Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Faculty of Natural Resources and Agricultural Sciences
Department of Food Science

Swedish native mountain cattle and their milk proteins

– Conventional compared to pasture operated farming for indoor and outdoor period

Fjällkor och deras mjölkproteiner

– Konventionell drift jämfört med fäboddrift för inne- och uteperiod

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Independent Project in Food Science• Bachelor Project • 15 hec • Ground G2E
Publication / Swedish University of Agricultural Sciences, Department of Food Science, no 451
Uppsala, 2016

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Credits: 15 hec
Level: Ground G2E
Course title: Independent Project in Food Science – Bachelor Project
Course code: EX0669
Programme/education: Agricultural Programme - Food Science

Place of publication: Uppsala
Year of publication: 2016
Title of series: Publication / Swedish University of Agricultural Sciences, Department of Food Science
Serie no: 451
Online publication: http://stud.epsilon.slu.se

Keywords: *WHEY, CASEIN, BOVINE, CAPILLARY ELECTROPHORESIS, COW*

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Abstract

Swedish native mountain cattle are a small breed from northern part of Sweden. They have existed as a breed since before the 1900-century. 1896 it was decided that one feature should be selected: hornless and white with black or brown features. The breed represents 0.3 % of dairy cows in Sweden and they are threatened by extinction. They have a small milk yield compared to other modern cows, but their milk proteins are of big interest.

The purpose of this bachelor study was to analyze the milk proteins from Swedish mountain cattle with emphases on outdoor and indoor period from samples from two different farms. The pasture operated farm had 6 cows and the conventional farm had 11 cows participating in the study.

The method was based on capillary electrophoresis (CE) and a percentage of different proteins in the milk samples was given.

The pasture operated farmed cow's milk had a 1.65-1.99 % unit higher total whey protein content. For total whey protein the difference between indoor and outdoor period was very similar for both farms cow's milk. The conventional farmed cow's milk decreased 3.39 % units from outdoor to indoor period for total casein proteins compared to pasture operated farmed cow's milk with 0.30 % units. Pasture operated farmed cows milk had a 0.23-0.51 % units higher β-CN B and 5.54-10 % units higher β-CN A2, but 3.68-4.41 % units lower β-CN A1 than conventional farmed cows milk. The β-CN B was decreasing 0.16-0.44 % units and β-CN A2 is decreasing 0.87-3.59 % units and β-CN A1 was increasing 0.23-0.96 % units when indoor period was compared to outdoor period. Pasture operated farmed cow's milk had 1 % units higher β-CN B than conventional farmed cow's milk and 2 % unit higher percentage than the control milk.

These differences could have many reasons. It does not seem likely that the differences were due to their feed intake. The cow's milk protein profile has a natural variation during the lactation months. The farms could have different genetic possibilities, since they could come from different blood lines.

Keywords: WHEY, CASEIN, BOVINE, CAPILLARY ELECTROPHORESIS, COW

Sammanfattning

Fjällkon är en liten ras från norra Sverige som har funnits som ras sedan 1800-talet. 1896 beslutades att fjällkon skulle ha ett utseende: ”den vita kostymen”. Det innebar att de skulle vara kulliga och vita med svarta eller bruna tecken. Rasen representerar 0.3% av mjölkkorna i Sverige och är utrotningshotad. De mjölkar ganska lite i jämförelse med andra raser, men deras mjölkproteiner är av stort intresse.

Syftet med arbetet var att analysera mjölkproteinerna från fjällkor med focus på inne och ute period från samlade mjölkprover från två gårdar. Fäboddrift gården hade 6 kor och den konventionella gården hade 11 kor med i studien.

Metoden som användes är baserad på kapillär elektrofores (CE) och ett procenttal av olika proteiner gavs från mjölk provet.

Fäboddrift gårdens kors mjölk hade 1,65–1,99 % enheter högre total vassle protein. For total vassle protein var skillnaden mellan inne och uteperiod väldigt lik för båda gårdar. Den konventionella gårdens komjölk sjönk med 3,39 % enheter från ute period till inne period för totalt kasein och fäboddrift gårdens komjölk med bara 0,30 % enheter. För β-kasein B hade fäboddrift gårdens komjölk högre värden med 0,23-0,51 % enheter och för β-kasein A2 högre med 5,54–10 % enheter än konventionella gårdens komjölk. Konventionella gårdens komjölk hade 3,68–4,41 % enheter högre β-kasein A1 än fäboddrift gårdens komjölk. β-kasein B minskade med 0,16-0,44 % enheter, β-kasein A2 minskade med 0,87-3,59 % enheter och β-kasein A1 ökade med 0,23-0,96 % enheter från ute period till inne period. Fäboddrift hade 1 % enhet högre β-kasein B än konventionella gårdens komjölk som i sin tur har 1 % enhet högre än kontrollen.

Skillnaderna som sågs mellan gårdarna kan ha olika anledningar. Det är inte troligt att skillnaderna berodde på olika foderintag. Kornas protein profil varierar naturligt med laktations månader. Gårdarna kan ha olika genetiska förutsättningar, då korna kan härstamma från olika avels linjer.

Nyckelord: VASSLE, KASEIN, KOR, KAPILLÄR ELEKTROFORES

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Abbreviations

SRB	Swedish red and white cattle
SLB	Swedish lowland cattle
SKB	Swedish hornless cattle
SJB	Swedish jersey cattle
CE	Capillary electrophoresis
CN	Casein
SSC	Somatic cells (cells/ml)
αS-CN	αS-Casein
β-CN	β-Casein
α-LA	α-Lactalbumin
β-LG	β-Lactoglobulin
PRF	“Projekt rädda fjällkon”
WWF	World wildlife fund

1 Introduction

1.1 Background

Milk is produced by female mammals in the mammary glands to feed their off-spring's (Christian 2014). The physical and chemical composition of milk varies depending on species, age, breed, activity level, stage of lactation, intervals between milking and usage of medicine (Christian 2014). Milk is a solution of water, fat, proteins, lactose, vitamins and minerals (Christian 2014; Coultate 2009).

In Sweden, the dairy cow population is dominated by Swedish red cattle (SRB) and Swedish lowland cattle (SLB) (Cattle statistics 2016). Swedish mountain cattle are a bread that belongs to Swedish hornless cattle (SKB) group together with red hornless cattle. Swedish mountain cattle are smaller, approximately 400 kg, compared to the more common breeds with 500-650 kg for SRB and SLB (Alskog *et al.* 1995). SKB represent only 0.3 % of the total number of dairy cows in Sweden. SKB has a milk yield of 5500 kg, with 4.45 % fat and 3.56 % protein. They have a protein and fat content similar to SRB, but with approximately half the milk yield. Swedish jersey cattle (SJB) have a higher fat, protein and milk yield than SKB. SLB has the highest milk yield, but lowest fat and protein content (Table I, see appendix 1; Cattle statistics 2016).

Swedish mountain cattle have existed as a breed since before nineteenth-century. Some had horns and some were hornless (Nilsson 2006). They used to have a big variation of color: black, brown, red, white-yellow, grey, multi-colored, dappled and spots that flout together on the side of the cow. In 1896 it was decided that hornless and white with features of black or brown should be the characteristics of this cow breed (Alskog *et al.* 1995; Nilsson 2006; Hallander 1989). Unfortunately, the selection of white cattle resulted in gonad hyperplasia, which is underdevelopment of female or male genitals. The source for the problem was a recessive gene. The gene was strongly related to the lack of color on the cattle, especially if the ears were white or predominant white (Nilsson 2006; Hallander 1989).

The breed was in 1993 threated by both extinction and inbreeding, because of the small number of cattle, especially purebred. World wildlife fund (WWF) starts project rescue Swedish mountain cattle (PRF) to try to save the breed. It was estimated that 300-500 cattle existed, but most were very close related. The Swedish Board of Agriculture (SJV) took over PRF and the association *Svensk fjällrasavel* was started by the farmers and have made it possible to preserve important lines of sperm from *Nordavel* (previously named NTC) to be used in breeding (Nilsson 2006). They are preserved in *Nordens ark*, together with other Swedish native animals. Swedish mountain cattle are not suited for the modern dairy systems in the way that the dairy companies pay by milk yield, not quality (Nilsson 2006). They are very important to preserve as a breed, but also to preserve them as a functioning dairy cow. The modern breeds have lower protein, compare to Swedish mountain cattle, since the yield has been increasing. It is possible that Swedish native mountain cattle may be a good resource in the future (Nilsson 2006; Ortman 2015). The Swedish mountain cow's milk seems to be preferable when making cheese (Nilsson 2006).

2 Pastures, milk components and purpose

2.1 Pasture operated farming

Pasture operated farming is an old form of farming. The cows are released in the morning to find food and would come home by themselves in the evening to get milked. They find food themselves in the outdoor period and eat hay in the indoor period.

2.2 Conventional farming

Conventional farming is the most common way of farming, following all the Swedish regulations. The cattle can be tied up in base or loose housing barn with milking pit or robot milking system. But when it comes to feed, the choice is up to the farmer and can therefore differ between farms. But common is to feed with grass, silage and feed concentrate in outdoor period and silage and feed concentrate in indoor period.

2.3 Grazing periods

In Sweden the animals have to be pastured and the law regulate the amount of days (Table II, see appendix 1). In northern part of Sweden animals must be greasing 60 days in pasture season (1 April-31 October). Of those 60 days, 30 must be within the period 1 May- 15 September. For middle Sweden, the cattle need to be outside for 90 days in pasture season, and 60 of them has to be within the period 1 May- 15 September. For the southern part of Sweden, the cows have to be outside 120 days in pasture season, whilst 60 days of them must be within the period 1 May- 15 September (Jordbruksverket).

2.4 Milk proteins

The cow milk´s protein fraction is approximately 3-4 % (Christian *et al.* 2014). This fraction consists of 80 % caseins and 20 % whey proteins. The casein proteins involve αS-casein (αS-CN), β-casein (β-CN), κ-casein (κ-CN). The most important whey proteins are α-lactalbumin (α-LA), β-lactoglobulin (β-LG), and immunoglobulins (Christian *et al*. 2014; Coultate 2009). There is a number of different versions of each protein, because of a small difference in amino acid sequence. Because of that it is possible to identify a breed of cow from a sample of its milk by comparing the protein variants proportions. (Coultate 2009).

2.4.1 Whey fraction

Whey proteins are α-lactalbumin, β-lactoglobulin, serum albumin and immunoglobulins. α-Lactalbumin is a co-enzyme in the synthesis of lactose. β-Lactoglobulins exist in milk as a dimer, since it is insoluble in water and therefore bind another α-lactalbumin. In low pH it can form an octamer. But the hydrophobic bonds break when heated (Walstra *et al.* 1999). Whey proteins are heat sensitive and denaturate at 60 ^{o}C. They are also important for fermented products, because during heating they form complex with casein and contribute to the wanted viscosity in yogurt and sour milk (Reivell *et al.* 2014).

2.4.2 Casein fraction

The caseins biological role is to transfer Ca^{2+} from mother to offspring (Reivell *et al.* 2014). Caseins are important for the dairy industry. The casein micelle is built up by αS-CN and β-CN. The κ-CN is present on the surface of the micelle and stabilize it (Christian et al. 2014).

γ-CN is a degradation product of β-CN. αS-CN consists of αS1-CN and αS2-CN. They are both phosphoproteins, but αS2-CN has sulfide bonds. αS-CN and β-CN are phosphoproteins with phosphate groups esterified with serine, which reacts with Ca^{2+}ions. κ-CN is a glycoprotein and protects the Ca^{2+} to not leak out. But κ-CN is an easy target for the rennet enzyme chymosin, and cause κ-CN to lose its protective property and the other caseins lose their Ca^{2+} and form a cheese curd (Walstra *et al.* 1999; Reivell *et al.* 2014)

CN are heat resistant and can tolerated 140 ^{o}C for 4 seconds. When fermented products are made, a starter culture is added which lower the pH, causing the CN-micelles negative charge to neutralize and they cannot repel other CN-micelles anymore. The CN-micelle lose its Ca^{2+} and the CN become a coagel and works as a protein network that build the structure of yogurt and sour milk (Reivell *et al.* 2014).

2.4.2.1 β-CN

Cheese making is an old way of preserving and concentrating the milk (Reivell *et al*. 2014). The protruding hairs of β-CN plays an important role for the colloidal stabilization of flocculation at lower temperature in cheese making (Walstra *et al.* 1999).

Swedish mountain cattle have according to farmers, a milk composition very suitable for cheese production, because only half the milk yield needs to be used to produce the same amount of cheese (Östensson 2013). Because of the combination of protein and fat, Swedish mountain cattle generally have a higher fat content than modern cow breeds (Table I, see appendix 1) they also have a higher percentage of CN B then more modern breeds (Table 1). Since the SKB cows produce half the milk yield as a SLB or SLB (cattle statistics 2016), they would produce the same amount of cheese. But since the SKB is cattle that can live on lean bait compared to the other two breeds, they would be good complement to the modern breeds. This could also be the future way of farming, when less feed would be needed for the cows (Nilsson 2006).

The cow can have four different β-CN alleles: A1, A2, A3 and B (Table 1). The A3 allele is rare, most breeds have A1 and A2, but only Swedish mountain cattle and Swedish red hornless cattle have the B allele (Lien *et al.* 1999).

Table 1. Allele frequencies for β-CN

Locus	**Allele**	**ROK**	**SFR**	**SLB**	**SRB**
	n	31	33	43	39
β-CN	**A1**	48	44	41	40
	A2	47	52	59	60
	A3	0	0	0	0
	B	5	5	0	0

ROK=Swedish red hornless cattle, SFR=Swedish native mountain cattle, SLB=Swedish lowland cattle, SRB=Swedish red and white cattle (Lien *et al*. 1999)

2.5 Purpose of this study

The purpose of this study was to analyze and compare the profile of milk proteins from Swedish mountain cattle and Swedish bulk milk based on modern dairy cow breeds. We emphasized also on outdoor and indoor period for two different farming systems in order to find out what impact the seasonal variation and effects of farming has on milk components from Swedish mountain cattle.

2.6 Purpose of results

The results will be included in a complex survey, where the quality of milk from Swedish mountain cattle is in focus. The work is performed in collaboration with SLU, Department of Animal Breeding & Genetics and *Svensk Fjällrasförening*.

2.6.1 Future work

Statistics on the data regarding correlation and significance will be performed in the future work.

3 Materials and Methods

3.1 Animals and milk sampling

Individual milk samples were obtained from two different farms in Sweden. One farm was pasture operated from Sparreholm (middle region of Sweden) and the second one was conventional farm from Boden (northern region of Sweden). The samples were collected during outdoor period (August and September) and indoor period (October and November). Six and eleven Swedish mountain cows were included from the pasture operated and conventional farm, respectively. The milk samples were preserved by bronopol, in order to inhibit the microbial activity. The samples were stored at -20°C prior to analysis. As a control, low pasteurized, not homogenized 4,3% fat bulk milk (Arla) available in the stores was used in addition to the other milk samples. The control milk was defatted and treated in the same way as the milk from Swedish cattle and analyzed for milk protein profile.

The conventional farming cattle in this study was fed regrowth lay in the day and silage with food concentrate in the night for outdoor period. For indoor period the cattle were fed silage and feed concentrate. Feed concentrate was crushed oat grains and rapeseed cake food concentrate.

The pasture operated farming cattle in this study was fed extended bait in August. Regrowth lay, grass, and birdsfoot trefoil in September. For indoor period (October and November) the cattle was fed hay, grass, birdsfoot trefoil and black medic.

The Swedish mountain cattle in this study for both conventional farming and pasture operated farming used tied up milking. Samples that was used in this study, were collected by farmers from Sparreholm and Boden. The cows calved in different months and was thereby in different lactation month when samples were collected (Table III, see appendix1).

3.2 Sample preparation

Frozen milk samples were thawed and placed in 45°C water bath for 15 minutes. Each sample was vortexed (Vortex-Genie 2, Scientific industries, inc., U.S.) and water bathed for further 15 minutes at the same temperature. Milk (150µL) was transferred by pipette into an Eppendorf safe lock tubes (Eppendorf, Germany) with 350 µL sample buffer (Table 10, see appendix 2) with ditioretinol (DTT) added and vortexed. The samples were incubated in room temperature for at least 1 hour and centrifuged (Himac CT15RE, Hitachi Koki Co., Ltd.) at 1500 rpm at 4°C for 10 minutes. The cream layers were removed with cotton swabs. The samples were filtered by using a syringe with a 45µm nylon membrane filter and transferred into new Eppendorf tubes. The samples, 30 µL, were transferred by pipette into conic vials (Agilent, Kista Sweden) for protein analysis by the capillary electrophoresis (CE) instrument.

3.3 Capillary electrophoresis

Protein separation was performed with a 7100 capillary electrophoresis (CE) system (Agilent Technologies Co. America) as described by Johansson *et al.* (2013). Separations were performed using unfused silica standard capillary, with 50µm inner diameter, and 0.4 m active length (Chrom Tech, Märsta, Sweden). Run buffer (Table 11, see appendix 2) was used. The calculation of relative concentrations of the individual proteins was based on the peak area and expressed as a percentage of the total areas recorded for all peaks in the electropherogram. Chemstation software version A 10.02 was used.

3.4 Milk composition data

Data over the milk components from individual cows during the four months were obtained from *Växa Sverige***.** Milk components included in the investigation were: urea, total fat (%), total protein (%) and somatic cells (SCC) (cells/ml).

3.5 Genotyping

Genotyping of individual cows with emphases on β-CN was done at Department of Animal Breeding and Genetics laboratory at SLU. These results can then be compared with the results of β-CN A1 and A2 presence given from the protein profile.

3.6 Statistics

Statistics of the data has not been performed and values described as higher or lower when compared in result and discussion, has not been examined to be significant.

4 Results

4.1 Milk composition data

The count of somatic cells in the milk for conventional farmed cows (Table 2) in the study was 3.08 x 10^4 cells/ml higher for outdoor period, than from indoor period, the same value in the milk from pasture operated farming cows (Table 3) was 3.5 x 10^4 cells/ml. The protein content was 0.11 % and 0.36 % units higher for indoor period, then for outdoor period for conventional (Table 2) and pasture operated (Table 3) farmed cow's milk, respectively. The fat content was 0.08 % units higher conventional farmed (Table 2) for outdoor than indoor, but 0.75 % units higher for indoor than outdoor for pasture operated farmed cow's milk (Table 3).

For all four periods conventional farmed cows had 9.77 x 10^4 cells/ml higher count of somatic cells in the milk, but 0.53 % units lower in fat and protein content compared to milk from pasture operated farmed cows (Table 4).

Table 2. Conventional farming for outdoor and indoor period

	SCC (x10^4 cells/ml)	**Fat %**	**Protein %**
Outdoor	23.34±4.23	4.14±0.44	3.53±0.28
Indoor	20.26±3.28	4.06±0.41	3.64±0.19

Values are present in means ± standard deviation. Somatic cells count (SCC), n=22

Table 3. Pasture operated farming for outdoor and indoor period

	SCC (x10^4 cells/ml)	**Fat %**	**Protein %**
Outdoor	13.85±3.10	4.25±0.47	3.73±0.52
Indoor	10.35±2.50	5.0±0.42	4.09±0.32

Values are present in means ± standard deviation. Somatic cells count (SCC), n=12 for outdoor period and n=10 for indoor period

Table 4. Conventional farming and pasture operated farming with mean for 4 months including both indoor and outdoor period

	SCC ($x10^4$ cells/ml)	Fat %	Protein %
Conventional farming	21.87±4.07	4.10±0.43	3.58±0.25
Pasture operated farming	12.10±3.28	4.63±0.58	3.91±0.46

Values are present in means ± standard deviation. Somatic cells count (SCC), n=44 for conventional farming and n=22 for pasture operated farming

4.2 Genotype data

The individual cows were previously genotyped with emphases on β-CN variants A1 and A2. The genetic variants of the cows were compiled with the findings about the protein profile obtained from CE.

For the pasture operated farming, all cows were gene sequenced for the β-CN. Three cows had genes for both β-CN A1 and β-CN A2. Three cows had genes only for β-CN A2. The result from gene sequencing was confirmed by the protein profile for the β-CN (Table 5).

Table 5. Cow genotype and protein content of β-caseins A1 and A2 of pasture operated farm. The protein content (%) in mean for 4 months

Cow ID	Genotype	Protein profile	
		β-Casein A1 (%)	**β-Casein A2 (%)**
69	A1/A2	21.57	21.45
151	A1/A2	19.44	20.44
158	A2	0	38.15
159	A2	0	36.62
160	A2	0	40.93
284	A1/A2	19.17	19.97

For the conventional farming, eight cows were gene sequenced for the β-CN (Table 6). Five cows had only genotype for β-CN A2 and three cows had gene type for both β-CN A1 and A2. For six cows, the expression of β-CN A2 was dominated. However, one individual (292) indicate presence of the both β-CN variants despite only presence of A2 genotype. The gene sequencing was not performed for cow 599, 650 and 735.

Table 6. Cow genotype and protein content of β-caseins A1 and A2 of conventional farm. The protein content (%) in mean for 4 months

Cow ID	**Genotype**	**Protein profile**	
		β-Casein A1 (%)	**β-Casein A2 (%)**
7	A2	4.96	31.35
292	A2	19.94	20.25
599	ND	18.67	19.07
600	A2	4.50	31.82
644	A1/A2	15.06	24.34
650	ND	19.12	21.61
654	A2	5.13	33.67
655	A1/A2	15.41	24.38
656	A1/ A2	18.13	18.94
665	A2	0	37.27
735	ND	16.89	20.62

ND = not done

4.3 Protein profile data

4.3.1 Whey protein

For conventional farmed cows the total whey protein content was 10.66 % in outdoor period compared to 10.88% in indoor period (Figure 1). For pasture operated farmed cow's whey protein content was 12.31% for outdoor period and 12.87% for indoor period. Conventional farmed cows had a total whey protein content that was 0.2% units higher for indoor period compared to outdoor period. The total whey protein content from pasture operated farmed cows was 0.56% units higher for the indoor period compared to the outdoor period. In pasture operated farmed cows the milk content was 1.65% and 2% units higher than conventional farmed cow's milk for outdoor and indoor period, respectively. Total whey protein content for the control milk sample was 12.53 %. The control was 1.87 % units higher in whey protein content than outdoor conventional farmed cow's milk samples and 1.65% units higher than indoor. The control was 0.22 % units higher than pasture operated farmed cow's milk samples for the outdoor period, but 0.34 % units lower than indoor. Pasture operated farmed cows had a whey protein content that was higher compared to conventional farmed cow's milk for both indoor and outdoor periods. Figure with whey protein for all four months can be found in Figure I, appendix 3.

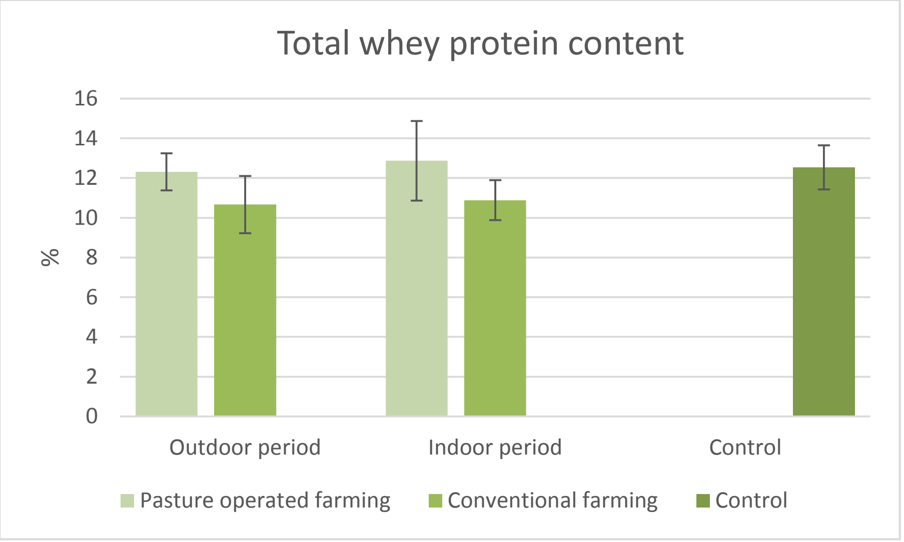

Figure 1. Total whey proteins for different periods for different farming systems and bulk milk (control) from modern cows. The bars indicate standard deviation.

4.3.2 Casein protein

For conventional farmed cow's milk, the total CN content was 83.82 % in outdoor period compared to 80.43 % in indoor period (Figure 2). For pasture operated farmed cow's milk the CN protein content was 80.68 % for outdoor period and 80.38 % for indoor period. In milk from the conventional farming the total CN decrease with 3.39% units from outdoor period to indoor period. Pasture operated farmed cow's milk decreased in casein with 0.30% units from outdoor period to indoor period. Both conventional and pasture operated farming result in decrease in CN protein during indoors compared to outdoor period. Only 0.05% difference for indoor period for conventional farmed cows compared to pasture operated farmed cows was observed. Total CN for control milk sample was 83.28 %. The control was 0.54% lower than conventional farmed cow's milk for outdoor period, but 2.85% higher than indoor period. The control was 2.6 % units higher for outdoor period and 2.9% units higher than indoor period compared to pasture operated farmed cow's milk. Figure with casein protein for all four months can be found in Figure II, appendix 3.

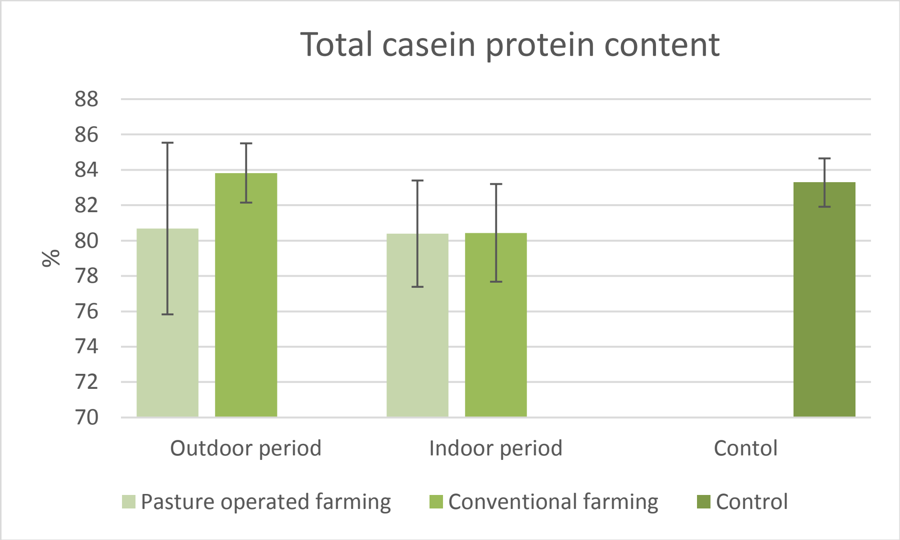

Figure 2. Total casein proteins for different periods for different farming systems and bulk milk (control) from modern cows. The bars indicate standard deviation.

4.3.2.1 β-CN

The β-CN B, A1 and A2 for pasture operated farmed cow's milk for outdoor period was 4.72 %, 8.36 % and 31.73 %, respectively (Figure 3). The β-CN B, A1 and A2 for conventional farmed cow's milk for outdoor period was 4.21 %, 12.04 % and 26.19 %, respectively. Pasture operated farmed cows had 0.51% units higher β-CN B compared with conventional for outdoor period. Conventional farmed cows had 3.68% units higher β-CN A1 than pasture operated farmed cows for outdoor period. Pasture operated farmed cows had 5.54% higher β-CN A2 than conventional farmed cows for outdoor period.

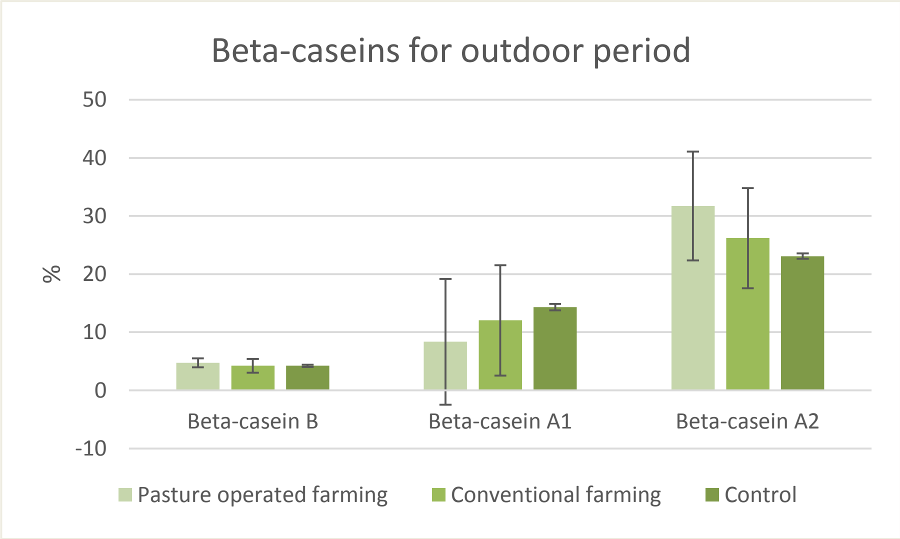

Figure 3. Comparison of β-caseins in milk from Swedish mountain cattle for outdoor period and bulk milk (control) from modern cows. The bars indicate standard deviation.

The β-CN B, A1 and A2 for pasture operated farmed cow´s milk for indoor period was 4.28 %, 8.59 % and 35.32 % units, respectively (Figure 4). The β-CN B, A1 and A2 for conventional farmed cow´s milk for indoor period was 4.05 %, 13.00 % and 25.32 % units, respectively. Pastured operated farmed cows had 0.23 % higher β-CN B content compared to conventional farmed cows for indoor period. Conventional farmed cows had 4.41 % units higher β-CN A1 compared to pasture operated farmed cows for indoor period. Pasture operated farmed cows had 10 % higher β-CN A2 compared with conventional farmed cows for indoor period.

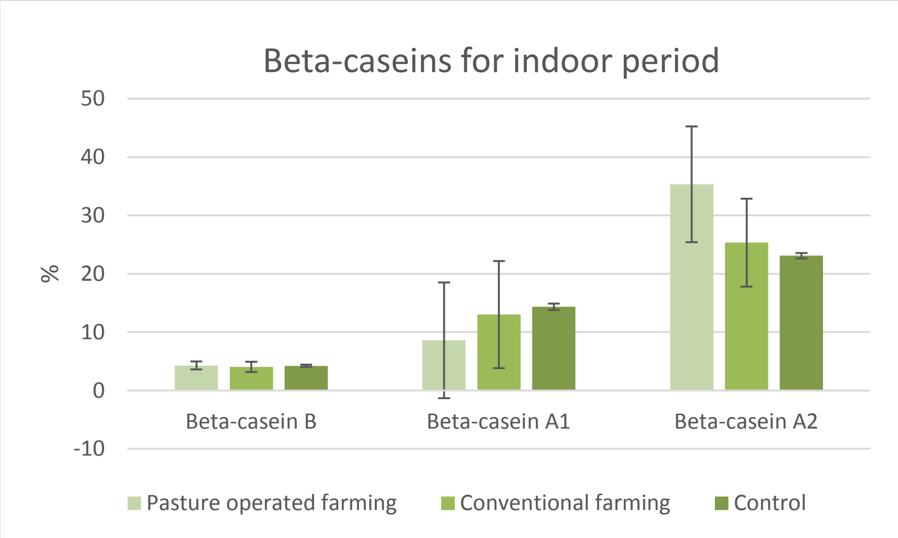

Figure 4. Comparison of β-caseins in milk from Swedish mountain cattle for indoor period and bulk milk (control) from modern cows. The bars indicate standard deviation.

Pasture operate farmed cow's milk had a higher content of β-CN B and A2, in contrast to conventional farmed cow's milk where to content of β-CN A1 is higher (Figure 3 and 4). In milk from the indoor period, conventional farmed cows had β-CN B decrease by 0.16%, β-CN A1 increase by 0.96% and β-CN A2 decrease by 0.87% units compared to outdoor period. In milk from indoor period, pasture operated farmed cows had β-CN B decrease by 0.44 %, β-CN A1 increase by 0.23% and β-CN A2 decrease by 3.59% units compared to outdoor period. The β-CN B and A2 was decreasing for indoors period compared to outdoor period. The β-CN A1 was increased indoors compared to outdoor period.

The β-CN´s for control milk sample are identical for indoor and outdoor period, since it is the same sample (Figure 3 and 4). The β-CN B has a value of 4.22 %. The β-CN A1 has a value of 14.32 %. The β-CN A2 has a value of 20.09 %. The β-CN B for control was approximately similar to both farms systems and periods (0.01-0.5 %). The β-CN A1 for the control was 5.73 and 5.96 % higher than pasture operated farmed cow's milk for indoor and outdoor period, respectively. The β-CN A1 for the control was 1.32 and 2.28 % higher than conventional farmed cow's milk for indoor and outdoor period, respectively. The β-CN A2 for the control was 15.23 and 11.64 % lower than pasture operated farmed cow's milk for indoor and outdoor period, respectively. The β-CN A2 for the control was 5.23 and 6.1 % lower than conventional farmed cow's milk for indoor and outdoor period, respectively.

Total β-CN (Figure 5) was 2.13% units higher for pasture operated farming compared to control and 1.26 % units higher compared to conventional farming. Total β-CN is 0.87% units higher for conventional compared to control milk.

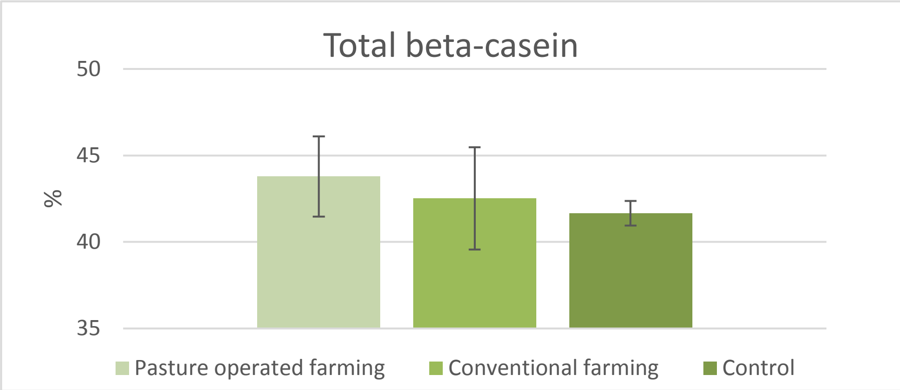

Figure 5. Comparison of β-caseins in milk from Swedish mountain cattle and bulk milk (control) from modern cows. The bars indicate standard deviation.

5 Discussion

This was an investigation of milk proteins from Swedish mountain cattle. The number of individuals in the study was 4-6 cows for pasture farming and 11 cows for conventional farming. Despite the low number of animals, this survey can give an indication of the parameters analyzed. For better significance however, bigger number of cows would be needed.

5.1 Milk composition data

The milk composition for SKB from cattle statistics was 4.45 % fat and 3.56 % protein (Table II, see appendix 1). The milk composition for the Swedish mountain cows in this study was 4.10 % total fat and 3.58 % total protein for conventional farming and 4.63 % total fat and 3.91 % total protein for pasture operated farming (Table 4). This is in agreement of the average levels of total- protein and fat shown by statistics of cattle breeds in Sweden (Table I, see appendix 1). The conventional cattle have double as high number of somatic cells in the milk than the pasture operated (Table 4). This could be due to differences in the farm management, where the conventional farm includes more individual's lead to a higher infection pressure.

5.2 Genotype data

The results from genotype analysis for pasture-operated farming were confirmed by protein profile analysis (Table 5). In two cases from the conventional farming, low expression of β-CN A1 was observed despite the A2 genotype. The levels of β-CN A2 were however 75% higher in this individuals. In one case the genotype did not coincide with the expressed proteins (Table 6). Possible explanation is that the samples were mixed up during handling. New milk samples for the protein analyses would be needed in order to clarify this issue.

5.3 Protein analysis

The pasture operated farming gave higher in total whey protein content for both indoor and outdoor period (Figure 1). The conventional farming decreased 3.39 % in CN and pasture operated farming decrease 0.30% from outdoor to indoor period (Figure 2). The conventional farmed cow's milk had 3.14% higher CN for outdoor period and only 0.05% higher for indoor period than pasture operated farmed cows. Pasture operated farmed cows had a higher β-CN B and A2, but lower β-CN A1 than conventional farmed cows. β-CN B and A2 decreased, but β-CN A1 increased for indoor period compared to outdoor period.

The differences between the periods can be explained by the stage of lactation (Table III, appendix 1). It has been shown previously that there is a variation in protein levels during lactation stage (Walstra 1999). The indoor period is connected to late lactation for pasture operated farm cows and for half of the conventional farms cows. For half of conventional farm cows, the indoor period is middle lactation. However, it is not known how the lactation period affects the milk composition in Swedish mountain cattle. However, these differences in protein profile in the milk between pasture operated farmed and conventional farmed cows seems unlikely to be caused by different feed intake, since different feed content has been shown to have no effect on protein profile according to the report from Johansson *et al.* (2013). One reason for the differences in protein levels could be that these two farms have genetic differences within their breed. It is known that there are a few different blood lines according to Nilsson (2006). Up to day however, the protein profile of the different lines has not been investigated and therefore the knowledge is poor. Interestingly, the Swedish mountain cattle showed to have higher amount of the total β-CN compare to the control milk (Table 5). Compared to the control (Table 3 and 4) Swedish mountain cattle had approximately the same amount of β-CN B, a higher amount of β-CN A2 and lower amount of β-CN A1 than the control bulk milk. For total whey protein, pasture operated farmed cows and control bulk milk sample showed no difference, but had approximately 15 % higher whey protein content than conventional farmed cows (Figure 1). The control milk was bulk milk available in the Swedish stores. As the amount of Swedish mountain cattle represent only 0.3 %, one can assume that the bulk milk represents the modern breeds.

6 Conclusion

This study investigated the milk protein profile in Swedish mountain cattle from two different farms for both outdoor and indoor periods. The pasture operated farmed cow's milk had a higher total whey protein content than conventional farming cow's milk. For total whey protein the difference between indoor and outdoor was very low for both farms. The conventional farmed cow's milk indicated a decrease from outdoor to indoor period for total caseins compared to pasture operated farmed cows. Milk from the pasture operated farm had a higher β-CN B and A2, but lower β-CN A1 than milk from conventional farming. The β-CN B and A2 decreased and β-CN A1 increased during the indoor period compared to outdoor period. Milk from pasture operated farm had higher β-CN B levels than milk from conventional farming. Despite the management of the farms, milk from Swedish native mountain cattle contains higher levels of total β-CN, lower of β-CN A1 and higher β-CN A2 compared to modern breeds (control).

7 Acknowledgements

I would like to thank the farmers Anna Jansdotter from Sparreholm and Robert Nilsson from Boden that participated with milk samples and information about what their cows were fed and info from the *Växa Sverige* milk analysis for the cows.

I would like to thank Kristina Belfrage from *Svensk fjällrasavel* for writing an application to be able to finance this project.

I would also like to thank for the data from gen-sequencing of these cows from Tomas Bergström.

I would also like to thank Jonas Ohlsson for preparing samples for august.

I would like to thank Monika Johansson for being my mentor in this study.

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Appendix 1

Statistics of cattle breeds in Sweden from *Växa Sverige* (Table I).

Table I. Statistics of cattle breeds in Sweden

Breed	Milk yield (Kg)	Fat (%)	Protein (%)	Dairy cows in Sweden (%)
SRB*	9 014	4.36	3.57	38.1
SLB*	10 133	4.09	3.40	54.0
SKB*	5 548	4.45	3.56	0.3
SJB*	6 963	5.87	4.06	0.7
Other	9 294	4.26	3.47	6.8

*SRB (Swedish red and white cattle), SLB (Swedish lowland cattle), SKB (Swedish hornless cattle), SJB (Swedish jersey cattle). (Cattle statistics 2016)

The Swedish regulations for grazing period (Table II).

Table II. Regulations for grazing periods

	Amount of days the animals must be outside during period: 1 April-31 October	Amount of days the animals must be outside during period: 1 May-15 September
Southern Sweden *	120 days	60 days
Middle Sweden **	90 days	60 days
North Sweden ***	60 days	30 days

* Blekinge, Skåne and Hallands län
** Stockholms, Uppsala, Södermanlands, Östergötlands, Jönköpings, Kronobergs, Kalmar, Gotlands, Västra Götalands, Värmlands, Örebro and Västmanlands län
*** Dalarnas, Gävleborgs, Västernorrlands, Jämtlands, Västerbottens and Norrbottens län
(Jordbruksverket)

The cows calved in different months and was thereby in different lactation month when the samples were collected (Table III).

Table III. Month of calving for cows and lactation month for collected samples from both farms.

	Number of cows calving in each month				
	February (6-9)	March (5-8)	April (4-7)	May (3-6)	June (2-5)
Pasture operated farm	3	3	-	-	-
Conventional farm	1	4	2	3	1

The lactation months that represent the collected samples (August-November) in this survey is in parenthesis

Appendix 2

Table IV. Sample buffer

	M	***C***
Triss	121.14	0.167
EDTA	372.24	0.067
MOPS	209.26	0.042
Urea*	60.06	6.00
MHEC**		0.05%
DDT***	154.25	0.017

*0.35L of 6M urea stock
**Add 0.05% MHEC (0.175g), 6.3 g ion exchange resin, mix, let stand for 2-4 hours, filter.
*** add 0,039g DDT /15ml sample buffer

Table V. Run buffer

	M	***C***
Trisodium citrate dehydrate	294.14	0.02
Citric acid	210.14	0.19
Urea*	60.06	6.00
MHEC		0.05%

Appendix 3

For conventional farming cows the total whey content in the cow milk for August, September, October and November was 10.26 %, 11.06 %, 10.37 % and 11.39 % respectively. For pasture operated farmed cows the whey protein content for August, September, October and November was 12.34 %, 12.27 %, 12.12% and 13.88 % respectively. For pasture operated farming during the indoor period the whey protein content in the milk increases 1.7%. For conventional farming the whey content is increased 0.7% from August to September. The same pattern was observed for the indoor period. Pasture operated farmed cows had a 2.08% higher whey protein content in August, 1.21% in September, 1.75% in October and 2.49 % in November compared to milk from conventional farmed cows. (Figure I).

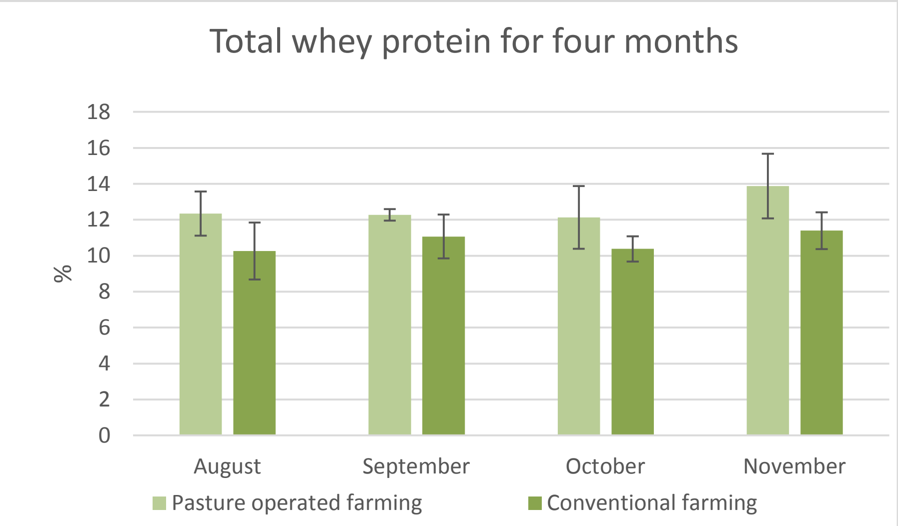

Figure I. Total whey protein from milk samples for four months. The bars indicate standard deviation.

For conventional farming the CN content in the milk for August, September, October and November was 83.45 %, 84.19 %, 78.31 % and 82.54 % respectively. For pasture operated farmed cows the casein content for August, September, October and November was 83.56 %, 76.36 %, 79.25% and 82.03 % respectively. For September the difference between pasture operated farmed and conventional farmed cows was 7.83%. Compared to the other months with a difference of 0.11% higher for conventional in August, 0.94% bigger for pasture operated farming in October and 0.51 % higher for conventional in November. (Figure II).

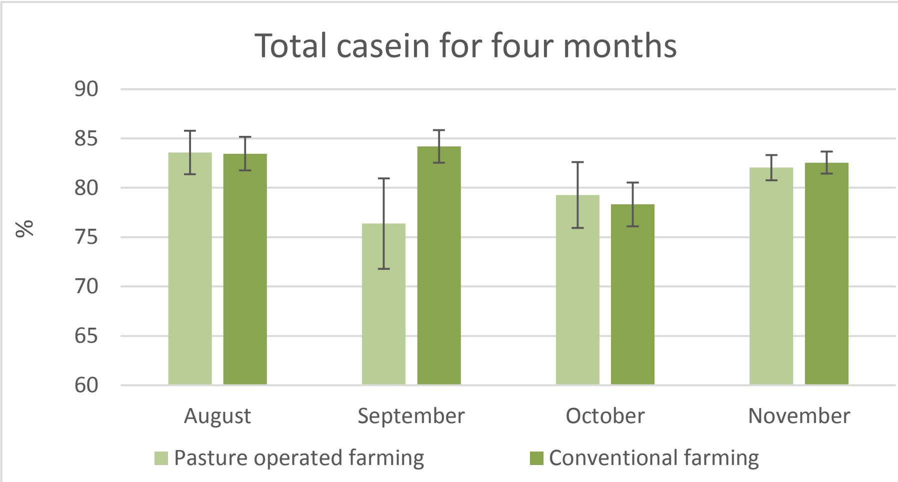

Figure II. Total casein protein from milk samples for four months. The bars indicate standard deviation.