Sveriges lantbruksuniversitet
Fakulteten för veterinärmedicin och husdjursvetenskap

Swedish University of Agricultural Sciences
Faculty of Veterinary Medicine and Animal Science

Determination of relation between α_{S1} casein concentration and coagulation properties of goat milk

Anna Talach

Examensarbete / SLU, Institutionen för husdjurens utfodring och vård, **452**

Uppsala 2013

Degree project / Swedish University of Agricultural Sciences,
Department of Animal Nutrition and Management, **452**

Examensarbete, 30 hp
Masterarbete
Husdjursvetenskap
Degree project, 30 hp
Master Thesis
Animal Science

Sveriges lantbruksuniversitet
Fakulteten för veterinärmedicin och husdjursvetenskap
Institutionen för husdjurens utfodring och vård

Swedish University of Agricultural Sciences
Faculty of Veterinary Medicine and Animal Science
Department of Animal Nutrition and Management

Determination of relation between α_{S1} casein concentration and coagulation properties of goat milk

Anna Talach

Handledare:
Supervisor: Monika Johansson, Department of Food science
Ämnesansvarig:
Subject responsibility:
Examinator:
Examiner: Kjell Holtenius, Department of Animal Nutrition and Management
Omfattning:
Extent: 30 hp
Kurstitel:
Course title: Degree project in Animal Science
Kurskod:
Course code: EX0551
Program:
Programme: Food of Life
Nivå:
Level: Advanced A2E
Utgivningsort:
Place of publication: Uppsala
Utgivningsår:
Year of publication: 2013
Serienamn, delnr: Examensarbete / Sveriges lantbruksuniversitet, Institutionen för husdjurens utfodring och vård, 452
Series name, part No:
On-line publicering: http://epsilon.slu.se
On-line published:
Nyckelord:
Key words: Goat milk, α_{S1} casein, coagulation

Acknowledgements

I would like to express my appreciation to all those who provided me with the help and assistance during my thesis work. A special gratitude I give to my supervisor Monika Johansson, but also Madeleine Högberg, Anders Adrén, Calle Brunius, Ulf Olofsson, Åse Lundh, Marie Backrud Ivgren, Peter Huaxing Wu, Karl-Gustav Jonahnsson, my parents, whose contribution, stimulating suggestions and encouragement, helped me in coordinating and writing of my thesis.

Abbreviations

CE	*Capillary Electrophoresis*
CF	*Curd Firmness*
CN	*Casein*
CMP	*Caseinomacropeptide*
CSN1S1	*Alpha S1 casein gene*
CT	*Coagulation Time*
DM	*Dry Matter*
G_{20}	*Gel firmness after 20 minutes of initiation of coagulation*
IMCU	*International Milk Clotting Units*
TS	*Total Solids*

Abstract

Most abundant goat milk proteins are caseins. Genetic protein polymorphism of α_{S1} casein is believed to affect cheese yield and production profitability of the cheese-makers. This thesis work aimed to investigate the relationship between concentration of α_{s1} casein and coagulation properties of goat milk with the use of rheological studies.

Milk samples from 58 Swedish Landrace goats from two herds were analyzed in terms of milk composition, α_{S1} casein concentration and coagulation properties. It was found that most prevalent variants were of goats with low (43%) and medium (34%) α_{S1} casein concentration, while high concentration of α_{S1} casein was noted only in 23% of goats.

It was observed that the concentration of α_{S1} casein in caprine milk influences its coagulation properties. Goats with low α_{S1} casein concentration in milk were characterized by weaker gel firmness, compared to medium and high concentration group and had longer coagulation time. Coagulation time was strongly associated with the pH value of the milk. Moreover, milks with higher levels of α_{S1} casein were characterized by a lower pH than groups with medium and low level of α_{S1} casein concentration.

Table of Contents

1. Introduction

1.1. General background

Goats are among the oldest domesticated animals and their milk has been consumed by humans for thousands of years. Goat herding tradition dates back to 8000 BC to middle-east region. Evidence of goat cheese making has been found in in Egypt, where traces of cheese pots were discovered in Pharaohs burial tombs.

Nowadays, total goat milk production on a global scale reaches 15,510,411 tons per year. In the last 20 years total goat population has increased by 20% and according to estimates world goat flock is the third largest after cattle and sheep (FAO, 2011; Haenlein, 2004). Goat milk consumption is predominant especially in developing countries, which stand for 80% of global goat milk production, where goat milk products are an important source of nutrients and are an important part of staple diet. Nonetheless, in recent years, developed countries showed a notable growth of goat dairy farming. This tendency is determined by the reluctance of urban consumers to industrialized agriculture and an increasing interest in ecological production (Dubeuf, 2005; Morand-Fehr *et al*., 2004). In Europe majority of goat milk is turned into cheese and caprine products are perceived as a high-end commodity. This is especially observed in countries such as France and Italy where goat cheeses are highly valued and priced more than cheeses from any other species. In southern Europe goat dairy farms have turned to a commercialized production and stand for a significant part of the dairy industry (Silanikove *et al*., 2010; Morand-Fehr et al., 2004).
In Scandinavia there is a growing interest in locally made goat milk products and the production is likely to increase in the years to come which is supported by the fact that the number of goats in Sweden in the last decade nearly tripled and currently, there are approximately 11650 goats (Jordbruksverket, 2012).

Sweden, unlike Mediterrean countries, has four goat breeds: Jämt goat, Göinge goat, Lapp goat and Swedish Landrace. The first three mentioned breeds are very rare and it is only Swedish Landrace *(Capra hircus)* that is popularized. *Svensk Lantras* is a medium size breed found in Sweden and Norway, used primarily for the small scale local milk and cheese production (Mason, 1996).

1.2. Properties of goat milk

Milk is a complex and diversified aqueous solution of proteins, carbohydrates, lipids, vitamins and minerals (Mastawet *et al.,* 2013). Goat milk is not only an excellent source of nutrients but it is also associated with various functional properties (Haenlein *et al*., 2004). An overview of the comparison between goat and other species milk composition is presented in the *Table 1.*

Goat milk has very good digestibility which might be justified by its slightly lower concentration of lactose and that fat droplets in caprine milk are of a smaller size and more homogenized than compared with milk from other species. Aforementioned characteristics also influence cheese making properties (Morand-Fehr *et al.*, 2004). As opposed to bovine milk, goat milk is a richer source of monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) and medium chain triglycerides (MCT), but lower in content of *trans* 18:1 fatty acids. Such a composition has number of human health benefits (Kumar *et al.,* 2012).

As far as the sensory parameters are concerned, caprine milk products and cheeses, compared to bovine cheeses are characterized by a distinct 'goaty' flavour, because of higher concentration of medium-chain fatty acids, in particular of caproic, caprylic and capric acid. Due to a lower content of β-carotene, caprine milk has a specific colour compared to bovine milk, which leads to obtaining cheeses with a brighter colour (Kumar *et al.,* 2012; Silanikove *et al*., 2010; Quéré *et al.,* 1998).

Table 1. Approximate average milk composition of different species (% w/w) adapted from Walstra *et al*., 2006

Animal	Species	Dry Matter	Carbohydrates	Casein	Serum protein	Fat	Ash
Cow	*Bos taurus*	12,7	4,6	2,6	0,6	3,9	0,7
Goat	*Capra hircus*	13,3	4,3	3,0	0,6	4,5	0,8
Buffalo	*Bubalus bubalis*	17,5	5,4	3,6	0,7	4,8	0,8
Sheep	*Ovis aries*	18,8	4,6	4,6	1,0	7,5	1,0
Reindeer	*Rangifer tarandus*	35,0	2,6	8,5	2,0	18,0	1,5
Zebu	*Bos indicus*	13,5	4,9	2,6	0,6	4,7	0,7
Yak	*Bos grunnies*	17,7	4,6	5,5	0,6	6,7	0,9
Donkey	*Equus asinus*	10,8	6,7	1,0	1,0	1,5	0,5
Horse	*Equus caballus*	10,8	6,0	1,3	1,2	1,7	0,5
Camel	*Camelus dromedarius*	13,4	4,5	2,7	0,9	4,5	0,8

One of the most valuable goat milk components is protein. In caprine milk, as in other domesticated ruminants, casein accounts for approximately 80% of total proteins, while whey proteins stand for the remaining 20% (Ambrosili *et al*., 1988). Those fractions can be further divided into: caseins consisting of α_{S1}-casein, α_{S2}–casein, β-casein, κ-casein and whey proteins that comprise of α-lactoalbumin and β-lactoalbumin. There is no distinct difference between caprine and bovine protein type, but it is the content and proportion of casein fractions that is at variance. Goat milk has similar amount of κ-casein and α_{S2}-casein, but higher content of β-casein and lower level of α_{S1}-casein opposed to bovine milk (Clark & Shebron, 2000a; Martin *et al*., 2002). *Table 2.* shows a comparison between composition of protein fractions in caprine and bovine milk.

Table 2. Composition of protein fraction in caprine and bovine milk (g/l) adapted based on Greppi *et al*., 2008

	Caprine milk	Bovine milk
Total proteins	28-32	32-34
Caseins	22-28	26-37
α_{S1}- casein	10	11-15
α_{S2} -casein	3	3-4
β-casein	11	9-11
κ-casein	4	2-4
Whey proteins	5,5-5,6	5,8-6,5
α-Lactoalbumin	1.2	0,6-1,5
β-Lactoalbumin	3,1	3-4
Serum albumin	0,5	0,4
Immunoglobulin	1,0	1,0
Lactoferrin	0,02-0,2	0,1

Typically, in regular milk at least 95 % of caseins are organized in porous structures called micelles. Apart from caseins, micelles are composed of citrate, ions, plasmin, milk serum and water. Despite the ongoing debate of casein micelle organization, so far it has been established that casein micelles are formed by aggregation of α_S- and β-casein fractions that are bound with phosphate bridges and stabilized by κ-casein. Any destabilization of the outer casein layer caused by reduction of pH or enzymatic activity leads to precipitation or aggregation of casein micelles (Mastawet *et al*., 2013).

Caprine casein micelles contain more calcium, inorganic phosphorus and are characterized by a bigger size ranging between 50-320 nm and peak of distribution at 225-294nm, compared to bovine micelle size of 35-400 nm and peak of distribution at 120-200nm (Mastawet *et al*., 2013; Silanikove *et al*., 2010).

Both quantitative and qualitative content of protein fractions is dependent on a range of environmental, nutritional, physiological (stage of lactation) and most importantly genetic (breed, genealogy) factors. All of these parameters also play an important role in cheese making and manufacture (Greppi *et al.,* 2008; Zullo *et al.,* 2005).

1.3 Genetic polymorphism of CSN1S1 locus

Genetic polymorphism can be described as an occurrence in a population of two or more possible nucleotides at a specific position in genome, which may result in quantitative discrepancy in gene product (Hallén, 2008). It was not until early 1980's when a research group of Boulanger *et al.,* (1984), has found that *CSN1S1* gene is polymorphic which leads to existence of multiple variants of α_{S1}-casein fraction. This discovery was a breakthrough in studies about protein genetic polymorphism that has opened the way for a research to find applications in dairy industry (Maga *et al.,* 2009; Cebo *et al.,* 2012; Feglini et al., 2005).

Initially seven variants of *CSN1S1* gene were identified, some of which were associated with synthesis level of α_{S1} casein in caprine milk. These variants were classified from the highest to the lowest expression type as A, B, C, D, E, F and O. shown in the Table 3.

Table 3. Polymorphism of *CSN1S1* based on Grosclaude & Martin, 1997

α_{S1} CN alleles	Type of α_{S1} casein expression	Approximate amount of α_{S1}-CN per allele (g/l)
A	Strong	3,6
B	Strong	3,6
C	Strong	3,6
E	Medium	1,6
F	Low	0,6
D	Low	0,6
O	Null	-

Further research proved the existence of even more genetic variants and numerous possible allelic combinations of *CSN1S1* gene. At present, 18 caprine *CSN1S1* allelic variants are known. They are linked with high (A, B1, B2, B3, B4, B*'*, C, H, L, M), medium (E and I), low (D, F, G) and null (01, 02, N) content of α_{S1} casein (Caravaca *et al.,* 2011; Roncada *et al.,* 2002).

Numerous studies aimed to evaluate how different genetic variants influence the functionality of the cheese milk (Maga *et al.,* 2009). It was shown that, in comparison with other genotypes, milk that has a strong genotype of *CSN1S1* locus has most desirable traits in dairy production.

There are indications that 'strong' milks are characterized by lower pH values, higher ion activity and have smaller diameter of casein micelles as opposed to weak and null variants. Furthermore, 'strong' variants have been reported to have this higher calcium content, greater propensity of enzymatic coagulation; thus, shorter activation period of coagulation. However, strong milks have a higher susceptibility to lipolysis and off-flavor formations (Tove *et al.,* 2011; Greppi *et al.,* 2008; Feligini *et al.*, 2005; Remeuf *et al*., 1993).

Positive correlation was drawn between high α_{S1} casein content and the amount of total solids, higher fat and protein content, resulting in alleviated cheese yield and cheese quality. Greater α_{S1} casein concentrations have been associated with faster curdling rate and greater gel firmness, while 'null' variants were linked with restricted or no ability to form curd. Some studies have also reported shorter coagulation time, whereas other noted longer coagulation time of milk with higher α_{S1} casein content (Ambrosoli *et al.,* 1988; Pirisi *et al.*, 1994; Clark & Sherbon, 2000; Cebo *et al.,* 2012; Tove *et al.,* 2011).

The great majority of goats across Europe are predominantly used for cheese manufacture. The presence of the strong variant has been dominant in the majority of economically important breeds, except of Spanish Canaria and Italian Frisa, which are known to have lower α_{S1} casein content. On the contrary, evidence from a Norwegian study, suggest that the most prevailing is the 'null' variant that accounts for nearly 70% of total goat population, which is considered to have a deleterious implications on the cheese production.

Goat population in Sweden has not yet been thoroughly studied, but there are indications that it may show similar tendency as in to the Norwegian case (Tove *et al.,* 2011). Therefore, it is of importance to examine and evaluate the specificity of the goat population in Sweden and possibly undertake actions that may balance the genetic background of Swedish goats and ameliorate their cheese quality.

1.4. Milk coagulation

Milk coagulation is a process of concentration of proteins, fat and mineral salts (Crucio *et al*., 2001). Coagulation properties of milk are considered to be one of the most important cheese making traits. There are two types of coagulation – acid and enzymatic coagulation, which result in obtaining different kinds of curd and have varied applications. Acid coagulation is commonly used in production process of fermented milk products with a liquid consistence, such as yoghurt and fil. Whereas, enzymatic coagulation is required in the cheese making process, as it allows obtaining elastic and firm curd.

1.4.1. Enzyme induced coagulation

Milk coagulation leading to gel formation is one of the most crucial and sensitive stage of production of rennet cheese varieties (Jovanović *et al.,* 2002). Rennet is a mixture of proteolytic enzymes: chymosin and pepsin that are used to induce milk coagulation. Traditionally rennet is obtained from abomasum of calf stomachs; however other ways of its production have been developed, such as fungal or microbial synthesis. Chymosin, such as Chymo Max (Christian Hansen), is typically used in industrial manufacture of cheese (Crabbe, 2000). Rennet induced coagulation can be divided into three phases. During the first, known also as primary phase κ-casein fraction is hydrolyzed into para-κ-casein, combined with the release of hydrophilic caseinomacropeptide (CMP) into whey. This results in destabilization of casein micelle and an increase of its susceptibility for calcium precipitation. The second phase encompasses of initiation of spontaneous aggregation, by cross link formation resulting in creation of three-dimensional network and gel formation the presence of Ca^{2+}. In the last phase, syneresis takes place, which results in expelling of whey from the casein network (Hallen, 2008; Walastra *et al*., 2006; Clark & Shebon, 2000).

Both of the coagulation phases may be affected by various factors, which may ultimately alter properties of the formed gel. Rennet coagulation is inversely correlated with pH, and its particularly susceptible for pH variations in the range between 6,5 - 7,0 (Mastawet *et al*., 2013). Among other important parameters are: type and concentration of enzymes used, heat treatment of milk, coagulation temperature, calcium concentration, total solids content and individual milk components (Jovanović *et al.,*2002; Clark &Shebon, 2000).

There is a strong correlation between the coagulation properties of the cheese milk and cheese yield. Poorly coagulating milk is less suitable for processing and may minimalize cheese yield (Frederiksen *et al.,* 2008).

1.5. Rheology

Rheology can be described as a study of deformation and flow of materials that are subjected to stress or strain. Rheological properties refer to intrinsic characteristics of materials such as elasticity of viscosity and viscoelasticity (O'Callaghan, 2000). Rheometeres are analytical instruments that are able to measure wide scope of properties of different kinds of materials from liquids, through semisolids and solids. The measurement spectrum in dairy science ranges from solid samples such as hard-cheeses, semi- solid creams and liquids such as milk. It can be also applied for observing changes in materials that undergo a shift of consistence, for example in clotting of milk (Herh *et al.,* 2000).

Rheometry is a technique commonly used for examining coagulation properties of milk .With its applications it is possible to measure CT (coagulation time), G' elasticity module and G''. From those parameters it is possible to determinate curd formation rate and curd firmness (Hallen, 2008 ; Frederiksen *et al.,* 2008).

This study will focus on: coagulation time (CT), which can be defined as the time from the enzyme addition until the start of the coagulation, and $G_{20,}$ which is the value of gel firmness measured after 20 minutes from the beginning of coagulation.

2. Objectives

This thesis work is a continuation of previously conducted research at Swedish University of Agricultural Sciences, Uppsala in 2012. In the former study 300 samples of milk from Swedish Landrace goats from Uppland, Sweden were subjected to analysis of α_{S1} casein level and divided into three groups: high, medium and low according to concentration of α_{S1} casein in milk. It was found that approximately 70% the goats had low α_{S1} casein concentration in their milk, which may have a detrimental impact on cheese making properties. While numerous studies focused on examining properties of milk from Mediterranean goat breeds, Swedish Landrace goats have not been an object of an extensive research. Therefore, it appeared to be of importance to also investigate the effect of the mutation of *CSN1S1* gene, leading to low α_{S1} casein concentration on milk coagulation from the Swedish goat breed.

The aim of this thesis work is to determine and evaluate the relation between α_{S1} casein concentration and coagulation properties of goat milk with the use of rheological studies and main focus on coagulation time (CT) and gel firmness (G_{20}).

3. Materials and methods

3.1. Animals

58 goats from Swedish Landrace breed (*Capra hircus*) were randomly selected for the analysis of coagulation properties. Eight lactating goats from the SLU herd in Uppsala and fifty from Löfsta Gårdsmejeri in Vallentuna, Sweden, were included in the experiment. Goats were at a similar age (1-2 years old) and stage of lactation, 50 out of which were selected for analysis. Goats were fed ad.lib with hay, water and mineral licks. Concentrate and oats (0,6-1 kg) was provided daily. Animals that were at the very early stage of lactation, or animals subjected to the antibiotic treatment were not included in the study.

3.2. Milk collection

Milk samples were collected between February and April, 2013. Samples of 50 ml were obtained from the morning milking. At Löfsta gårdsmejeri milk collection took place prior to

milking for cheese production. After collection, samples were kept at the stabilized refrigeration temperature. In order to stop the deterioration process of the milk, bronopol solution (17%, 2µl/ml) was added to samples that were to be analyzed in the scope longer than 48 hours after milk collection.

3.3. Milk analysis

Measurmnet of milk fat, lactose, protein and dry matte of goat milk samples were analyzed with the use Miris Farm Milk Analyser, (Miris AB, Uppsala, Sweden). Analyzes were performed in duplicates at the same day or the day after milk collection.

3.4. Capillary electrophoresis analyses

Capillary electrophoresis (CE) was run on all of the collected milk samples. Before the protein analyses, the milk samples were defatted by centrifugation at 4000 rpm at 4º C for 10 minutes. The relative α_{S1}-casein concentration was counted as a percentage of all detected proteins. The samples were analyzed by CE (G-1600AX, Agilent Technologies Co., SE-164 94, Kista, Sweden), controlled by Chemstation software version A 10.02. (Agilent Technologies). Separation of the proteins was performed as described by Åkerstedt, Wredle, Lam & Johansson (2012), using unfused silica standard capillary column, 50 µM inner diameter, 40 cM active length (Chrom Tech, SE-195 30, Märsta, Sweden).

3.5. Coagulation analysis

Milk samples were centrifuged at 4000 rpm for 10 minutes and defatted. Prior to rheological measurements, samples were heated up to 35° C and maintained at this temperature for a minimal time of 30 minutes and maximum of 2 hours. Measurements of the pH (check which pH meter) value at 35ºC, were carried out before analysis of each sample.

Coagulation was induced by the addition of enzyme Chymosin Ultra Christian Hansen® diluted to 1:10 and added at the concentration of 75 IMCU. Each sample was subsequently vortexed for 5 seconds before being poured to the bob. Coagulation time was measured from the point of the enzyme addition and was determined based on a time of reaching the 1 Pa value. G_{20} was determined based on G' at 1200 seconds from the beginning of measurement.

Coagulation properties including rennet coagulation time and gel-strength were investigated with the use of Bohlin CVOR-150-900 rheometer (Malverin Instruments), Figure 1. Rheometer was set to osciallation mode. Measurments of G' and G'' were conducted continuously for test time 1320-1800s, angular frequency 1 rad/s and integration time 6.283, strain 0.01. Rheometer was calibrated before each day of the measurements.

*Figure 1.*Bohlin CVOR Rheometer

3.6. Statistical analysis

Effects of the variables on coagulation time and gel firmness were statistically analyzed with use of SAS (SAS institute, Inc, Cary, USA) and ANOVA software using MIXED and GML procedure. Values of α_{S1} casein concentration were transformed into their natural logarithms (ln) in order to improve their linear relationship. Statistical analysis was initially performed with the use of division into different 3 groups (low, medium and high) of α_{S1} casein concentration. However, it was observed that more reliable results will be obtained when the division into groups is eliminated. Therefore, in order to obtain most credible results, statistical analysis was conducted twice, with group division and without. The significance level was set as p= 0,005.

4. Results

4.1. Milk composition

Results of conducted measurement of milk composition are presented in Table 4. As milk composition was not the main focus of this thesis work, these data is included in the thesis for informative purposes. Statistical analysis was not conducted on data below.

Table 4. Comparison of average milk composition from group with low, medium and high α_{S1} casein concentration.

TYPE	N	Fat (%)	Protein (%)	Lactose (%)	TS (%)
Low	27	3,85±1,24	2,88±0,32	4,49±0,27	12,01±1,33
Medium	20	3,40±1,04	3,10±0,38	4,54±0,20	11,82±1,11
High	15	3,45±0,89	3,48±0,30	4,66±0,15	12,30±1,18
Total	62	3,60±1,12	3,09±0,41	4,55±0,23	12,01±1,23

±Standard error is indicated, TS- Total solids

4.2. Capillary electrophoresis

The concentration of α_{S1} casein was estimated based on eletropherograms from capillary electrophoresis. Division into three groups (Low, Medium and High) of α_{S1} casein according to the concentration was calculated as a percentage of total protein detected. Figures 3 and 4 illustrate the representative results of the capillary electrophoresis for goats with low- and high amount of α_{S1} casein respectively.

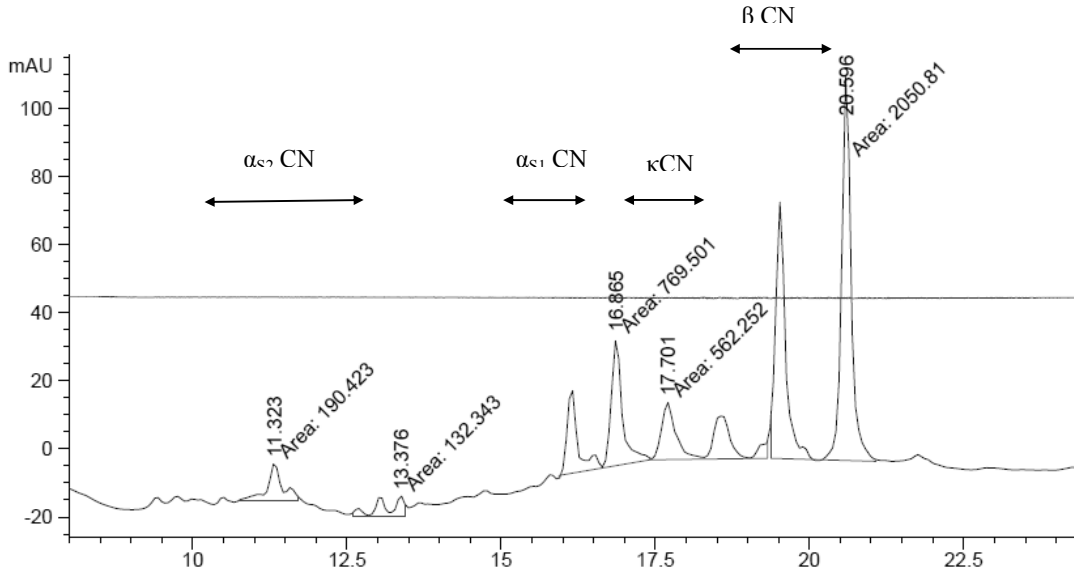

Figure 2. Capillary electrophoresis electropherogram for goat with high with α_{S1} casein concentration. Identified caseins are indicated: α_{S1}- CN, α_{S2}- CN, β-CN, κ-CN in the figure.

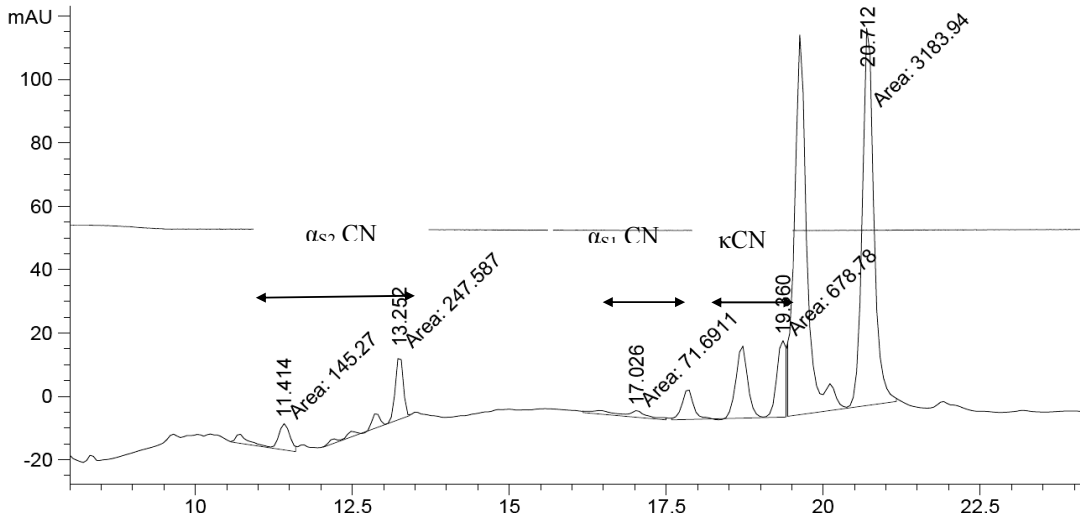

Figure 3. Capillary electrophoresis results for goat with low α_{S1} casein concentration. Identified caseins are indicated: α_{S1}- CN, α_{S2}- CN, β-CN, κ-CN in the figure.

With the use of the capillary electrophoresis results, discrimination into three groups of α_{S1} casein concentration was done. Group division was based on the classification from the previously conducted study and was as follows in Table 5. :

Table 5. Group division based on α_{S1} casein concentration

Type	Concentration of α_{S1} casein in total protein
Low	0-6,9%
Medium	7-14,9%
High	>15%

It was shown that goats with low α_{S1} casein concentration in milk represent 43% of the analyzed population, while medium 34% and high 43% stand for the remaining part. Figure 4. shows the distribution of group size according to α_{S1} casein concentration. It is apparent, that most prevalent variants are those with low and medium α_{S1} casein concentration.

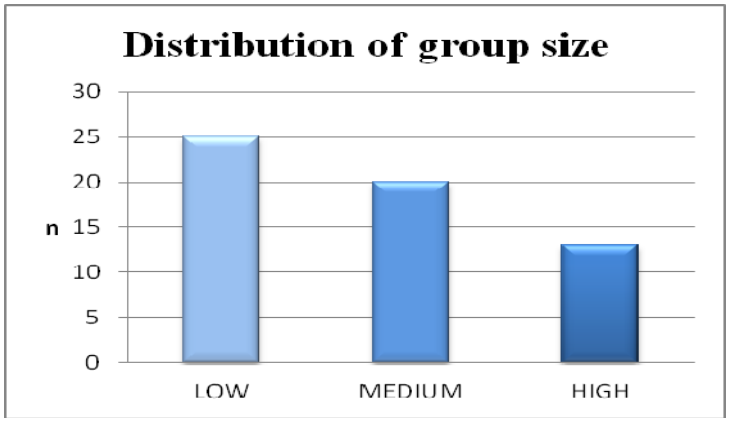

Figure 4. Distribution of a group size according to α_{S1} casein concentration

4.3 Coagulation results

Coagulation time is the time of reaching 1 Pa value for the elastic modulus G' from the rennet addition to the milk sample. Notwithstanding the uniform treatment of the samples coagulation time proved to be very highly variable parameter, it ranged from 511 to 1987 seconds. Figure 5. illustrates the difference between the coagulation curve for a high and low a_{S1} casein concentration representatives. Distinct differences for the gel firmness can be observed after 1200 seconds (Figure 5). Milk with lower α_{S1} casein concentration showed significantly lower values of gel firmness, compared to the milk with high α_{S1} casein concentration.

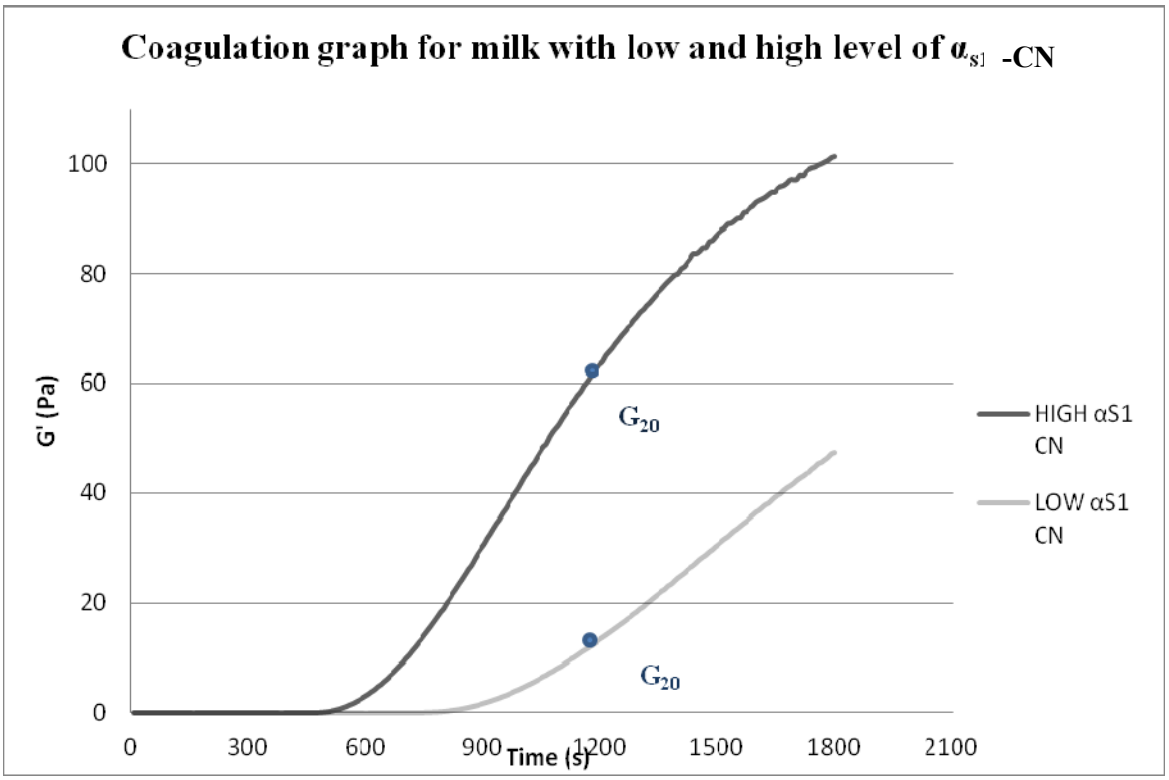

Figure 5. Coagulation time for milk with low and high level of α_{S1} casein. G_{20} - gel firmness after 20 minutes of enzyme addition, G'- elasticity module, CN-casein.

4.5. Statistical analysis

4.5.1. Statistical analysis with group division

Groups with low, medium and high α_{S1} casein concentration were analyzed and compared between each other with statistical analysis. It was found that coagulation time was on average the longest in the group with low α_{S1}casein concentration and shortest in group with high a_{S1} casein concentration. Group with low α_{S1} casein concentration had the highest mean pH value, while group with high α_{S1} casein concentration had lowest mean pH value. However, neither of those parameters had a significance level greater than p<0,005. Comparing these results, it can be seen that gel firmness was distinctly higher in group with high concentration of α_{S1} casein with the level of significance p= 0,0018.

Table 6. Average coagulation time, G_{20} and pH. Group with low (1), medium (2), high (3) α_{S1} casein concentration are indicated

Variable	1	2	3	p
Coagulation time	905,2 ±49,32	779,9±55,43	766,5±67,01	0,1437
G_{20}	$18,09^{a}$±4,055	$28,13^{a}$±4,534	$44,13^{b}$±5,623	0,0018
pH	6,56±0,017	6,52±00,019	6,51±0,023	0,2422

±SE Standard error, G_{20}- gel firmness after 20 minutes of enzyme addition

When coagulation time was correlated with casein concentration, pH and interaction between α_{S1} casein and pH (Table 7), it was shown that only pH has a significant impact on coagulation time (p=0,0005).

Table 7. Coagulation time versus α_{S1} casein concentration, pH and interaction between α_{S1} casein and pH

Solution for Fixed Effects					
Effect	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept	-15314	4405,31	52	-3.48	0,0010
α_{S1} casein	3080,08	2196,55	52	1.40	0,1668
pH	2478,48	671,37	52	3.69	0,0005
α_{S1} casein * pH	-476.79	335,71	52	-1.42	0,1615

In contrast to coagulation time, the results of statistical analysis showed that both α_{S1}casein content (p=0,0120) and interaction between α_{S1}casein and pH (p= 0,0134) have a significant influence on G_{20} – gel firmness (Table 8.). The impact of pH of milk on gel firmness was shown not to be significant (p=0,3416).

Table 8. Gel firmness versus α_{S1} casein concentration, pH and interaction between α_{S1} casein and pH

Solution for Fixed Effects					
Effect	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept	331,60	334,18	54	0,99	0,3255
α_{S1} casein	433,04	166,57	54	2,60	0,0120
pH	-48,8696	50,9311	54	-0,96	0,3416
α_{S1} casein *pH	-65,0786	25,4574	54	-2,56	0,0134

To determine dependence between α_{S1} casein concentration, pH, gel firmness and coagulation time Pearson and Spearman correlation efficiencies were calculated and compared (Table 9.). The closer the R value to 1, the more correlated are parameters.

Table 9. Correlation between α_{S1} casein concentration, coagulation time, pH and G_{20} gel firmness, according to Pearson (above diagonal) and Spearman (below diagonal).

	α_{S1} casein	Coagulation time	pH	G_{20}
α_{S1} casein		-0,29 *	-0,26**	0,43***
Coagulation time	-0,24 *		0,58***	-0,67***
pH	-0,27*	0,61***		-0,68***
G_{20}	0,42***	-0,83***	-0,71***	

a) Correlation performed on logarithmic transfer of α_{S1} casein concentration
b) Significance levels at *p< 0,10, **p < 0,05***p<0,001 are indicated

4.5.2 Statistical analysis without group division

Statistical analysis was also conducted without group division of goats to low, medium and high α_{S1} casein concentration. When the group division was eliminated significant correlation (p=0, 0455) between concentration between of α_{S1} casein concentration and coagulation time was shown (Figure 6).

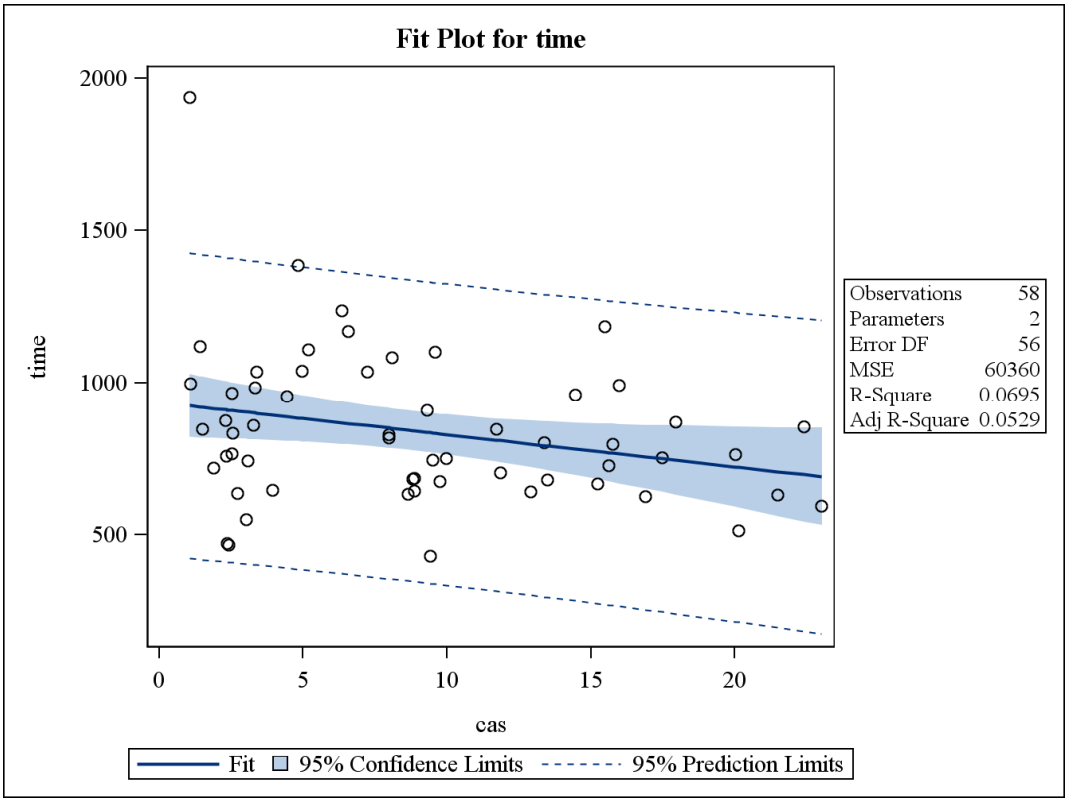

Figure 6. Relation between coagulation time and α_{S1} casein concentration of milk

Also a significant (p< 0,001) positive correlation between coagulation time and pH was shown (Figure 7).

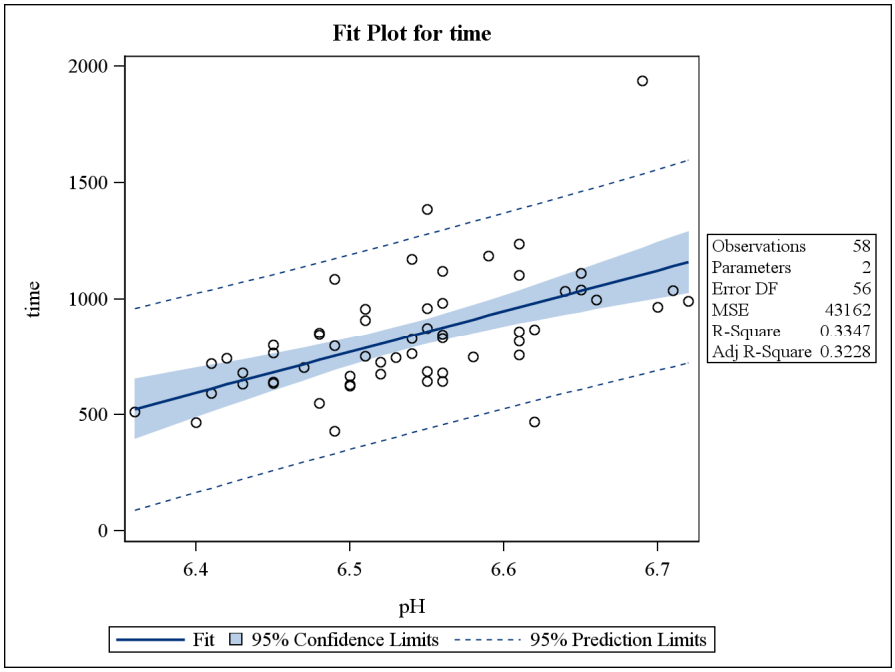

Figure 7. Relation between coagulation time and pH

Furthermore, significant correlation between gel firmness and the level of α_{S1} casein concentration ($p < 0{,}001$) was found. Correlation has a linear character, the higher α_{S1} casein content, the greater gel firmness and better quality of gel is formed (Figure 8).

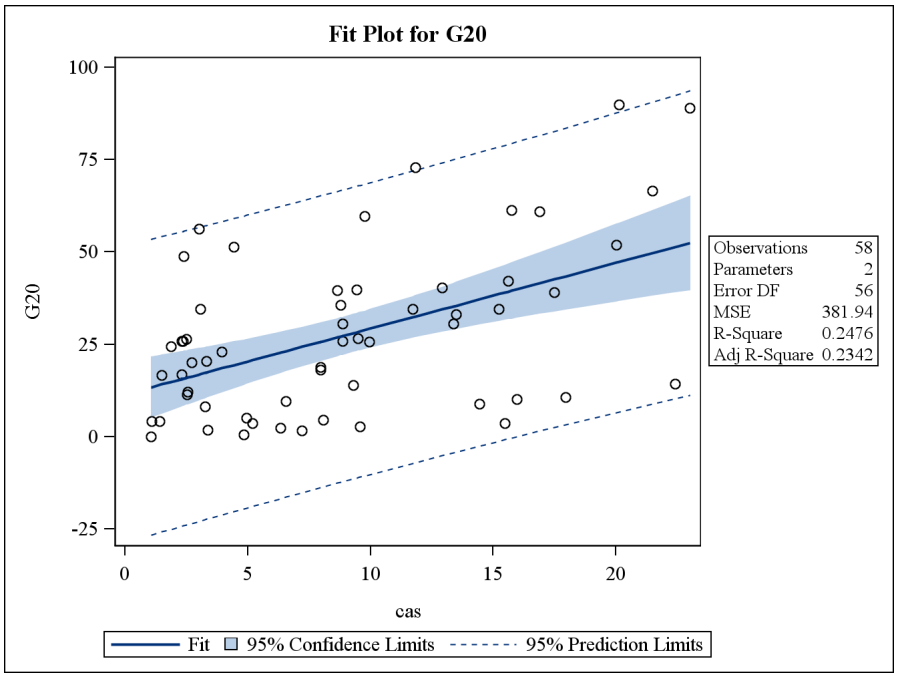

Figure 8. Relation between gel firmness (G_{20}) and α_{S1} casein concentration

5. Discussion

The results of identification of α_{S1} casein among analyzed Swedish Landrace goats showed high incidence of animals with low α_{S1} casein concentration (43 %), followed by medium (34%), and high α_{S1} casein concentration (23%). That proved that the variants with low and medium α_{S1} casein concentration are prevalent and may account for > 70%. Those results are in accordance with the outcome of previous research at SLU, Sweden in 2012 that analyzed goat milk samples from various farms in terms of α_{S1} casein concentration and discovered high incidence of low α_{S1} casein concentration variant. It also bears similarity to studies conducted in Norway by Devold *et al.,* 2011, who found very high frequencies of the null variant, present in 70% of the population. Even though, the occurrence of null variant is not as high as in Norwegian goats, still the incidence of variants with high α_{S1} casein concentration is lower than in southern European breeds.

The findings of the current study regarding the coagulation properties are consistent with those of Clark and Sherbon (2000a, 2000b) who found that high level of α_{S1} casein can be associated with better coagulation properties, better gel firmness and quality of formed coagula.

The influence of α_{S1} casein on coagulation time has been a point of a debate in the literature. Unlike, Ambrosili *et al.,* 1988 who reported that the coagulation time for milk with high α_{S1} concentration was elongated compared to milk with low α_{S1} concentration, this work found that coagulation time of for milk samples with high α_{S1} casein concentration was shorter.

Divergence between results regarding the influence of α_{S1} casein concentration on coagulation time can be possibly explained by the different ways of experimental design. In the first case, where the goats were looked at as three different groups that were compared with each other no significant differences in terms of α_{S1} casein and coagulation time were observed. When division into groups was eliminated and overall amount of α_{S1} casein in all analyzed goats was taken into consideration, the results showed a significant correlation between those parameters. This leads to the conclusion that in order to obtain more precise results of statistical analysis, the group sizes need to be bigger.

The results of conducted study indicate that goat milk composition and coagulation properties are dependent on the genetic factors. This signifies that there is a possibility of improvement

milk cheese quality of Swedish dairy goats by introducing suitable breeding strategies. It would be most beneficial from the economical point of view, since the higher total solids content the higher the cheese yield.

6. Conclusion

From the findings of the study it was concluded that the coagulation properties of goat milk depend on α_{S1} casein content. If the concentration of α_{S1} casein is high, it is accompanied by lower pH, better gel firmness and shorter coagulation time and may be related higher content of total solids. Therefore, goat milk higher level of α_{S1} casein demonstrates desirable technological properties and is better- suited for cheese production purposes than milk with low α_{S1} casein content.

Goat dairy market has a significant growth potential in Sweden and other Scandinavian countries. Genetic selection for the high α_{S1} casein concentration may be a valuable tool for the local dairy industry and of economic interest for goat cheese producers. Introduction of animal breeding schemes may ultimately lead to improvement of the cheese milk quality and, obtaining higher cheese yield and increasing the profitability of the goat cheese production (Dagnachew *et al*., 2013; Clark & Sherbon, 2000; Schmidely *et al.,* 2002).

7. Future research

While there are numerous studies focusing on properties of goat milk from Mediterranean breeds and its cheese-making traits Swedish Landrace goats have not been an object of an extensive research. Hence, it would be beneficial for Swedish producers and consumers to focus on local goat breed. Further research is needed in order to explore ways of producing superior caprine cheese milk quality from Swedish Landrace breed.

To obtain more reliable results the analysis of *CSN1S1* expression and α_{S1} casein concentration should be conducted on a greater number of individuals, which ought to be accompanied by analysis of milk composition. Additionally, to eliminate pH variation its optimization with the use of buffer, acid or a starter culture should be taken into consideration. It would be also advisable to investigate and measure other factors that may influence the coagulation properties such as Ca^{2+} in relation to α_{S1} casein concentration.

Moreover, it would be of interest to investigate whether other casein fractions may have a greater impact on coagulation time, such as α_{S2} casein, which so far has been identified in 3 allelic variants, but this has not yet been evaluated in terms of coagulation properties (Moioli *et al.,* 1998)

8. References

1. Ambrosoli R. 1988. Content of α_{S1}-casein and Coagulation Properties in Goat Milk. *Journal of Diary Science* 71, 24-28
2. Boulanger A., Grosclaude F., Mahé M.F.1984. Polymorphisme des caséines α_{S1} et α_{S2} de la chèvre (Capra hircus), *Genetic Selection Evolution* 16, 157–176
3. Caravaca F., Ares J.L., Carrizosa J., Urrutia B., Baena F., Jordana J., Badaoui B., Sànchez A., Angiolillo A., Amills M., Serradilla J. M. 2011. Effects of α_{S1}-casein (CSN1S1) and κ-casein (CSN3) genotypes on milk coagulation properties in Murciano-Granadina goats. *Journal of Dairy Research* 78 32–37
4. Cebo C., Lopez C., Henry C., Beauvallet C., Ménard O., Bevilacqua C., Bouvier F., Caillat H., Martin P. 2012. Goat α(S1)-casein genotype affects milk fat globule physicochemical properties and the composition of the milk fat globule membrane. *Journal of Dairy Science* 95, 6215-29.
5. Clark S., Sherbon J.W.2000a. Alpha$_{S1}$-casein, milk composition and coagulation properties of goat milk. *Small Ruminant Research* 38, 123-134
6. Clark S., Sherbon J.W. 2000b. Genetic variants of alpha$_{S1}$-CN in goat milk: breed distribution and associations with milk composition and coagulation properties. *Small Ruminant Research* 38, 135-143
7. Crabbe M.J.C., 2000. Rennets: General and molecular aspects. *In* Fox F. Patrick, McSweeney P.L.H., Guinee P., *Cheese. Chemistry, Physics and Microbiology*. Vol 1 General Aspects. Third Edition, Elsevier Academic Press, 511-523
8. Crucio S.,Gabriele I., Giordano V., Calabro V., Cindo B., Iorio G. 2001. A rheological approach to the study of concentrated milk clotting. *Rheologica Acta* 40, 154-161
9. Dagnachew B. S., Kohler 1 A., Ådnøy T. 2013. Genetic and environmental information in goat milk. Fourier transform infrared spectra. *Journal of Dairy Science* 96, 1–13
10. Devold, T.G., Nordbo R., Langsrund T., Svenning C., Brovold M.J., Sørensen E.S., Christiansen B., Ådnøy, Veragud G.E.2011. Extreme frequencies of the α_{S1} – casein 'null' variant in milk from Norwegian dairy goats- Implications for milk composition, micellar size and renneting properties. *Dairy Science and Technology* 91,39-51
11. Dubeuf, J.P., 2005. Structural, market and organizational conditions for developing goat dairy production systems. *Small Ruminant Research* 60, 67–74.
12. Feligini M., Frati1 S., Cubric Curik V., Brambilla A., Parma P., Curik I., Greppi G.F., Enne1 G. 2005. Caprine αS1-Casein Polymorphism: Characterisation of A, B, E and F variants by means of various biochemical and molecular techniques. Caprine α_{S1}-casein Polymorphism, *Food Technology and Biotechnolgy* 43 (2), 123–132
13. Frederiksen P.D., Hammershøj M., Nygaard, Andersen P., Bakman M., Larsen L.B. 2008. Coagulation ability of cheese milk determined by a ReoRox4 instrument. Annual Transactions of the Nordic Rheology Society 16
14. Greppi G.F., Roncada P., Fortini R. 2008. Protein Components of Goat's Milk. *Dairy goats feeding and nutrition.* CAB International, Oxfordshire, UK ,71-94
15. Grosclaude F., Martin P. 1997. Casein polymorphism in the goat. International Dairy Federation. Proceedings of the IDF seminar. In Milk protein polymorphism. Milk protein polymorphism in other species. Palmerston North, Nouvelle-Zelande, 241-253
16. Haenlein G.F.W. 2004. Goat milk in human nutrition. *Small Ruminant Research* 51, 155-163
17. Hallén E. 2008. Coagulation Properties of Milk Association with Milk Protein Composition and Genetic Polymorphism. Doctoral Thesis Swedish University of Agricultural Sciences ,Uppsala
18. Herh P. K.W., Colo S.M., Roye N., Hedman K. 2000. Rheology of foods: New techniques, capabilities, and instruments. Circle Reader Service.
19. Jovanović S., Maćej O., Djurdjević J. D. 2002. The influence of various factors on milk clotting time. *Journal of Agricultural Sciences* 47, 57-73
20. Kumar S., Kumar B., Kuma R., Kuma S. 2012. Nutritional Features of Goat Milk-A Review. *Indian Journal of Dairy Science* 65
21. Maga E. A., Daftari P., Kültz D. Penedo M. C. T. 2009. Prevalence of α_{S1}-casein genotypes in American dairy goats. *Journal of Animal Science* 87, 3464-3469
22. Martin P., Szymanowska M., Zwierzchowski L., Leroux C. 2002. The impact of genetic polymorphisms on the protein composition of ruminant milks. *Reproduction Nutrition Development* 42, 433–459
23. Mason I.L. 1996. A World Dictionary of Livestock Breeds, Types and Varieties. Fourth Edition. C.A.B International 273

24. Mastawet T.A. 2013. Ethiopian Goat breeds and their potential for high quality milk products. Doctoral thesis. Norwegian University of Life Sciences. Department of Chemistry
25. Moioli B., Pilla F., Tripaldi C. 1998. Detection of milk protein genetic polymorphisms in order to improve dairy traits in sheep and goats: a review. *Small Ruminant Research* 27, 185–195
26. Morand-Fehr P., Boutonnet J.P., Devendra C., Dubeuf J.P., Haenlein G.F.W., Holst P., Mowlemg L., Capote J. 2004. Strategy for goat farming in the 21st century. *Small Ruminant Research* 51, 175-183
27. O'Callaghan, Guinee T.P. 2000. Rheology and Texture of Cheese. In Fox F. Patrick, McSweeney P.L.H., Guinee P. *Cheese. Chemistry, Physics and Microbiology*. Vol. 1 General Aspects. Third Edition, 511-523 Elsevier Academic Press
28. Pirisi A., Colin O., Laurent F., Scher J., Parmentier M. 1994. Comparison of milk composition, cheesemaking properties and textural characteristics of the cheese from two groups of goats with a high or low α_{S1}-casein synthesis. *International Dairy Journal* 4, 329-345
29. Quéré J.L., Pierre A., Riaublanc A., Demaizières D. 1998. Characterization of aroma compounds in the volatile fraction of soft goat cheese during ripening. *Lait* 78, 279-290
30. Roncada P., Gaviraghi A., Liberatori S., Canas B., Bini L., Greppi G.F. 2002. Identification of caseins in goat milk. *Proteomics 2*, 723–726
31. Silanikove N., Leitner G., Merin U., Prosser C.G. 2010. Recent advances in exploiting goat's milk: Quality, safety and production aspects. *Small Ruminant Research* 89, 110–124.
32. Schmidely P., Meschy F., Tessier J., Sauvant D. 2002. Lactation Response and Nitrogen, Calcium, and Phosphorus Utilization of Dairy Goats Differing by the Genotype for α_{S1}-casein in Milk, and Fed Diets Varying in Crude Protein Concentration. *Journal of Dairy Science* 85, 2299–2307.
33. Walstra P., Geurts T.J., Noomen A., Jellema a., Boekel M.A.J.S. 2006. *Dairy technology*. Principals of milk properties and processes. Marcel Dekker, New York.
34. Zullo A., Barone C.M.A., Chianese L., Colatruglio P., Occidente M., Matassino D. 2005. Protein polymorphisms and coagulation properties of Cilentana goat milk. *Small Ruminant Research* 58, 223-230
35. FAO.2011.<http://faostat.fao.org/site/569/default.aspx#ancor>.

Appendix

List of figures

List of tables

I denna serie publiceras examensarbeten (motsvarande 15, 30, 45 eller 60 högskolepoäng) vid Institutionen för husdjurens utfodring och vård, Sveriges lantbruksuniversitet. Institutionens examensarbeten finns publicerade på SLUs hemsida *www.slu.se*.

In this series Degree projects (corresponding 15, 30, 45 or 60 credits) at the Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences, are published. The department's degree projects are published on the SLU website *www.slu.se*.

Sveriges lantbruksuniversitet Fakulteten för veterinärmedicin och husdjursvetenskap Institutionen för husdjurens utfodring och vård Box 7024 750 07 Uppsala Tel. 018/67 10 00 Hemsida: www.slu.se/husdjur-utfodring-vard	*Swedish University of Agricultural Sciences* *Faculty of Veterinary Medicine and Animal Science* *Department of Animal Nutrition and Management* *PO Box 7024* *SE-750 07 Uppsala* *Phone +46 (0) 18 67 10 00* *Homepage: www.slu.se/animal-nutrition-management*