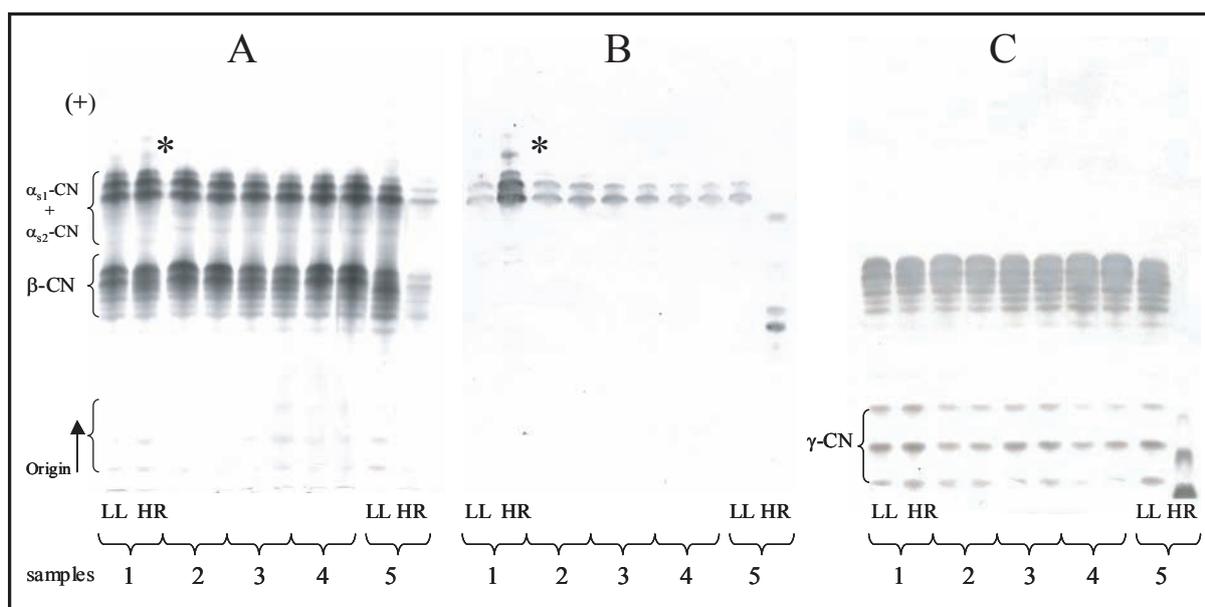


Figure 1. Blue Coomassie staining (A) of PAGE analysis at pH 8.6 of half udder caseins, left with low SCC $67 \times 10^3/mL$ (LL), and right with high SCC $204 \times 10^3/mL$ (HR), from 5 milk samples throughout three months of lactation, followed by immunospecific staining with polyclonal antibodies against: α_s1 -CN (B) and β -CN (C).

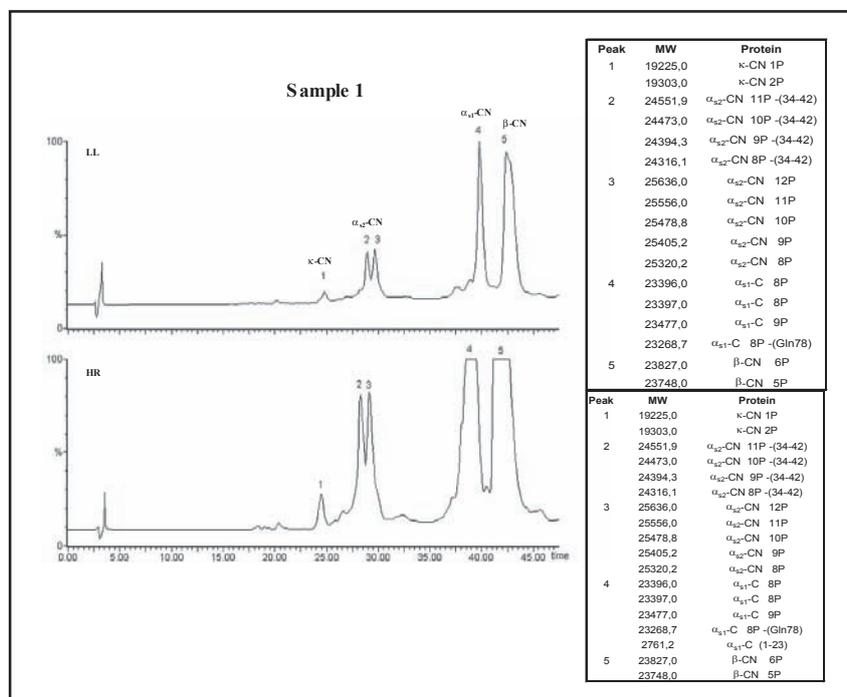


Figure 2. LC/ESI/MS of half udder caseins, left with low SCC 67×10^3 /mL (Low Left, LL), and right with high SCC 204×10^3 /mL (High Right, HR) from milk sample 1 at beginning of three months.

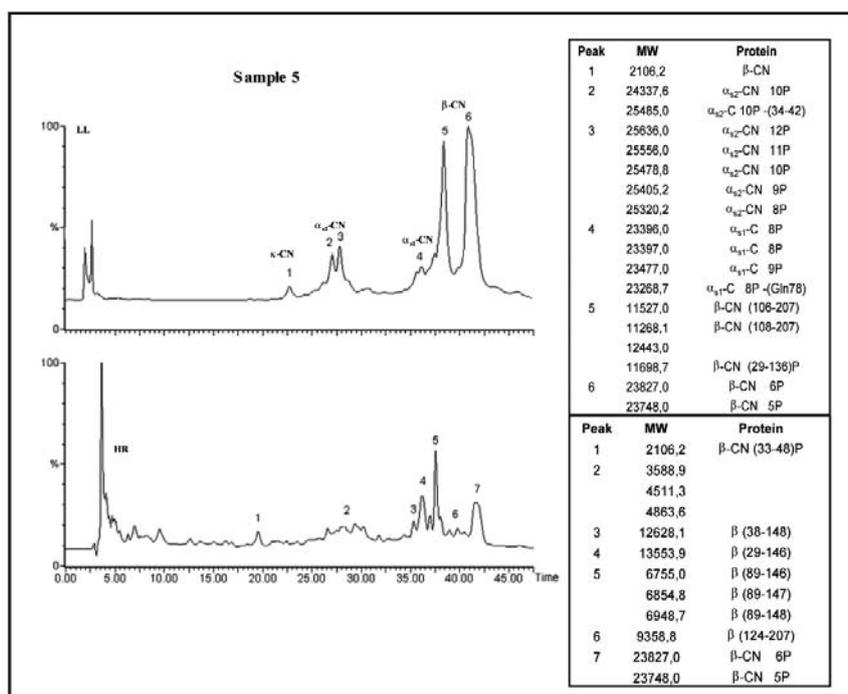


Figure 2. LC/ESI/MS of half udder caseins, left with low SCC 67×10^3 /mL (Low Left, LL), and right with high SCC 204×10^3 /mL (High Right, HR), from milk sample 5 at the end of three months.

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I-P061: Polyamines in Ovine and Caprine Colostrum and Mature Milk

A. Galitsopoulou¹, A.M. Michaelidou¹, A. Polychroniadou¹

Summary

The objective of this research effort was the monitoring of the polyamine content in ovine and caprine milk at the colostrum, transitional and mature stages of lactation. For this purpose samples of 20 dairy sheep and 20 dairy goats were collected at the 1st, 2nd, 3rd, 4th, 5th and 15th day post partum. Putrescine, spermidine and spermine were determined by HPLC as dansylated derivatives.

The total polyamine levels ranged from 3.61 to 4.94 nmole/ml for ovine samples and 1.85 to 7.21 nmole/ml for caprine samples. Both animals provided similar qualitative profiles, but quantitatively significant differences were sometimes observed.

1. Introduction

Polyamines (PA), namely putrescine, spermidine, and spermine are aliphatic, low-molecular polycations with polybasic character that can interact with negatively charged cell constituents (DNA, RNA, proteins). PA may be involved in various processes of cell growth and cell differentiation. Their importance in cell function is reflected in a strict regulatory control of their intracellular levels [2].

The PA requirements that cannot be met by biosynthesis have to be satisfied by exogenous PA consumed from the food. Organs, such the gastrointestinal tract, pancreas and spleen, with a high cell turnover rate are especially dependent on dietary polyamines [3]. Thus, PA have attracted considerable interest as their presence in human breast milk may have trophic effects on the maturation of the infant gut. Furthermore, PA may be important for the fidelity of the enhanced DNA transcription and RNA translation, that occurs in response to infection and during tissue repair, gut growth after surgery, and in gut barrier functions [1].

Data on the concentration of polyamines in ovine and caprine milk are limited. Hence, it was interesting to record their values, in animals of Greek breeds, at the colostrum, transitional and mature stage of lactation and to investigate whether caprine and ovine milk could be considered as a good natural source of PA for specific population groups.

2. Materials and methods

Samples from 20 dairy sheep and 20 dairy goats of Greek breeds were collected on the 1st, 2nd, 3rd, 4th, 5th and 15th day post partum. Polyamines were determined by RP-HPLC as dansylated derivatives.

3. Results and discussion

The total concentration of PA ranged from 3.61 nmol/ml to 4.94 nmol/ml in ovine milk and from 1.85 nmol/ml to 7.21 nmol/ml in caprine (Table 1). High inter-individual variation was observed. Samples from ewes and goats provided similar qualitative profiles of PA, but quantitatively significant differences were sometimes recorded (Figure 1).

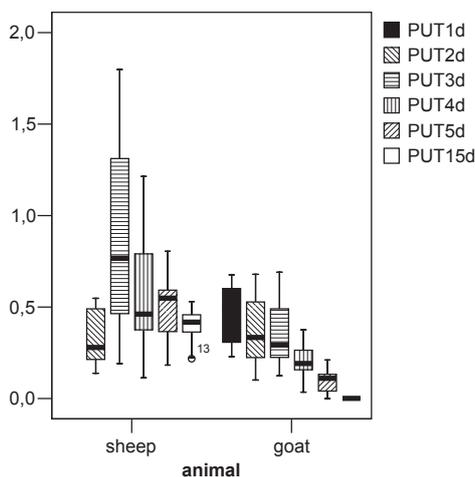
Mature sheep's milk (15th day) was always richer in PA compared to goat's, but caprine colostrum had a higher PA content than ovine.

The level of putrescine was higher in ovine samples than in caprine; furthermore, no putrescine was detected in mature goat's milk. Spermidine was the prevailing polyamine in caprine samples, whereas in ovine samples spermidine and spermine levels were comparable throughout the sampling period.

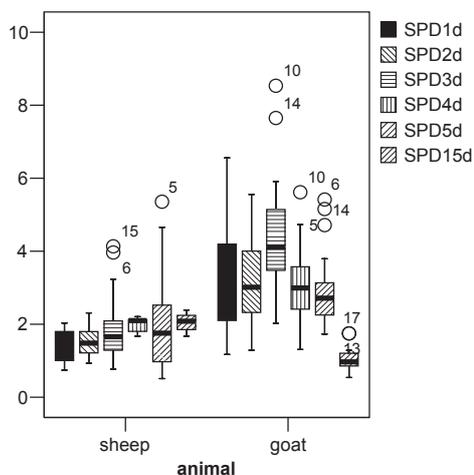
¹ Department of Food Science and Technology, School of Agriculture, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece

Table 1: Mean values and standard error of the total polyamine concentration in sheep and goats milk throughout 15 days of lactation. (nmole/ml)

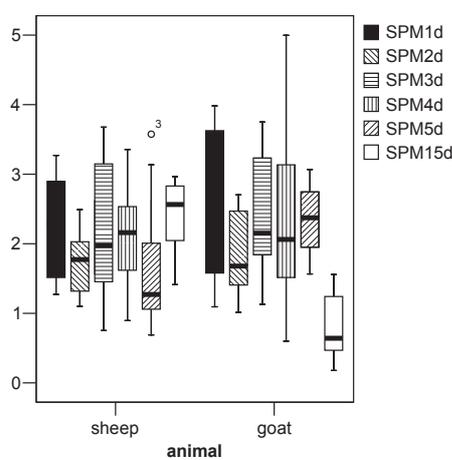
Days post partum	Sheep milk		Goats milk	
	Mean	SE	Mean	SE
1	3.92	0.26	6.15	0.51
2	3.61	0.18	5.35	0.39
3	4.94	0.44	7.21	0.48
4	4.70	0.20	5.72	0.52
5	4.17	0.48	5.38	0.32
15	4.85	0.14	1.85	0.17



(a)



(b)



(c)

Figure 1. Boxplots of (a) putrescine, (b) spermidine, and (c) spermine concentration (nmole/l) in sheep and goats milk on the 1st, 2nd, 3rd, 4th, 5th and 15th day post partum. PUT: putrescine; SPD: spermidine; SPM: spermine

4. Conclusions

Considerable quantitative interspecies variation was recorded as far as the polyamine pattern in ovine and caprine milk is concerned. Also, the time elapsed after parturition considerably influenced PA concentration, presumably because the needs of the young animal change with age. Further investigations would be of interest.

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I-P062: Nucleotides and Nucleosides in Ovine and Caprine Colostrum and Milk

S. Plakantara¹, A. Polychroniadou¹, A.M. Michaelidou¹

Summary

The aim of this study was to monitor the nucleoside/nucleotide content in ovine and caprine milk at the colostrum, transitional and mature stages of lactation. Samples were collected 1, 2, 3, 4, 5 and 15 d *post partum* and analysed by HPLC. A high inter-individual variation was observed. The total nucleoside content ranged from 58 to 185 µmol/l and from 54 to 119 µmol/l in ovine and caprine milk, respectively. The major nucleoside in both kinds of milk was uridine, representing *per se* more than 70% of the total. The total nucleotide content ranged from 294 to 836 µmol/l in ovine milk and from 166 to 366 µmol/l in caprine milk. Uridyl-5'-mono-phosphate was the dominant nucleotide in the milk of both species. Inter-species differences were significant ($P < 0.05$) for total nucleotides but not for total nucleosides.

1. Introduction

Nucleotides (NT) have long been recognized to be important especially for preterm and small gestational age infants. Rapidly growing tissues in the gastro-intestinal mucosa, the immune system and nervous tissues, immature metabolic systems in the newborn make nucleotides essential during this period of life [1]. Recently, NT became of clear and attractive value also to other clinical situations. Literature on the nucleoside (NS) and NT content of the milk of small ruminants is very limited [2, 3]. It was therefore interesting to study the NS and NT content of ovine and caprine milk and, if appropriate, to investigate the eventual use of this milk for specific clinical applications.

2. Materials and methods

Samples from 18 dairy sheep and 18 dairy goats of Greek breeds were collected 1, 2, 3, 4, 5 and 15 d *post partum*. Determination of the NS and NT was performed by RP-HPLC using a phosphate buffer-acetonitrile gradient elution. Results were evaluated by ANOVA

3. Results and discussion

The total NT content was at least 3-fold higher than the total NS content for both small ruminant species. This tendency was observed throughout all the sampling period. Inter-species differences were more obvious for total NT.

ANOVA showed that the total NT content was significantly higher ($P < 0.05$) in ovine milk than in caprine. As far as total NS is concerned, the interaction between day *post partum* and animal species found as significant ($P < 0.05$).

The relative concentrations of the individual NT and NS for both species were also investigated (Tables 1 and 2). The predominance of uridyl-5'-monophosphate and uridine in all samples, regardless origin and lactation day, was recorded.

Regarding NT, the origin of milk was not significant for AMP, although caprine samples exhibited relatively high concentrations one day *post partum*. However, the origin of milk was significant for UMP and especially for GMP and CMP ($F = 17,74$ and $F = 36,68$, respectively). CMP percentage of total NT was higher in ovine milk than in caprine.

With regard to the influence of animal species, data pertaining to the NS studied, indicate significant differences ($P < 0.05$) especially for cytidine and adenosine, ($F = 124,39$ and $F = 144,86$ respectively). By plotting adenosine against cytidine, as percentages of total NS, on the 3rd day *post partum* ovine colostrum samples were separated from caprine ones.

¹ Department of Food Science and Technology, School of Agriculture, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece.

Table 1: Level of individual NS, expressed as percentage of the total NS, (mean \pm standard error) in sheep and goats milk throughout 15 days of lactation

Days post partum	Uridine	Cytidine	Inosine	Guanosine	Adenosine
Sheep milk					
1	58.74 \pm 2.42	16.27 \pm 1.01	14.00 \pm 1.53	1.79 \pm 0.23	9.21 \pm 0.65
2	70.30 \pm 1.64	14.41 \pm 1.45	8.26 \pm 1.05	1.31 \pm 0.36	5.71 \pm 0.96
3	76.28 \pm 1.26	11.11 \pm 0.96	6.88 \pm 0.56	2.52 \pm 0.22	3.21 \pm 0.37
4	67.85 \pm 2.82	23.69 \pm 2.57	3.39 \pm 0.43	2.17 \pm 0.28	2.90 \pm 0.27
5	80.92 \pm 1.97	12.17 \pm 1.94	3.97 \pm 0.46	1.86 \pm 0.27	1.09 \pm 0.18
15	86.83 \pm 1.23	5.79 \pm 1.00	5.05 \pm 0.83	0.94 \pm 0.11	1.40 \pm 0.21
Average	73.48	13.90	6.93	1.77	3.92
Goats milk					
1	64.76 \pm 1.59	11.82 \pm 1.14	14.07 \pm 1.20	1.04 \pm 0.15	8.21 \pm 0.71
2	80.28 \pm 1.59	4.46 \pm 0.32	5.61 \pm 0.61	1.43 \pm 0.11	8.22 \pm 1.06
3	80.37 \pm 1.00	4.24 \pm 0.39	4.29 \pm 0.46	1.50 \pm 0.24	9.60 \pm 0.63
4	79.62 \pm 1.50	3.67 \pm 0.32	5.60 \pm 0.70	1.46 \pm 0.29	9.64 \pm 0.75
5	78.61 \pm 0.95	3.53 \pm 0.30	6.17 \pm 0.57	1.93 \pm 0.21	9.76 \pm 0.44
15	71.01 \pm 1.31	4.35 \pm 0.32	7.21 \pm 0.58	1.18 \pm 0.17	16.25 \pm 1.06
Average	75.78	5.34	7.18	1.42	10.28

Table 2: Level of individual NT, expressed as percentage of the total NT, (mean \pm standard error) in sheep and goats milk throughout 15 days of lactation

Days post partum	UMP*	CMP*	GMP*	AMP*
Sheep milk				
1	86.53 \pm 1.03	7.47 \pm 0.60	1.64 \pm 0.15	4.35 \pm 0.51
2	89.06 \pm 0.68	5.23 \pm 0.40	1.46 \pm 0.11	4.24 \pm 0.50
3	85.65 \pm 0.78	7.24 \pm 0.46	3.44 \pm 0.30	3.67 \pm 0.44
4	82.94 \pm 0.97	9.88 \pm 0.66	3.24 \pm 0.33	3.94 \pm 0.64
5	84.35 \pm 0.78	8.02 \pm 0.62	2.34 \pm 0.23	5.33 \pm 0.63
15	84.54 \pm 1.01	7.44 \pm 0.44	1.41 \pm 0.13	6.61 \pm 0.94
Average	85.51	7.55	2.25	4.69
Goats milk				
1	82.89 \pm 0.73	6.00 \pm 0.31	1.67 \pm 0.27	9.44 \pm 0.62
2	86.07 \pm 0.82	5.92 \pm 0.28	3.22 \pm 0.51	4.79 \pm 0.43
3	86.90 \pm 0.32	6.20 \pm 0.31	2.96 \pm 0.29	3.94 \pm 0.23
4	88.15 \pm 0.48	5.44 \pm 0.35	2.71 \pm 0.25	3.71 \pm 0.24
5	88.68 \pm 0.52	4.87 \pm 0.27	2.90 \pm 0.28	3.56 \pm 0.26
15	87.42 \pm 0.75	5.17 \pm 0.28	4.02 \pm 0.38	3.39 \pm 0.27
Average	86.68	5.60	2.91	4.81

*UMP, uridylyl-5'-monophosphate; CMP, cytidyl-5'-monophosphate; GMP, guanosyl-5'-monophosphate; AMP, adenosyl-5'-monophosphate

4. Conclusions

The composition of the NS/NT fraction of the milk is species-specific. Furthermore, it depends on the time after parturition and, eventually, on other factors. Further investigations would be of interest.

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I-P063: Effect of Prepartum Photoperiod on Milk Production of Dairy Ewes

C.M. Mikolayunas¹, D.L. Thomas¹, Y.M. Berger², T.F. Gressley³, G.E. Dahl⁴

Summary

Dairy ewes exposed to short days (8 h light: 16 h dark) for approximately 6 weeks prepartum produced 0.14 kg/d more ($P < 0.05$) milk during the first eight weeks of lactation than ewes exposed to long days (16 h light: 8 h dark) prepartum. In addition, ewes exposed to short days produced more ($P < 0.05$) milk fat and milk protein. The response to photoperiod may be mediated by an increased sensitivity of the mammary gland to circulating prolactin. Short day photoperiod ewes had lower levels of circulating prolactin prepartum, possibly influencing lactogenesis in the mammary gland.

1. Introduction

Seasonal changes in day-length regulate many aspects of mammalian physiology, including reproduction, growth, and fattening. Increased photoperiod during lactation increases milk production in dairy cattle (Peters et al., 1978) and dairy ewes (Bocquier et al., 1997), but decreased photoperiod prepartum has a positive effect on subsequent milk production in dairy cattle (Auchtung et al., 2005). The objective of this study was to determine the effect of prepartum photoperiod on milk production, milk composition, and prolactin levels of multiparous dairy ewes.

2. Materials and methods

Twenty-two multiparous, 4-yr-old dairy ewes were randomly assigned to one of two photoperiod treatments: LDPP (16 h light; 8 h dark; $n=11$) or SDPP (8 h light; 16 h dark; $n=11$). Due to variations in lambing date, photoperiod treatments were applied for 44 to 78 d prepartum. Ewes were housed in a climate controlled environment (17° C and 30 to 70 % relative humidity). Light exposure averaging 365 lux at sheep-eye level (0.76 m above the floor) was provided by fluorescent lights and controlled by an automatic timer. Lights were turned on at 0700 h daily and turned off at 1500 h and 2300 h for the SDPP and LDPP treatments, respectively.

Ewes were blood sampled biweekly from 1 day prior to the start of the light treatments to 1 week after lambing. Plasma was harvested by centrifugation within 1 hr of collection and stored at -20° C. Prolactin concentration was analyzed by PRL radioimmunoassay as

described by Miller et al. (1999). Following parturition, lambs were removed within 12 h of lambing, and ewes were moved from photoperiod treatment rooms to a common room, exposed to 12 h of light followed by 12 h of darkness. Ewes were machine milked within 12 h of lambing and subsequently twice per day at 0700 h and 1700 h. Ewes were fed ad libitum alfalfa haylage and 0.9 kg corn/d during prepartum and postpartum periods. Milk yield was measured twice per week and milk solids percentage (fat and protein) was measured once per week.

Milk production and milk composition was analyzed using the PROC MIXED method of SAS (v.8) with autocorrelation for repeated measures on ewes. Prolactin levels were analyzed using the PROC MIXED model of SAS using pretrial prolactin levels as a covariate.

3. Results and discussion

Ewes exposed to short-day photoperiods (8 h light; SDPP) for approximately six weeks prepartum produced an average of 0.14 kg/d more ($P < 0.05$) milk on each test day over the first eight

¹ University of Wisconsin-Madison, 1675Observatroy Drive, Madison, Wisconsin 53706, USA.

² Spooner Agricultural Research Station, W6646 Highway 70, Spooner, Wisconsin 54801, USA.

³ University of Illinois, 1207 West Gregory Drive, Urbana, Illinois 6180, USA. Current address: University of Delaware 531 College Ave., Newark, Delaware 19716, USA

⁴ University of Illinois, 1207 West Gregory Drive, Urbana, Illinois 6180, USA. Current address: University of Florida, PO Box 10910, Gainesville, Florida 32611-0910, USA

weeks of lactation than ewes exposed to long-day photoperiods (16 h light; LDPP; Figure 1). Average daily milk fat yield was higher (+ 24 g/d; $P < 0.01$) in SDPP ewes, resulting from greater milk production and a higher milk fat percentage. Average daily milk protein percentage also was higher (+ 10 g/d; $P < 0.05$) in SDPP ewes. The effect of prepartum photoperiod on milk composition in early lactation appears unique to sheep, as studies with dairy cattle do not support an increase in milk solids.

Both treatments experienced a prolactin surge at lambing, but SDPP ewes had lower circulating prolactin ($P < 0.05$) than LDPP ewes from 7 d before to 0.5 d after lambing (Figure 2). Previous studies in dairy cattle also found that prepartum prolactin levels were inversely related to subsequent milk production.

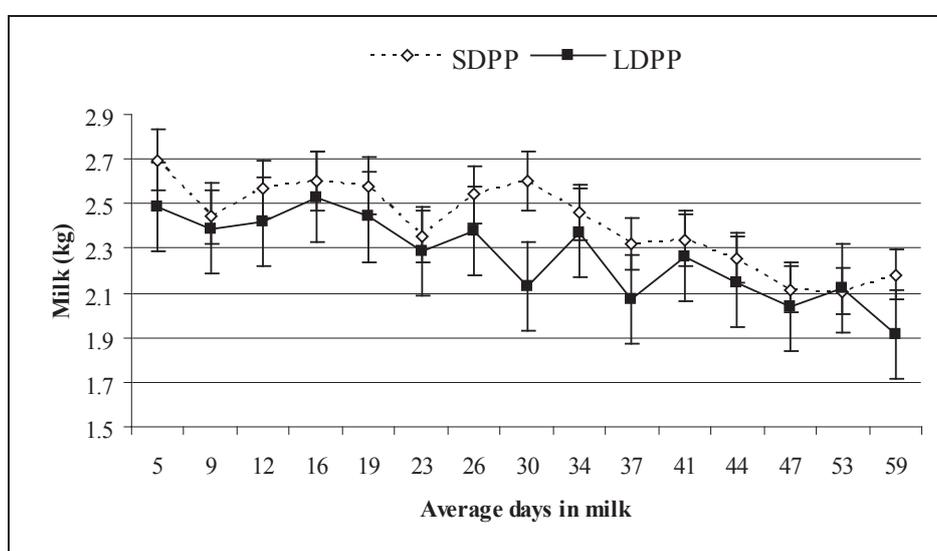


Figure 1. Milk production of ewes exposed to short (SDPP) or long days (LDPP) prepartum.

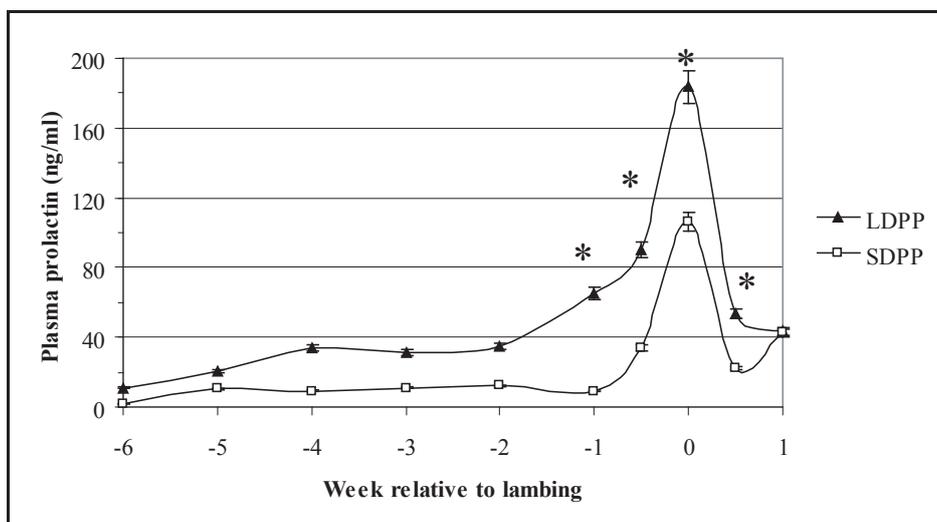


Figure 2. Plasma prolactin levels of ewes exposed short (SDPP) or long days (LDPP) prepartum. * Indicates $P < 0.05$

4. Conclusions

Decreased prepartum photoperiod can increase milk production in dairy ewes, supporting previous studies in dairy cattle (Auchtung et al., 2005). Exposure to short days prepartum resulted in a decrease in circulating prolactin levels, which may contribute to an increased sensitivity of the mammary gland to prolactin. The prolactin surge at parturition may have a greater influence on the mammary glands of SDPP ewes, resulting in an increase in milk production in the subsequent lactation.

This work may have implications for dairy sheep flocks, many of which lamb once per year. Producers may be able to utilize this response to photoperiod by breeding to minimize prepartum day length (i.e. winter lambing). This breeding scheme will take advantage of the effects of prepartum photoperiod and increasing daylength during established lactation on milk production. Berger (2005) observed greater milk production from North American dairy ewes lambing in January (winter) than from ewes lambing in April (spring). While changes in genetics, length of lactation, management, and feed quality may all have contributed, prepartum photoperiod may be one of the factors influencing this difference.

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I-P066: Microbiological Methods for the Detection of Inhibitors in Goat Milk

M.C. Beltran¹, R.L. Althaus², I. Berruga³, A. Molina³, M.P. Molina¹

Summary

Drug residues in milk may have public health and technological implications. Thus, this work studies the microbiological methods for the detection of inhibitors (BRT[®], Copan[®] and Eclipse[®]) in goat milk analysing “false positive” results (specificity), the effect of the sample pre-heating, the use of preservative, acidol, and test incubation time upon specificity. The selectivity for beta-lactams and tetracyclines in comparison with the MRLs is also studied.

The specificity of preservative-free milk samples without heat treatment was high. In general the pre-heating of the samples did not favour the results. When acidol was used, the specificity of the samples was lower given that the presence of the preservative increased the frequency of doubtful results. In all the methods, the increase in incubation time, reduced the frequency of doubtful and positive results. The sensitivity of the test allows the detection of beta-lactam antibiotics whereas none of the tests are suitable for detecting tetracyclines in goat milk according to EU-MRLs.

1. Introduction

The presence of antimicrobial substances in raw milk can have serious toxicological and technical consequences. To ensure human food safety, maximum residue limits (MRLs) have been set out for many antimicrobial agents. It is therefore of fundamental importance to avoid the presence of residues in milk in order to reduce problems during processing as well as to prevent their transmission to the consumers.

Microbiological inhibitor tests are widely utilised for the screening of inhibitors in milk, being quick, easy to use and economical. The microbiological inhibitor tests were developed to verify the quality of cow milk, although few studies have been carried out on goat milk (Contreras et al. 1997)

This work studies the characteristics of the microbiological methods (BRT-AiM[®], Copan[®] and Eclipse[®]) for the detection of inhibitors in goat milk, analysing how milk composition, use of acidol, sample pre-heating and test incubation time affect the specificity of the methods. The method's sensitivity to beta-lactams and tetracyclines is also evaluated.

2. Material and methods

The study was performed with Murciano Granadina goat milk samples from the experimental farm at the Department of Animal Science of the UPV (Spain). The goats did not receive any drugs throughout lactation.

The test specificity was calculated analysing milk samples from 65 goats in the period 30 to 115 days postpartum. Before analysis, the samples were divided into 4 aliquots: preservative-free (untreated and heated at 83°C for 10 min); acidol (untreated and heated at 83°C for 10 min). As preservative, 5 µl solution of acidol (150 mg chloramphenicol, 20 ml ethanol, 36 g sodium azide, 90 g sodium citrate 5H₂O, in 2000 ml of distilled water and 0.70 g bromophenol blue) was added to 1.5 ml milk.

To study the sensitivity, 30 samples from individual animals taken from the central period of lactation fortified with 8 beta-lactam and 3 tetracyclines at LMR concentration were used. Milk samples were analysed by BRT[®] (AIM-Analytik in Milch, Munchen, Germany), Copan[®] (Copan Italia, Brescia, Italy) and Eclipse[®] (ZEU Inmunotec, Zaragoza, Spain) microbial tests. The methods

¹ Instituto de Ciencia y Tecnología Animal. Universidad Politécnica. 46022 Valencia, Spain.

² Facultad de Ciencias Veterinaria, Universidad Nacional del Litoral, 3080 Esperanza, Argentina.

³ Departamento de Ciencia y Tecnología Agroforestal, ETSIA-IDR, Universidad de Castilla-La Mancha, 02071 Albacete, Spain.

were carried out according manufacturer's instructions. The colour changes of the methods were classified visually as either negative, doubtful or positive.

3. Results and discussion

The results of test specificity (negative results/total samples x 100) are in Table 1. The specificity of preservative-free milk samples without heat treatment was high (96.3 %), with results improving for those samples thermally treated at 83 °C/10 min (99.0 % for BRT® and 98.7 % for Delvotest®). When acidol was utilised, the specificity was lower (90.2 %), given that the presence of the preservative increased the frequency of doubtful results.

In ewe milk, Molina et al. (2003a) also observed a decrease in the specificity of the BRT® and Delvotest® when using milk samples preserved with acidol (96.3 and 97.7 vs 90.2 and 91.0).

Table 2 shows the sensitivity of beta-lactams and tetracyclines calculated for the different tests. As can be seen sensitivity was generally very good (> 90%) for almost all beta-lactams apart from amoxicillin in the Eclipse® and cephalixin in the BRT-AiM® which was low (20%). The sensitivity for tetracyclines was inexistent or very low.

In ewe milk, some authors (Althaus et al., 2002, Molina et al.; 2003b and Montero et al, 2005) using Delvotest®, BRT-AiM® and Eclipse® respectively, found good beta-lactam sensitivity but low detection potential for other antimicrobials.

Table 1: . Specificity (%) of BRT-AiM®, Copan® test and Eclipse® in goat milk

Preservative	Heat treatment	Incubation Time	Specificity (%)		
			BRT-AiM®	Copan®	Eclipse®
Free preservative	Untreated	IT-1	88,5	94,8	98,5
		IT-2	98,1	99,8	99,6
	83°C-10 min	IT-1	89,0	95,0	98,8
		IT-2	98,7	99,8	99,8
Acidol	Untreated	IT-1	72,3	86,9	83,8
		IT-2	91,2	94,4	97,1
	83°C-10 min	IT-1	74,6	88,3	90,6
		IT-2	93,8	94,2	98,5

Specificity : (negative results/total samples x 100)

IT-1 = manufacturer's recommended incubation time (BRT and Copan: 3 h; Eclipse: 2.5 h)

IT-2 = prolonged incubation time (BRT and Copan: 4 h; Eclipse: 3 h)

Table 2: Sensitivity (%) of BRT AiM®, Copan® and Eclipse® test

Antibiotic	MRL (µg/L)	BRT-AiM®	Copan®	Eclipse®
Penicillin G	4	90	97	97
Amoxicillin	4	80	70	47
Cloxacillin	30	90	90	77
Oxacillin	30	90	97	90
Cefoperazone	50	93	100	100
Cephalexin	100	20	100	97
Cefadroxil*	100	90	100	100
Cefuroxime*	100	90	100	93
Chlortetracycline	100	3	0	0
Oxytetracycline	100	37	10	20
Tetracycline	100	17	0	0

Selectivity (%): positive results/30 milk samples x 100;

* 100 µg/L has been considered as these have not been established

4. Conclusions

In goat milk, the addition of acidol increased the rate of doubtful and positive results in all the microbiological tests. Pre-heating the samples did not reduce the doubtful and positive results, except in Eclipse® when samples with acidol were used. Prolonging the incubation time increased the negative results in all the tests. In general, the methods presented a high selectivity, around 99%, for preservative-free unheated goat milk samples after a prolonged incubation period.

As concerns test sensitivity, it may be concluded that the BRT®, Copan® and Delvotest® tests are suitable for detecting residues of beta-lactam antibiotics according to EU-MRLs, whereas the sensitivity for tetracyclines in goat milk were very low.

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I-P067: Estrous Cycles and Correlation with Milk Parameters and Somatic Cell Counts in Dairy Goats

G. Pisoni¹, S. Acuña⁴, G. Savoini², E. van Lier³, J.P. Damián⁴, A. Meikle⁴, P. Moroni¹

Summary

The study was conducted to assess the effect of the stage (proestrus/estrus, metaestrus and diestrus) of a spontaneous estrous cycle on SCC, milk yield and composition (fat, protein, caseins, lactose and urea) in dairy goats. The major components of milk and SCC were monitored weekly in 80 lactating Saanen goats for 6 week while estrus detection was daily. Milk casein, and protein percentages were significantly affected by the stage of estrous cycle; during proestrus/estrus, these variables were higher (3.32 ± 0.06 and 4.44 ± 0.08) than during metestrus (3.03 ± 0.07 and 4.07 ± 0.1) but not higher than during diestrus (3.23 ± 0.06 and 4.35 ± 0.09). SCC during proestrus/estrus stage was higher than metaestrus stage (4393 ± 508 vs $2548 \pm 676 \times 10^3$ cell/mL, $P < 0.05$). The increase of SCC and higher levels of protein and casein were found at proestrus/estrus, suggesting a positive effect of estrogens.

1. Introduction

Somatic cell count is a widely implemented tool for indirect diagnosis of IMI in cows and it is used as a milk quality indicator. Intra-mammary infections caused by bacteria (Moroni et al., 2005a, b) or by caprine arthritis-encephalitis virus (Sanchez et al., 2001) are a well known factor that causes a significant increase in SCC, but SCC in dairy goats is influenced by many other factors such as duration of lactation, stage of lactation and parity (Wilson et al., 1995), number of kids born (Luengo et al., 2004). Moreover, 75% of the variation in SCC in does free of IMI could not be explained (Wilson et al., 1995) and vast individual variation in daily SCC in free-mastitis goats was reported (Zeng et al. 1997). The influence of estrus on SCC and milk parameters has been studied in dairy cows, but the results were contradictory. The aim of the present work is to study the effect of spontaneous estrus on milk parameters and SCC in dairy goats.

2. Material and methods

Lactating goats ($n = 80$) with 1 to 4 lactations and a similar BCS (2.73 ± 0.12 , mean \pm SEM) were housed in external paddocks. Animals were classified by lactation number: first ($n = 18$), second ($n = 28$), third ($n = 17$) and more than three lactations ($n = 17$). Animals were 7 to 9 mo in milk (266 ± 17 d, mean \pm SD). Estrus was recorded daily during 6 wk in 80 lactating goats. Stages of the cycle were defined taking into consideration the day of detection of estrus (d 0) as follows: Proestrus/estrus: d -3 to 0; Metestrus: d 2 to 5; and Diestrus: d 7 to 15. Milk samples not included in these definitions were excluded for consideration for the analysis. Once weekly, during the morning milking, 100 mL of milk were sampled from all animals for determination of SCC, fat, protein, caseins, lactose and urea. Milk samples were preserved with sodium azide and kept refrigerated (4°C) until analysis. Milk yield was recorded for all animals.

3. Results and discussion

Of the 80 goats, 74 exhibited cyclic activity as determined by visual observation of estrus during the 6 wk of study. The mean length of the estrous cycle was 20.9 ± 0.24 d with a range from

¹ Department of Veterinary Pathology, Hygiene and Public Health, University of Milan, via Celoria 10, 20133 Milan, Italy.

² Department of Veterinary Sciences and Technologies for Food Safety, University of Milan, via Celoria 10, 20133 Milan, Italy.

³ Animal and Forage Sciences Department, Faculty of Agriculture, University of Uruguay, Avda. E. Garzón 780, Montevideo, Uruguay.

⁴ Department of Molecular and Cellular Biology, Veterinary Faculty, University of Uruguay, Lasplacas 1550, Montevideo, Uruguay.

19 to 22 d. Data from the 6 goats that did not show estrus were excluded from the statistical analysis. Parity ($P < 0.01$) and period of lactation ($P < 0.05$) affected somatic cell count. Third lactation goats had higher SCC ($5,293 \pm 732 \times 10^3$ cells/mL, mean \pm SEM) than first and second lactations ($3,066 \pm 649$ and $3,437 \pm 563 \times 10^3$ cells/mL), which had also higher SCC than fourth lactation goats ($1,864 \pm 666 \times 10^3$ cells/mL). A higher SCC was found in postpartum period 1 (234 to 259 days postpartum; $4,589 \pm 577 \times 10^3$ cells/mL) than in period 3 (285 to 309 days postpartum; $2,287 \pm 765 \times 10^3$ cells/mL). SCC during proestrus/estrus stage was higher than metaestrus stage ($4,393$ vs $2,548 \times 10^3$ cell/mL, $P < 0.05$). Milk Yield and Composition: only a significant effect of parity ($P < 0.001$) on milk yield was found; primiparous goats produced more milk (838 ± 53 mL) than second and fourth lactation (670 ± 46 and 692 ± 55 mL), which produced more than third lactation goats (513 ± 60 mL). Fat and lactose percentages were not affected by parity, stage of the estrous cycle or postpartum period. Mean percentage of fat and lactose were $5.2\% \pm 0.14$ and $4.5\% \pm 0.1$, respectively. Protein and casein percentages were affected by the stage of the cycle ($P < 0.01$); a decrease in the percentage of both variables was observed during metaestrus (Figure 1). The urea contents were affected by parity ($P < 0.001$) and postpartum period ($P < 0.001$); milk of third lactation goats contained less urea (34.6 ± 0.9 mg/100 mL, mean \pm SEM) than that of first (38.9 ± 0.8 mg/100 mL), second (37.3 ± 0.7 mg/100mL) and fourth (39.9 ± 0.8 mg/100mL) lactation goats. The urea content increased as DIM increased: 35.2 ± 0.7 mg/100mL (mean \pm SEM; postpartum period 1), 37.5 ± 0.5 (postpartum period 2), 40.2 mg/100 mL (postpartum period 3).

4. Conclusion

Milk SCC for uninfected goats is higher than SCC for uninfected cows (cows: 40 to 80×10^3 /mL; goats: 50 to 400×10^3 /mL; Dulin et al., 1983). The relationship between SCC and the microbiological quality of small ruminant milk remains controversial (Park and Humphrey, 1986). The SCC always seems influenced by various factors such as stage of lactation, parity, time of sampling (before, during or after milking), stress and kidding season in dairy goats (Haenlein, 2002) and sheep (Sevi et al., 2004). Moreover, Wilson et al. (1995) reported that more than 90% of the variation in milk SCC in goats was not due to IMI; increasing DIM and month of the year were among the most important factors contributing to increased cell count in the absence of IMI, which our results support. In this study we have shown that SCC in goats is affected by the day of the estrous cycle presenting maximum levels at estrus. The SCC profile is associated with the endocrine environment, e.g., higher SCC levels were accompanied with high E2 and basal P4 concentrations. Milk composition is important to the goat cheese manufacturer, since any factor that influences milk composition (especially protein and casein content) also influences cheese properties, quality, yield and economic returns (Storry et al., 1983). In this study we observed higher levels of protein and casein at proestrus/estrus, suggesting a positive effect of E2.

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I-P068: Effect of Heat, Formaldehyde and Tannic Acid Treated-soybean Meal on Gas Production and Rumen Fermentation *In Vitro*

M. Nasser¹, A. Hagino², K. Katoh², Y. Obara²

Summary

The effect of soybean meal treated by heat, formaldehyde and tannic acid at three levels (1, 3 and 5 % of DM) on *in vitro* gas production and rumen fermentation was assessed by incubation of untreated and treated soybean meal in buffered rumen fluid using an *in vitro* gas technique. *In vitro* gas production was recorded at 3, 6, 9, 12, 24, 48 and 72 h incubation. The maximum gas volume was highest for SBM followed by heating SBM, SBM treated with 1 or 3% of TA and lowest for SBM treated with formaldehyde and 5% of TA, respectively. The concentrations of NH₃-N and VFA's were decreased ($P<0.05$) when SBM treated with formaldehyde or 5% of TA. The prediction of OMD, ME, NE and MN were decreased when SBM treated by formaldehyde. The present study concluded that the treatment of SBM by formaldehyde decreased the gas production. In addition, no significant effect of the three levels of TA (1, 3 and 5 % of DM) or heating on gas production and rumen fermentation, *in vitro*.

1. Introduction

Soybean meal (SBM) is the most commonly used protein supplement in beef and dairy diets. It is very palatable and has a good amino acid balance and high availability. SBM has relative low protein efficiency because of extensive ruminal degradation. Improvement in ruminal escape characteristics of SBM is of major importance to ruminant nutritionists, especially ruminant animals have high requirements for undegradable dietary protein in certain physiological states. Although, various methods have been used for reducing protein degradation in the rumen only, some of these methods have been fully tested *in vivo* as well as *in vitro*. In fact, there is still need to perfect conditions for protein protection. Therefore, the objective of the current study was to determine the effects of soybean meal treated by heating, formaldehyde and tannic acid at three levels (1, 3 and 5 % of DM) on *in vitro* gas production, rumen fermentation, energy content and microbial nitrogen.

2. Materials and methods

Untreated soybean meal (SBM), heating SBM (HSBM) {heated for 2 h at 110 °C}, formaldehyde treated SBM (FSBM) was prepared according to Subuh et al., (3) and treated SBM with three levels of tannic acid (1%, 3% and 5 % of DM) were ground to chemical analysis and *in vitro* gas production measurements. Rumen contents were collected before the morning feeding from three rumen-fistulated sheep fed on timothy hay and commercial concentrate mixture diet. Rumen fluid was mixed with buffer solution (1:2 v/v), flushed with CO₂ and maintained in a water bath at 39 °C. Samples (200±10 mg) of air-dry feedstuffs were accurately weighted into syringe fitted with plungers. Buffered rumen fluid (30 ml) was pipetted into each syringe, containing the feed samples, and the syringes were immediately placed into the water bath at 39 °C. The gas production was recorded after 3, 6, 9, 12, 24, 48, and 72 h of incubation. Total gas values were corrected for the blank incubation, and reported gas values are expressed in ml per 200 mg of DM. Cumulative gas production was fitted to the exponential model. The energy values of feedstuffs can be calculated from the amount of gas produced at 24 h of incubation with supplementary analysis of crude protein, ash and ether extract. Other syringes containing 400 mg protein sources samples and 45 ml Buffered rumen fluid were incubated for

¹ Animal Production Dept., Faculty of Agriculture, Alexandria University, El-Shatby, Alexandria, Egypt, e-mail: menassero@yahoo.com

² Animal Physiology Dept., Graduate School of Agricultural Science, Tohoku University, Amamiyamachi, Aoba-ku, Sendai 981-8555, Japan.

determination ammonia nitrogen (NH₃-N) and volatile fatty acids (VFA's) concentrations at 16h of incubation. Data were subjected to analysis of variance (ANOVA) using the General Linear Model.

3. Results and discussion

The cumulative gas production "corrected for blank fermentation" of SBM, HSBM, FSBM and SBM treated with 1 %TA, 3 % TA and 5% TA is showed in Figure (1). The maximum gas volume was highest for SBM followed by HSBM, SBM treated with 1 or 3% of TA and lowest for FSBM and 5% of TA, respectively. The soluble fraction (a) of SBM was significantly ($P<0.05$) decreased when SBM was treated by heating or formaldehyde. The gas production from the insoluble fraction (b) was significantly ($P<0.05$) decreased when SBM was treated by formaldehyde. The similar results were obtained for the gas production rate constant for the insoluble fraction (c) in SBM or FSBM. The concentrations of NH₃-N and VFA were decreased ($P<0.05$) when SBM treated with formaldehyde or 5% of TA. The present results are in agreement with the results of Stern et al. (2) and Tice et al. (4) they reported that no decline in ruminal NH₃-N of cows fed diets containing heat-treated soybeans compared to cows fed whole raw soybeans, but disagree with El-Waziry et al., (1). The prediction of OMD, ME, NE and MN were decreased when SBM treated by formaldehyde (Table1).

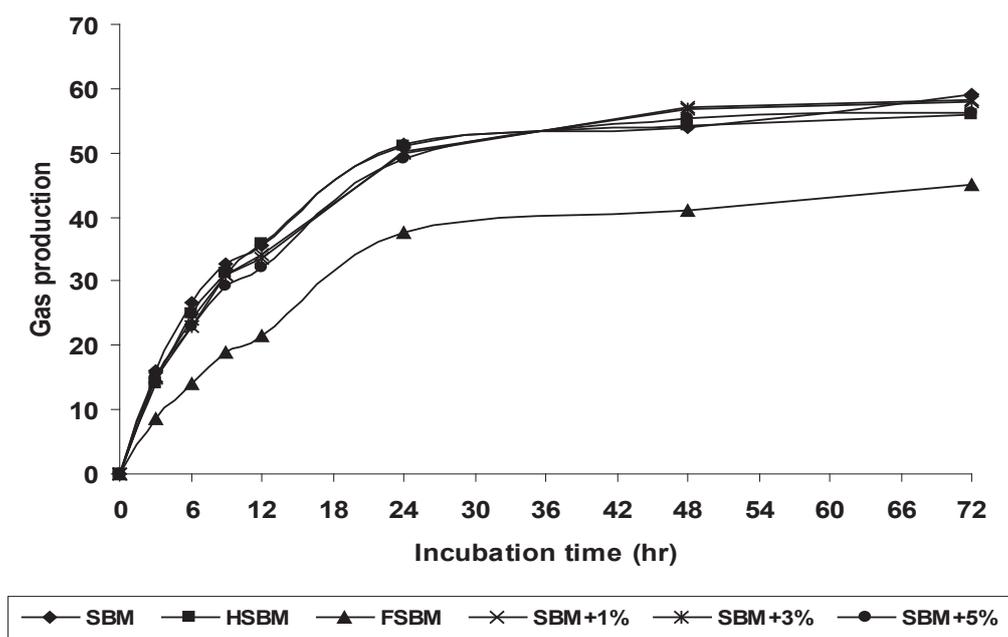


Figure 1. Effect of different treatments on soybean meal (SBM) on cumulative gas production (ml/200 mg DM) for 72 h incubation *in vitro*

Table 1: Effect of soybean meal treatment on the predicted of metabolizable energy (ME), net energy (NE), organic matter digestibility (OMD) and microbial protein (MN) *in vitro*

Feedstuffs	ME (MJ/Kg) DM	NE (MJ/kg DM)	OMD %	MN g/kg OMD
SBM	13.185±0.39 ^a	7.796±0.22 ^a	82.999±2.22 ^a	16.019±0.43 ^a
HSBM	13.133±0.29 ^a	7.767±0.14 ^a	82.703±1.45 ^a	15.961±0.28 ^a
FSBM	10.830±0.32 ^b	6.501±0.18 ^b	69.664±1.83 ^b	13.446±0.35 ^b
SBM +1%T	12.976±0.00 ^a	7.681±0.00 ^a	81.814±0.00 ^a	15.787±0.00 ^a
SBM +3%T	13.028±0.20 ^a	7.710±0.11 ^a	82.110±1.11 ^a	15.845±0.22 ^a
SBM +5%T	12.819±0.26 ^a	7.595±0.14 ^a	80.925±1.45 ^a	15.614±0.28 ^a

^{a,b} Means within a column bearing different superscripts differ ($P<0.05$).

4. Conclusion

The present study concluded that the treatment of SBM by formaldehyde decreased the gas production, rumen fermentation and energy value while, no significant effect of the three levels of TA (1, 3 and 5 % of DM) or heating.

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I-P069: The Welfare of Lactating Ewes: Guidelines for Farm Workers

P. Nicolussi¹, S. Dore¹, S. Masala¹, E. Piras¹, S. Piredda², E.A. Cannas¹

Summary

The Istituto Zooprofilattico della Sardegna "G. Pegreffi" developed three different guidelines for the lactating ewes as a part of animal welfare training programme.

Welfare of lactating ewes: description of necessary measures for ensuring welfare in farm management

Welfare in milking of lactating ewes: correct use of milking machine and milking routine for safety and welfare of lactating ewes.

Somatic cells and welfare of lactating ewes: advice to farmers about this problem which affected welfare and health of ewes and hygienic and sanitary quality milk.

1. Introduction

The Regulations (EC) 178/2002 and 852/2004 of the European Parliament on food hygiene apply to the food business from primary production up to and including sale to the final consumer. Farm workers now become legally responsible for primary production food safety and they should comply with the principles of Good Agricultural Practices (GAP) prescribing requirements to be met throughout the cycle of production including respect of animal welfare.

Farmers are now required to maintain animal welfare, because it is accepted as an integral part of the Community's "from farm to fork" policies and is one of the priorities related to the development of more sustainable food production policies. Animal welfare, indeed, is closely connected to the milk production quality and quantity and, therefore, to the food safety. Animal welfare is identified as high priority for European Community and it is one of the requirements for CAP payments (E.C. Reg. 1782/2003).

The Istituto Zooprofilattico della Sardegna "G. Pegreffi" developed the following guidelines for the lactating ewes as a part of animal welfare training programme.

The aim of these three guidelines is to give a contribution for stakeholders' training about welfare lactating ewes, using simple and concise language.

a) Welfare in lactating ewes

Main requirements in sheep breeding are described particularly about sheepfold, equipment, management, features and behavioural needs.

In particular:

a) Sheep farmer

The skills to safeguard the welfare of the animal and know the signs of ill health in sheep.

b) Space and accommodation

Floors with surfaces that avoid the risk of injury.

The protection of effects of climatic extremes.

c) Feed and water

Food required for growth, pregnancy and lactation.

Unlimited access to water.

d) Management practices

Features of the practices as the shearing, the tail docking and castration.

¹ Istituto Zooprofilattico Sperimentale della Sardegna "G. Pegreffi", Sassari, Italy, paola.nicolussi@izs-sardegna.it, agnese.cannas@izs-sardegna.it, simonedore@hotmail.com

² Ente Regionale di sviluppo e Assistenza Tecnica della Sardegna, Cagliari, Italy.

e) The animal health

The features of good and ill health .

Prevention and treatment of lameness, parasites and mastitis.

b) welfare in milking of lactating ewes

The diffusion of the milking machine determined a radical change in the bosom of the dairy sheep farms, especially in the Sardinian rural economy. The less demanding job for the milking operations allows the farmer to dedicate more time for the other activities and to improve the farm management. After the milking machine introduction in the dairy sheep farm caused significant advantages in milk hygienic characteristics, particularly in the Total bacterial Count decrease. However, the malfunction of the milking machine or an incorrect milking routine can adversely affect udder health and milk production, determining mastitis outbreak and its transmission through the animals. In these guidelines are described:

- pre-milking room and milking room structural characteristics and their hygienic requisites
- the principles of the milking machine functioning and a brief description of the basic components
- the main operations of the milking routine and their correct management
- kind and frequency of the milking machine maintenances
- milking machine washing procedures and microbiological characteristic of the rinsing water and disinfectants
- udder health and hygiene

The good management of the milking machine along with a perfect milking routine represent the necessary conditions to assure animal welfare and udder health, improving milk production on quality and yield.

c) somatic cells and welfare of lactating ewes

Somatic cells represent the main animal welfare parameter for dairy animals and it is the basic useful tool for the mastitis control programmes. These guidelines informs the farmers about somatic cells in milk and their role as health indicator in a dairy sheep farm. The improvement of the Somatic Cell Count (SCC) determines economic losses because it is strictly related to the udder pathologies, to the milk quality and yield decrease and, finally, to the differential milk quality payment. In this book general information to know, to prevent and to resolve somatic cell problem are provided.

In particular:

e) What are somatic cells?

The main cell typologies that can be found in physiological and in pathological udder condition are described.

f) When do somatic cells increase?

SCC increase is due either to natural factors (age, after lambing and in late stage of lactation) or to bad management (incorrect feeding, worse climatological conditions, mistreatments, transportation, udder injuries, irregular milking procedures, water pollution and udder inflammation).

Mastitis, subclinical especially, represent the most important and frequent cause of the SCC increase in milk.

c) How are somatic cells determined?

SCC can be evaluated by indirect (*California Mastitis Test*) or direct (Fluoro-opto-electronic method) tests. *California Mastitis Test* is carried out on the farm and it can be performed directly by the farmer as long as he is trained properly. The fluoro-opto-electronic method is carried out only in specialized laboratories; it determines somatic cell content in a precise way.

d) Milk sampling

SCC test can be carried out on:

- Bulk milk: it allows to know the mean of the SCC of the herd.
- Individual/half-udder milk: it is sampled directly from each single animal.

e) How can somatic cell problem resolve?

High values of SCC in milk show animal health problems. A prompt intervention is useful to find the cause and to avoid the diffusion of the pathology in the herd. Reduction of the SCC is neither simple nor immediate and, along with the continuous attention of the farmer, the veterinary assistance is needed because it represents a sanitary problem.

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I-P071: Studies on Hemoglobin Polymorphism of Apulian Native Dairy Goats and its Relationship to Hematocrit Value and Hemoglobin Concentrations

E. Pieragostini¹, I. Alloggio¹, A. Caroli², F. Petazzi³

Summary

The functional effect of hemoglobin (Hb) phenotype on the hematological pattern has been demonstrated in humans as well as in mammalian species. Particularly positively charged variants both at the alpha globin (HBA) and beta globin (HBB) loci are somehow related to a decreased mean corpuscular volume (MCV), hematocrit value (HCT) and hemoglobin levels (HGB) which in turn may have an adaptive value in arid climates. The aim of this work is to extend the number of species investigated for this issue by analyzing Apulian native dairy goats which are highly polymorphic at the HBB alleles; the high frequency of alleles, whose isoelectric point (pI) range around pH 7.3, is particularly relevant to this purpose. The results obtained on 327 individuals divided in three main groups and classified as A, AD and D on the basis of their major band pattern, are in agreement with previous findings and highlight a highly significant effect ($P < 0.001$) of Hb phenotype on PCV and HGB.

1. Introduction

Goats exhibit a very complex hemoglobin polymorphism due to the presence of a number of allelic and non allelic chains both at the alpha and beta globin systems [1]. However, all the possible tetramers resulting from the combination between this heterogeneity of alpha and beta globins resolve in two main electrophoretic zones, namely the A and B/or D zone, according to their different electrical properties. A group of positively charged tetramers such as, HbB, HbD and HbD^{Malta} migrate in the B/D zone, whose isoelectric point (pI) range is around pH 7.2. HbB results from an alpha chain variation, due to the presence of a rare allele at the HBA1 locus whereas the HbD and HbD^{Malta} exhibit variation in the beta chain as a consequence of different point mutations at the HBB locus [1] (Table 1).

The functional effect of the hemoglobin (Hb) phenotype on the hematological pattern has been demonstrated in humans as well as in mammalian species. Particularly positively charged variants are somehow related to a decreased MCV and PCV which in turn may have an adaptive value in arid climates. In sheep, a significant effect was detected for the HBB locus on haematocrit (HCT), hemoglobin content (HGB) and mean corpuscular volume (MCV), with decreasing HCT, HGB and MCV for a decreasing number of HBBA alleles in the genotype [2, 3]. This work aims to extend the number of species investigated with regard to this issue. Thus Apulian native dairy goats have been investigated to estimate both the frequency of positively charged variants and their functional effect on hematological values.

Table 1: Beta globin chains relevant to the identification of the Hb electrophoretic zones [4, 5, 6]

Beta globin chain	Mutation vs β A globin chain	MW	pI
β A	wild type 86 ^{Gln>His} ;	16021.4	6.75
β E	103 ^{Lys>Arg} ; 124 ^{Leu>Val}	16044.4	6.79
β D	20 ^{Asp>His}	16043.46	7.17
β D ^{Malta}	69 ^{Asp>Gly}	15963.37	7.14

¹ Dept. PROGESA- Università degli Studi di Bari. Italy.

² Dept. SBB Università degli Studi di Brescia - Italy.

³ Dept. SBA - Università degli Studi di Bari - Italy.

2. Material and methods

Hemolysates belonging to 183 Garganica and 166 Jonica goats reared in 12 different farms were available subsequent to two different experimental trials whose aim was the hematological characterization of Apulian native goat breeds. Hb phenotypes were analyzed with isoelectric focusing in a pH range of 6.7-7.7 (PAGIF). Heterogeneity of globin chains was evidenced both by AUT-PAGE and RP-HPLC [1]. The 349 individuals in the sample were divided into three main groups and classified as A, AD and D on the basis of their major band pattern assuming that the intraband variation included functionally equivalent tetramers. Hematological variables of animals typed at the globin systems were previously evaluated using a hematology analyzer.

Table 2: Data set and sample size

Breed	Sampling	Farms	N	Hb phenotype		
				A	AD	D
Garganica	Winter (W)	5	143	42	63	38
	Spring (S)	5 bis	90	32	36	22
Jonica	Winter	7	146	71	49	26
Total		12	379	145	148	86

The data were analyzed by a linear model including the effects of Hb phenotype, flock, sex, and age as covariates, as well as the effect of the farm management on the hematological values. Gene frequencies were estimated by the GENEPOP program [7].

3. Results and discussion

As expected, HbB hemoglobin was not found in Apulian native goat breeds[1] thus table 3 reports only the frequency values of the positively charged variants at the HBB locus showing the high frequency value of HbD^{Malta} both in Garganica and Jonica goats. The alleles and genotypes in each breed sample were in HW equilibrium. As to the effect of the Hb phenotype on the hematological values, both HGB and HCT gave significant results, as shown in table 4 and 5. In particular, in the winter sample of Garganica goats, a significant decreasing trend was found as to the HGB values related to the number of HBB^{Malta} gene in the genotype. This phenomenon was confirmed by the HGB values recorded in the spring sample of Garganica goats (table 4) as well as by the HGB and HCT values of Jonica goats (table 5).

Table 3: Frequency of the positively charged variants at the HBB locus in Apulian native goat breeds

	N	β^D	β^D Malta
Garganica	183	0	0.49
Jonica	166	0.008	0.43
Maltese	327		

The lower HCT values correspond to lower blood viscosity and thus to a greater availability of water, which seems to be of particular adaptive significance in habitats characterized by an arid climate, such as Apulia [5]. These general comments are substantiated and supported by the results obtained after analyzing the haemoglobin phenogroups as in table 5, where D goats fit particularly well with the above adaptive features.

Table 4: Least square mean values and standard error (s.e.) of haemoglobin (HGB) for β globin phenotype in Garganica goats. W and S indicate the two different data sets as in table 2

Hb type	W	HGB g/dl	s.e.	S	HGB g/dl	s.e.
A	42	9.15a	0.17	32	9.39a	0.22
AD	63	8.93	0.15	36	9.03	0.23
D	38	8.53b	0.2	22	8.38b	0.28

4. Conclusions

Based on these results three points deserve attention: i) the high frequency of the positively charged variants in two Apulian native goat breeds; ii) the fact that similar frequency values have been found only in Maltese goats [7] supporting the hypothesis of a latitudinal relationship and then of an adaptive significance; iii) the further evidence provided regarding the effect that the Hb phenotype has on hematological values.

Table 5: Least square mean values and standard error (s.e.) of hematocrit values (HCT) and haemoglobin (HGB) for β globin phenotype in Jonica goats

Hb type	N	HCT %	s.e.	HGB g/dl	s.e.
A	71	26.7 a	0.47	9.58a	0.16
AD	49	25.1 b	0.51	8.96b	0.17
D	26	25.0	0.73	9.14	0.25

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I-P072: Basic Hematological and Serological Parameters in the Jonica Goat (*Capra Hircus*)

G.T. Rubino¹, R. Lacinio¹, A. Caroli², E. Pieragostini³, F. Petazzi¹

Summary

An investigation was carried out to evaluate the variability of the basic hematological and serological values in the Jonica goat. An additional aim was to elucidate the relationships between these parameters and the production environment.

A total of 144 Jonica goats of both sexes were sampled from 7 farms in the area around Taranto, in southern Apulia. The basic hematological parameters were evaluated. The biochemical, enzymatic, and colorimetric analyses were also carried out together with serum protein electrophoresis.

Highly significant differences ($P < 0.001$) were found among farms for 31 of the 33 parameters considered. A multivariate analysis clearly separated the flocks into two groups, mainly on the basis of the first principal component which was highly related to the herd management level.

1. Introduction

The Jonica goat is a Southern Italian breed resulting from crosses of the local Apulian goat population and the Maltese breed. Jonica goats are almost exclusively raised in the Southern area of Apulia and are physically suited for living on arid soils. Average lactation milk production is about 300 kg in 215 d. The breed is mainly used for milk and typical cheese production, which are strongly affected by the health and management conditions. This paper reports the results of an investigation carried out to evaluate the variability of the basic hematological and serological values in the Jonica goat. It also elucidates the relationships between these parameters and the production environment.

2. Material and methods

A total of 144 Jonica animals of both sexes reared under semi-extensive system were sampled from 7 farms in the area around Taranto, in southern Apulia. The sampled animals appeared to be clinically healthy.

The basic hematological parameters were evaluated by means of a hematology analyzer (CELL DYN 3700® ABBOTT). The biochemical, enzymatic, and colorimetric analyses were carried out using specific kits and an automatic clinical chemistry analyser equipped with interference filters. Serum protein electrophoresis was carried out according to the method described by Petazzi et al. [1]. The parameters considered and their abbreviation codes are listed in Table 1. The data were analysed by a linear model and included the effects of flock, sex, and age as covariates, as well as the effect of the farm management on the hematological and serological values. A multivariate analysis was also carried out on the entire data-set by the PRINCOMP procedure [2].

3. Results and discussion

Descriptive statistics of the hematological and serological parameters in the Jonica breed are shown in Table 1. Highly significant differences ($P < 0.001$) were found among farms for 31 of the 33 parameters considered (all except MCV and ALP). The multivariate analysis clearly separated the flocks into two groups, mainly on the basis of the first principal component which was highly related to the herd management level, as shown in Figure 1 depicting the distribution of the first principal component (Prin1) as a function of flocks. The distribution is binomial, with two flocks

¹ DSBA, Università degli Studi di Bari, Bari, Italy.

² DSBB, Università degli Studi di Brescia, 25123 Brescia, Italy.

³ Dip. PROGESA, Università degli Studi di Bari, Bari, Italy.

(labelled as C and D) showing the lowest Prin1 levels, two flocks (F and G) showing the highest Prin1 levels, and the other flocks (A, B, and E) in an intermediate position. The Prin1 score highly correlated with the general management conditions of the different flocks.

Table 1: Descriptive statistics of the hematological and serological parameters.
SD = standard deviation; Min = minimum value; Max = maximum value

Variable	Code	Mean	Dev
Hematocrit (dl/dl) (%)	PCV	25.47	4.41
Hemoglobin (g/dl)	Hb	9.19	1.58
Red Blood Cells (x10 ⁶ /μl)	RBC	15.93	2.73
White Blood Cells (x10 ³ /μl)	WBC	9.86	2.70
Mean Corpuscular Volume (fL)	MCV	15.92	1.39
Mean Corpuscular Hemoglobin (pg)	MCH	5.86	0.57
Mean Corpuscular Hemoglobin Concentration (g/l)	MCHC	36.19	2.39
Total proteins (g/dl)	PT	8.68	1.49
Albumin %	Alb_p	38.15	5.33
Albumin (g/dl)	Alb_g	3.29	0.60
Alpha globin %	Alfa_p	9.79	2.66
Alpha globin (g/dl)	Alfa_g	0.83	0.21
Beta1 globin %	Beta1_p	11.56	1.91
Beta1 globin (g/dl)	Beta1_g	1.01	0.25
Beta2 globin %	Beta2_p	5.37	1.71
Beta2 globin (g/dl)	Beta2_g	0.46	0.15
Gamma globin %	Gamma_p	35.13	5.87
Gamma globin (g/dl)	Gamma_g	3.09	0.90
Glucose (mg/dl)	GLU	51.27	10.64
Cholesterol (mg/dl)	CHO	69.35	19.25
Triglycerides (mg/dl)	TRI	29.96	11.51
Albumin (g/dl)	ALB	3.04	0.35
Creatinine (mg/dl)	CRE	0.91	0.19
Urea (mg/dl)	BUN	25.33	9.19
Calcium (mg/dl)	Ca	9.70	2.04
Phosphorus (mg/dl)	P	5.36	1.37
Magnesium (mg/dl)	Mg	3.43	0.78
Aspartate aminotransferase (u/l)	AST	106.01	29.27
Alanine aminotransferase (u/l)	ALT	24.79	8.38
γ-Glutamyltransferase (u/l)	γ-GT	59.39	13.00
Lactic dehydrogenase (u/l)	LDH	513.70	123.84
Creatinine kinase (u/l)	CK	182.65	77.17
Alkaline phosphatase (u/l)	ALP	932.87	1222.40

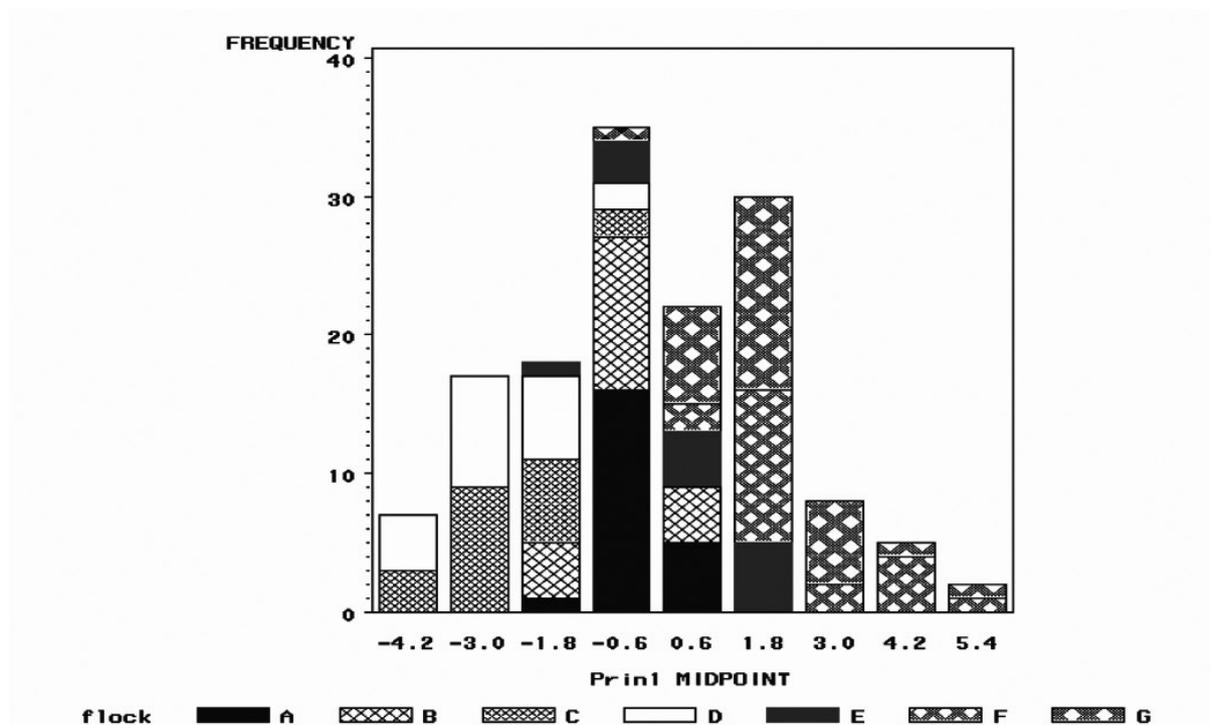


Figure 1. Frequency distribution % (FREQUENCY) of the first principal component obtained from the multivariate analysis (Prin1 MIDPOINT) as a function of flocks.

4. Conclusion

The results obtained highlighted that hematological and serological analysis is a useful tool to check animal health and herd management in goat breeding systems. The score obtained on the basis of the first component from multivariate analysis permitted ranking of the different flocks within a range that was highly related to the production environment.

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I-P073: Sheep Hemoglobin I in Sicilian Dairy Breeds

I. Alloggio¹, P. Loizzo¹, E. Pieragostini¹

Summary

At the beginning of the last decade, in Sarda and Altamura sheep a beta globin gene (HBBI), was found to be responsible for an electrophoretically silent hemoglobin band referred to as the HbI. This work reports the results obtained from a screening program in two Sicilian dairy breeds reared in Apulia. Blood samples were taken from 156 Comisana sheep and 150 Valle del Belice sheep. Hemolysates were obtained following the traditional method, the tetramers were analysed by PAGIF and globin chains were separated by AUT-PAGE. PAGIF detects HbA and HbB bands, HbI comigrating with HbB. In AUT-PAGE beta globin I migrates close to beta globin A. The results showed that both Sicilian breeds exhibit an HBBI allele frequency of about 0.2, which is more than double the previous frequency data. These findings confirm HBBI as a rather common allele in Southern Italian sheep breed and suggest checking whether there is the same frequency latitudinal cline as with the HBBB gene.

1. Introduction

At the beginning of the last decade, in Sarda and Altamura sheep (a rare Apulian native breed) a beta globin gene (HBBI), was found to be responsible for an electrophoretically silent hemoglobin band referred to as the HbI [1]. The two breeds showed not only the same variant due to the 13 Gly→Ser point mutation, but also almost the same frequency [2, 3]. This finding brought up interesting questions on the origin and spread of this mutation because, owing to the distance between the respective breeding sites, the occurrence of gene flow was rather improbable. Later, the only records collected on this issue concern i) breeds from Corsica [4] and ii) a recent detection in another Apulian native breed Gentile di Puglia [5]. With the conviction that understanding the dynamics and fate of a mutation is fundamental to explain how it contributes measurably to the "standing" variation of populations, this work reports the results obtained from a screening program implemented in two Sicilian dairy breeds reared in Apulia.

2. Material and methods

Blood samples were taken from purebred Comisana and Valle del Belice sheep in different farms in Apulia (table 1).

Table 1: Sampling scheme and sample size

Farm	Breed	F	M	N° of samples	N° of samples per breed	Flock size	Purebred group
A	C	20	1	21		26	
B	C	18	1	19		20	
C	C	35	7	42		400	
D	C	36	5	41		150	
E	C	18	2	20		200	
F	C	12	1	13	156	13	810
G	VB	52	38	90		800	
H	VB	33	7	40		320	
I	VB	9	11	20	150	300	1420
Total		367	98	465		3910	

Hemolysates were obtained following the traditional method. The tetramers were analysed by PAGIF2 and globin chains were separated by AUT-PAGE. PAGIF detects HbA and HbB bands,

¹ PROGESA- Università di Bari, Italy.

with HbI comigrating with HbB. In AUT-PAGE beta globin I migrates close to beta globin A. Gene frequencies were calculated by gene counting and allele counting. The estimates of the standard deviation were computed with the following formula:

$$s = \sqrt{\frac{pq}{2Ne}} \quad \text{where} \quad Ne = \frac{4MF}{N} \quad \text{was obtained considering that the males in the sample were all the males in the flock (Tab.1).}$$

3. Results and discussion

Table 2 shows Gene frequency and the standard deviation of the alleles at the beta globin locus (HBB) found in Comisana and Valle del Belice sheep breeds. The alleles and genotypes in each breed sample were in HW equilibrium.

Table 2: Gene frequency and standard deviation (s) of the alleles at beta globin locus (HBB) found in Comisana and Valle del Belice sheep breeds

Breed	HBBA	s	HBBB	s	HBBI	s
Comisana	0.053	0.018	0.784	0.033	0.165	0.030
Valle del Belice	0.047	0.017	0.740	0.035	0.213	0.033

4. Conclusion

Once these findings were compared with the literature (Table 3) they not only provided further evidence that HBBI is a rather common allele in Southern Italian sheep breed but also suggested checking whether there is the same frequency latitudinal cline as with the HBBB gene.

Table 3: Frequency of HBBI allele as reported in the literature

Breed	HBBA	HBBB	HBBI	References
Altamura (126)	0.095	0.882	0.083	Pieragostini et al., 2001
Corsican (36)	0.000	0.985*	0.015*	*Estimated value from Serreri et al., 1998
Gentile di Puglia (294)	0.117	0.832	0.051	Pieragostini et al., 2006
Sarda (258)	0.030	0.890	0.080	Hadjjsterkotis et al., 1995

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I-P074: Detection by ELISA of Caprine Mastitis Due to *S. Aureus*

F.B. Gilbert¹, B. Poutrel¹, A. Fromageau¹, V. Lictevout², C. Dubuc-Forfait³, J.L. Champion³, R. de Crémoux⁴

Summary

An enzyme-linked immunosorbent assay (ELISA) for detecting *Staphylococcus aureus* antibodies in caprine milk samples (n=566) from 13 farms was evaluated by comparison with bacteriological examination. The results for the sensitivity and specificity of the test were 84% and 82%, respectively.

1. Introduction

The detection of mastitis caused by *S. aureus* remains a challenge for milk producers and the dairy industry, especially for raw milk-derived products. Indeed, *S. aureus* is a public health concern as some strains have the ability to produce enterotoxins and cause food-poisoning. Currently, the diagnosis of mastitis caused by *S. aureus* relies on SCC (somatic cell count) and bacteriological examination of aseptically collected milk samples. However, bacteriological analyses are expensive and not applicable for routine surveillance plans of herds. Our objective is to develop an ELISA test able to detect putative *S. aureus* mastitis by using individual milk samples collected without special precautions. The strategy consists in the detection of specific antibodies elaborated in response to the infection.

2. Material and methods

A *S. aureus* antigen was purified and evaluated by ELISA with individual milk samples collected in 13 caprine farms encountering contamination problems of milk and/or cheese by *S. aureus*. The bacteriological analysis of aseptically collected milk samples from half-udders was used as reference method.

ELISA tests were performed with 96-well plates coated with a 4 µg/ml solution of antigen. Briefly, for all the farms, 100 µl of undiluted or 10x diluted individual milk, i.e. a mixture of milk from the two half udders of goats, were deposited on the plates. After 45 min at room temperature, each well was washed, 100 µl of peroxidase conjugate was added, and the plate was incubated for 45 min at 37°C. The microtiter plate was then washed, and revelation was performed with tetramethylbenzidine for 20 min in the dark. The optical density (OD) at 450 nm was read with a microplate reader. For each breed, the arithmetic mean (M) of all ODs as well as the standard deviation (sd) were determined to define positive threshold values.

3. Results and discussion

Among the 566 lactating goats implicated in the study, 50 excreted *S. aureus* in milk according to bacteriological examinations.

Overall, the best concordance between ELISA and bacteriological results was obtained with the ELISA using diluted milks and M+sd as threshold value after elimination the 3 highest ODs. The sensitivity and the specificity of the test were 84% and 82%, respectively. Some goats displaying *S. aureus* in milk samples without any inflammation (SCC<250 000 cells/ml) were negative in the ELISA test. For one of these goats, additional milk samples were subjected to bacteriological analysis and found sterile. It should be noticed that i) *S. aureus* colonization restricted to the teat canal will lead to negative ELISA results but possibly to positive bacteriological analysis of milk ii) recent mastitis will not be detected until sufficient antibody levels

¹ INRA, UR1282, Infectiologie Animale et Santé Publique, IASP, 37380 Nouzilly, France.

² Touraine Avenir Lait, 38 rue A. Fresnel, BP 50139, 37171 Chambray Les Tours Cedex, France.

³ Groupement de Défense Sanitaire, 66 Boulevard Gassendi, BP 117, 04004 Digne Les Bains, France.

⁴ Institut de l'Élevage, Chambre d'Agriculture du Tarn, BP 89, 81003 Albi Cedex, France.

have been reached (2 to 3 weeks) iii) milk from recovering mammary glands after cure of *S. aureus* mastitis will lead to false positive results.

The limitation of the ELISA for goats displaying high somatic cell counts is conceivable. A preliminary study performed with 5 breeds for which SCC records were available is presented in Table 1. When the ELISA was performed with milk samples from all the goats belonging to these 5 farms, the sensitivity and specificity values were 78% and 85%, respectively. Seven goats for which *S. aureus* were isolated in milks, among them 4 goats without any inflammation, were found negative with the ELISA (recent infections, teat canal colonisation?). Unfortunately, we did not get the opportunity to collect additional aseptic samples for bacteriological examination. The restriction of the ELISA to goats with SCC threshold of 750 000 appeared the most appropriate as 25/28 *S. aureus* excretory goats were detected by ELISA. An interesting point is that the same goats were detected positive when the ELISA was conducted for all the goats or restricted to goats demonstrating an inflammation (SCC > 750 000 cells/ml). Indeed, this result suggests that the use of the ELISA test could be modulated according to specific herd management (restriction to animals demonstrating an inflammation when SCC records are available, global analysis when such records do not exist).

Table 1: ELISA screening of goats belonging to 5 farms according to SCC score and bacteriological status

No of goats (total)				SCC > 750 000 cells/ml		SCC > 1 000 000 cells/ml	
N = 286				N = 101		N = 76	
SC+ = 32 Other = 254				SC+ = 28 Other = 73		SC+ = 26 Other = 50	
				ELISA positive goats		ELISA positive goats	
Breed	Total	SC+	Other	SC+	Other	SC+	Other
A	46	1	45	1	3	1	2
B	41	6	35	5	2	4	1
C	45	4	41	4	8	4	7
D	92	20	72	14	4	13	4
E	62	1	61	1	5	1	3
Total	286	32	254	25	22	23	17
			Sensitivity	89.3%			88.5%
			Specificity			69.9%	66.0%

SCC = somatic cell count

SC+ = goats for which *S. aureus* was detected in aseptically collected milk samples.

Other = goats for which milk samples bacteriological analysis was negative or revealed the presence of a pathogen different from *S. aureus* (coagulase negative staphylococci or streptococci).

The sensitivity and specificity values of the test performed with milk samples from all the goats of these breeds were 78% and 85%, respectively.

4. Conclusion

This simple and inexpensive ELISA test that does not require aseptic milk samples could be useful for detection of infected goats in field conditions. In a *S. aureus* mastitis detection protocol, this ELISA test could be used as a primary step, preferentially in combination with SCC determination, followed by bacterial analysis of milk samples of ELISA positive goats.

This work is part of a ACTA/ACTIA project granted by the French government aiming at improving S. aureus control in caprine breeds.

I-P075: Goat's and Sheep's Milkfat: Characterization of Triglycerides by Ag⁺SPE/GC/MS

M. Povalo¹, V. Pelizzola¹, A. Avalli¹, G. Contarini¹

Summary

The knowledge of the molecular species that constitute the triglyceride fraction of milkfat can provide important information regarding different aspects, such as physical properties, effects on human nutrition and mechanisms of biosynthesis in the ruminant mammary gland. In addition, the study of milkfat triglycerides of different species could help in the characterization and recognition of these fats. In our research the fat obtained from goat and sheep milk was pre-separated by silver ion solid phase extraction (Ag⁺-SPE). Four fractions were obtained according to the degree of unsaturation. The principal triglycerides of each fraction were identified by GC/MS. Differences were observed between the two ovine species in the distribution of some molecules of triglycerides, particularly within the groups having from 32 to 40 total carbon number. These results seem to be promising for the setting up of a method able to characterize non-bovine milkfats.

1. Introduction

The triglyceride (TG) composition of milkfat has been studied with different purposes, for example knowledge of physical properties, biosynthesis mechanisms. Due to the presence of several molecular species a satisfactory separation requires the adoption of more than one technique (Fontecha et al., 2000; Christie, 2003). The study of the composition of triglycerides that constitute the milkfat from different species could help in the identification of those fats, whose fatty acid composition is very similar. At present no validated methods are available to check the purity of non-bovine milkfats. In this research milkfat from sheep and goat was pre-separated by Ag⁺-SPE and then analyzed by GC, using both FID and MS detector.

2. Material and methods

Fat was extracted from 100 ml milk according to the ISO 14156:2001 method. Twenty mg of fat were dissolved into 0.3 ml of dichloromethane. The separation into the four fractions was performed by Ag⁺-SPE: a normal phase silica SPE cartridge of 1 g (Supelco, Bellefonte, USA) was modified with AgNO₃ according to Christie (1989). The fractions were separated by elution of the solvent mixtures reported in brackets: saturated (pentane : dichloromethane 50:50 v/v, 8 ml), monounsaturated (dichloromethane : acetone 99:1 v/v, 7 ml), diunsaturated (dichloromethane: acetone 95:5 v/v, 8 ml) and polyunsaturated (acetone, 7 ml). The fractions were analyzed by both GC/FID and GC/MS. A Trace GC and a Trace GC coupled with Trace MS plus mass spectrometer (ThermoElectron, Rodano, Italy) were used. The capillary column adopted was a CB-TAP (Varian, Middelburg, NL). For the GC/FID analysis hydrogen was used as carrier gas at 1 ml/min, in constant flow. The MS acquisition was performed in Electron Impact mode (70 eV) and mass range *m/z* 70-900. Source and interface temperatures were 250°C and 360°C respectively. The identification of fatty acids esterified in the triglyceride was carried out by the evaluation of the presence of fragments [RCO]₊, [RCO+74]⁺, [RCO+128]⁺ and [M-RCOO]⁺.

3. Results and discussion

As regards the separation of triglycerides according to the degree of unsaturation, it was noticed that there was a not complete fractionation, for example, some saturated molecules were found in the fraction containing monounsaturated acids. This behaviour, observed also by Fontecha et al. (2000) by adopting AgNO₃-TLC technique, is particularly evident for triglycerides having up to 40 carbon atoms. The presence in these TGs of short chain fatty acids influences

¹ CRA- Istituto Sperimentale Lattiero-Caseario, Lodi - Italy.

the separation properties of the molecules (Christie, 2003). Differences were observed in the percentage composition of TG classes within the four fractions: in general, higher values of TGs having 48-54 carbon number (CN) were detected in sheep milkfat than goat, that, on the other hand, showed higher percentages of TGs having CN 24-32. As regards the molecular species, the same TGs were detected in sheep and goat milkfat, but the distribution of these molecular species within their own triglyceride class was different. In particular this was observed for TGs having from 32 to 40 CN (Table 1). Goat milkfat was characterized by higher proportions of TGs containing C10 fatty acids than sheep milkfat. Figure 1 shows the enlarged view from CN 32 to CN 36 of the saturated fraction. This behaviour was similar in all the four fractions separated.

Table 1: Distribution of the molecular species within the most interesting triglyceride classes for the four fractions

SATURATED		goat	sheep
32	10,10,12 (a)	11.4	4.0
	8,10,14 (b)	12.4	7.7
	6,10,16 (c)	30.3	24.0
	4,12,16 (d)	39.7	57.5
	4,14,14 (e)	6.3	6.8
34	10,10,14 (a)	16.9	6.9
	8,10,16 (b)	13.7	5.5
	6,12,16 (c)	16.6	9.5
	6,10,18 (d)	3.7	1.9
	4,14,16 (e)	49.0	76.1
36	10,10,16 (a)	32.6	11.8
	6,14,16 (b)	23.6	17.4
	4,16,16 (c)	43.8	70.8
MONOUNSATURATED		goat	sheep
36	10,10,16/10,12,14	17.8	7.6
	6,14,16	17.4	11.7
	4,16,16	47.6	50.5
	4,14,18:1	17.1	30.2
38	10,12,16	10.7	2.7
	8,14,16/10,14,14	8.2	3.1
	10,10,18:1/6,16,16	27.3	12.4
	4,16,18	22.5	24.0
	4,16,18:1	31.3	57.8

DIUNSATURATED		goat	sheep
36	8,10,18:1	21.8	8.7
	6,12,18:1	17.9	7.5
	4,14,18:1	50.3	67.7
	4,16,16:1	10.0	16.1
38	10,10,18:1/10,10,18:2	20.3	4.5
	6,14,18:1/6,14,18:2	19.2	8.8
	4,16,18:1/4,16,18:2	60.5	86.7
40	10,12,18:1	22.1	4.3
	8,14,18:1	17.4	10.9
	6,16,18:1	45.3	44.1
	4,18,18:1	15.2	40.7
POLYUNSATURATED		goat	sheep
36	8,10,18:2	27.1	9.7
	8,10,18:3	15.5	6.3
	4,14,18:2	57.5	84.0
38	10,10,18:3	19.5	2.9
	6,14,18:3	18.1	11.8
	4,16:1,18:2/4,16:1,18:3	62.4	85.3
40	10,12,18:3	11.0	1.3
	8,14,18:2	5.9	1.2
	6,16:1,18:2	6.0	3.1
	6,16,18:3	15.1	6.1
	4,18,18:2/4,18,18:3	52.2	75.4
	4,18:1,18:2	9.8	13.0

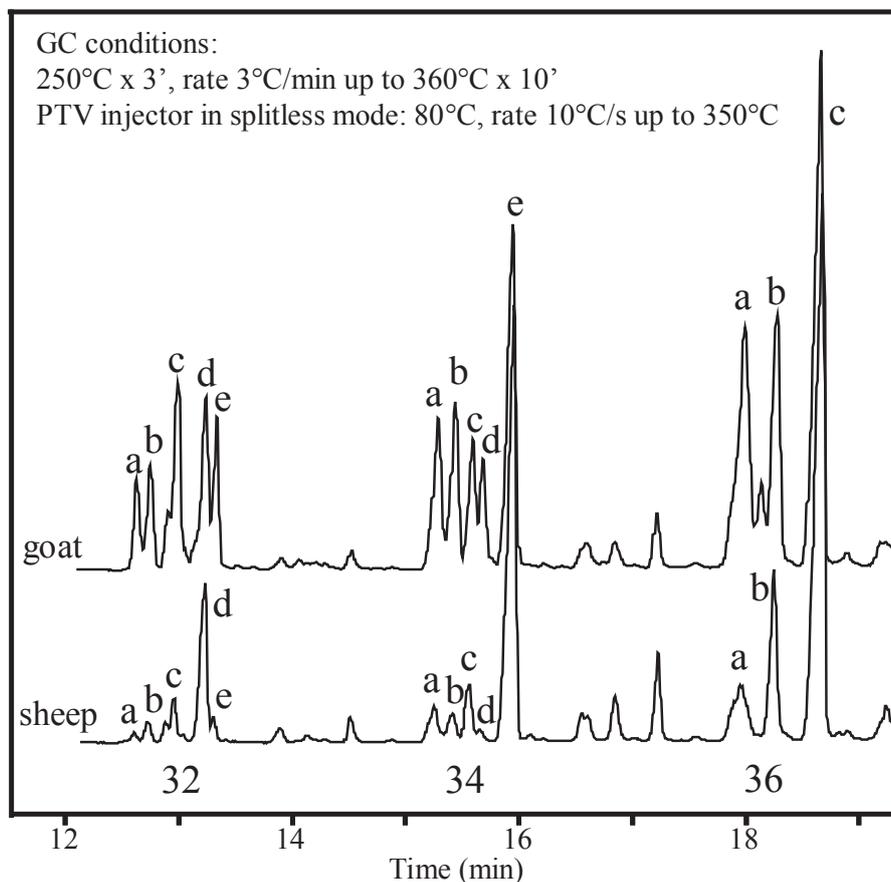


Figure 1. Enlarged view from CN 32 to CN 36 of the saturated fraction (peak letters: see Table 1).

4. Conclusion

The study of triglyceride composition of milkfat by separation into fractions by Ag⁺-SPE showed differences between goats and sheep milkfat. These results suggest that this analytical approach could help the set up of a method able to identify and characterize these milkfats.

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I-P076: An Accurate Determination of Amino Acids in Goat Milk

S.M. Rutherford¹, P.J. Moughan², M. Kruger¹, D. Lowry³, C.G. Prosser³

Summary

Multiple hydrolysis times were used to gain an accurate measure of the amino acid composition of goat milk. The analysis was conducted on a composite of three spray-dried goat whole milk powders produced by Dairy Goat Co-operative (N.Z.) Ltd at different times of the year. Isoleucine, cysteine, aspartic acid, proline and tryptophan were underestimated by more than 5%, if using a single 24 h hydrolysis showing that this analysis is not accurate for all amino acids in goat milk.

1. Introduction

The accurate analysis of the amino acid composition of proteins is important for determining the nutritional quality of foods, including milk. Amino acid analysis of milk usually employs a single 24h acid or base hydrolysis to liberate amino acids from milk proteins. However, not all amino acids are stable under these conditions and the peptide bonds between hydrophobic amino acids are difficult to break. Consequently, a single 24 h hydrolysis may under or over estimate some amino acids (Rutherford and Moughan, 2000). To overcome this we have used nonlinear least-squares extrapolation of multiple hydrolysis time curves (Darragh et al., 1998; Darragh and Moughan, 2005).

2. Materials and methods

A composite of three spray-dried goat whole milk powders produced by Dairy Goat Co-operative (N.Z.) Ltd were used. The powders were produced at three different times of the year to account for seasonal variation in amino acid composition. This powder was subjected to acid and base hydrolysis for 20 time points ranging from 0 to 170h (AOAC 1990). The concentration of the released amino acids, measured by HPLC and post-column ninhydrin derivatisation, was plotted against the hydrolysis time and the following equation used to fit curves to the plot for each amino acid.

$$B(t) = \frac{A_0h(e^{-lt}-e^{-ht}) + B_0(e^{-lt})}{h-l}$$

Where

- $B(t)$ is the amino acid concentration at each time point
- B_0 is the free amino acid concentration measured before hydrolysis
- h is the hydrolysis rate
- l is the loss rate
- A_0 is the actual protein bound amino acid content

3. Results and discussion

Good agreement between the multiple hydrolysis and the single 24 h hydrolysis method was obtained for approximately half of the amino acids (Table 1). However, isoleucine, cysteine, aspartic acid, proline and tryptophan were underestimated by more than 5%, if using a single 24 h hydrolysis. Taurine and glutamic acid were the most abundant free amino acids in goat milk.

¹ Institute of Food, Nutrition and Human Health, Massey University, New Zealand.

² Riddet Centre, Massey University, New Zealand.

³ Dairy Goat Co-operative (N.Z.) Ltd, Hamilton, New Zealand.

Table 1: Comparison of concentrations of amino acids in goat whole milk powder estimated by multiple time point hydrolysis compared with a single 24 h hydrolysis

Amino acid (mg/g protein)	Multiple time point hydrolysis		24 h hydrolysis	% difference 24 h vs multiple time point
	Free amino acids (Bo)	Total amino acids		
Tryptophan	0	16	14	-11.2
Proline	0	127	119	-6.4
Aspartic acid	0.24	85	80	-5.8
Cysteine	0.03	9	9	-5.4
Isoleucine	0.07	50	47	-5.4
Tyrosine	0.08	42	41	-4.2
Valine	0.19	75	72	-3.7
Threonine	0.09	52	50	-3.6
Alanine	0.27	33	32	-3.0
Serine	0.37	53	52	-1.9
Methionine	0	26	26	-0.4
Glutamic acid	1.39	200	202	0.8
Glycine	0.81	18	19	1.6
Leucine	0.09	95	96	1.9
Phenylalanine	0	51	53	2.7
Histidine	0	29	29	3.2
Lysine	0.38	80	84	4.5
Arginine	0.22	27	29	5.5
Taurine	3.3	N/A	N/A	

N/A not applicable

4. Conclusion

Amino acid analysis using a single time point hydrolysis (24 h) is not accurate for all amino acids, particularly tryptophan. This result highlights the importance of considering the amino acid analysis procedure when using the amino acid composition of milk proteins to assess their suitability for human nutrition. The present study provides highly accurate reference data on the gross amino acid composition of New Zealand goat milk whole milk powders.

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I-P077: Influence of Ovine β -casein Phenotype on Milk Production and Composition from Merino Ewes

S. Caio³, M. Izquierdo², J. Gonzalez¹, F.I. Hernández², J.M. Corral², L. Pinto³, J. Rodríguez¹, I. Roa¹

Summary

The aim of this study was to evaluate the different variants of β -casein and their relationship with milk composition in a population of Merino ewes from the research institute *La Orden-Valdesequera* in the South West of Spain. After lambing, milk samples were collected monthly to evaluate milk composition and one of the samples were use to determine the different β -casein variants. After isoelectric precipitation at pH 4,6, the casein fractions were individually submitted to IEF at pH 2,5-6,5, in polyacrilamide gels in the presence of urea. Three variants, Type 1, Type 2 and Type 5 were identified for β -casein in 2,96%, 96,45% and 0,59%, of the ewes studied, respectively. There was an influence of β -casein variants on milk composition. Significant differences ($P < 0,05$) were found in the percentage protein, dry matter and non-fatty solids. Type 1 showed to have higher percentages of protein, dry matter and non-fatty solids than Type 2.

1. Introduction

The possible effect that milk protein polymorphisms may have in milk production and composition is very interesting in animal production not only for the potential application in the technological properties of milk but also for the potential benefits in animal breeding [2].

There have been some research on milk protein polymorphisms in sheep, and some were based in the application of techniques like electrophoresis and isoelectric focusing [4][2]. In this study, by using electrophoretic the aim was to identify the β -casein variants present in the milk of a Merino ewes population, and to find any association between milk production and milk composition. The objective of studying the best phenotype to produce sheep milk will improve the cheese production.

2. Material and methods

A group of 176 ewes from a population of 742 Merino ewes from the research institute *La Orden-Valdesequera* in the South West of Spain was used for this study. After lambing, ewes were milked, and milk samples were collected monthly to evaluate milk composition (by using a MilkoScan; Foss Electric, Denmark) and to determine the different β -casein variants. The casein fractions were separated from the whole milk samples by isoelectric precipitation at pH 4,6 [3]. Then, every casein sample, were individually submitted to IEF at pH 2,5-6,5, in polyacrilamide gels (260 x 100 x 1mm) in the presence of urea. The 2,5 - 6,5 pH gradient of the gels was achieved by using three different carrier ampholytes: 2,5-5,0 (Pharmalyte), 4,5-5,4 (Pharmalyte) and 4,0-6,5 (Pharmalyte). IEF was performed in a horizontal electrophoresis apparatus (Pharmacia LKB, Multiphor II), with the following carrier conditions: 1200V, 16W and 15 mA and constant 12°C. There was a pre-focalisation of the gel in the first hour, than were applied 12 μ l of lyophilized sample of casein on the Whatman n° 1 paper (10 x 5 mm) near the anode. Focalization occurred during two more hours.

To test the relationship between the β -casein variants and milk composition, 2690 results from the composition samples collected in spring and fall lactations were obtained from these ewes between 1999 and 2006. The phenotype effect on milk composition was considered a fixed

¹ Departamento de Productos Lácteos. Instituto Tecnológico Agroalimentario, 06071 Badajoz, Spain.

² Centro de Investigación La Orden-Valdesequera, Junta de Extremadura. Carretera Nacional V, Km 374, Guadajira, 06071 Badajoz, Spain.

³ Escola Agrária de Castelo Branco do Instituto Politécnico de Castelo Branco, Quinta de Senhora de Mércules, Ap. 119, 6001-909 Castelo Branco, Portugal.

effect, and was analysed using a mixed model including the various composition results of the same lactation as repeated measures using PROC MIXED of SAS (SAS Institute, 1998). Were included in the model fixed effects like the season-year of lambing (15: since Autumn of 1999 until Spring of 2006), type of birth (2: one lamb or two lambs), milking hour (2: morning and afternoon), number of lambings (1 to 3) and phenotypes. Ewe and residual effects were included as random effects. The model was:

$$Y_{ijklmn} = \mu + X_i + T_j + P_k + NP_l + Phen_m + Ov_n + \epsilon_{ijklmn}$$

3. Results and discussion

Three different isoelectrophoretic patterns were observed. Those patterns corresponded to Type 1, Type 2 and Type 5 identified by Chianese et al. 1995, and, therefore, here, the nomenclature of these authors is maintained. Type 1 was found in 2,96%, Type 2 in 96,45% and Type 5 was found only in 0,59%, of the ewes studied. When analysing the statistical result to know the β -casein variants influence in milk composition, significant differences ($P < 0,05$) were found in the percentage protein, dry matter and non-fatty solids, due to the differences in the β -casein variants. Type 1 showed to have higher percentages of protein, dry matter and non-fatty solids than Type 2. Type 5 was excluded from the statistical analyses because it was only found in one sheep (Table 1).

These results cannot be compared with others authors because, until now, no research in this area has been done. In fact there are some doubts on the origin of β -casein variants. Factors like stage of lactation, health, age of individuals, and an altered phosphate availability may change the level of phosphorylation of β -casein, and, therefore, determine different variants [4]. According to this theory, the presence of any β -casein variant would be explained, not by genetic factors, but by factors that also affect milk composition during a female life. Furthermore, new studies of β -casein variants will indicate whether associations between β -casein variants and production traits will be suitable for performance improvement.

Table 1: Least square means (LS Means) and standard error (SE) for fat, protein, lactose, dry matter and non-fatty solids of ovine β -casein variants

		β -Casein	
		Type 1	Type 2
Fat	n	56	2634
	LSMeans \pm SE (%)	8,14 \pm 0,27 ^a	7,74 \pm 0,07 ^a
Protein	n	56	2634
	LSMeans \pm SE (%)	6,86 \pm 0,25 ^a	6,42 \pm 0,06 ^a
Lactose	n	40	2184
	LSMeans \pm SE (%)	4,40 \pm 0,09 ^a	4,41 \pm 0,02 ^a
Dry matter	n	56	2634
	LSMeans \pm SE (%)	12,31 \pm 0,17 ^a	11,80 \pm 0,04 ^b
Non-fatty solids	n	56	2634
	LSMeans \pm SE (%)	20,17 \pm 0,35 ^a	19,29 \pm 0,09 ^b

n: number of samples;

^{a,b} different letters indicate significant differences to $P < 0,05$.

4. Conclusion

We believe that the research on this protein must continue, to understand if these protein variants are really genetically determined or if they are products of posterior reactions at Golgi apparatus [5] and influenced by the nutrient intake to mammary gland. It is very important to understand how protein polymorphism affect sheep milk production and composition, and how we can use it to produce better and more suitable milks for cheese production.

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I-P080: Comparison of Udder Health and Cell Count Pattern in Swiss Goats and Milking Ewes

S. Ryffel¹, J. Maurer¹, W. Schaeren¹

Summary

In two comprehensive studies, covering full lactation periods of 136 goats and 105 milking ewes each of three different herds, we evaluated the relationship between the results of the California Mastitis Test (CMT), the somatic cell counts (SCC) and the bacteriological status in udder halve foremilk samples for goats and milking ewes.

On the one hand, for ewe's milk, we found a good correlation between the three parameters, similar to cow's milk samples. On the other hand, even though the CMT and SCC results correlate well for goat's milk too, the bacteriological status of goats can hardly be predicted just on the basis SCC results.

1. Introduction

Direct and indirect methods to assess the cell counts in milk are appropriate means to monitor the udder health of individual animals and the quality of milk supplied. For cow's milk, the legal threshold limit is set at 350'000 cells/ml in Switzerland. However the interpretation of cell counts and the results of the California Mastitis Test (CMT) may be a problem in the case of small ruminants, particularly goats. Therefore no comparable limit values of cell counts for goat's or ewe's milk have yet been established.

2. Animals, material and methods

A total of 2152 and 1624 udder halve foremilk samples were taken at monthly intervals over a full lactation period from 136 goats and 105 milking ewes each on three different farms. The udder health status was assessed at the farms by the California Mastitis Test (CMT). The enumeration of somatic cells (SCC) was done in the laboratory by optoelectronic fluorescent detection with a Fossomatic 5000. All milk samples were further analysed bacteriologically according to the guidelines of the National Mastitis Council.

3. Results

The correlation between the CMT scores and the SCC was high for both species. The geometric means of SCC for CMT negative samples (neg) were 60'000 cells/ml for ewes and 95'000 cells/ml for goats. Samples showing distinctively positive reactions (+ to ++++) had SCC of 900'000 to 10 millions cells/ml for ewes and 500'000 to 7 millions cells/ml for goats (fig. 1).

However, the relationship between cell counts and bacteriological results was different for both species: Cell counts in about 95% of ewe's milk samples from uninfected udder halves were \leq 350'000 cells/ml, whereas only 42% of goat milk samples from uninfected udder were below this limit. Therefore, 25% of the foremilk samples from uninfected goats showed a positive CMT score (+ to ++++) and more than 20% of udder halves infected by coagulase negative staphylococci (CNS) were tested CMT negative (neg). In contrast, we found a good correlation between CMT results and udder infections for ewes (fig. 2).

The only exceptions were infections with *Staphylococcus aureus*: All samples from udder halves infected with *S. aureus* showed a strongly positive CMT reaction (++ or ++++).

¹ Agroscope Liebefeld-Posieux Research Station ALP, Schwarzenburgstrasse 161, CH-3003 Bern, Corresponding author: Tel.: +4131 323 82 22; fax.: +4131 323 82 27 stephan.ryffel@alp.admin.ch

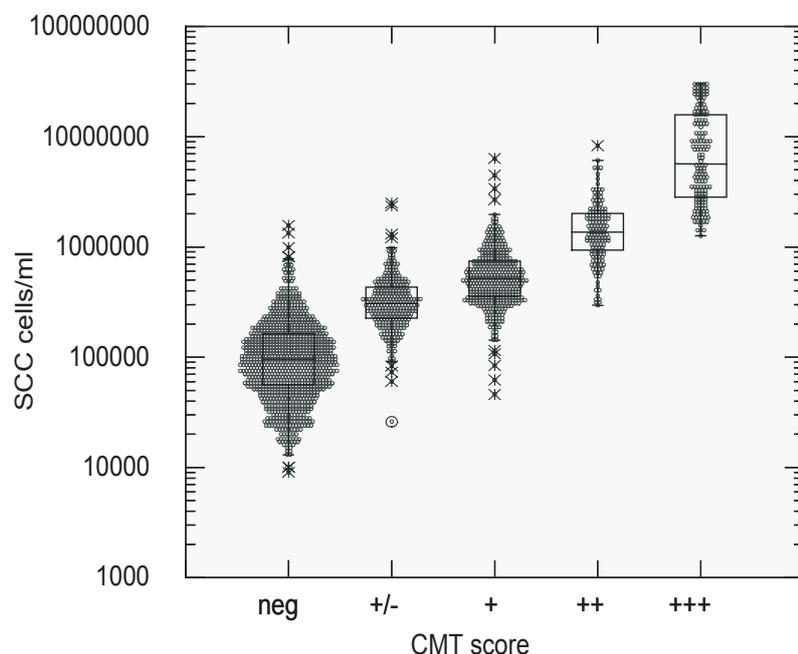


Figure 1. Comparison between CMT scores and SCC in foremilk samples from goats.

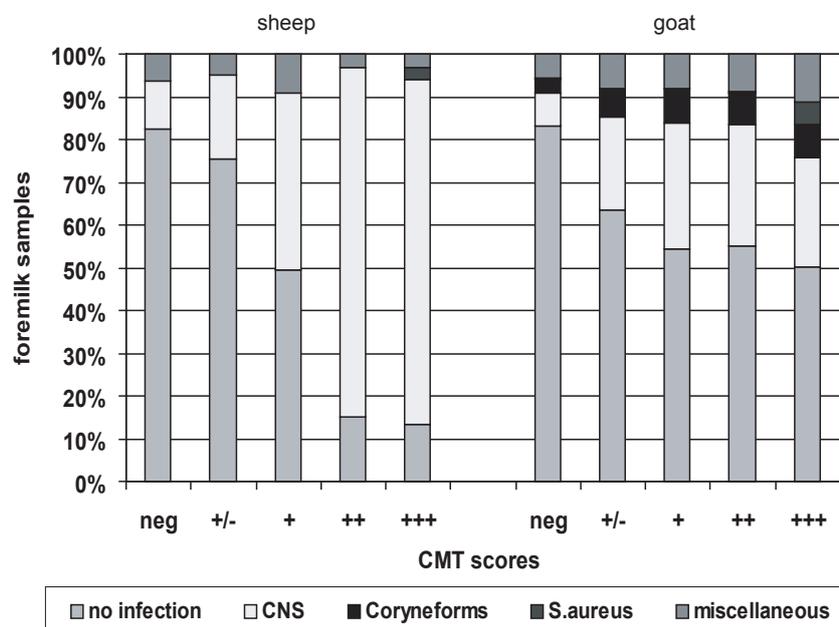


Figure 2. Comparison between CMT scores and udder infections for ewes.

4. Conclusions

The results showed a close relationship between the CMT reaction scores, the SCC and bacteriological infections for ewe’s milk. Therefore, a cell count limit of 500’000 cells per ml for milk from healthy ewes can be suggested. As for goat’s milk this relationship seems not to be very close and the diagnostic reliability for udder infection of the CMT is very poor, it would be difficult to argue for the introduction of a non-compliance cell count limit below 1’000’000 cells per ml.

I-P081: Induced Lactation in Nulliparous Dairy Goats During Winter

A.A.K. Salama¹, G. Caja¹, X. Such¹, E. Albanell¹, S. Carné¹, R. Casals¹

Summary

Lactation was induced in 14 nulliparous goats by s.c. injections of estradiol-17 β and progesterone for 7 d. Goats were divided into 2 groups and i.m. injected with reserpine (RES; n=7) or vehicle (CON; n=7) on d 12, 14, 16, 18 and 20. Dexamethasone was injected on d 18 to 20. Goats were milked once daily from d 21 to 120, at which goats were mated jointly with herdmates. Goats initiated lactation on d 21 (100%) and difference in milk yield between CON and RES increased as lactation advanced. Milk of CON contained greater non protein nitrogen than RES. Distance between teats, and volume and depth of the udder increased similarly in both groups during mammogenesis and lactation. The 82% of herdmates became pregnant, whereas only 21% of the experimental goats conceived. In conclusion, reserpine improved milk yield, but neither the milk production nor the side effects on fertility support its recommendation.

1. Introduction

Smith and Schanbacher (1973) proposed a protocol in which lactation was induced in dairy cows by a 7-d estradiol and progesterone treatment. Compared to what occurs during pregnancy, mammary growth during lactation induction is considered insufficient and varies widely between animals.

Induced lactation has received little attention in dairy goats (Hart and Morant, 1980; Chilliard et al., 1986) compared to dairy cows and ewes. First attempts to induce lactation in dairy goats (Hart and Morant, 1980) used long time steroid priming (35 to 140 d), which is unpractical and may be uneconomic.

The aim of this study was to evaluate milk yield, milk composition, and udder morphology changes in nulliparous dairy goats induced to lactate with or without reserpine treatment.

2. Material and methods

Animals and Treatments

A total of 14 nulliparous Murciano-Granadina goats (17 months of age and 38 kg BW) from the herd of the Universitat Autònoma de Barcelona were allocated in 2 balanced experimental groups. Estrus was synchronized (February 1st) using 40 mg fluorprogesterone vaginal sponges (Chronolone, Intervet, Salamanca, Spain) for 14 d. Seven d after sponges withdrawal, all goats received daily s.c. injections of estradiol-17 β (0.5 mg/kg BW) and progesterone (1.25 mg/kg BW) in 2 portions at 8 and 18 h on d 1 to 7. Goats were i.m. injected with 1 mg/d reserpine (**RES**; n = 7) or vehicle as controls (**CON**; n = 7) at 900 h on d 12, 14, 16, 18 and 20. Lactation in all goats was triggered by dexamethasone (10 mg/d) injected s.c. at 900 h on d 18 to 20, and machine milking was initiated on d 21 and lasted for 14 wk (d 21 to 120).

Samples, Analyses, and Measurements

Milk yield was recorded until wk 14 of lactation. Milk composition was evaluated daily during the wk 1, weekly from wk 2 to 8, and biweekly from wk 10 to 14. Milk samples were analyzed with a near infra-red spectrometer (FOSS NIRSystems 5000, Hillerød, Denmark) according to Albanell et al. (2003).

Udder morphology traits were measured 2 d before the hormonal treatment, and 7 h after milking, at wk 2 and 12 of induced lactation (d 35 and 105, respectively) as described by Peris et al. (1999). Data were analyzed by the PROC MIXED for repeated measurements of SAS. The

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

statistical model contained the random effect of the animal within the treatment; the fixed effects of treatment and week of lactation; the interaction between treatment and week of lactation; and the residual error.

3. Results and discussion

Data of milk yield are shown in Figure 1. Daily milk yield did not vary ($P = 0.253$) between CON (659 ml/d) and RES (793 ml/d) goats from wk 1 to 7 of lactation.

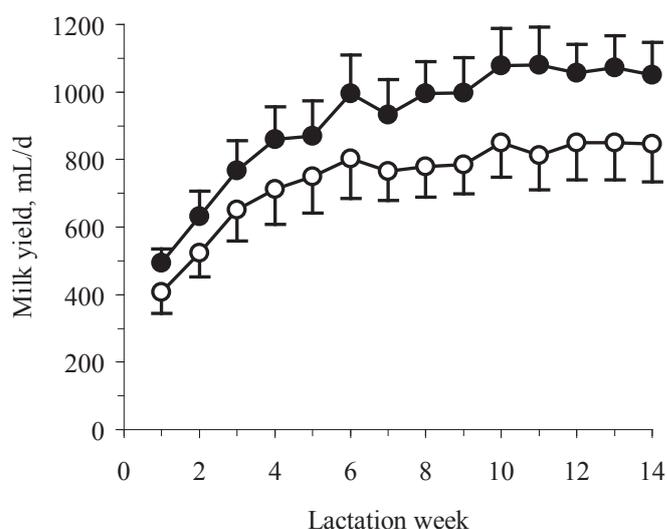


Figure 1. Milk yield of control (○) and reserpine treated (●) nulliparous dairy goats.

However, the difference between groups increased as lactation advanced, and from wk 8 to 14 RES goats (1,052 ml/d) produced 28% greater milk ($P < 0.10$) than CON goats (825 ml/d). Peak milk yield in both groups was at wk 10 of lactation and differed ($P = 0.084$) between CON (850 ml/d) and RES (1,079 ml/d) goats.

Using milk yield at peak ≥ 0.5 L/d as an arbitrary criterion of lactation induction success, 100% of goats were successfully induced to lactate. When this criterion was increased to ≥ 1 L/d, success rate was 29 and 71% in CON and RES goats, respectively. Collier et al. (1977) reported that success rate was 55 and 100% in control and reserpine-treated cows, respectively.

Peak milk yield values in CON and RES goats were 49 and 60% of peak milk yield values obtained after kidding in primiparous goats milked once daily from the same breed and herd (Salama et al., 2003). Similarly, multiparous goats induced into lactation by Chilliard et al. (1986) produced 55% of the milk during natural lactations.

Milk composition on the first day of lactation was similar between CON and RES goats and averaged: 17.0% total solids, 5.66% fat, 6.61% protein, 3.96% casein (60% milk protein), 2.62% whey protein, and 0.33% non protein nitrogen. Milk fat, protein, casein and total solids percentages in both groups were high during the first 2 d, decreased by d 3 to standard milk values and remained constant thereafter.

Throughout the experimental period, milk composition was similar between CON and RES goats (Table 1). However, milk of CON goats contained greater ($P < 0.05$) non protein nitrogen than milk of RES goats.

At wk 2 of induced lactation (d 35) there were no differences between CON and RES groups for volume and depth of the udder, which averaged 768 ml and 14.2 cm, respectively. Despite milk yield differences ($P = 0.107$) between CON (850 ml/d) and RES (1,057 ml/d) goats at wk 12, no differences were detected in udder volume or udder depth. Thus, increased milk yield in RES goats seems to be due to increased mammary cell differentiation rather than to mammary growth.

Of the 14 experimental goats, only 3 (1 RES and 2 CON goats) became pregnant after mating (fertility, 21.4%). The low fertility observed was not attributed to the buck utilized for inseminating the experimental goats because the same buck had also mated 17 herdmates; 14 of them kidded (fertility, 82.4%). This result indicates a negative side-effect of the hormonal treatment used on the reproduction of nulliparous goats which was probably related to the high level of estrogens used.

Table 1: Milk composition in dairy goats induced to lactate with or without reserpine

Component, %	Control	Reserpine	SD
Total solids	13.9	13.6	0.25
Fat	4.62	4.36	0.24
Protein	4.29	4.21	0.16
Casein	2.83	2.74	0.09
Whey protein	1.46	1.47	0.08
Non protein N	0.48 ^a	0.41 ^b	0.02

^{a,b} Means with different superscripts differ ($P < 0.05$).

4. Conclusions

The 7-d hormonal protocol was efficient to induce lactation in nulliparous goats and milk yield was improved by using reserpine as a prolactin stimulator. Nevertheless, the obtained milk yield was only one half of that obtained after normal kidding in primiparous goats indicating that induction protocol needs to be improved. Moreover, the reduced fertility observed after the treatment suggests the need for a substantial reduction of estrogen doses. This technique is not recommended for dairy goat in practice.

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I-P082: Milk Yield and Milk Composition During Normal or Induced Lactation in Dairy Ewes

B. Ramirez-Andrade¹, A.A.K. Salama², G. Caja², E. Albanell², X. Such²

Summary

Eighteen Lacaune dairy ewes were used to evaluate milk yield and milk composition produced during natural (N) or hormonally induced (I) lactation. Nine ewes were mated and lambd in January. The other 9 ewes were estrus synchronized and hormonally induced to lactate in January by using steroids and corticoids for 20 d. Machine milking ($\times 2$ daily) was initiated after lambs weaning (wk 4) in N or after the hormonal treatment (d 21) in I ewes, and lasted for 13 wk. Milk yield was 62% lower in I than in N (0.90 vs. 2.37 L/d; $P < 0.001$), but milk composition did not differ. However, casein content tended to be lower in I than in N (3.2 vs. 3.4%; $P < 0.10$). In conclusion, artificially induced ewes were able to produce normal milk although in a lesser amount (38%) than naturally lactated ewes. These results indicate that mammary cell proliferation was incomplete during induction.

1. Introduction

Compared to pregnancy, mammary growth during artificially induced lactation is smaller and varies widely between animals. Therefore, induced lactation has variable success rate (58 to 80%) and milk yield (25 to 106% of natural lactation) in cows (Smith and Schanbacher, 1973), goats (Chilliard et al., 1986; Fowler et al., 1991) and ewes.

Most studies on lactation induction in ewes were carried out in Prealpes du Sud ewes (Head et al., 1980; Kann, 1997; Kann et al., 1999), which is not a high yielding dairy breed. Researchers tried to improve the response of induced ewes by the inclusion of placental lactogen (Kann et al., 1999) or somatotropina (Kann, 1997).

There are no studies comparing milk composition of dairy ewes artificially or naturally induced to lactate. The aim of this work is to study the performance of Lacaune dairy ewes natural (**N**) or hormonally induced (**I**) lactation.

2. Material and methods

Animals and Treatments

A total of 18 Lacaune dairy ewes (10 nulli- and 8 multi-parous) from the experimental farm of the Universitat Autònoma de Barcelona in Bellaterra, Spain (41° 30' North and 2° 5' East) were used. Half of the ewes (N group: 5 nulli- and 4 multi-parous) were submitted to ram effect and mated in August. The N ewes lambd and suckled their lambs (4 wk) in January. The other half (I group: 5 nulli- and 4 multi-parous) were also induced to lactate in January. The I ewes were estrus synchronized with 40 mg fluoroprogestosterone vaginal sponges for 12 d and 400 IU PMSG (Chronolone, Intervet, Salamanca, Spain). Five d after sponge withdrawal (d 0), the I ewes received s.c. injections of estradiol-17 β (0.5 mg/kg BW) and progesterone (1.25 mg/kg BW) in 2 portions at 8 and 18 h (d 1 to 7). Lactation in I ewes was triggered by hydrocortisone acetate (50 mg/d) injected s.c. during 3 d (d 18 to 20). Machine milking (800 and 1700 h) was initiated after the weaning of the lambs (wk 4 after lambing) in N ewes and on d 21 in I ewes.

Samples, Analyses, and Measurements

Milk yield was recorded weekly from the start of milking until wk 13. Milk composition was evaluated daily during the wk 1, and then on wk 2, 4, 7, 10, and 13. Milk samples were analyzed with a near infra-red spectrometer (FOSS NIRSystems 5000, Hillerød, Denmark) according to Albanell et al. (1999). Data were analyzed by the PROC MIXED for repeated measurements of

¹ Facultad de Agronomía, Universidad Autónoma de San Luis Potosí, México.

² Grup de Recerca en Remugants. Facultad de Veterinaria. Universidad Autónoma de Barcelona. 08193 Bellaterra, Barcelona, Spain.

SAS. The statistical model contained the random effect of the animal within the treatment; the fixed effects of treatment (N vs. I), parity, and experimental week; the interactions treatment \times week and treatment \times parity; and, the residual error.

3. Results and discussion

Data of milk yield are shown in Figure 1. Using milk yield at peak ≥ 0.5 L/d as an arbitrary criterion of lactation induction success, induction success was 100%. However, throughout the 13-wk experimental period I ewes (0.9 L/d) produced only 38% ($P < 0.001$) of milk produced by N ewes (2.37 L/d). This percentage falls within the range of 25 to 50% reported by Head et al. (1980) in Prealpes du Sud. Milk yield decreased normally from 2.81 to 1.93 L/d from wk 1 to 13 in N ewes.

Milk yield in I ewes was 0.52 L/d at wk 1 and then increased gradually, peaking at wk 8 (1.10 L/d). The delayed peak during I induced lactation could be partially due to continued mammary proliferation after the start of lactation. Similarly, peak milk yield during induced lactation was at wk 9 to 11 in dairy cows (Collier et al., 1975), wk 7 in ewes (Head et al., 1980) and wk 9 in dairy goats (Fowler et al., 1991). There were no differences in milk yield between nulli- (0.94 L/d) and multi-parous (0.86 L/d) ewes in the I group. Nevertheless, multiparous ewes (2.74 L/d) produced greater ($P < 0.01$) milk than primiparous ewes (2.00 L/d) in the N group.

Milk on the first day of lactation in I ewes contained 16.5% total solids, 4.02% fat, 8.52% protein, 3.85% casein, 4.66% whey protein, and 0.48% NPN. By d 3, these components reached standard milk values and remained constant thereafter. Throughout the experimental period, milk composition was similar between I and N ewes (Table 1). However, casein content tended to be lesser in I than in N (3.17 vs 3.36%; $P = 0.083$), which corresponded to 64 and 70% of milk protein, and indicate that casein synthesis was impaired in I ewes. Despite this negative effect, I ewes were able to produce normal milk although in a lesser amount than naturally lactated ewes. These results could indicate that mammary cell proliferation, rather than mammary cell differentiation, was incomplete during the hormonal treatment used, and the induction protocol should be improved. Short-day photoperiod during the induction might also have had a negative effect due to reduced prolactin secretion.

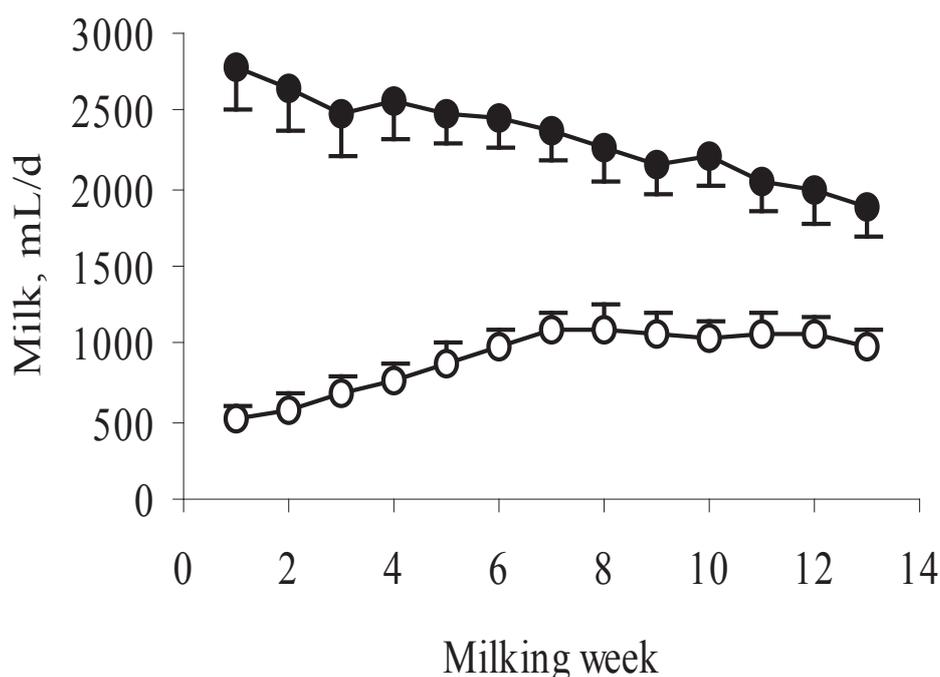


Figure 1. Milk yield in dairy ewes naturally (●) or artificially (○) induced to lactate.

Table 1: Milk composition in naturally or artificially induced to lactate dairy ewes.

Component, %	Lactation		SD
	Natural	Induced	
Total solids	15.8	15.7	0.34
Fat	6.05	5.99	0.26
Protein	4.82	4.94	0.12
Casein	3.36 ^a	3.17 ^b	0.11
Non protein N	0.19	0.20	0.03

^{a,b} Means with different superscripts differ ($P < 0.10$).

4. Conclusions

Dairy ewes artificially induced to lactate only produced 38% of milk produced by naturally lactating ewes, although milk composition was normal. Impaired milk yield seems to be a consequence of a reduced cell proliferation during mammogenesis.

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I-P083: Detection of Enterotoxins and Virulence Genes in *Staphylococcus Aureus* Isolated from Goat's Bulk Milk

C. Scarano¹, S. Viridis¹, A.L. Pilo¹, P. Marongiu¹, E.P.L. De Santis¹, M. Bes²

Summary

Thirty *S. aureus* strains isolated from goat's bulk milk were analysed by multiplex PCR for the following genes: *gyrA* (gyrase); *agr* I-IV alleles (accessory gene regulator); *sea-e*, *seh*, *sek*, *sel*, *sem*, *seo* (enterotoxins genes); *tst* (TSST-1); *eta*, *etb*, *etd* (exfoliatins); *lukF-PV* and *lukS-PV* (Panton-Valentine leukocidin); *lukE* and *lukD* (lukE-lukD leukocidin); *lukM* (lukM leukocidin); *hlg*, *hlgv* and *hly* (γ , γ variant and β hemolysins); *edin* (epidermal cell differentiation inhibitor); *mecA* (methicillin resistance determinant). SEA-E production was also tested using ELISA method. Genes *agr* III, *sec*, *sel*, *tst*, *LukE-lukD*, *lukM*, *hlgv* and *hly* were found in majority of the strains, while *agr* IV, *seb*, *seh*, *mecA*, *eta*, *etb*, *etd*, *lukF-PV* and *lukS-PV* were not found. Ten different profiles on the bases of the association of *agr* alleles, SEs and virulence genes were observed.

1. Introduction

S. aureus is one of the most important pathogens for the dairy chain and affects animal health and food safety. In large and small ruminants it is well known as a mastitis causing agent [3]. Staphylococcal enterotoxins (SEs) may also contaminate milk and milk products and cause foodborne diseases [1]. *S. aureus* virulence factors profiles are needed to understand their epidemiological implications, but little data are available on the strains isolated from sheep and goat [8]. *S. aureus* has a wide pattern of virulence factors including hemolysins, nucleases, proteases, lipases, hyaluronidase and collagenase. Some strains produce other specific factor such as toxic shock syndrome toxin-1 (TSST-1), SEs, exfoliative toxins, leukocidins, epidermal cell differentiation inhibitor (EDIN) and methicillin resistance (*mecA*) [3]. The purpose of the paper is to perform an in depth characterization of virulence factor profile of *S. aureus* isolated from goat's bulk milk.

2. Material and methods

Thirty *S. aureus* strains were isolated from goat's bulk tank milk samples, each collected from different farms in Sardinia [7]. All strains were identified on the basis of conventional phenotypic characters: Gram staining, cell and colony morphology, catalase and oxidase activity, coagulase production in rabbit plasma (bioMerieux, France), production of clumping factor (Staphylect Plus, Oxoid, England) and thermonuclease. The isolates were identified by means of ID 32 Staph (bioMerieux, France). SEA-E production was tested by ELISA (r-Biofarm), according to manufacturing instruction. All strains were analysed by multiplex PCR for staphylococcal enterotoxins (SEs) and genes encoding virulence factors [4]. Sequences specific for accessory gene regulator allele (*agr* I-IV), SE genes (*sea*, *seb*, *sec*, *sed*, *see*, *seh*, *sek*, *sel*, *sem* and *seo*), TSST-1 (*tst*), haemolysin γ (*hlg* and *hlgv*), haemolysin β (*hly*), leukocidins LukE-LukD (*lukE* and *lukD*), Panton-Valentine leukocidin (*lukF-PV* and *lukS-PV*), leukocidin LukM (*lukM*), exfoliative toxin genes (*eta*, *etb*, *etd*), epidermal cell differentiation inhibitor gene (*edin*), methicillin resistance determinant (*mecA*) were detected by PCR as previously described [4,9]. Amplification of *gyrA* was used to confirm the quality of each DNA extract and the absence of PCR inhibitor.

3. Results and discussion

All thirty strains belonging to *S. aureus* on the basis of the ID 32 STAPH identification and the detection of *gyrA*. The isolates were set into 3 of the 4 allelic *agr* groups. In this respect, *agr* I was found in 11 (36.6%) strains, *agr* II in one (3.3%) strain and *agr* III in 18 (60%) strains.

¹ Dipartimento di Biologia Animale, Università di Sassari, 07100 Sassari, Italia.

² National Reference Centre for Staphylococcal Toxaemia, Faculté de Médecine Laennec, Lyon, France.

Hemolysins genes *hlg* and *hlgv* were detected in 27 (90%) strains while in the other isolates were founded respectively *hlg* and *hly*, *hlgv*, and *hlg*. In *S. aureus* strains were also detected *edin* in 17 (56.6%) strains, *luk-E* and *luk-D* in 25 (83.3%) strains, and *lukM* in 14 (46.6%) strains. In none of the strains exfoliatins genes, Pantone-Valentine leukocidin genes and methicillin resistance gene were detected. Fourteen strains harboured either one or more SEs genes. Genes *sec*, *sel* and *tst* were detected in 11 strains (36.6%). Other genes associations were respectively found only in one strain (table 1). SEs genes *seb* and *seh* were not found. The results of the ELISA test and PCR analysis were in agreement in thirteen out of fourteen enterotoxigenic strains. In one strain the ELISA test showed SEC production but the PCR test was unable to detect the *sec* gene. One strain showed the *sed* gene but no SED production was detected by means of the ELISA test. Ten different profiles (T1-T10) on the bases of the association of *agr* alleles, SEs and virulence genes were observed.

Table 1: Pathogenic profiles and accessory genes regulator allele of *S. aureus* isolated from goat's bulk milk

profiles	<i>agr</i> group	enterotoxins	TSST-1	exfoliatins	leukocidins	hemolysins	Edin factor	mecA	n.
T1	<i>agr</i> III	-	-	-	-	<i>hlgv</i> , <i>hly</i>	<i>edinABC</i>	-	4
T2	<i>agr</i> III	<i>sec</i> , <i>sel</i>	<i>tst</i>	-	<i>lukD-E</i> , <i>lukM</i>	<i>hlgv</i> , <i>hly</i>	<i>edinABC</i>	-	10
T3	<i>agr</i> III	-	-	-	<i>lukD-E</i> , <i>lukM</i> ,	<i>hlgv</i> , <i>hly</i>	<i>edinABC</i>	-	2
T4	<i>agr</i> III	<i>sed</i>	-	-	<i>lukD-E</i> , <i>lukM</i> ,	<i>hlgv</i> , <i>hly</i>	<i>edinABC</i>	-	1
T5	<i>agr</i> III	<i>sea</i> , <i>see</i> , <i>sek</i>	<i>tst</i>	-	<i>lukD-E</i> ,	<i>hlgv</i>	-	-	1
T6	<i>agr</i> I	<i>sec</i> , <i>sel</i> , <i>sem</i> , <i>seo</i>	-	-	<i>lukD-E</i>	<i>hlg</i>	-	-	1
T7	<i>agr</i> I	-	-	-	<i>lukD-E</i> ,	<i>hlgv</i> , <i>hly</i>	-	-	8
T8	<i>agr</i> I	-	-	-	-	<i>hlg</i> , <i>hly</i>	-	-	1
T9	<i>agr</i> I	<i>sec</i> , <i>sel</i>	<i>tst</i>	-	<i>lukD-E</i> , <i>lukM</i>	<i>hlgv</i> , <i>hly</i>	-	-	1
T10	<i>agr</i> II	-	-	-	<i>lukD-E</i>	<i>hlgv</i> , <i>hly</i>	-	-	1

4. Conclusion

All strains were identified as *S. aureus* according to the phenotypic and genotypic characteristics. Almost all the strains showed *hly* and *hlgv* genes. *hly* finding gives evidence for the animal origin of the strains [3]. The *agr* locus is characterized by a polymorphism which results in 4 major *agr* groups [4]. Group *agr* III was the most prevalent (60%). Eight (26.6%) of the strains harboured *lukM*. In previous works *lukM* was detected in two-thirds of caprine strains [6]. *lukM* findings in sheep isolates ranged between 25% to approximately 100% of the strains while it was detected only in 35% of bovine strains [3,6]. Exfoliatin genes were not found according to the results of other authors, which were unable to find them at all in cow mastitis strains or, if they did, at level not higher than 1% [3]. None of strains harboured Pantone-Valentine leukocidin genes, which agrees with the other authors [6]. Twelve (40%) strains harboured *sec* and *sel*, *sec* and SEC production is a common findings in *S. aureus* isolates from sheep and goat [3]. The association between *sec*, *sel* and *tst* was found in eleven strains and it was also previously detected in cattle and human strains. Indeed *sec*, *sel* and *tst* were localised in the same pathogenic island [5]. The strains were placed in ten profiles depending on the arrangement of virulence factors.

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I-P084: Enzymatic Activity of Some Ewe's Milk Fat Globule Membrane Proteins: Preliminary Studies

M. Martini¹, C. Scolozzi¹, F. Salari¹, R. Pesi², M.G. Tozzi²

Summary

It is well known that milk fat, in lactating animals, is secreted by the mammary gland as myriads of lipid droplets of a size ranging from 0,1 to 15 μm encircled by a membrane consisting of a lipid bilayer and proteins (MFGM). Some of these proteins are enzymes, whose role has not yet been completely understood. Individual milk samples from 14 pluriparous Massese ewes were evaluated for qualitative analysis, morphometric characteristic of the fat globules (n°/ml, diameter) and the enzymatic activity of some of the membrane proteins. A highly significant negative correlation was found between the activity of *xanthine oxidase* (XO) and *xanthine dehydrogenase* (XDH) and fat globule size, between γ -glutamyl transpeptidase (γ -GT) and XO and XDH, between γ -GT and the total percentage of milk protein, and between γ -GT and somatic cells content. In addition, a positive correlation between *alkaline phosphatase* (AP) activity and the number of globule/ml and medium milk fat globules (3-6 μm) was observed.

1. Introduction

In recent years there has been a growing interest in the factors that may help to promote good health and the possible role of novel ingredients and a potential nutraceutical attributed to the membranes of fat globules in milk (MFGM) (Correding et al., 2003; Singh, 2005; Spitsberg, 2005; Harrison, 2006; Fong et al., 2007). Among the MFGM proteins, some, such as xanthine oxidase, show an enzymatic activity whose role has not yet been completely understood. Various hypotheses have been put forward, such as the natural antimicrobial agent of milk thanks to its capacity to form reactive species. In addition the role of xanthine oxidase, has been suggested as a hypothesis, together with other proteins of the apical membrane of the mammary gland, in the process of secreting the fat globules not as enzymes, but as proteins (Mather and Keenan, 1998; Michalski et al., 2001; Vorbach et al., 2002; Heid and Keenan, 2005; Harrison, 2006).

The aim of this study was to research and measure the activity of some enzyme of the fat globule membranes from sheep's milk and to define the relationship between the morphometric characteristics of the fat globules and various parameters relating to the quality of the milk.

2. Material and methods

The trial was carried out on 14 pluriparous Massese ewes reared in the same herd and homogeneous in terms of lactation phase and diet. Individual milk samples were analyzed for fat, protein, lactose and dry matter content by infrared analysis (Milkoscan, Italian Foss Electric), and somatic cell count (SCC) (Fossomatic). For each milk sample, a morphometric analysis of the fat globules (number of milk fat globules/ml and diameter) was performed using the methods of Scolozzi et al. (2003). A frequency distribution of the total number of measured milk fat globules was evaluated according to size. They were divided into three categories of fat globules: small globules (SG) with diameter <2 μm , medium-sized globules (MG) with a diameter of 2-5 μm and large globules (LG). The enzymatic activity of the membrane, isolated from fresh milk according to the partially modified method of Patton and Huston (1986) were evaluated. Statistical analysis was performed using the SAS Institute (2002) JMP software, vers. 5.0 per PCs.

3. Results and discussion

The statistical analysis showed negative correlations ($P < 0.05$) between the total percentage of milk protein and γ -GT activity, and between the latter and the somatic cell content. According

¹ Dipartimento di Produzioni Animali, Università di Pisa, Viale delle Piagge 2, 56124, Pisa Italy.

² Dipartimento di Biologia, Università di Pisa, Via S. Zeno 51, 56127, Pisa Italy.

to Johnston et al. (2004), the γ -GT activity in the mammary glands of lactating ruminants plays an important role in the protein synthesis of milk.

Wiking et al (2004) revealed an association between γ -GT activity and the size of the fat globules in cow's milk. They proposed that this protein could be used as a marker for the amount of membrane material. Alkaline phosphatase showed significant and positive correlations ($P < 0.01$) between the number of globules per millilitre of milk and medium globules MG ($P < 0.05$); the latter is an enzyme whose activity is used as a marker for the pasteurization of milk (Harding and Garry, 2005). The correlations that we observed between the number of fat globules and the size below $5\mu\text{m}$ may be further proof in this regard. Xanthine oxidase activity showed a significant negative correlation between the mean diameter of the globules and the large globules (LG), and positive between the small globules (SG); xanthine dehydrogenase activity showed the same trend but with a larger significance ($P < 0.01$).

Regarding the xanthine oxidase and xanthine dehydrogenase activities, as has already been mentioned, they are carried out by the same protein and are active in two different forms. Both were found to be negatively correlated between the mean diameters of the fat globules and the globules larger than $5\mu\text{m}$, and positively between the smaller fat globules. This result suggests that there may be a relation between the enzyme and the surface of the fat globules since, as is well known, the smaller fat globules have a relatively greater quantity of membrane material than the larger fat globules. Xanthine oxidase is in fact the second protein, after butyrophilin, that is most represented in the MFGM (Spitzberg et al., 2005), and with the latter would seem to form a complex that is involved in envelopping the membrane around the lipidic droplet during secretion by the alveolar mammary cells (Mc Manaman et al., 2003; Vorbach et al., 2002; Heid and Keenan, 2005).

Table 1: Correlation between enzymatic activity and various milk quality parameters

	X.O.	X.DH.	γ -GT	A.P.	5'-N
Milk					
Fat	-0.266	-0.257	0.186	0.297	-0.215
Protein	0.222	0.336	-0.577*	0.480	0.132
Lactose	0.121	-0.044	0.263	-0.023	0.063
Dray Matter	-0.172	-0.151	0.040	0.477	-0.162
Non Fat Dry Matter	0.360	0.392	-0.509	0.511	0.219
CCS	0.054	0.120	-0.581*	-0.262	-0.343

Table 2: Correlation between enzymatic activity and morphometric characteristics of milk fat globules

	X.O.	X.DH.	γ -GT	A.P.	5'-N
Globules					
N°/ml	-0.288	-0.124	0.050	0.721**	-0.034
Diameter	-0.621*	-0.715**	0.459	-0.068	-0.075
Small	0.660*	0.595*	-0.234	0.391	0.023
Medium	0.256	0.494	-0.428	0.551*	0.103
Large	-0.541*	-0.690**	0.454	-0.219	-0.091

** $P < 0.01$; * $P < 0.05$

4. Conclusion

The relationship between γ -GT and the protein content in milk, and any implication that this enzyme may have in the definition of the hygienic quality of milk suggest that a further investigation should be carried out. With regard to the role of xanthine oxidase during the formation of the globule, our results highlighted a correlation between the activity of this

enzyme and the size of the globule. Further studies will be needed to assess the relation between the activity of xanthine oxidase and the enveloping of the membrane around the lipidic droplet, and to find out whether this link has a physiological significance and is also confirmed by other studies conducted on the milk of other species.

Acknowledgements

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I-P086: Evaluation of Antibiotic Residue Screening Test for Beta-Lactamic Detection in Goat's Milk

D. Sierra¹, A. Sánchez², C. Luengo¹, F. San Eustaquio¹, B. Agüera¹, J.C. Corrales², C. de la Fe², C.T. Morales¹, A. Contreras²

Summary

To estimate the detection limits in comparison with the MRL of the antibiotic residues stabilized by the UE, we had carried out 3456 analysis using composite goat milk samples antibiotic-free and added with different dilution of beta-lactamic antibiotics. The kits used were BRT AiM®; Delvotest MCS®; Eclipse 100® and CMT-Copan Milk. The results demonstrated that under analytical rules specified by the IDF, milk goat is an adequate substrate to detect beta-lactamic residues with these kits and most of them detected the different antibiotics below the maximum residue limit (MRL).

1. Introduction

Most of the screening methods for antibiotic detection in milk are based on the inhibition of *Bacillus stearothermophilus var calidolactis* and has been developed and evaluated for cow's milk. Instead that some authors had studied also the detection limit of some screening kits for sheep milk (Althaus et al., 2003, Molina et al., 2003, Montero et al., 2005). Regarding goat milk, little information is available in relation to antibiotic residues detection test (Contreras et al., 1997) and no previous studies to establish the detection limit has been published. The objective of this work is to establish the detection limit of four commercial screening tests, using six betalactamic antibiotic in goat milk samples.

2. Material and methods

Composite milk from thirty primiparous Murciano-Granadina goats from an organic farm with good health status, free of antibiotic administration for life and free of intramammary infections (including *Mycoplasma* spp) was collected to prepare the substrate, following the IDF recommendations (1999, FIL-IDF Standard No. 183). The SCC of the goat milk samples ranged from 20 to 256 x 10³/ml and the SCC of the milk substrate was 67 x 10³/ml, with a total bacteria viable count lower than 1000 UFC/ml. The screening tests studied were BRT AiM®; Delvotest MCS®; Eclipse 100® and CMT-Copan Milk Test. Six beta-lactamic antibiotics were studied: Penicillin-G; Ampicillin; Amoxicillin; Cloxacillin; Oxacillin and Dicloxacillin using eight different antibiotic dilutions from each one (Table 1), including a blank. Eighteen replicates of each dilution were analyzed, so a total of 3456 analysis were performed. The detection limit was considered as the concentration where 95% of the test results was positive (IDF,1999)

Table 1: Antibiotic and dilutions utilised, Maximun Residue Limits (LMRs) stabilised and Detection Limits obtained (95%) for the four residual detection methods studied

Antibiotic	Dilutions (µg/Kg)	LMRs (µg/Kg)	Detection Limit (µg/kg)			
			Delvotest	CMT-Copan	Eclipse	BRT
Penicilin G	0, 1, 2, 3, 4, 5, 6, 8	4	2	2	3	2
Ampicilin	0, 1, 2, 3, 4, 5, 6, 8	4	4	2	5	3
Amoxicilin	0, 1, 2, 3, 4, 5, 6, 8	4	4	3	6	4
Cloxacilin	0, 5, 7.5, 10, 20, 30, 40, 50	30	30	30	50	30
Oxacilin	0, 5, 7.5, 10, 20, 30, 40, 50	30	7,5	7,5	20	20
Dicloxacilin	0, 5, 7.5, 10, 20, 30, 40, 50	30	30	20	30	10

¹ Laboratorio Agroalimentario y de Sanidad Animal. Consejería de Agricultura y Agua. Murcia (Spain).

² Dpto. de Sanidad Animal. Facultad de Veterinaria. Universidad de Murcia (Spain).

3. Results and discussion

The results are expressed in Table 1. Most of the tests detected the different antibiotics below the maximum residue limit (MRL). Similar results have been obtained by other authors studying sheep milk as a substrate with different residues detection methods as Delvotest (Althaus et al., 2003); BRT (Molina et al., 2003) and Eclipse (Montero et al., 2005). No previous data about Copan CMT test evaluation has been published for sheep milk. Instead that one of the screening tests (Eclipse) failed to detect three antibiotics (ampicillin, amoxicillin and cloxacillin) below the LMR, we could conclude that under analytical rules specified by the IDF the goat milk is an adequate substrate to detect beta-lactamic residues for most of the residues detection methods studied.

4. Conclusions

Under the analytical conditions specified by the IDF, milk goat is an adequate substrate to detect beta-lactamic residues, using the four residues detection methods studied.

Acknowledgements

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I-P087: Evaluation of MilkoScan FT 6000 Milk Analyzer for Determination of Freezing Point in Goat Milk

D. Sierra¹, A. Sánchez², C. Luengo¹, M. Beltrí¹, I. Guirao¹, J.C. Corrales², C. de la Fe², C.T. Morales¹, A. Contreras², C. Gonzalo³

Summary

The MilkoScan FT 6000 (Foss Electric, Hillerød, Denmark) was evaluated for its ability to determine the freezing point of goat's milk, comparing it with the thermistor cryoscope method under different analytical conditions: five different preservation strategies in 8 different amounts of water added (0, 1, 2, 3, 4, 5, 6 or 7% total volume). The small difference between methods (1.5 m°C) allows the MilkoScan method to be used with goat's milk for screening purpose. The type and concentration of the preservative used influenced significantly the freezing point and they should be taken into account when interpreting it. The best regression coefficient between methods corresponded to samples preserved with bronopol at 0.020 g/100 mL.

1. Introduction

The freezing point (FP) of milk is measured to determine the presence of added water in the bulk tank milk because of certain milking operations or as a sign of fraud. Currently, a number of preservation strategies are used with milk samples destined to undergo SCC analysis using fluoro-opto-electronic counters (IDF, 2006) as well as in the determination of milk fat, protein and lactose contents by mid-infrared measurements (IDF, 2000). The effect of these preservatives on goat's milk SCCs and the milk composition values have been studied (Sánchez et al., 2005). The aims of the present study were to determine the overall accuracy of the MilkoScan FT 6000 method for determining FP in goat's milk, comparing it with the reference thermistor cryoscope method (IDF, 2002) under different analytical conditions.

2. Material and methods

On 1,800 milk aliquots obtained from 45 bulk tank milk samples of ten Murciano-Granadina goat herds, the FP values were determined in duplicate in both, reference and automatic method, with a thermistor cryoscope (The Advanced Cryoscope model 4D3, USA) and with a MilkoScan FT 6000 (Foss Electric, Denmark), respectively. Five different preservation strategies were evaluated: no preservative (NP), preservation with azidiol at 0.006 g of sodium azide/100 mL (AZ6) and at 0.018 g of sodium azide/100 mL (AZ18), and preservation with bronopol at 0.020 g/100 mL (BR20) and at 0.040 g/100 mL (BR40). For each preservative studied, eight milk aliquots were added with 0, 1, 2, 3, 4, 5, 6 and 7% of water.

3. Results and discussion

Under most analytical conditions, the FP was recorded as lower by the MilkoScan method, with a mean difference of 1.5 m°C compared to the reference method. Both methods showed similar repeatabilities (the overall sr% was 0.22% for the MilkoScan method and 0.20% for the reference method). In comparisons of the two methods, the highest regression coefficients were obtained with aliquots containing >3% added water. The best regression coefficients (0.85 to 1.02) were obtained for milk samples preserved with bronopol at 0.020 g/100 mL (Figure 1). The factors of added water, preservative, analytical method, lactose concentration, and the effect of the bulk tank milk sample within each lactose group contributed significantly to the observed variation in FP. For practical purposes, either of the bronopol concentrations used could be employed when determining the FP of goat's milk with the methods tested. However, the increase in the

¹ Laboratorio Agroalimentario y de Sanidad. Animal. Consejería de Agricultura y Agua. Murcia, Spain.

² Dpto. de Sanidad Animal. Facultad de Veterinaria. Universidad de Murcia, Spain.

³ Dpto. Producción Animal. Facultad de Veterinaria. Universidad de León, Spain.

concentration of sodium azide in the azidiol formula contributed to an important reduction in the FPs recorded (Figure 2).

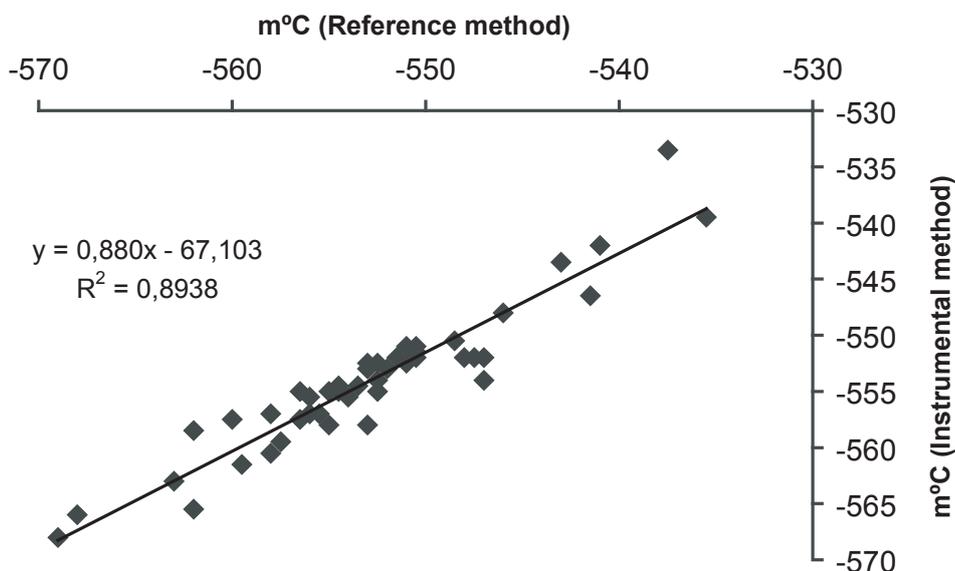


Figure 1. Linear regression between the FP values obtained by the reference (The Advanced Cryoscope model 4D3, USA) and instrumental (MilkoScan FT 6000, Foss Electric, Denmark) methods for the milk aliquots without added water and preserved with bronopol at 0.020 g/100 mL

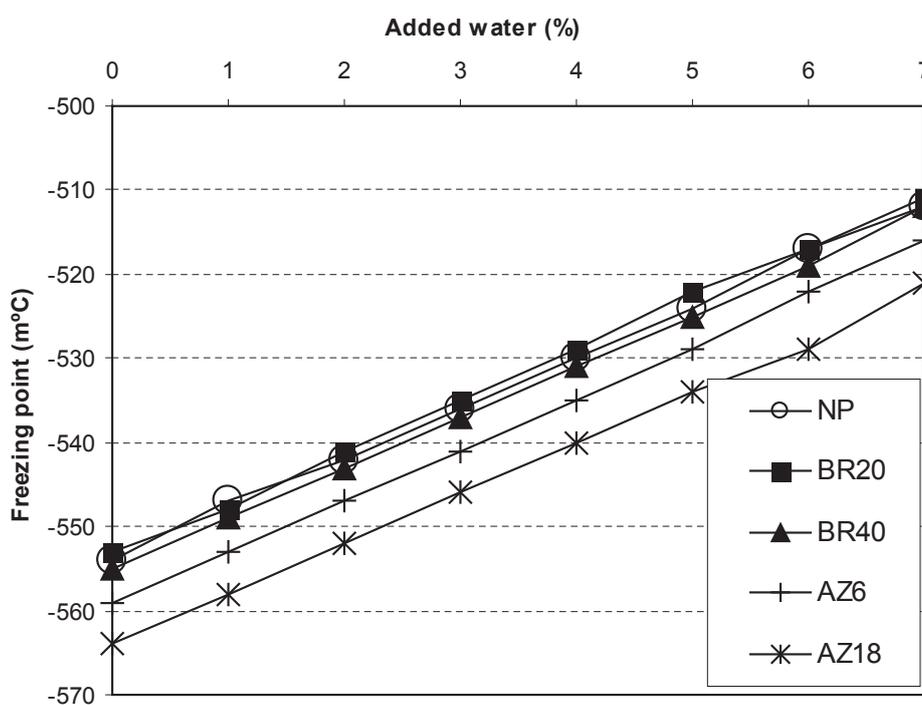


Figure 2. Least square means of freezing point according to the interaction added water x preservative

4. Conclusions

The MilkoScan method can be used for screening FP in bulk tank goat's milk samples. Within each method, the FP of goat's milk must be interpreted taking into account the type and concentration of the preservative used; if not, the effect of the preservative could lead to misinterpretations regarding the water content.

Acknowledgements

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I-P088: Efficacy of Dry Period Therapy as a Mean to Reduce Somatic Cell Counts in Goats Herds

G. Leitner¹, N. Silanikove², U. Merin³

Summary

Subclinical intramammary infection (IMI) by coagulase negative staphylococci (CNS) is the major single factor affecting flock IMI and consequently milk and cheese yield loss in small ruminants. If no antibiotic dry-off therapy is used in such herds, animals that became infected with CNS remain infected throughout their lactations, reflecting an overall accumulation of infected animals. Treating goats with cattle dry-off treatment resulted in 78.6% cure. However, every curative treatment in a given flock, certainly including dry-off treatment, should be executed only after consulting with veterinarian and utmost care, keeping in mind that dry-off treatment is only one option and the alternative risk of evolution of antibiotic-resistant or violent bacteria.

1. Introduction

Subclinical IMI reduces milk production and curd yield and causes loss of income to the farmer. Therefore, simple estimate of the flock's IMI status may serve the farmer as a tool to reduce economic losses and improve flock management by motivating him to apply a range of veterinary (dry therapy treatment) and management means (e.g., improving milking hygiene) to improve health problems in his herd. Subclinical IMI by CNS was found to be the major single factor affecting flock IMI and consequently milk and cheese yield loss in small ruminants (Bergonier et al., 2003; Leitner et al., 2007a). We have shown recently that it possible to predict accurately milk and cured yield in sheep and goats in a given herd from somatic cell counts (SCC) and presented the following scheme for grading small ruminant's milk: i. High-quality milk <800,000 SCC/mL, associated with <25% IMI; ii. Medium quality milk <1,500,000 SCC/mL, associated with IMI of 25-50%; iii. Low-quality milk >1,500,000 SCC/mL, associated with >50% IMI; and iv. Milk containing >3,500,000 SCC/mL, which should not be accepted for human consumption (Leitner et al., 2007b). However, caution in using these schemes should be paid to animals immediately after parturition and at the end of the lactation, because there is a natural increase of SCC during these two periods regardless of IMI.

2. Materials and Methods

The study was conducted in a large Israeli dairy goat farm. Goats were machine milked twice daily. Forty-nine goats were tested for pregnancy within 60 d after consumption and were dried-off 35-60 days before the expected next parturition. Milk samples from each udder half were taken 1-2 weeks before drying-off, at day of drying-off and 21 and 28 days postpartum for bacteriological testing and CMT. At drying off, each of the goats received intramammary treatment with a commercial cattle dry-off treatment (VETIPEN DC, Vetimex, Bladel, Holland), which is a combination of procaine benzyl penicillin (300 mg), Nafcillin (109.65 mg) and dihydrostreptomycin (125 mg). A whole tube was administered to each udder half.

3. Results and Discussion

Out of the 98 udder-halves tested 54 were found uninfected while 44 (42.9%) were infected by various CNS. All the CNS's found were susceptible to components of the antibiotic used. Postpartum testing revealed that 51/54 (94.4%) of the udder-halves which tested negative before

¹ National Mastitis Reference Center, Kimron Veterinary Institute, P.O. Box 12, Bet Dagan 50250, Israel.

² Institute of Animal Science, Agricultural Research Organization, The Volcani Center, P.O. Box 6, Bet Dagan 50250, Israel.

³ Department of Food Science, Agricultural Research Organization, The Volcani Center, P.O. Box 6, Bet Dagan 50250, Israel.

treatment remained negative and only 3 udder halves were infected during the dry period or immediately postpartum (5.5%). It is important to note that in dairy herds with high IMI, ~15% of the new infections accrued before first parturition and at the dry periods between lactations. Of the 44 udder-halves which were found infected and dry treated, 33 recovered (i.e., no bacteria was identified and CMT was 0-1 in the next lactation), while 9 udder-halves remained infected with the same bacteria, meaning that treatment success was 78.6%. These results are similar to our previous study conducted with Asaff sheep, where 71% of the treated udder halves (63-88%) were cured compared to 8% of the udder halves (0-30%) of the control animals. Inversely, 29% of the udder halves remained infected in a chronic stage in the treated sheep in comparison to 92% in the control animals (Shwimmer et al., 2007).

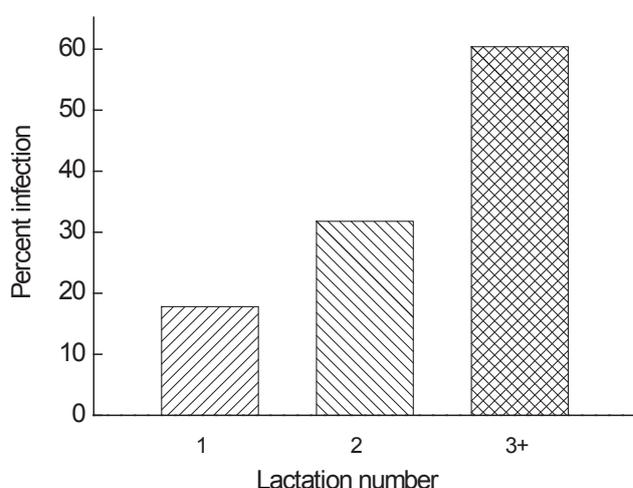


Figure 1. Increase of infected udder halves throughout lactations during the study. The purpose of this study was to evaluate dry-off antibiotic therapy in a goatherd with high subclinical IMI. As can be seen in Fig. 1, when no antibiotic dry-off therapy was used in the herd, animals that became infected with CNS remained infected for their entire lactations, so the overall accumulation of infected animals in the herd increased.

4. Conclusion

Proper flock management and meticulous care, especially before parturition and in the days close to it, including teat dipping after milking, could maintain the flock at a relatively low level of IMI without the need for such treatments. Dry-off antibiotic therapy of animals entering their dry period cost money, raises the risk of inhibitory substances in the milk and could cause an increase in the number of violent bacteria, even if not antibiotic resistant. Therefore, it is advisable that dry-off treatment be recommended for flocks where there are large number of animals with subclinical infection with a high bulk milk tank SCC. However, every curative treatment of the flock, including dry-off treatment, should be applied only after consulting and with extreme care, keeping in mind that dry-off treatment is an option and not a must!

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I-P089: Efficiency of Milking Machines for Dairy Ewes in Central Macedonia, Greece

B. Skapetas¹, V. Laga¹, I. Katanos¹, E. Sinapis², I. Hatziminaoglou²

Summary

The aim of this work was to study the efficiency of milking machines of different types for dairy ewes in the region of Central Macedonia, Greece. Thirty nine sheep farms of medium and large size were used. Five visits were carried out in each farm during the first 5 months of the milking period of ewes. Two questionnaires were used for registering parlors' throughput, total time of milking, time of hand stripping, milking machines type and size, farms' size, sheep breeds etc. Generally, the average parlors' throughput per hour and per milker and per hour ranged at low or medium levels because of the great variation on the milkability of ewes and the unsatisfactory technical level of operators. The total milking time of flocks ranged at satisfactory levels.

1. Introduction

Sheep production in Greece constitutes the most important sector of livestock production contributing 35,9% to the total output of country's animal production. All the ewes are milked. During the last decade machine milking of ewes is applied in many dairy flocks mainly in the lowland regions. Today in Greece there are representatives of many construction companies of milking machines for dairy sheep like Strangko, Westfalia, Hector, De Laval, Flaco, Manovac, Intermilk etc. However there is a lack of information regarding the efficiency of milking machines and their working conditions.

For this reason the objective of this work was to study the efficiency of milking machines for dairy ewes in the region of Central Macedonia, Greece.

2. Material and methods

In our study thirty-nine dairy sheep farms were used during the years 2005 and 2006 in the Central Macedonia area of Greece. All the flocks were of medium and large size (above 300 ewes in milk). In each farm 5 visits were carried out during the first 5 months of the milking period of ewes (1 visit per month). All visits took place at the evening milking. Two types of questionnaire were used for registering different measurements and technical data in the milking parlors such as: starting and finishing hour of milking, number of ewes in each milking group, milking time of each group of ewes, hand stripping time, total bulk milk, milking machine's type and size, working parameters of machines etc. Based on the above measurements the following parameters were calculated: total milking time of flocks, parlors' throughput per hour and per milker and hour, machine milking efficiency etc.

3. Results and discussion

Of the total of milking machines that were examined 46.15% of it was of «Casse system» of various sizes (1 x 24, 2 x 24, 1 x 30 and 2 x 30), 43.59% of modern «Casse system» or «Fast exit system» of various sizes (1 x 24, 1 x 32, 2 x 32 and 2 x 33), 5.13% of Rotary system (Carrousel) and 5.13% of bucket system.

Table 1 gives the results of efficiency of milking machines of different types. These results are considered as unsatisfactory for the modern milking machines. From field experiments in other countries was confirmed that the average throughput in the «Casse system» parlors is fluctuated from 100 to 350 ewes/h, in the «modern Casse system» from 320 to 410 ewes/h and in the Rotary system from 420 to 650 ewes/h (Billon, 1998, Berger, 2001).

The main causes of low or medium efficiency of milking machines for dairy ewes in this study are: the great variability inside the breeds and the flocks of ewes as regards milk yield, milk

¹ Department of Animal Production, Technological and Educational Institution of Thessaloniki, Greece.

² Department of Animal Production, School of Agriculture, Aristotle University of Thessaloniki, Greece.

ejection reflex, conformation and morphological characteristics of the udders and the teats. The above factors lead to overmilking of some ewes and oblige operators to apply the hand stripping which is a very time-consuming procedure (Caja et. al., 2000) . Hand stripping suppression in some cases increased milking machines' efficiency by 45.3% and decreased the total milking time by 36.6% (data not shown). Furthermore, the ability and skills of operators during the milking procedure, the habituation of the animals to the milking machines, the correct regulation and the regular service of milking machines have influenced their efficiency (Marnet, 1997). The total milking time of flocks ranged from 1.2 to 2.2 hours. This parameter was fluctuated in normal limits having in mind that the farmers are applying time-consuming milking routines.

Table 1: Efficiency of milking machines for dairy ewes in Central Macedonia, Greece.

Parlors' type	Average throughput (ewes/Øh)	Average throughput (ewes/milker/Øh)	Efficiency of machine milking (Øl milk/Øh)
«Casse system»	97 – 227.9	57 – 130.8	78.8 – 140.6
Modern «Casse system»	127.5 – 256.9	63.5 – 128.4	64.3 – 138.9
Rotary system	279.3 – 388.2	64.7 – 93.1	152.4 – 172
Bucket system	44.7 – 56.8	28.4 – 44.7	19.6 – 20.9

4. Conclusion

Efficiency of milking machines in the region of Central Macedonia, Greece according the type and size of milking parlors ranged in unsatisfactory levels because of the great variability of ewes' milkability and also the unsatisfactory technical level of operators. Genetic improvement for ewes' milking ability and udder conformation must be applied. On the other hand, farmers must improve their knowledge on milking techniques.

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I-P091: Milk Composition and Milk Yield of Goats Fed Sugar Cane Silage

R.S. Gentil¹, A.V. Pires¹, I. Susin¹, L.G. Nussio¹, C.Q. Mendes¹, O.C. de Almeida¹, M.A.A. Queiroz¹, IU Packer¹

Summary

Thirty-six lactating Saanen goats (15 ± 3 DIM) were assigned to a complete randomized block design (according to milk production, DIM and number of lactation) to evaluate the effects of feeding sugar cane silage treated with microbial (*Lactobacillus buchneri*) or chemical (urea) additives on milk yield and milk composition. Goats were housed individually in a tie stall for a period of 12 weeks. Does were fed a 50:50 (concentrate:roughage ratio) TMR with 16% crude protein. Experimental treatments were the roughage source: fresh sugar cane (FSC), sugar cane silage with *Lactobacillus buchneri* (SCS+Lb), 5x10⁴ cfu/g wet basis, sugar cane silage with 1% urea (SCS1%) or sugar cane silage with 1.5% urea (SCS1.5%). Means were compared across treatments: FSC vs SCS+Lb or SCS1% vs SCS1.5%. There were no differences (P>0.05) on dry matter intake, milk production, 3.5% fat corrected milk, milk fat, milk protein and milk urea for FSC vs SCS+Lb and SCS1% vs SCS1.5%, respectively. Sugar cane silage added with *L. buchneri* or urea had no detrimental effect on lactation performance of Saanen goats.

1. Introduction

Sugar cane is an important roughage source and an alternative feed for ruminants. Ensiling sugar cane may contribute to improve field management. However, the high levels of ethanol found in sugar cane ensiled without additive may reduce voluntary feed intake and may affect animal performance. This experiment was assigned to evaluate the effects of fresh sugar cane and sugar cane silage treated with microbial (*Lactobacillus buchneri*) or chemical (urea) additives fed to goats.

2. Material and methods

Thirty-six early lactating (15 ± 3 DIM) Saanen goats were used to evaluate dry matter intake, milk production and milk composition. Goats were assigned to a complete randomized block design (according to milk production, DIM and number of lactation) and housed individually in a tie stall during 12 weeks. Does were fed a 50:50 (concentrate: roughage ratio) TMR. Experimental treatments were the roughage source: fresh sugar cane (FSC), sugar cane silage with *L. buchneri* (SCS + Lb), 5x10⁴ cfu/g wet basis, sugar cane silage with 1% urea wet basis (SCS1%) or sugar cane silage with 1.5% urea wet basis (SCS1.5%). Means were compared across treatments: FSC vs SCS+Lb or SCS1% vs SCS1.5%. Milk production was measured twice a week. Once a week, milk samples were collected from each goat and preserved with 2-bromo-2-nitropropane-1,3-diol for later determination of milk fat, protein, lactose and urea using an equipment Bentley 2000.

3. Results and discussion

Results of dry matter intake, milk yield and composition are given in Table 1. There was no difference (P>0.05) on dry matter intake among treatments FSC vs SCS+Lb and SCS1% vs SCS1.5%. These results might have contributed to similar values (P>0.05) on milk yield. Milk fat and milk protein were similar (P>0.05) among overall comparisons. This result differs from those obtained by Mendes (2006), who reported higher values (P<0.05) of milk fat and milk protein in goats fed sugar cane silage inoculated with *L. buchneri*. Measurements of milk urea nitrogen could be used to assess the adequacy of protein feeding in dairy goats and the

¹ Department of Animal Science, Escola Superior de Agricultura "Luiz de Queiroz", University of São Paulo, Piracicaba - SP, Brazil, ivasusin@esalq.usp.br

efficiency of N utilization for milk production (Broderick and Clayton, 1997). In the present experiment, milk urea was similar ($P>0.05$) among compared contrasts.

Table 1: Dry matter intake (DMI), milk yield and milk composition for dairy goats fed fresh sugar cane or sugar cane silage treated with additives

Item	Treatments ¹				SEM ³	Contrasts	
	FSC	SCS+Lb	SCS1%	SCS1.5%		FSC x SCS+Lb	SCS1% x SCS1.5%
DMI, kg/day	2.43	2.16	2.25	2.10	0.13	ns ⁴	ns
Milk yield, kg/day	2.07	2.04	1.99	1.92	0.19	ns	ns
3.5% FCM ² , kg/day	1.98	2.03	2.04	2.04	0.15	ns	ns

¹Treatments: fresh sugar cane (FSC), sugar cane silage with *L. buchneri* (SCS + Lb), 5×10^4 cfu/g wet basis, sugar cane silage with 1% urea (SCS1%) or sugar cane silage with 1.5% urea (SCS1.5%);

²FCM: fat corrected milk;

³SEM: Standard error of the mean;

⁴ Not significant ($P>0.05$).

Table 2: Milk composition for dairy goats fed fresh sugar cane or sugar cane silage treated with additives

Item	Treatments ¹				SEM ²	Contrasts	
	FSC	SCS+Lb	SCS1%	SCS1.5%		FSC x SCS+Lb	SCS1% x SCS1.5%
Fat							
%	3.23	3.43	3.65	3.86	0.08	ns ³	ns
g/d	66.92	72.49	70.91	74.77	4.38	ns	ns
Protein							
%	3.02	2.88	3.01	2.99	0.05	ns	ns
g/d	62.47	57.26	61.48	57.26	3.51	ns	ns
Urea							
mg/dL	26.83	25.52	25.21	22.77	0.71	ns	ns
mg/d	5.48	4.99	5.05	4.36	0.26	ns	ns

¹Treatments: fresh sugar cane (FSC), sugar cane silage with *L. buchneri* (SCS + Lb), 5×10^4 cfu/g wet basis, sugar cane silage with 1% urea (SCS1%) or sugar cane silage with 1.5% urea (SCS1.5%);

²SEM: Standard error of the mean;

³Not significant ($P>0.05$).

4. Conclusion

Sugar cane silage added with *L. buchneri* or urea had no detrimental effect on lactation performance of Saanen goats.

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I-P092: Kappa Casein and Beta Lactoglobulin in the Czech Sumava Breed

Z. Sztankoova¹, V. Matlova¹, C. Senese², T. Kott¹, M. Milerski¹

Summary

Polymorphism of milk proteins affects dairy production in ruminants. Gene of milk protein are organized as a cluster including α_s -, β - and κ -casein. Among caseins, κ -casein plays an important role in formation, stabilization and aggregation of micelles. Genetic polymorphism of ovine κ -casein is characterized both, at protein level, at 104 position (Ser→Leu) and at DNA level at position 443 C →T. β -lactoglobulin is a major whey protein in milk of ruminant, it is able to bind and transport a small hydrophobic molecule. Three genetic variants A, B and C have been found in sheep species. κ -casein and β -lactoglobulin fraction have been determined in the Czech native sheep breed Sumava using Light Cycler analysis and PCR-RFLP technique, respectively. For analysis of the κ -casein gene no polymorphism was observed (C=1.00). The value of allelic frequencies at the β -lactoglobulin were A=0.747 and B=0.253, genotype distribution: AA=0.624, AB=0.247 and BB=0.129.

1. Introduction

Approximately 80 of the ruminant milk is composed of the acid-precipitable-phosphoproteins, the caseins. Ovine milk proteins polymorphism is less extensive investigate. κ -casein plays an important role in formation, stabilization and aggregation of the casein micelle. Recently molecular analysis of exon 4 showed two different patterns, where C is more common (GeneBank accession number X51822) compared with the pattern T. This new pattern revealed the presence of the transition C→T at position 443 of the referring sequence (Ceriotti et al. 2004). Ovine β -lactoglobulin gene has been described with three genetic variants: A, B (Bell and Mc Kenzie, 1967) and C (Erhardt, 1989). Allele B and A are differ only in a single AA change His→Tyr at position 20. The C allele is a subtype of the A allele with a single exchange of the Arg→Glu, at position 148. The aim of this work was evaluate κ -casein and β -lactoglobulin gene in the Czech Sumava sheep breed.

2. Material and methods

We analyzed 85 animals of Sumava sheep population. Genomic DNA was extracted from whole blood using the NucleoSpin Blood Kit (CLONTECH Laboratories, Palo Alto, CA). The genetic polymorphism of the κ -casein (Ceriotti et al., 2004) and β -lactoglobulin (Anton et al., 1999), were detected by using Light Cycler analysis and PCR-RFLP, respectively

3. Results and discussion

Results of the molecular analysis of the κ -casein and β -lactoglobulin are presented in Table 1. In Sumava sheep breeds, no polymorphism was observed at the κ -casein locus. The distribution of the pattern T appears to be absence or with very low frequency at the κ -casein locus (Amigo et al., 2000; Ceriotti et al., 2004). At the β -Lg locus, showed the prevalence of the variant A=0.747 compared to the variant B=0.253. However, at the β -Lg gene, allele frequency are similar to those results are postulated by Amigo et al., (2000); Macha and Novackova, (1974), in the Czech sheep breeds. Sumava population did not confirm the expectation to fit the Hard-Weinberg equilibrium.

¹ Institute of Animal Science, Pratelstvi 815, 104 00 Prague Uhřetín, Czech Republic, e-mail: sztankoova@seznam.cz

² Dipartimento di Scienze delle Produzioni Animali, Università degli Studi della Basilicata, Potenza, Italy.

Table 1: Genotype frequency at the κ -casein and the β -Lg loci in the Sumava sheep populations

Locus	Genotype	Frequencies
κ -casein	CC	1.00
β -Lg	AA	0.624
	AB	0.247
	BB	0.129

4. Conclusion

Two genetic polymorphisms were evaluated in Sumava sheep breed, kept in Czech Republic. Results showed that the κ -casein is monomorphic. This event confirms, that polymorphism at the κ -casein in exon 4 is less polymorphic in sheep species compared with cow and goat species (Ceriotti et al., 2004). Ovine the β -Lg showed similar distribution as proposed Macha and Novackova (1974) (in the Czech sheep breeds), Amigo et al., (2000).

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I-P094: Estimates of Genetic Parameters for Milk Production Traits and Litter Size of U.S. Dairy Ewes

J. Casellas¹, D.L. Thomas², Y.M. Berger³

Summary

Estimates of genetic parameters for litter size and lactation traits were obtained from an analysis of 2,554 lactation records of 1,068 ewes collected at the University of Wisconsin-Madison, USA between 1996 and 2005. The flock was crossbred and in the process of being graded-up to high percentage dairy breeding from a meat sheep base. Heritabilities were highest for protein % and fat % (0.49 and 0.42, respectively), moderate for yield of milk, protein, and fat (0.30, 0.24, 0.21, respectively), and lowest for lactation length, litter size, and log somatic cell count (0.12, 0.10, 0.09, respectively). Significant genetic correlations were observed between milk yield and fat yield (0.94), protein yield (0.94), lactation length (0.87), protein % (-0.38), and fat % (-0.22).

1. Introduction

Commercial dairy sheep production in North America (NA) started approximately 25 years ago, and it is still a very small industry [1]. Genetic improvement of domestic meat flocks for dairy production has been accomplished primarily through the introduction of improved dairy breeds (East Friesian (EF) and Lacaune (LA)) from Europe and grading-up [1]. Rates of genetic improvement in the future will depend upon well-designed selection programs; requiring knowledge of genetic parameters and effects of non-genetic factors on lactation traits. This paper reports the first estimates of genetic parameters for lactation traits in dairy sheep in North America.

2. Materials and methods

The University of Wisconsin-Madison operates the only dairy sheep research farm in NA at the Spooner Agric. Res. Station in northwestern Wisconsin, USA (latitude: 45° 49'; longitude: 91° 53'; northern temperate climate).

A total of 2,554 lactation records of 1,068 ewes collected at the station between 1996 and 2005 were analyzed. The distribution of records by age of ewe was: 1 yr = 1,043, 2 yr = 793, 3 yr = 428, and over 3 yr = 290. The flock was initially composed of crossbred ewes of several meat breeds. The flock was gradually graded-up to a higher percentage of dairy breeding by mating with semen or rams of EF-cross (n = 5), EF (n = 15), or LA (n = 6) breeding.

Ewes grazed improved orchard grass – kura clover pastures during the grazing season; generally from May through October. During the non-grazing season, ewes received alfalfa haylage, and concentrates were provided during late gestation. During lactation, ewes received approximately .91 kg of concentrate per day; fed in two feedings in the parlor during milking.

Ewes were milked twice per day in a double-12 parlor. Test day milk yield, percentage of milk fat and protein, and somatic cell count (SCC) were measured at least once per month. Ewes were removed from milking when their test day milk yield dropped below .5 kg or if their SCC was extremely high.

Analyses were based on a multivariate linear animal model, and parameters were inferred using a Bayesian approach [2]. The model for lactation traits included the additive genetic effect of each animal, the permanent environmental effect of each ewe, age of ewe, weaning management system [3], number of lambs born, and breed composition of the ewe. Coefficients

¹ Grup de Recerca en Remugants, Departament de Ciència Animal i dels Aliments, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain, Joaquim.Casellas@irta.es

² Department of Animal Sciences, University of Wisconsin-Madison, 1675 Observatory Dr., Madison, Wisconsin 53706, USA, dlthomas@wisc.edu

³ Spooner Agricultural Research Station, University of Wisconsin-Madison, W6646 Hwy. 70, Spooner, Wisconsin 54801, USA, ymberger.wisc.edu

for individual heterosis and gametic recombination were fitted in the model as covariates. The model for litter size was similar, but without weaning management system and number of lambs born.

3. Results and discussion

Mean performance and heritabilities are presented in Table 1. Heritabilities were highest for protein % and fat % (0.49 and 0.42, respectively), moderate for yield of milk, protein, and fat (0.30, 0.24, 0.21, respectively), and lowest for lactation length, litter size, and log somatic cell count (0.12, 0.10, 0.09, respectively). Significant genetic correlations were observed between milk yield and fat yield (0.94), protein yield (0.94), lactation length (0.87), protein % (-0.38), and fat % (-0.22) (Table 2). These heritabilities and genetic correlation estimates are in close agreement to estimates from European sources [4].

Litter size and SCC were not genetically correlated with any other trait, with the exception of litter size and % protein ($r_g = .51$) (Table 2).

The significant negative environmental correlations between SCC and the yield traits and lactation length (Table 2, -0.29 to -0.35) may be a result of management practices in the flock. Ewes with extremely high SCC were removed from milking for the season and were often culled. This would result in shorter lactation lengths and lower yields for ewes with very high SCC. If these ewes had been allowed to stay in milking, the environmental correlations may still have been negative, but they probably would have been smaller.

Heterosis effects were positive for all traits and significantly different from zero for milk yield and litter size. Recombination effects were negative for six of the eight traits and significantly different from zero for fat yield and protein yield (Table 1).

Table 1: Unadjusted means, heritability estimates, heterosis effects, and recombination effects for litter size and lactation traits

Item	Litter size, no.	Lactation length, d	Milk yield, kg	Fat %	Fat yield, kg	Protein %	Protein yield, kg	SCC, no./ml
Mean	1.84	150.5	231.7	5.67	13.4	4.96	11.5	144 544
Heritability	.10	.12	.30	.42	.21	.49	.24	.09
Heterosis, units of the trait ¹	.11**	5.33	34.98**	.11	.35	.09	.34	1023
Recombination, units of the trait ¹	-.05	-2.11	-5.16	.07	-1.88*	-.05	-1.22**	1023

¹Null value outside of the highest posterior density region at 90 % (*) or at 95 % (**).

Table 2: Genetic correlations (above the diagonal) and residual environmental correlations (below the diagonal)

Trait	Trait							
	LS	LL	MY	F%	FY	P%	PY	SCC
LS		-.40	.01	.09	-.02	.51**	-.01	-.01
LL	-.32		.87**	-.01	.81**	-.39**	.83**	.05
MY	-.01	.64**		-.22*	.94**	-.38**	.94**	.17
F%	.39*	.29*	.18**		.01	.55**	-.05	.22
FY	.06	.66**	.93**	.40**		-.41**	.92**	-.02
P%	.39*	.09*	.02	.37**	.27**		-.36**	.21
PY	.02	.81**	.93**	.14**	.95**	.19**		.32
SCC	-.01	-.35**	-.31**	.07	-.29**	.16**	-.31**	

*, **Null value outside of the highest posterior density region at 90 % (*) or at 95 % (**)

4. Conclusions

Yield of milk, fat, and protein were moderately heritable, and percentage of milk fat and milk protein were highly heritable. Each trait should show reasonable progress from selection. However, the negative correlations between the yield traits and the percentage of milk fat and milk protein suggest that selection on yield will generally result in a decrease in percentage of fat and protein. It may be desirable to develop selection indexes that aim to maximize increases in milk yield while minimizing the decrease in fat and protein percentage.

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I-P095: Antibiotic Susceptibility of *Staphylococcus* spp Strains Isolated from Sub-clinical Mastitis in Goat Milk

S. Viridis¹, G. Corgiolu¹, C. Scarano¹, A.L. Pilo¹, P. Marongiu¹, E.P.L. De Santis¹

Summary

3,000 half-udder milk samples from 8 goats flocks reared in Sardinia were collected and analysed for mastitis pathogens. From 469 positive samples, 421 Coagulase Negative Staphylococci, 25 *S. aureus*, 4 *Micrococcus* spp, and 21 other species were isolated and identified. Four of the strains were not identified. Antibiotic susceptibility tests were carried out on 25 *S. aureus* and 75 CNS randomly selected strains. Minimum Inhibitory Concentrations (MICs) were determined by using the broth micro-dilution method according to the guidelines and standards of The Clinical and Laboratory Standards Institute (CLSI). The MIC values were compared to break-points of ampicillin (AMP), cephalothin (KF), cefoperazone (CFP), ceftriaxone (CRO), cloxacillin (OB), kanamycin (K), novobiocin (NV), ofloxacin (OFX), oxytetracycline (OT) and vancomycin (VA). More than 96 % or over of the strains were susceptible to all the antimicrobial agents tested, with the exception of AMP (70.0%), K (74.0%), OT (92.0%) and OFX (94.0%). The MIC₉₀ for these antimicrobial agents were, respectively, 4.0, 0.25, 4.0, 8.0, 1.0, 64.0, 0.5, 1.0, 2.0 and 4 µg/ml. Multiple resistance was detected in 8 strains.

1. Introduction

Resistance to antibiotics has become one of the most relevant health problems in the last years [19]. This trouble has developed together with the widespread use of antibiotics in both the medical and the veterinary fields [17, 12]. There are many aspects to the problem, from the typically nosocomial to those linked to the production of foodstuffs of animal origin [1, 10]. Raw bulk milk and raw milk cheese are among the foodstuffs which have the highest risk of spreading resistant pathogens [14]. These can originate either from the farm environment or from sub-clinical mastitis (SM). In half-udder milk of goats affected by SM the prevalence of *S. aureus* ranged between 4.1% and 18.0% while for Coagulase Negative Staphylococci (CNS) it was between 61.1 and 95.9% [5]. On goat farms the spreading of Methicillin-Resistant Staphylococci (MRS) associated to antibiotics multiple resistance is of great concern [11]. Determining the antibiotics susceptibility pattern is notable for the choice of which should be used for mastitis therapy. Little research has been carried out on the susceptibility to antibiotics of *Staphylococcus* spp isolated in goats [2, 13], and most of the data has been collected using the Bauer et al (1966) method [18]. The aim of this research was to determine the Minimum Inhibitory Concentration (MIC) and the susceptibility of *Staphylococcus* spp isolated in goats SM to 10 antibiotics used in human and veterinary medicine. The single and multiple antibiotic resistance of each isolated strain were also evaluated.

2. Materials and methods

Farms and sampling – milk samples were collected in 8 farms in Sardinia (Italy) from 1,500 Sarda or Sarda-Maltese goats. Half-udder milk samples (3,000) were taken during the first stage of lactation (from January to April) from goats which did not exhibit clinical signs of mastitis [11]. **Microbiological analysis and somatic cell count (SCC)** – Microbiological analysis was carried out streaking 10 µl of each milk sample on blood agar (5%). Each plate was incubated for 48-72 hours at +37°C. The isolates were identified using standard microbiological procedures and API ID 32 STAPH system (bioMérieux, Marcy l'Etoile, France). SCC was determined with the Fossomatic 5000 system (Foss Electric, Hillerød, Denmark). **Antimicrobial susceptibility** – It was tested on 25 *S. aureus* and 75 CNS strains. The latter were randomly selected from CNS isolated strains. On each isolates the MIC of 10 antibiotics (AMP, KF, CFP, CRO, OB, K, NV, OFX,

¹ Department of Animal Biology, 07100 Sassari, Italy.

OT and VA) was determined, at concentrations of between 0.06 and 128 µg/ml by using broth microdilution method [3]. The susceptibility of each strain was determined comparing MIC values with the reference breakpoints [4, 15]. For each antibiotic the MICs mode and range, MIC₅₀ and MIC₉₀ were also evaluated. The quality control of the test was established by evaluating susceptibility of the reference strains *S. aureus* ATCC 29213 and *E. faecalis* ATCC 29212.

3. Results and discussion

Microbiological analysis and SCC - From 469 bacteriological positive samples, 421 (88.9%) CNS, 25 (5.3%) *S. aureus*, 4 (0.9%) *Micrococcus* spp and 21 (4.0%) other species were isolated and identified. Four of the strains were not identified. In bacteriological positive samples the average SCC (6.3 log ml⁻¹) was higher (P<0.001) than in negative ones (5.7 log ml⁻¹).

Antimicrobial susceptibility tests (MIC) - the staphylococci tested for susceptibility to antibiotics were *S. aureus* (25), *S. caprae* (25), *S. warneri* (16), *S. simulans* (15), *S. chromogenes* (7), *S. epidermidis* (6), *S. equorum* (2), *S. cohnii* (1), *S. haemolyticus* (1), *S. lugdunensis* (1), and *S. xylosus* (1). Table 1 shows MIC₅₀, MIC₉₀, mode and range of the MIC values for each antibiotic and the isolates susceptibility.

Penicillins - Ampicillin (AMP): 30.0% of the strains were resistant and, respectively, 36% of SCN and 12% of *S. aureus*. - Cloxacillin (OB): 98.0% of the strains were susceptible. The MIC₉₀ was comparable to that observed by aa [13]. The higher efficiency of OB rather than AMP is due to its resistance to staphylococcal β-lactamase.

Cephalosporins - Cephalothin (KF) - Cefoperazone (CFP) - Ceftriaxone (CRO). The susceptibility of the strains tested was, respectively 98.0%, 99.0% and 96.0%. Resistance to the KF and CRO antibiotics was detected in 1 (4.0%) *S. aureus* and 1 (1.3%) *S. epidermidis* respectively. The results of our work confirm the efficiency of cephalosporin with respect to Gram positive bacteria [16].

Aminoglycosides - Kanamycin (K): 74 strains were susceptible, 12 resistant and 14 intermediate. The resistant strains consisted of 7 (28.0%) *S. aureus*, 1 *S. caprae*, 2 *S. simulans* and 2 *S. epidermidis*.

Tetracyclines - Oxytetracycline (OT): 92 strains were susceptible. The resistant strains were 2 *S. caprae*, 1 *S. epidermidis*, 4 (16.0%) *S. aureus* and 1 *S. warneri*. In previous works on *Staphylococcus* spp strains isolated from goat's milk lower rates of susceptibility are reported [2, 13].

Fluoroquinolones - Ofloxacin (OFX): 94.0% of the strains were susceptible. The resistant strains were *S. epidermidis* (2) and *S. caprae* (1). The susceptibility of *Staphylococcus* spp isolated by SM in sheep in previous works was 92.1% [9].

Glycopeptides - Vancomycin (VA): all the strains (100.0%) were susceptible, as was reported in previous studies on strains isolated in sheep and goat breeds [2, 7].

Novobiocin (NV): 97.0% of the strains were susceptible.

Table 1: In vitro antimicrobial resistance of 100 *Staphylococcus* spp strains isolated from goats subclinical mastitis using the broth microdilution method

antibiotics	n°	Susceptible %	Intermediate %	Resistant %	MIC ₅₀ µg/ml	MIC ₉₀ µg/ml	Mode µg/ml	Range µg/ml
AMP ^a	100	70	-	30	0.12	4.00	0.06	0.06 - 16.00
CFP ^b	100	99	1	-	2.00	4.00	2.00	0.06 - 32.0
CRO ^b	100	96	3	1	2.00	8.00	2.00	0.06 - 128.0
KF ^a	100	98	1	1	0.12	0.25	0.25	0.06 - 128.0
K ^a	100	74	14	12	8.00	64.0	16.0	0.06 - ≥128
NV ^b	100	97	-	3	0.12	0.50	0.06	0.06 - 32.00
OB ^a	100	98	-	2	0.25	1.00	0.25	0.06 - ≥128
OFX ^b	100	94	3	3	0.50	1.00	0.50	0.06 - 32.00
OT ^a	100	92	-	8	0.50	2.00	0.25	0.06 - ≥128
VA ^a	100	100	-	-	2.00	4.00	2.00	0.06 - 4.00

AMP=Ampicillin; CFP=Cefoperazone; CRO=Ceftriaxone; KF=Cephalothin; K=Kanamycin; NV=Novobiocin; OB=Cloxacillin; OFX=Ofloxacin; OT=Oxytetracycline; VA=Vancomycin (^a = Breakpoints NCCLS, 2002; ^b = CLSI, 2006).

Antibiotic resistance to NV is useful for taxonomic purpose and in CNS it is also associated with a low pathogenic activity [8]. The resistant strains were *S. xylosum* (1) and *S. equorum* (2). Multiple resistances to antibiotics were observed in 8 strains. The species which were contemporaneously resistant to two or more antibiotics were: 1 *S. aureus* (K and OT), 3 *S. caprae* (AMP, OFX and OT), 1 *S. simulans* (AMP and OB), 1 *S. warneri* (AMP and OT), and 2 *S. epidermidis*, one of whose strains was resistant to four different antibiotics (AMP-OB-OFX-OT).

4. Conclusions

Overall CNS and *S. aureus* strains susceptibility ranged between 96% and 100% to OB, CFP, CRO, KF, NV and VA. The susceptibility to OFX (94%) and OT (92%) was lower. Resistance and intermediate responses were mainly observed towards AMP and K. Multiple resistance was found in 8 of the isolated strains. SCN were more resistant to AMP than *S. aureus* (36% vs. 12%). The latter was more resistant than SCN to K (16% vs. 5,3%) and OT (28% vs. 6,7%).

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I-P096: Milk Production and Quality of Some Croatian Sheep Breeds

V. Pavić¹, B. Mioč¹, N. Antunac², D. Samaržija², V. Sušić³, I. Vnučec¹, Z. Prpić¹, Z. Barać⁴

Summary

The aim of this research was to determine the effect of the breed (genotype) on lactation length, milk yield, fat and protein yield. The data relating to the milking ability controls of 1544 ewes (336 indigenous Istrian sheep, 677 indigenous Pag sheep, 444 East Friesian (EF) sheep and 87 crossbreed ewes (Istrian x Awassi x EF, IAEF) were used for this research. The EF sheep and IAEF crosses had approximately the same values for lactation milk yield, the lactation period, the milking and the suckling periods (289.91 vs. 289.03 kg, 195 vs. 186 days, 136 vs. 133 days and 59 vs. 53 days, respectively). The Pag sheep had the lowest lactation milk yield (143.28 kg) with the highest average fat and protein content (7.73 and 6.19%, respectively). These results suggest that EF and Awassi sheep can be used to upgrade the indigenous sheep breeds for increased milk production, especially in areas with higher herbage availability.

1. Introduction

In Croatia sheep are primarily bred for meat production. The production of lamb meat is mostly represented in the coastal areas where there is a lot of rocky ground and stone, poor vegetation, thicket and underbrush, and there are very few possibilities of breeding other types of livestock (except goats). However, for the past ten years the production and processing of sheep milk has been growing. Sheep milk in Croatia is mainly produced by autochthonous sheep breeds and their crosses (located in the Adriatic coastal area), and recently by East Friesian sheep (EF), located in continental area. During the year 2005, 1.808,478 kg of sheep milk have been purchased and processed. Considering the growing economic importance of sheep in the entire Croatian livestock, especially in the production and processing of milk, the goal of this work is to show the most important characteristics of the sheep milk production in Croatia.

2. Material and methods

The data relating to the milking ability controls [1] for five lactations of 336 Istrian sheep, 677 Pag sheep, 444 EF sheep and 87 IAEF crosses (Istrian sheep x Awassi x EF) were used for this research. All animals within one individual herd, regardless of the number of lactation and milking ability were kept in equal feeding and living conditions. The milk production control was performed using the AT method [2] with a single milking by hand (morning or evening) once a month (every 28-34 days), with the measuring of the quantity of milked milk. The amount of milk (kg) is calculated by multiplying the quantity of milk shown in litres (l) with the average density of goat milk 1.030 [2]. The milk yield in the suckling period (from lambing to weaning or slaughtering of lambs) is calculated by multiplying the amount of milk determined in the first milk production control with the days of suckling. The amount of milk in the period of milking (since weaning or slaughtering of lambs to dry-off) is obtained through calculations based on the data of the monthly milk production controls. By adding the amounts of milk in the two mentioned periods, one can get the total amount of milk in lactation [2]. The content of milkfat and proteins [3] were obtained using the IR spectrophotometer (Milkoscan 4400). The acquired data were analyzed using SAS statistical software [4].

¹ Department of Animal Production, Faculty of Agriculture, University of Zagreb, Svetošimunska 25, 10 000 Zagreb, Croatia.

² Dairy Science Department, Faculty of Agriculture, University of Zagreb, Svetošimunska 25, 10 000 Zagreb, Croatia.

³ Department of Animal Husbandry, Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, 10 000 Zagreb, Croatia.

⁴ Croatian Livestock Center, Ilica 101, 10 000 Zagreb, Croatia.

Table 1: Review of recorded lactations per breed in 2005

Breed	Lactation period (days)	Suckling period (days)	Milking period (days)	Total milk yield in lactation (kg)	Milk yield in suckling period (kg)
Istrian sheep	188	55	133	204.49	77.19
Pag sheep	162	31	131	143.28	28.67
EF sheep	195	59	136	289.91	95.67
IAEF sheep	186	53	133	289.03	93.17

Table 1: Continued

Breed	Milking period					
	Milk yield (kg)	Milk yield (kg/day)	Fat (%)	Fat (kg)	Proteins (%)	Proteins (kg)
Istrian sheep	127.18	0.95	6.99	8.87	5.96	7.58
Pag sheep	114.61	0.87	7.73	8.78	6.19	7.07
EF sheep	194.25	1.42	5.87	11.29	5.09	9.87
IAEF sheep	195.85	1.47	6.16	11.91	5.09	9.75

Source: Croatian Livestock Centre, 2006.

3. Results and discussion

The considerable differences in the values of lactation milk yield between certain sheep genotypes were determined (Table 1). The EF sheep and the IAEF had the highest average lactation milk yield. EF sheep had the longest average lactation period which is in accordance with results of earlier research [5]. Pag sheep had the lowest lactation milk yield and with the highest average content of milkfat and proteins. The highest average daily milk yield during milking period was determined in IAEF and EF ewes (1.47 and 1.42 kg, respectively) The Pag lambs suckled the lowest quantity of milk during the suckling period, while the EF and IAEF lambs suckled the greatest quantity of milk. However, during the milking period, the crosses produced the highest quantity of milkfat, while the EF sheep produced the highest quantity of proteins.

4. Conclusion

There are considerable differences among the investigated genotypes in the total milk yield in lactation and the chemical composition of the milk, as well as in the protein and fat yield. These results suggest that imported dairy sheep can improve milkability of local sheep breeds as well as they are adaptable as pure breeds to Croatian environmental conditions.

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Published in four separate parts

[Part 2: Posters I-P044 to I-P096](#)

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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