UNIVERSITÀ DEGLI STUDI DI PADOVA

Department of Agronomy, Food, Natural resources, Animals and Environment (DAFNAE)

Second Cycle Degree (MSc) In Italian Food and Wine

"Investigation of milk quality differences between young and old cows."

Supervisor
Dr. Sara Pegolo
University of Padova | UNIPD
Department of Agronomy, Food, Natural Resources, Animals and the Environment (DAFNAE)

Co - Supervisor
Dr. Monika Johansson
Swedish University of Agricultural Sciences | SLU
Department of Molecular Sciences

Submitted by
Dinuka Rachinder Kumarasinghe
Student ID. 122979

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Dedication

This research is lovingly dedicated to Massimo Bottura, Lara Gilmore and Francescana family for giving me a wonderful dream to come to Italy and learn about Italian food.

"Not everyone can be a truffle. Most of us are potatoes. And potato is a very good thing to be"

Massimo Bottura

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1. ABSTRACT

This study aimed to investigate if the number of lactations or breed, affects milk quality parameters, i.e. protein profile, gross composition, pH, somatic cell count, and processability, i.e. rennet coagulation time (RCT), gel firmness, and curd yield for the two major breeds of Swedish dairy cows. In this study, 116 milk samples from Swedish Red and White (SRB) and Swedish Holstein (SLB) cows were collected in herds participating in an ongoing research project. Fresh milk samples of the individual animals were pooled separately for young and old cows. pooled milk samples (PM) were used in each analysis. The protein composition of milk samples was investigated by capillary electrophoresis, to evaluate the variation in protein profile from different lactation numbers and breeds. In milk from SRB cows, the content of β_{A1}-caseins was significantly higher than in milk from SLB cows, whereas SLB milk had a significantly higher content of β_{A2}-caseins. Additionally, milk from SRB cows had a numerically higher content of fat and total solids, and higher relative concentrations of SFA, UFA, MUFA, PUFA, C14:0, C16:0, C18:0, and C18:1C9, compared to milk from SLB cows. Milk from older SLB cows had a higher gel firmness and shorter rennet coagulation time compared to milk from young cows, however, opposite results were observed within milk from SRB cows. There was no significant difference in ethanol stability, and SCS between young and old cows, but SLB had significantly higher ethanol stability compared to SRB. Several statistically significant correlations were found in old cows within the data, including SCS and C18:0 (0.688, $P<0.05$); rennet coagulation time (RCT) and gel firmness (-0,967, $P<0.001$); whey and protein (0.761, $P<0.05$); RCT and β_{A1}-CN; (-0.815, $P<0.01$); RCT and β_{A2}-CN (0.829, $P<0.01$). Overall, there were few variations in milk composition and processibility between young and old cows. These results demonstrate that older cows could be retained in dairy production but more extensive studies are needed in order to support the results.

Keywords: milk quality, lactation number, gel firmness, rennet coagulation time, Swedish Holstein, Swedish Red.

Abbreviations

SRB	Swedish Red
SLB	Swedish Holstein
SCC	Somatic cell count
SCS	Somatic cell score
PM	Pooled milk samples
G_{20}	Gel firmness after 20 min
RCT	Rennet coagulation time
CE	Capillary Electrophoreses
FA	Fatty acid
κ-CN	κappa-casein
β_{A1}-CN	beta A1-casein
β_{A2}-CN	beta A2-casein
α_{S1}-CN	alfa S1-casein
α_{S2}-CN	alfa S2-casein
β-LG	beta-lactoglobulin
β_{B}-CN	beta-casein B

2. INTRODUCTION

Sustainability is a key topic in modern day dairy farming. In recent years, dairy farming is integrated with major sustainability challenges emerging from the growing public interest in animal welfare and health, and the impact on the environment. Modern dairy farming leads to various problems including emission of greenhouse gases, resource depletion, and pollution of watercourses, simultaneously production costs are increasing but profit from milk production is very low, Therefore Impact of these problems has given special attention to sustainable dairy production over the past few years (Segerkvist et al., 2020). The dairy sector is an important sector in agriculture in Sweden and it contributes to sustainable food production. The recent development of the dairy industry reflects more economic, social, and environmental aspects. In addition, it also considers ethical aspects like animal health and welfare. (Gunnarsson et al., 2009). Recent trends in sustainable dairy farming in Sweden focus more on sustainability goals to achieve more profit enhance productivity, resource efficiency and climate benefits. One of the most important goals is to increase resource efficiency and focus more on animal health, welfare and waste management. (Diary-Sweden-Sustainability-Goal, 2019).

Dairy cattle represent an important part of livestock production. Cow longevity is an important factor in dairy farming. Increasing longevity by reducing culling and keeping older cows more lactation is key to improving farm profit and animal welfare. Dairy producers cull cows because of fertility problems, mastitis, and locomotive disorders (Van Knegsel et al., 2014). Culling is the process of removing an animal from the herd due to death, damage, trade, or slaughtering. Culled dairy cows are replaced by more profitable cows but replacement cost is one of the most expensive costs in dairy farming. The high cost is due to feeding, medical treatments and health. (Fetrow et al., 2006). The cost of replacement and rearing of heifers has been estimated to be around 20% of the total operating cost (Grohn et al., 2003). Replacement cost can be reduced by increasing the longevity by keeping older cows more lactation. Economic benefits that can be generated from replacement of a heifer may exceed keeping an older cow in a herd (TW & JW, 1998). Keeping older cows in the herd may provide advantages in the long-term run. In typical weather conditions, older cows typically make more milk than their younger herd mates (Lin et al., 1974). Older animals feed intake is high compared to younger animals, they do not use nutrients for growth. Both of these factors increase their milk production potential when compared to their younger herd mate. The high cost of rearing calves forces many dairy farmers to keep their animals for longer lactation.

The natural life expectancy of dairy cattle is approximately 15-20 years but in the commercial system lifespan often shorter than the natural life expectancy which is around 5- 6 years. In Sweden, dairy cows have 2.5 lactation cycles during their 5 years of life expectancy. Increased longevity is associated with many benefits that include reducing the climate impact caused by milk production, reducing involuntary culling and increasing the profitability of the farm. When increasing the longevity and reducing involuntary culling there will be fewer substitutions of heifers that produce no milk. De Vries & Marcondes 2020 stated that a herd with cows that have given birth more than once excretes less phosphorus and emits less methane per unit of milk and meat compared to a herd with a high number of heifers. Therefore, the present study was carried out to examine whether keeping older animals longer will have a significant benefit.

2.1. Dairy Cattle Farming in Sweden

Livestock production is an important part of Swedish agriculture. Dairy farming is the largest sector in economic standings accountable for 20 % of the value of Swedish agricultural production (Kuylenstierna et al., 2019). In 2019, there were 305 600 dairy cows and 3253 animal holdings. The yield of Swedish dairy cows is one of the highest in the EU, the average production is 10 232 kg of milk per year. The average fat percentage is 4.25 and the average protein percentage is 3.57 (Cattle statistics, 2021).

Sweden's dairy industry (farming and dairy production) is concentrated in the southern regions of Sweden. Together, these regions account for 73% of the milk production, with Halland and Gotland being the most important regions. The Swedish “milk belt” is situated in Southern Sweden where nearly 70% of all Swedish milk is produced on only 20% of the entire land. Swedish dairy farmers produce about 2.8 billion kg of milk per year (Wille-Sonk & Lassen, 2013). The total quantity of milk delivered to diaries in 2020 decreased compared to the year 2019 but the delivery of organic milk increased marginally. The number of animals and animal holdings continuously decreased from 2010 to 2019 (Jordbruksverk & Scb, 2020).

2.2. Dairy Cattle Breeds in Sweden

In Sweden, Swedish Red cows (SRB) and Swedish Holstein cows (SLB) are the two high-producing dairy breeds. Currently, Swedish Holstein cows are the most common breed (50% of the dairy cow population in Sweden), followed by Swedish Red (SRB; 44%), rest only 5 % for Jersey, Swedish polled (SKB) and crossbred cows (Two Competing Swedish - Milkproduction.Com, 2004), (Växa Sverige, 2017) and Swedish Mountain cattle are related to native breeds in Sweden (Poulsen et al., 2017). The following table summarizes the average milk yield, fat %, and protein % per breed in 2020. Swedish Holstein has the highest milk yield but SRB has high fat and protein content compared to SLB. Swedish Jersey cattle (SJB) have the highest fat and protein contents compared to other breeds (Cattle statistics, 2021).

Table 1. Average milk yield, fat and protein percentages per breed

Breed	Milk (Kg)	Fat %	Protein %
SRB	9471	4.41	3.68
SLB	10787	4.14	3.51
SKB	5968	4.40	3.61
SJB	7065	5.88	4.16

Swedish Red and White cows (SRB), Swedish Holstein cows (SLB), Swedish polled cows (SKB) and Swedish Jersey (SJB).

2.3. Milk composition

The composition of milk is based on various factors such as breed, age, lactation stage, milking intervals, and health status. These factors are vitally important in determining the specific technological and physicochemical properties of milk (Christian 2014). The principal components of cow milk are water, protein, fat, lactose and minerals. Milk composition and characteristics are vital factors that can directly Influence the quality and quantity of dairy products. (Murphy et al., 2016)

Milk contains a higher percentage of water than any element, about 87.7 %. Bovine milk contains on average 3.5% protein and consists of two major protein groups, which are casein and whey protein. Casein is the most dominant milk protein which covers 80% of total protein and whey protein covers nearly 20% (Yalcin, 2006).

Milk fat is the most variable component in milk. (Emery, 1988). Fat constitutes represents nearly 3-4% of the total solid content in milk. Milk fat has high levels of saturated fatty acids and low levels of polyunsaturated fatty acids. Unsaturated fatty acids are most important in human nutrition and health. (Singh et al., 2018). Triglycerides are the major fat in milk and it consist of short, intermediate, and long chain fatty acids. In cows, about one-half of the fatty acids are produced in the mammary gland, and the remaining are derived directly from the blood plasma. (Linn, 1988). Milk fat also consists of other lipids such as phospholipid, Cholesterol, free fatty acids and trace amounts of fat-soluble vitamins, B carotene, and flavoring compounds. (Taylor & MacGibbon, 2002). According to Hanuš et al. (2018), several factors influence the variability in fat profile in bovine milk. These factors are breed, genetic factors, lactation stage, milk yield, methods of animal rearing, and feeding. Milk fat highly influences on Texture and consistency of dairy products (Wright et al., 2011). Furthermore, it acts as an energy source and gives flavor and palatability values to dairy products therefore it is highly important in determining the pricing of Milk. (Laben, 1963). Lactose is the major carbohydrate found in milk and is commonly referred to as milk sugar. (Gambelli, 2017). It gives significant value to dairy products but it does not have any nutritional value (Laben, 1963). Bovine milk contains on average 4.6% lactose and it is the least variable component in milk. Lactose synthesis is carried out in epithelial cells of the mammary gland and it involves osmoregulation of milk, it is mainly linked to milk production (Osorio et al., 2016). Milk is an excellent source of macro and micronutrients. It provides many minerals and vitamins, In particular, calcium and phosphorus, potassium, magnesium, zinc, selenium, and both water-soluble vitamins (riboflavin and B12) and fat-soluble vitamins (e.g., A and E) (FAO, 2013).

2.4. Protein Profile in Milk

Casein and whey proteins are complete protein sources that contain all essential amino acids and are an important source of bioactive compounds. (COZMA et al., 2011). Milk protein classifies into soluble and insoluble proteins. Casein is an insoluble protein that comprises numerous forms of caseins such as αs1-, αs2-, beta-, gamma-, and kappa-casein. Whey or serum proteins are soluble milk proteins. Major whey proteins are α−lactalbumin and β-lactoglobulin. Other minor proteins like immunoglobulin and lactoferrin also can be found in milk. (Park et al., 2007).

Casein is the primary protein group in milk. Cow milk contains on average 24–29 g L of casein, depending on breed and lactation stage. Caseins exist in milk as micelles. Casein micelles are proteinaceous colloidal particles with an average diameter of 120 nm and an average mass of 105 kDa (Semenova et al., 2010, Fox et al., 2015). The micelles comprise casein molecules together with calcium, inorganic phosphate, magnesium, and citrate ions. (Petrotos et al., 2014). The structure of the casein micelle is shown in figure 1. As observable, the casein submicelles are incorporated together to construct a bigger casein micelle. These submicelles are bound together by calcium phosphate. The surface of the submicelles directly cooperates with calcium phosphate and forms linkage sites between the submicelles. Kappa-Casein peptide chains are connected to the outer surfaces of the micelle structure. (Fox, 2003). Some studies reported that Casein micelle size can influence several technological properties of milk such as coagulation time (Ekstrand et al., 1980). Casein micelle size is influenced by several factors such as A and B genetic variants of κ-casein, and glycosylation of κ-casein (Bijl et al., 2014).

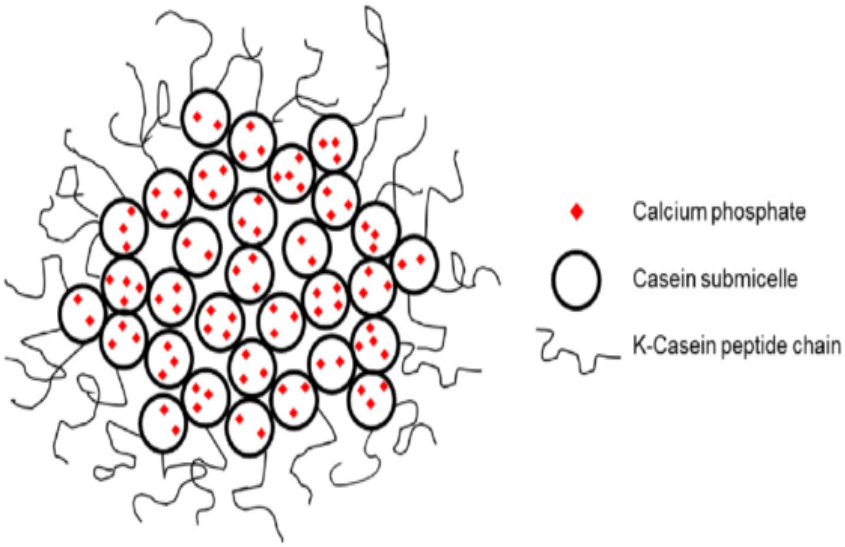

Figure 1 - Schematic diagram of casein micelle structure (Fox et al., 2015).

The primary function of the casein micelle is to allow fluidity to casein molecules and solubilize calcium and phosphate. Tong & Berner (2016) describe that casein micelle contain 6.6% inorganic material, 35.6% αs1-casein, 9.9% αs2-casein, 33.6% β-casein, 11.9% κ-casein, and 2.3% casein protein in dry basis. Alpha casein represents a major protein fraction among bovine milk proteins. αs1-casein and αs2-casein have four genetic variants A, B, C, and D. β-Casein is the most hydrophobic casein and it occurs in five gene/tic variants (A1, A2, and A3, B, C, D, and E) (Kalyankar et al., 2016). κ-CN has genetic variants A, B, and E (Lisson et al., 2013).

Whey proteins are the proteins that remain in solution after coagulation of the casein. β-lactoglobulin (β-Lg),α-lactalbumin (α-La), and protease peptone are abundant forms that can be found in whey (Tavares & Malcata, 2013). β-Lactoglobulin is the major whey protein in milk and it represents one-half of the proteins in whey. It has four genetic variants while α-Lactalbumin has three genetic variants (Kalyankar et al., 2016).

Casein and whey protein are important in the dairy industry, especially in cheese production. Previous research showed that casein and whey proteins influence milk coagulation properties. Jõudu et al. (2008) stated that κ-casein (κ-CN) and α-casein (α-CN) amounts have positive effects on rennet coagulation time and curd firmness. Higher contents of αS1-, β-, and κ-Cn, decrease the rennet coagulation time and formed a firmer curd. Further, his study has reported that whey proteins influence curd properties such as α -lactalbumin (α-LA) helps to improve the rate of curd firming and curd firmness.

The analysis of the different individual protein profiles is widely used in dairy research. There are several techniques for the identification and quantification of individual protein profiles in milk and dairy products. Quantification of the individual proteins involved ELISA and Western blot techniques (Bär et al., 2019). Milk proteins can be identified by chromatographic and electrophoretic techniques. Liquid chromatography is a great technique, particularly together with mass spectrometry. This technique can be used to identification of proteins and their genetic variants accurately. Capillary electrophoresis also used quantification of milk protein especially whey protein. Liquid chromatography and mass spectrometry are the best methods for the identification of protein compare to other methods (Dupont et al., 2018).

2.5. Proteases in Milk

The proteolytic activity of milk is a result of external and internal factors. Proteases are enzymes produced by bacteria that contribute to degrading milk proteins. Protease is considered as an external factor that causes proteolytic activity in milk. These proteases can cause unpleasant flavors and odor's in milk, therefore it strongly affect dairy product quality. Proteolytic enzymes in bovine milk have obtained important attention because these proteases strongly affect milk quality (Fox, 1981, Veisseyre, 1988, Mitchell and Ewings, 1985). Bovine milk contains various Indigenous enzymes such as PL, PG and lysosomal proteinases of somatic cells. They are considered as internal factors that contribute to proteolysis of milk proteins. Milk also comprises several proteolytic enzymes, which are derived from somatic cells in the milk such as collagenase and cathepsin (Kelly and McSweeney, 2003).

2.6. Technological properties and processability of Milk

Milk composition together with favorable technological properties and processability can influence the quality of milk and dairy products. Different quality attributes contribute to milk processing characteristics, such as rennet coagulation time (RCT), curd-firming time (k20), (a30 and a60) - curd firmness 30 or 60 min after addition of rennet to milk, pH, heat coagulation time (HCT), casein micelle size (CMS), and pH. Rennet coagulation time, k20, a30, and a60 are commonly referred to as milk coagulation properties or Indicators of milk processability (Visentin et al., 2017). Milk coagulation properties are the outcome of some interrelating factors such as milk composition, somatic cell count, and calcium and phosphorus concentrations. (De Marchi et al., 2007). Somatic cell count is used as indicator of milk quality. Inflammation of the udder results in a high somatic cell count in milk and it has a significant effect on milk yield, milk protein, milk lactose, and total solids except for milk fat. (Cinar et al., 2016). Higher SCC in milk changes the protein profile. It increases the level of whey protein but decreases α- and β-caseins (Le Maréchal et al., 2011). The main effects of SCC in dairy products include lower cheese yield, fat and protein loss, high humidity, shorter shelf life, and lower sensory quality in mozzarella cheeses (Andreatta et al., 2007, 2009). High SCC increases the rennet coagulation time resulting in low curd firmness and reducing the rate of curd firming (Raynal-Ljutovac et al. 2007). Le Maréchal et al., (2011) also reported that high SCC could influence on renetting steps in cheese production and lower the production yields of some cheese. According to Fernandes et al. (2008), a high SCC in raw milk surges the proteolysis of UHT milk because of beta-casein degradation, which can lower nutritional value and affect the quality of dairy products in an undesirable way.

3. AIM OF THE THESIS

Dairy cows in Sweden have an average life span of five years, which means that each cow undergoes about 2.5 lactation cycles throughout her life. Most dairy cows are culled involuntary before their full potential is reached. Culled dairy cows are replaced with heifers but it takes dairy farmers an average of 1.5 lactations to repay the cost of rearing a heifer to calving. Therefore, it is important to investigate whether older cows can be retained in production for a longer period of time, thus increasing productivity and longevity of cows, without having milk quality attributes negatively affected. For this purpose, we examined the difference in composition, technological properties, and cheese-making ability of the milk from older cows and younger cows and if there any positive outcomes in milk from older cows. Previous studies were carried out on Individual cows and pooled milk samples whether the number of lactations has an effect on raw milk composition therefore this study was carried out as an advantage for those studies and also to obtain higher reliability. The aim was also to evaluate if the milk composition differed between two breeds, Swedish red and Swedish Holstein.

4. MATERIALS AND METHODS

4.1. Milk Samples

Representative milk samples from separate cows were collected from the dairy farms in Uppland and Södermanland County in Sweden. The selection of cows was based on the number of lactations. Milk samples were collected from 11 different farms and 116 dairy cows. The two common breeds in Sweden, Swedish Red (SRB) and Swedish Holstein (SLB) were used. The cows were divided into two groups based on breed. Each group consisted of five younger i.e., ≤ 2 lactations and five older i.e., ≥ 3 lactations. Except for two groups consisting of four younger i.e., ≤ 2 lactations and four older i.e., ≥ 3 lactations. Milk (300-500ml) from each cow was directly transferred into a glass bottle and kept in a cooling box until arrival at the dairy science lab.

Table 2. Number of young and old cows in Swedish Red and Swedish Holstein

Breed	**SRB**	**SLB**
Young	44	14
Old	44	14

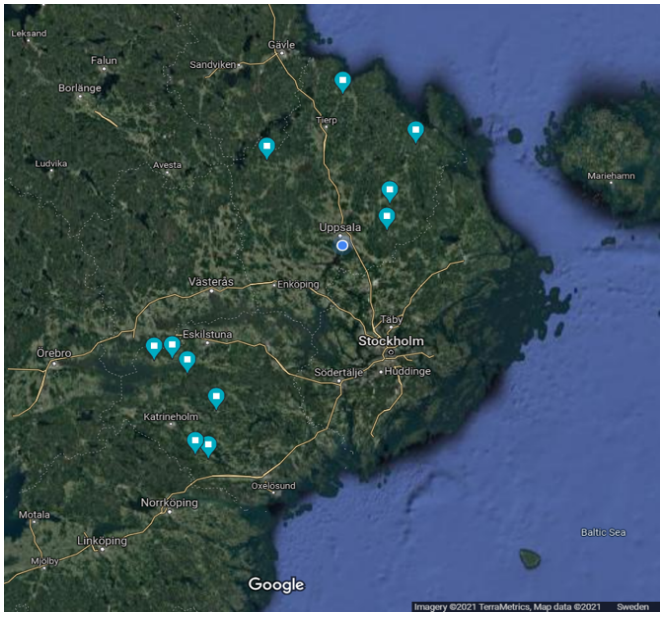

Figure 1. Location of the farms participating in the trial.

The cows taking part in this study were in the lactation interval of a minimum of 8 weeks after calving and have 12 weeks until the next one. Milk sampling took place in the morning for five different months (February, March, September, October, and November) in 2020. The study was conducted in the research facilities at the Swedish university of agriculture (SLU) in Uppsala, Sweden.

Table 3. The proportions of pooled milk samples (PM) from young and old cows.

Pooled Milk Samples	Milk from young cows (%)	Milk from old cows (%)
PM 1	0	100
PM 2	30	70
PM 3	50	50
PM 4	70	30
PM 5	100	0

Fresh milk samples of the individual animals were pooled separately for young and old cows from each of the two breeds. Pooled milk aliquots of 200 ml were created, based on five different concentrations of young and old cows' milk. The different concentrations intended to examine whether pooling milk with different proportions of milk from younger and older cows affects the outcome. In all analysis five concentrations of this pooled milk samples (PM) were used.

4.2 Milk Analysis

4.2.1 Preparation of Samples

The fresh whole pooled milk (PM) were used for micro cheese production and gross composition analyses. An aliquot of 50ml from each pooled milk was used for protein profile analyses, rheology measurement, and ethanol stability. These milk samples were centrifuged and defatted at 3,000 RPM at 4°C for 10 min. After centrifugation, the fat layer on the surface was removed by a cotton stick. Other remaining milk was stored at -20°C for further analyses.

4.2.2 Milk Gross Composition Analysis

Individual milk samples were analysed for gross composition at the Department of Animal Nutrition and Management, SLU (Sweden). Total protein, total casein, total fat, lactose concentrations, saturated FA (SFA), unsaturated FA (UFA), monounsaturated FA (MUFA), polyunsaturated FA (PUFA), myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), and oleic acid (C18:1c9) were analysed by a mid-infrared spectroscopy method (Fourier Transform Infrared Spectroscopy; FTIR); (FOSS Electric A/S (Hilleröd, Denmark). Somatic cell count (SCC) in milk was examined by electronic fluorescence-based cell counting (Fossomatic Foss FT 120, Hilleröd, Denmark). SCC was converted to SCS by logarithm transformation to achieve normality [SCS = log2 (SCC/100,000) +3] according to Ali and Shook (1980).

4.2.2.1 Buffer samples preparation

Sample buffers and running buffer formulations were prepared for capillary electrophoresis. The sample buffer consisting 0.167M Tris base (Triss; Mw 121.14), 0.067M EDTA disodium dihydrate (EDTA; Mw 372.2), 0.042M 3-(N-Morpholino) propane sulfonic acid (MOPS; Mw 209.26), and w/w 0.05% hypromellose (HPMC) and 6.3g ion exchange resin (AG 501-X8 Resin, Bio-Rad Laboratories, Inc, CA) was dissolved in 0.35L of 6M urea solution (Mw 60.06) overnight. After dissolution, the sample buffer was filtered through a 0.45 µm Nylon Membrane Filter into 15 ml falcon tubes, with aliquots of 10 ml. The sample buffer aliquots were stored at – 20˚C.

The running buffer comprised of 0.02 2M Trisodium citrate dihydrate (Mw 294.10), 0.19M citric acid (Mw 210.14), and w/w 0.05% Hypromellose (HPMC) and was dissolved in 0.35L of 6M urea solution. The running buffer was filtered through a 0.45 µm Nylon Membrane Filter into aliquots of 2 ml which were stored at –20˚C together with the sample buffer aliquots until used for analysis. mL? 0.017M DTT (DL-dithiothreitol, anhydrous Mw 154.25) was added to the Sample buffer before the analysis of samples for reduction or disruption of the natural disulphide bonds of the milk proteins. Materials were purchased from Sigma Aldrich Chemicals (St. Louis MO, USA) unless mentioned otherwise.

4.2.2.2 Milk sample preparation

From each pooled milk sample, 2ml of defatted milk was thawed at room temperature for 30min. From each sample, 200µl of milk was transferred in an Eppendorf tube, and then 400 µl of sample buffer with DTT (D, L-dithiothreitol (DTT) was added to the sample buffer to disrupt disulphide bridges of the milk proteins. The mixed solution was vortexed and kept for one hour at room temperature. The samples were centrifuged for 10 min at 10,000 RPM and 4°C (VWR Hitachi CT15RE). The surface fat layer was removed by cotton bud and a clean sample was filtered through a 0.45µm nylon syringe filter into a new Eppendorf tube. Then, 30µl of the filtrate was transferred to the 250µl polypropylene conical vials for CE. Running buffers were added to snap polypropylene 1 ml vials. Prepared samples were inserted into NAOH vials and then run through the machine according to standard procedures.

4.2.3 Milk Protein Profile Analysis

4.2.3.1 Capillary electrophoresis method

Electrophoresis is a method that explores the use of an electric field to separate charged species in the liquid phase. Capillary electrophoresis is the high-performance version of this method that has been used for the determination of a large variety of food-related molecules, In our case milk proteins. These milk proteins have different mobility under the influence of an electric field, depending on their size, shape, and net charge. CE carries the advantages of lack of complication in setup and miniaturization, fast separation with high resolution and efficiency, and need a very small amount of sample compared to the traditional separation techniques like liquid chromatography and gel electrophoresis (Gao, Z., & Zhong, 2018). Compared to high-performance liquid chromatography (HPLC), Capillary electrophoresis has a low operative cost, higher resolving power, and shorter analysis time (Xu 1995; Perrett 1999). A wide range of biologically active molecules can be separated by CE, i.e., proteins, AA, peptides, hormones, steroids, vitamins, carbohydrates, etc.

4.2.3.2 Analysis of protein profile

Analysis of milk protein profile was performed with a 7100-capillary electrophoresis (CE) system (Agilent Technologies Co., Santa Clara, USA), using an unfused silica standard capillary. This method was performed according to Johansson et al (2013).

Figure 2. Capillary electrophoresis (CE) system

The casein fractions (α_{S1}-CN, α_{S2}-CN, β–CN, and κ-CN) and whey proteins (α-LA and β-LG) were measured as a percentage of total area, based on the peak area expressed in percentage in the electropherogram. The peaks in the capillary electropherogram were identified by comparing the migration time of molecular weight standards. The area of each identified peak was calculated from the electropherogram using a valley to valley method. The total casein concentration was defined as the totality of α_{S1}-CN, α_{S2}-CN, β_{B}-CN, β_{A1}-CN, β_{A2}-CN, and κ-CN. The total whey protein concentration was defined as the sum of α-LA and β-LG.

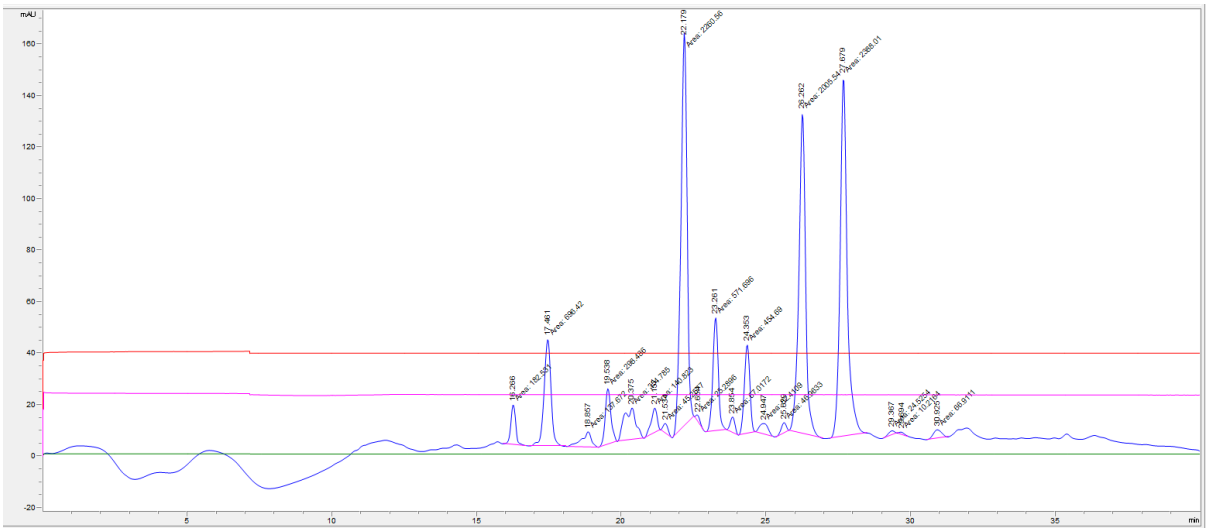

Figure 3. Electropherogram of a milk sample on capillary electrophoresis

4.2.4 Milk pH measurement

The pooled milk samples were thawed in a water bath for 15 minutes (min) at 30˚C before measuring the pH of the milk. The pH was determined using Mettler Toledo, Seven Compact pH meter S210.

4.3. Micro cheese making procedure

Micro cheeses were produced according to Othmane et al.(2002) and Högberg (2016), with some changes.

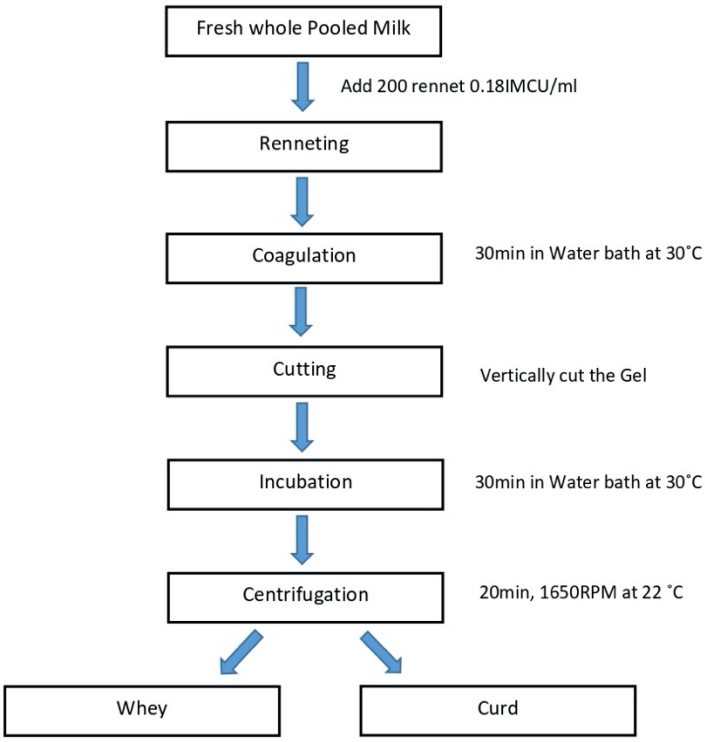

Figure 4. Micro cheese production flowchart (Othmane et al. 2002; Högber, 2016).

Briefly, 200 µl rennet (strength 18 IMCU) was added to 20 ml of fresh milk (30°C) and vortexed. The samples were kept to coagulate for 30 min before the curd was vertically cut into four equally sized pieces. After another 30 min of incubation at 30°C, the samples were centrifuged at 1,650 RPM for 20 min at 22°C. (Sorvall, Super T21, Sorvall Products L.P., Newton, Connecticut, USA). Expelled whey was transferred to 50ml conical falcon tubes and curd and whey were weighted individually. The weight of the conical falcon tube was deducted from the weighted curd in order to get the curd weight. The whey was frozen at –20˚C for further analyses of the protein content.

The curd yield was determined from the weight difference between the initial milk sample and the expelled whey, expressed as grams of curd per 100 g of milk, and presented as a percentage. Whey was analyzed by mid-infrared technique to determine the casein content in milk. The following equation was used to calculate casein content (Casein content = Total protein content – Whey protein content). The casein number (Cn No%) was calculated as (casein/total protein) *100.

4.4. Ethanol stability test

The Ethanol stability test is used on fresh milk to indicate whether it will coagulate during thermal processing. Ethanol stability was expressed as the maximum ethanol concentration that could be added to the sample without causing visual coagulation of the milk. Ethanol stability was measured by mixing equal volumes of 0.5ml of a milk sample and ethanol solution (water/ethanol ranging from 10 to 100%, v/v) at room temperature. During each sampling occasion, one biological replicate of the ethanol stability test was carried out on each pooled milk sample.

4.5. Rheological measurements

Milk coagulation properties were measured according to the method reported by Johansson et al. (2015) using a rheometer (Bohlin CVOR 150, Malvern instruments) equipped with a cup (Ø 25 mm) and a concentric cylinder (Ø 28 mm) at a height of 40 mm. In brief, skimmed milk mixed with bovine rennet 75/25 chymosin/pepsin, 180 IMCU (Scandirenn Kemikalia AB, Skurup, Sweden), at a concentration of 0.18 IMCU/ml. The rennet coagulation time (RCT) was measured from the addition of the rennet until the elastic modulus (G') reached 1 Pa. The gel firmness (G20, Pa) was determined twenty minutes from the rennet addition. The RCT and G20 in each pooled milk sample were analyzed.

4.6 Statistical Analysis

Mean values and standard deviations (SD) were calculated for each parameter. One way analysis of variance (ANOVA) was performed separately for the effect of lactation number and breed (since groups were not balanced for running a two-way ANOVA) on the different milk quality parameters. Differences between means were calculated with Tukey's test and the *P* value threshold for significant effects was set at $P<0.05$.

The effect of lactation number on milk quality variables and milk proteins and cheesemaking traits were analyzed by a one-way ANOVA according to the following model: **M1**

$$\textbf{M1 } \boldsymbol{y_{ij}} = \mu + \boldsymbol{Lact_i} + \boldsymbol{e_j},$$

where y represents the phenotype, μ is the overall mean, $Lact_i$ is the fixed effect of the *i*th lactation number in classes (class1: PM1; class2: PM2; class3: PM3; class4: PM4; class5: PM5) and e is the residual effect. The random effect of e was deemed to be normally distributed as $e \sim N(0, I\sigma_e^2)$, where σ_e^2 is the residual variance component.

The effect of breed on milk quality variables and milk proteins and cheesemaking traits were analyzed by a one-way ANOVA according to the following model: **M2**

$$\textbf{M2 } \boldsymbol{y_{ij}} = \mu + \boldsymbol{Breed_i} + \boldsymbol{e_j},$$

where y represents the phenotype, μ is the overall mean, $Breed_i$ is the fixed effect of the *i*th breed in classes (class1: SRB; class 2: SLB) and e is the residual effect. The random effect of e was deemed to be normally distributed as $e \sim N(0, I\sigma_e^2)$, where σ_e^2 is the residual variance component.

Relationships between milk quality and cheesemaking traits and milk proteins were analyzed with Pearson's correlation ($P<0.05$). All the statistical analysis were performed using the software Minitab® Version 20 (Minitab Inc., in the United States). Graphical illustrations were made in Microsoft® Excel® version 2016.

5. RESULT AND DISCUSSION

5.1 Analysis of Protein Profile

Protein profile were measured as a percentage of total area, based on the peak area expressed in percentage in the electropherogram. The peaks in the capillary electropherogram were identified by comparing the migration time of molecular weight standards. The area of each identified peak was calculated from the proportion of the detected peak area of a milk protein make up out of the total area of all detected peaks (See Figure 5).

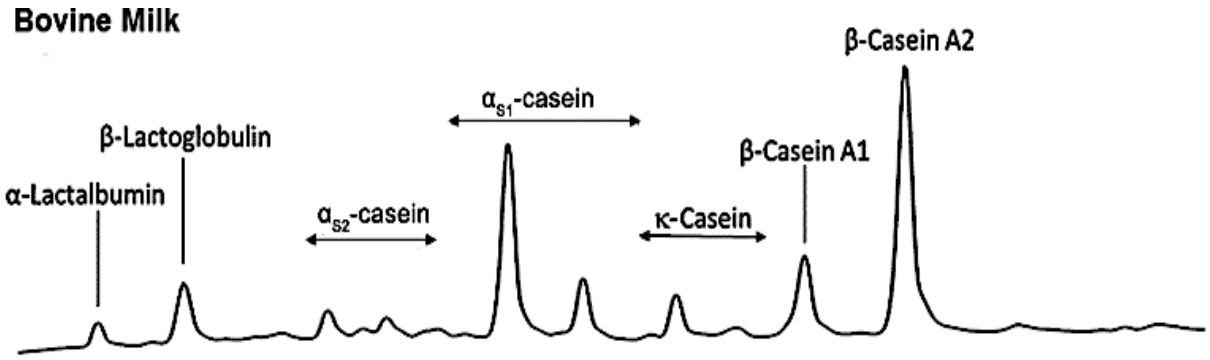

Figure 5. Representative electropherogram of bovine milk determined by capillary electrophoresis (Mohamed et al., 2020).

5.1.1. Individual proteins in Pooled milk samples for Swedish Red cows

In milk from SRB cows, the relative concentrations of four variables κ-CN, β_B-CN, β_{A1}-CN, and Total β-casein, were shown to differ when comparing pooled milk levels but it was significant only for Total β-casein (Table 4). The relative content of Total β-casein in milk from SRB cows was significantly higher in PM5 compared to older cows (PM1) (P<0.05).

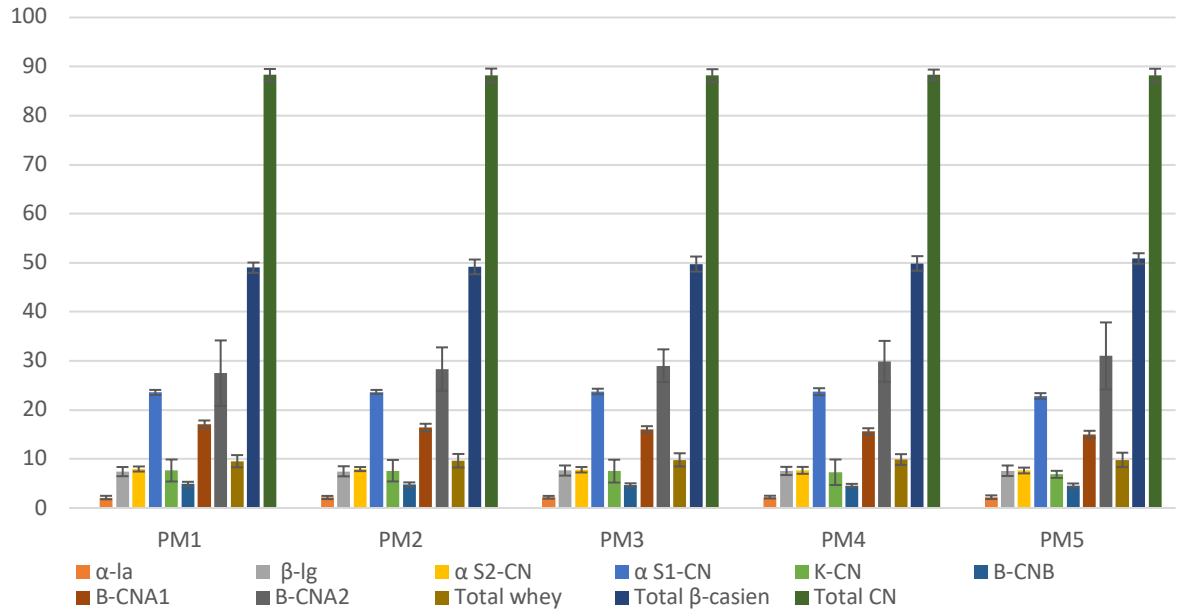

Figure 6. The Mean values of an individual protein in pooled milk samples for Swedish red cows.

Table 4. Individual proteins percentages in pooled milk samples from Swedish red cows(n=9)

SRB Means ± SD	PM1 (0%) Old cows	PM2 (30%)	PM3 (50%)	PM4 (70%)	PM5 (100%) Young cows
α-La	2.13±0.35	2.14±0.34	2.18±0.30	2.22±0.29	2.20±0.40
β-Lg	7.41±0.94	7.47±1.09	7.63±1.10	7.55±0.89	7.60±1.14
α S_2-CN	7.95±0.52	7.93±0.42	7.80±0.56	7.68±0.71	7.65±0.59
α S_1-CN	23.56±2.25	23.66±2.19	23.76±2.33	23.72±2.61	22.86±0.72
κ-CN	7.65±0.78	7.61±0.77	7.47±0.61	7.24±0.66	6.86±0.73
β_B-CN	4.88±0.46	4.80±0.43	4.68±0.35	4.59±0.33	4.58±0.42
β_{A1}-CN	17.09±6.86	16.40±4.04	16.07±2.87	15.73±3.55	15.28±6.14
β_{A2}-CN	27.49±6.68	28.32±4.14	28.98±3.33	29.90±4.17	31.00±6.83
Total whey	9.54±1.24	9.61±1.39	9.82±1.34	9.78±1.10	9.80±1.47
Total β-casein	48.99±1.05[a]	49.16±1.50[ab]	49.34±1.53[ab]	49.86±1.49[ab]	50.86±1.08[b]
Total CN	87.99±1.20	88.17±1.42	88.16±1.30	88.29±1.09	88.22±1.34

Abbreviations: SD=standard deviation; PM=pooled milk sample.
The percentage in parentheses in pm 1-5, indicates the proportion of milk from younger cows in the PM
[a,b] Different letter superscripts in the same row indicate significance difference within breed (P<0.05).

5.1.2. Individual Proteins in pooled milk samples for Swedish Holstein cows

In milk from Swedish Holstein cows, none of the proteins were differed significantly between different pooled milk samples but κ-CN and β_{A1}-CN were numerically increased in a higher proportion of milk from older cows. β_B-CN and α S_1-CN were increased numerically in a higher proportion of milk from younger cows.

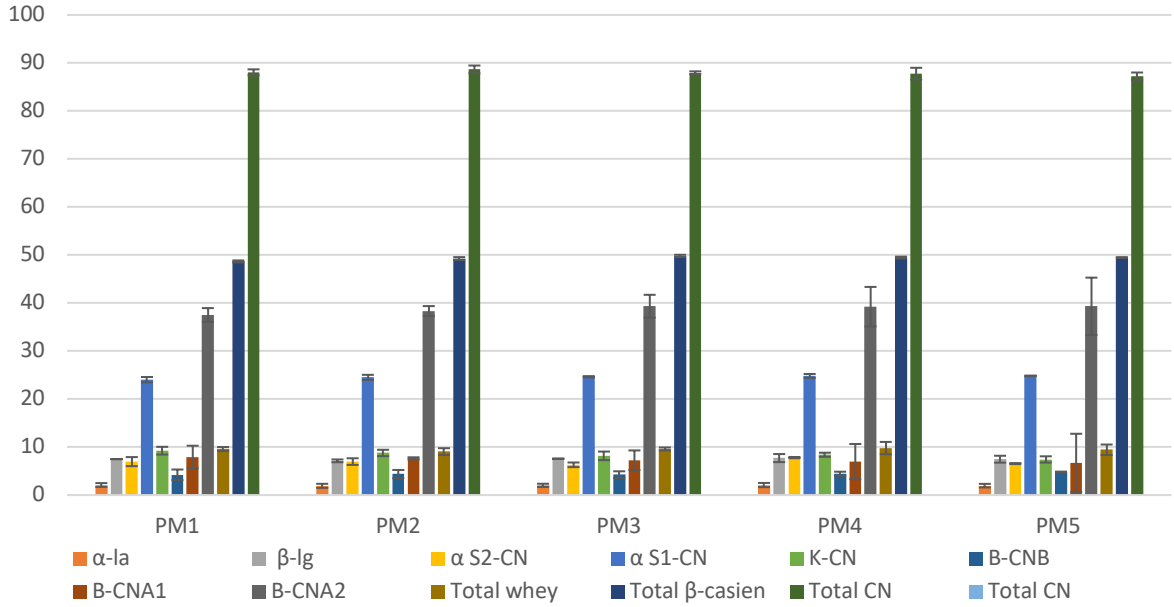

Figure 7. The Mean values of an individual protein in pooled milk samples for Swedish Holstein cows.

Table 5. Individual proteins percentages in pooled milk samples from Swedish Holstein cows (n=3)

SLB Means ± SD	PM1 (0%) Old cows	PM2 (30%)	PM3 (50%)	PM4 (70%)	PM5 (100%) Young Cows
α-La	2.10±0.29	1.92±0.32	2.03±0.25	2.09±0.31	1.96±0.28
β-Lg	7.49±0.12	7.13±0.41	7.58±0.30	7.70±0.60	7.48±0.56
α S_2-CN	6.97±1.28	6.98±1.46	6.31±2.26	6.54±2.18	6.56±2.41
α S_1-CN	24.04±4.70	24.51±4.39	24.65±4.26	24.81±4.26	24.83±3.78
κ-CN	9.25±2.06	8.78±1.54	8.17±1.16	8.02±0.60	7.42±0.72
β_B-CN	4.23±0.80	4.38±0.61	4.25±0.55	4.43±0.31	4.78±0.16
β_{A1}-CN	7.92±2.39	7.65±1.43	7.26±2.75	6.98±4.45	6.69±6.87
β_{A2}-CN	37.51±2.00	38.32±2.13	39.34±4.16	39.24±5.20	39.32±6.41
Total whey	9.59±0.30	9.05±0.68	9.60±0.43	9.79±0.91	9.44±0.79
Total β-casein	48.66±0.88	49.26±1.55	49.81±0.47	49.47±1.40	49.47±3.03
Total CN	88.05±0.88	88.65±1.61	87.95±1.13	87.77±1.68	87.24±2.95

Abbreviations: SD=standard deviation; PM=pooled milk sample.
The percentage in parentheses in pm 1-5, indicates the proportion of milk from younger cows in the PM
[a,b] Different letter superscripts in the same row indicate significance difference within breed (P<0.05).

5.1.3. Comparison between Swedish red cows and Swedish Holstein cows for individual proteins

Milk from SRB cows contained more **β_{A1}-caseins** and it was significant for PM2, PM3 and, PM4 compared to SLB cows. Milk from SLB cows contained more **β_{A2}-caseins** compared to SRB cows and it was significant for PM1-PM4.

Table 6. The relative content % for individual proteins between pooled milk samples (PM 1-5) from Swedish Holstein and Swedish Red cows

.

	PM1 (0%) Old cows		PM2 (30%)		PM3 (50%)		PM4 (70%)		PM5 (100%) Young Cows	
Object %	SLB Mean± SD	SRB Mean±SD	SLB Mean± SD	SRB Mean±SD	SLB Mean± SD	SRB Mean±SD	SLB Mean± SD	SRB Mean±SD	SLB Mean± SD	SRB Mean±SD
α-La	2.10±0.29	2.13±0.35	1.92±0.32	2.14±0.34	2.03±0.25	2.18±0.30	2.09±0.31	2.22±0.29	1.96±0.28	2.20±0.40
β-Lg	7.49±0.12	7.41±0.94	7.13±0.41	7.47±1.09	7.58±0.30	7.63±1.10	7.70±0.60	7.55±0.89	7.48±0.56	7.60±1.14
α S_2-CN	6.97±1.28	7.95±0.52	6.98±1.46	7.93±0.42	6.31±2.26	7.80±0.56	6.54±2.18	7.68±0.71	6.56±2.41	7.65±0.59
α S_1-CN	24.04±4.70	23.56±2.25	24.51±4.39	23.66±2.19	24.65±4.26	23.76±2.33	24.81±4.26	23.72±2.61	24.83±3.78	22.86±0.72
κ-CN	9.25±2.06	7.65±0.78	8.78±1.54	7.61±0.77	8.17±1.16	7.47±0.61	8.02±0.60	7.24±0.66	7.42±0.72	6.86±0.73
β_B-CN	4.23±0.80	4.88±0.46	4.38±0.61	4.80±0.43	4.25±0.55	4.68±0.35	4.43±0.31	4.59±0.33	4.78±0.16	4.58±0.42
β_{A1}-CN	7.92±2.39	17.09±6.86	7.65±1.43[a]	16.40±4.04[b]	7.26±2.75[a]	16.07±2.87[b]	6.98±4.45[a]	15.73±3.55[b]	6.69±6.87	15.28±6.14
β_{A2}-CN	37.51±2.00[a]	27.49±6.68[b]	38.32±2.13[a]	28.32±4.14[b]	39.34±4.16[a]	28.98±3.33[b]	39.24±5.20[a]	29.90±4.17[b]	39.32±6.41	31.00±6.83
Total whey	9.59±0.30	9.54±1.24	9.05±0.68	9.61±1.39	9.60±0.43	9.82±1.34	9.79±0.91	9.78±1.10	9.44±0.79	9.80±1.47
Total β-casein	48.66±0.88	48.99±1.05	49.26±1.55	49.16±1.50	49.81±0.47	49.34±1.53	49.47±1.40	49.86±1.49	49.47±3.03	50.86±1.08
Total CN	88.05±0.88	87.99±1.20	88.65±1.61	88.17±1.42	87.95±1.13	88.16±1.30	87.77±1.68	88.29±1.09	87.24±2.95	88.22±1.34

Abbreviations: SD=standard deviation; PM=pooled milk sample.
The percentage in parentheses in pm 1-5, indicates the proportion of milk from younger cows in the PM for each pooled milk sample proportion pm1-5 between breeds, means values within the row with different superscripted (a,b) presented significance difference. ($p \leq 0.05$)

5.2. Ethanol stability

The comparison of the ethanol (EtOH) stability between all sampling occasions is shown in figure 8. In SLB cows PM1 7.0%, PM2 7.3%, PM3 5.8%, PM4 7.3% and PM5 5.8% numerically higher in ethanol stability compared to SRB cows. The highest values for EtOH stability, 90%, were observed in milk from both SLB and SRB breeds. In milk from SRB, the lowest value observed was 56% and 82%. Karlsson et al. (2017) stated that in their research the average value of ethanol stability was 79% and 81% during the indoor and outdoor periods, respectively. In our research average value of ethanol stability is also similar to Karlsson's study.

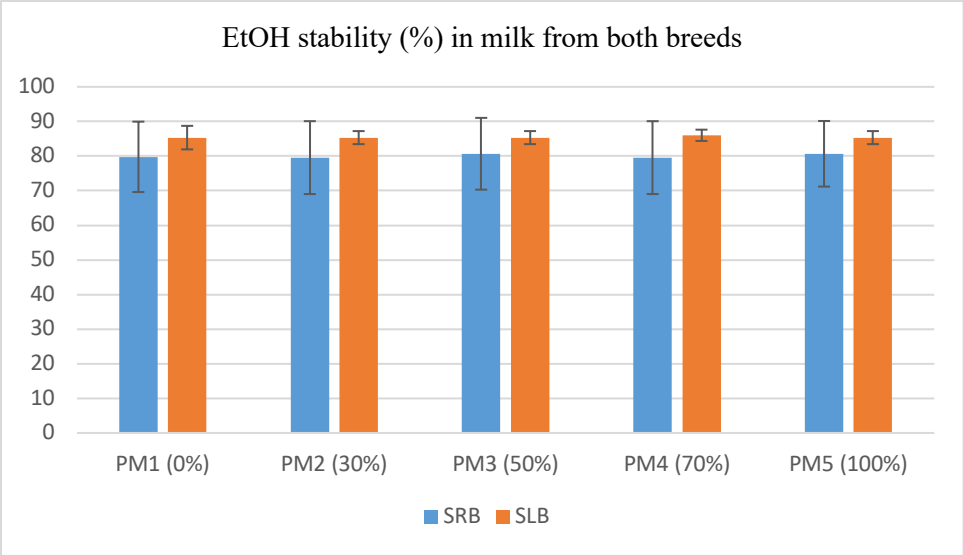

Figure 8. The mean values of ethanol stability (%) in pooled milk samples from Swedish red and Swedish Holstein cows.

PM1–5 is the different pooled milk samples, and the percentage indicates the proportion of milk from young cows in the PM. Standard deviation is indicated.

5.3. Milk gross composition

The mean values of gross composition from the milk sampling in both breeds are shown in the tables. In SLB milk, Fat and Total solids (TS) were numerically higher at 14.9 % and 5.4 % respectively for milk from younger cows, but it was not significant. There was no significant milk composition factor for both breeds.

Table 7. Average Milk composition in pooled milk samples (PM1-5) for Swedish Holstein(n=3) cows. Values presented are mean and standard deviation

SLB Means ± SD	PM1 (0%) Old cows	PM2 (30%)	PM3 (50%)	PM4 (70%)	PM5 (100%) Young Cows
Object	**Means ± SD**	**Means ± SD**	**Means ± SD**	**Means ± SD**	**Means ± SD**
Fat%	4.29±0.20	4.53±0.13	4.68±0.23	4.82±0.32	5.04±0.45
Protein%	3.43±0.20	3.45±0.21	3.48±0.21	3.50±0.22	3.53±0.23
Lactose%	4.78±0.08	4.77±0.06	4.78±0.05	4.79±0.06	4.79±0.04
Total Solids%	13.19±0.38	13.41±0.25	13.57±0.25	13.73±0.31	13.95±0.38
SCS	3.42±0.51	3.32±0.55	3.29±0.69	3.15±0.63	2.61±0.85
Whey%	0.85±0.07	0.86±0.06	0.87±0.05	0.89±0.04	0.90±0.03
Casein%	2.69±0.14	2.73±0.16	2.77±0.16	2.61±0.19	2.63±0.23
CN	75.30±1.71	75.40±1.77	75.41±1.46	74.56±1.08	74.53±1.86
Curd %g	57.38±4.97	56.38±3.36	58.12±4.82	58.41±6.99	60.24±3.49

Abbreviations: SD=standard deviation; PM=pooled milk sample.
The percentage in parentheses in pm 1-5, indicates the proportion of milk from younger cows in the PM
[a,b] Different letter superscripts in the same row indicate significance difference within breed (P<0.05)

Table 8. Average Milk composition in pooled milk samples (PM1-5) for Swedish Red (n=9) cows. Values presented are mean and standard deviation

SRB Means ± SD	PM1 (0%) Old cows	PM2 (30%)	PM3 (50%)	PM4 (70%)	PM5 (100%) Young Cows
Object	**Means ± SD**	**Means ± SD**	**Means ± SD**	**Means ± SD**	**Means ± SD**
Fat%	5.09±0.67	5.01±0.72	5.05±0.68	5.04±0.79	4.95±0.84
Protein%	3.78±0.24	3.77±0.21	3.76±0.19	3.72±0.23	3.73±0.26
Lactose%	4.62±0.10	4.64±0.10	4.66±0.10	4.65±0.13	4.68±0.14
Total Solids%	14.16±0.76	14.07±0.83	14.11±0.75	14.06±0.93	13.97±0.97
SCS	3.57±1.07	3.51±0.83	3.36±0.77	3.29±0.78	2.73±1.39
Whey%	1.00±0.11	0.99±0.09	1.00±0.07	0.99±0.07	0.99±0.08
Casein%	2.78±0.15	2.78±0.14	2.77±0.14	2.72±0.17	2.74±0.19
CN	73.54±1.57	73.75±1.44	73.50±1.35	73.23±0.90	73.45±1.12
Curd %g	57.58±7.88	57.76±6.83	57.98±6.80	60.21±7.36	61.98±9.80

Abbreviations: SD=standard deviation; PM=pooled milk sample.
The percentage in parentheses in pm 1-5, indicates the proportion of milk from younger cows in the PM
[a,b] Different letter superscripts in the same row indicate significance difference within breed (P<0.05).

5.3.1. Comparison between Swedish Red cows and Swedish Holstein cows for milk composition

Milk from old SLB cows (PM1) had a significantly lower content of whey protein (P<0.05) compared to milk from old SRB cows. Pooled milk samples with 30% and 50% milk from young SRB cows had significantly higher protein content compared to the corresponding sample from SLB. Old SLB cows had a significantly higher lactose content compared to old cows from SRB.

Table 9. The relative content % for Milk composition between pooled milk samples (PM 1-5) from Swedish Holstein and Swedish Red cows0

	PM1 (0%) Old cows		PM2 (30%)		PM3 (50%)		PM4 (70%)		PM5 (100%) Young Cows	
Object %	SLB Mean± SD	SRB Mean±SD	SLB Mean± SD	SRB Mean±SD	SLB Mean± SD	SRB Mean±SD	SLB Mean± SD	SRB Mean±SD	SLB Mean± SD	SRB Mean±SD
Fat%	4.29±0.20	5.09±0.67	4.53±0.13	5.01±0.72	4.68±0.23	5.05±0.68	4.82±0.32	5.04±0.79	5.04±0.45	4.95±0.84
Protein%	3.43±0.20	3.78±0.24	3.45±0.21[a]	3.77±0.21[b]	3.48±0.21[a]	3.76±0.19[b]	3.50±0.22	3.72±0.23	3.53±0.23	3.73±0.26
Lactose%	4.78±0.08[a]	4.62±0.10[b]	4.77±0.06	4.64±0.10	4.78±0.05	4.66±0.10	4.79±0.06	4.65±0.13	4.79±0.04	4.68±0.14
Total Solids%	13.19±0.38	14.16±0.76	13.41±0.25	14.07±0.83	13.57±0.25	14.11±0.75	13.73±0.31	14.06±0.93	13.95±0.38	13.97±0.97
SCC(10^3/mL)	139.30±50.80	186.80±126.30	130.70±45.80	166.00±100.30	131.00±54.10	147.20±91.30	118.00±44.20	141.90±94.80	85.70±51.80	114.80±94.90
SCS	3.42 ±0.51	3.57±1.07	3.32±0.55	3.51±0.83	3.29±0.69	3.36±0.77	3.15±0.63	3.29±0.78	2.61±0.85	2.73±1.39
Whey%	0.85±0.07[a]	1.00±0.11	0.86±0.06[a]	0.99±0.09[b]	0.87±0.05[a]	1.00±0.07[b]	0.89±0.04[a]	0.99±0.07[b]	0.90±0.03	0.99±0.08
Casein%	2.69±0.14	2.78±0.15	2.73±0.16	2.78±0.14	2.77±0.16	2.77±0.14	2.61±0.19	2.72±0.17	2.63±0.23	2.74±0.19
CN	75.30±1.71	73.54±1.57	75.40±1.77	73.75±1.44	75.41±1.46	73.50±1.35	74.56±1.08	73.23±0.90	74.53±1.86	73.45±1.12
Curd %g	57.38±4.97	57.58±7.88	56.38±3.36	57.76±6.83	58.12±4.82	57.98±6.80	58.41±6.99	60.21±7.36	60.24±3.49	61.98±9.80

Abbreviations: SD=standard deviation; PM=pooled milk sample.
The percentage in parentheses in pm 1-5, indicates the proportion of milk from younger cows in the PM for each pooled milk sample proportion pm1-5 between breeds, means values within the row with different superscripted (a,b) presented significance difference. ($p \leq 0.05$)

5.4. Fatty Acid Composition

The mean values of fatty acid composition from the milk sampling in SRB and SLB are shown in Tables 11-12. SFA, UFA, MUFA, PUFA, C16:0, C18:0, C18:1C9, and C14:0 were increased as the proportion of milk from young cows increased in pooled milk but it was significant only for SFA acid in Swedish Holstein cows (P<0.05). In Swedish red cows pooled milk sample values were similar for each fatty acid and also there was no significance difference. When comparing SRB and SLB, SRB had higher Fatty acids compared to SLB but there was no significance difference in any fatty acids between breeds.

Table 10. Milk fatty acid composition in pooled milk samples (PM1-5) for Swedish Holstein (n=3) cows. Values presented are mean and standard deviation.

SLB Means ± SD	PM1 (0%) Old cows	PM2 (30%)	PM3 (50%)	PM4 (70%)	PM5 (100%) Young Cows
Object	**Means ± SD**	**Means ± SD**	**Means ± SD**	**Means ± SD**	**Means ± SD**
SFA	2.88±0.10 [a]	3.00±0.02 [ab]	3.06±0.05 [ab]	3.14±0.14 [ab]	3.27±0.20 [b]
UFA	1.11±0.23	1.21±0.23	1.28±0.25	1.33±0.25	1.40±0.29
MUFA	0.83±0.18	0.92±0.18	0.98±0.20	1.02±0.20	1.08±0.24
PUFA	0.09±0.06	0.11±0.05	0.13±0.06	0.13±0.05	0.14±0.05
C16:0	1.34±0.19	1.39±0.15	1.41±0.11	1.44±0.09	1.50±0.09
C18:0	0.33±0.09	0.34±0.11	0.38±0.12	0.40±0.15	0.44±0.17
C18:1C9	0.68±0.17	0.73±0.17	0.78±0.17	0.81±0.17	0.85±0.21
C14:0	0.57±0.03	0.60±0.03	0.61±0.04	0.62±0.05	0.64±0.06

Abbreviations: SD=standard deviation; PM=pooled milk sample.
The percentage in parentheses in pm 1-5, indicates the proportion of milk from younger cows in the PM
[a,b] Different letter superscripts in the same row indicate significance difference within breed (p≤0.05)

Table 11. Milk fatty acid composition in pooled milk samples (PM1-5) for Swedish red (n=9) cows. Values presented are mean and standard deviation.

SRB Means ± SD	PM1 (0%) Old cows	PM2 (30%)	PM3 (50%)	PM4 (70%)	PM5 (100%) Young Cows
Object	**Means ± SD**	**Means ± SD**	**Means ± SD**	**Means ± SD**	**Means ± SD**
SFA	3.41±0.46	3.34±0.51	3.36±0.50	3.37±0.60	3.29±0.65
UFA	1.31±0.22	1.30±0.23	1.32±0.23	1.31±0.27	1.29±0.28
MUFA	1.00±0.18	1.00±0.20	1.01±0.20	1.00±0.23	0.98±0.24
PUFA	0.12±0.04	0.13±0.04	0.13±0.04	0.13±0.04	0.12±0.05
C16:0	1.55±0.24	1.53±0.26	1.54±0.24	1.53±0.29	1.50±0.30
C18:0	0.41±0.09	0.41±0.10	0.42±0.11	0.44±0.12	0.44±0.14

C18:1C9	0.84±0.15	0.83±0.15	0.83±0.17	0.83±0.20	0.81±0.21
C14:0	0.67±0.10	0.66±0.10	0.66±0.10	0.64±0.12	0.63±0.12

Abbreviations: SD=standard deviation; PM=pooled milk sample.
The percentage in parentheses in pm 1-5, indicates the proportion of milk from younger cows in the PM
[a,b] Different letter superscripts in the same row indicate significance difference within breed (P<0.05).

5.5. Rheological measurements

5.5.1. Gel firmness

The gel firmness (G20) in the different PMs of milk from old and young cows of the SRB breed did not vary as much compared to milk from SLB (see Figure 6 a–b). The mean values of gel firmness from sampling occasions in both breeds are shown in Figure. A numerical stronger gel was observed in PM 1–3 in SRB compared to SLB, in contrast to a weaker gel in PM 4–5. It was a greater variation in G20 within the pooled SLB milk samples compared to SRB, especially for PM1 (see Figure 9).

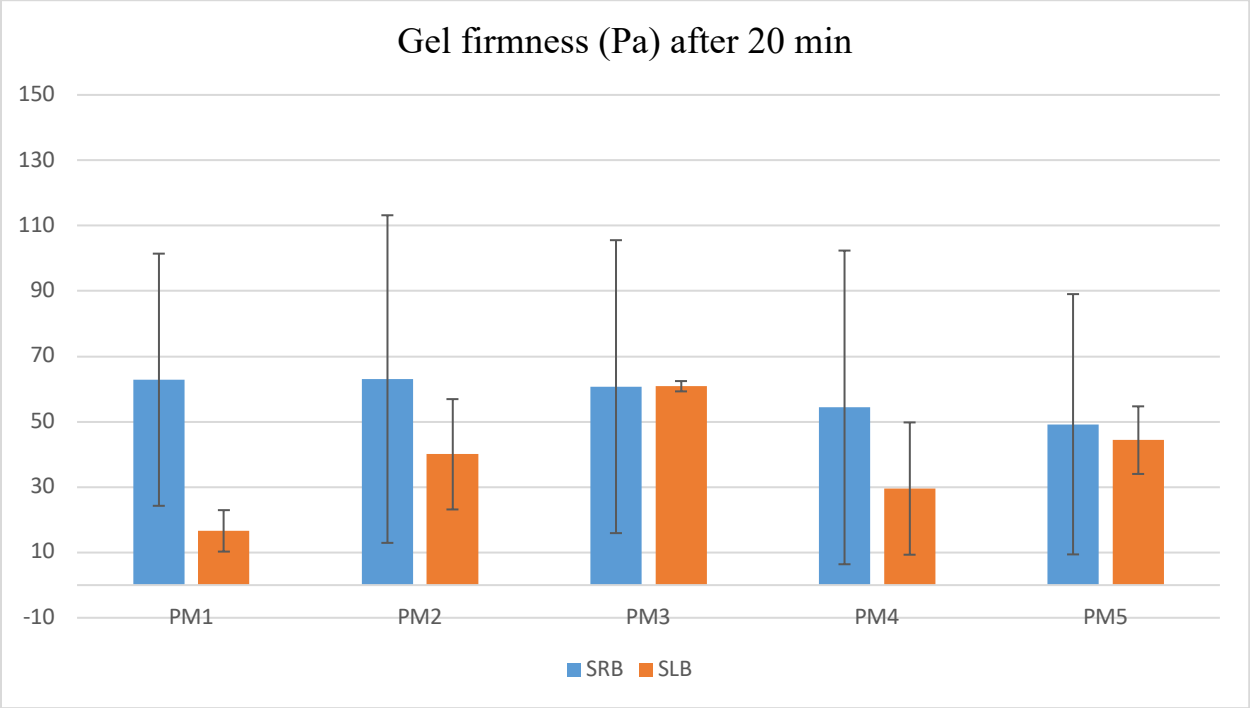

Figure 9. The mean values (n=12) of gel firmness (Pa) in milk from Swedish red and Swedish Holstein cows.

PM1–5 is the different pooled milk samples, and the percentage indicates the proportion of milk from young cows in the PM. Standard deviation is indicated.

5.5.2. Rennet coagulation time for milk from Swedish red and Swedish Holstein cows.

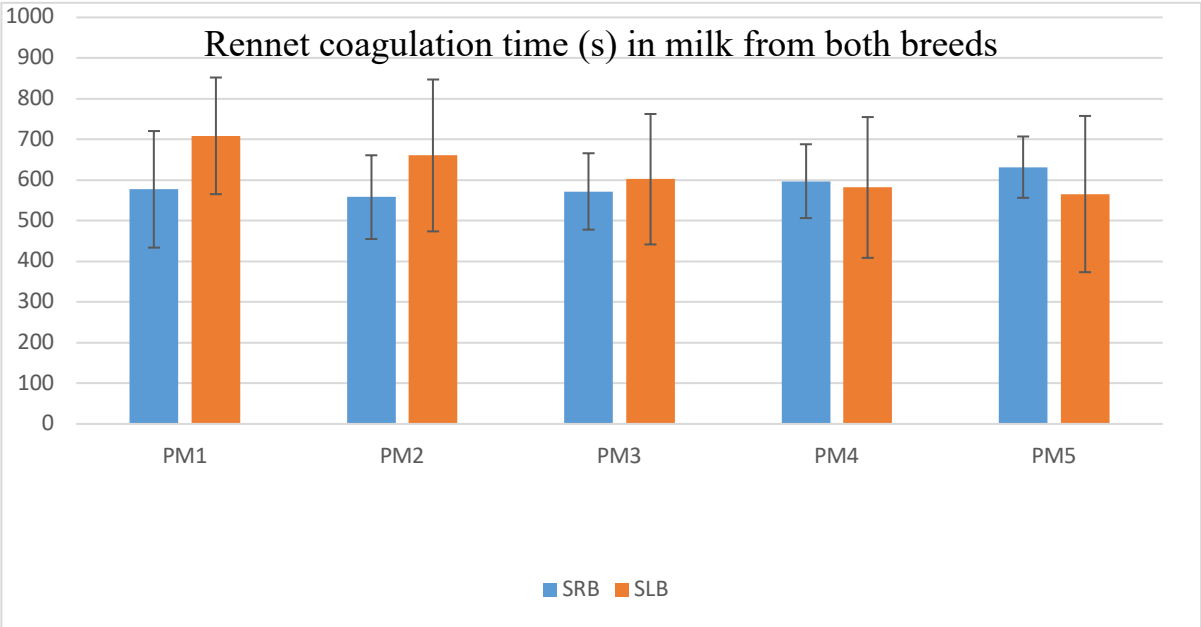

Figure 10. The mean values (n=12) of rennet coagulation time(s) in milk samples from Swedish red and Swedish Holstein cows.

PM1–5 is the different pooled milk samples, and the percentage indicates the proportion of milk from young cows in the PM. Standard deviation is indicated.

The mean values of RCT from sampling occasions in both breeds are shown in Figure 10. Milk from older cows had 22.8% numerically higher RCT in SLB compared to SRB older cows and milk from younger cows observed the opposite of this where the younger SRB cows had 11.7% numerically higher RCT compared to SLB younger cows. Besides there was a higher difference between breeds but there was no significance difference. The proportion of milk from younger cows increased, and the RCT decreased in the milk of SLB cows but the decrease was not significant between pooled milk samples.

6. DISCUSSION

6.1. Milk composition

According to cattle statistics 2021, SLB cows had higher numerical content of total protein compared to SRB cows. Our study also had a higher numerical value of total protein and total casein in SRB cows compared to SLB cows. Considering the result of Wedholm (2008) total protein is expected to be higher in milk from SRB cows compared to SLB cows. The protein content in the milk is largely determined by the availability of amino acids in the feed and proper activity in the ruminant system (Nilsson, 2017). Therefore along with the genetic factors, the feed could be the one reason why there was a variance between SRB and SLB for the total protein content. Previous studies results show that the concentration of milk fat was significantly higher in SRB milk compared to SLB milk. In our study same result was observed, the fat content of SRB milk was 7.75 % higher compared to SLB milk. According to previous studies allele frequencies and effects of the DGAT1 K232A variants in the Swedish dairy breeds could be the reason for the difference in milk fat between SRB and SLB (Näslund et al., 2008). In the present results, Average lactose content was higher in SLB compared to SRB. Other studies also mentioned average lactose content is also higher in SLB. However, the lactose content between breeds was not significant in our study. Wedholm (2006a) reported significant higher concentrations of lactose in milk from SLB than in milk from SRB, thus in agreement with our result. α-Lactalbumin acts as the regulatory protein of lactose synthase (Walstra et al. 2005). Therefore, the concentration of α-Lactalbumin in milk affects the concentration of lactose (Wedholm 2008). But there was no positive correlation between 'α-LA' and 'lactose' in our research. In this study, milk from SRB had a higher proportion of fat, total solids, SFA, UFA, MUFA, PUFA, C14:0, C16:0, C18:0, and C18:1C9, compared to SLB. But it was significant only for SFA between two breeds. Somatic cell score (SCS) was decreased numerically with a higher proportion of milk from younger cows. Therefore milk from older cows contained more SCS compared to the milk from young cows. This was observed for both breeds, but there was no significance difference between pooled milk samples only numerically higher difference were observed. Somatic cell score is used as an indicator trait for intramammary infection (Shook & Schutz (1994).In our research Somatic cell score ranged between 2.6 – 3.6 for both breeds which seemed to indicate that they were healthy during the time of sampling.

There was significant correlation between SCS with other quality parameters in pooled milk samples. C18:0 and SCS positively correlated in PM1(0.688, $P<0.05$), PM2(0.747, $P<0.05$) and PM3(0.689, $P<0.05$) in SRB cows. In milk from younger SRB cows (PM5) Total solids, fat, protein, whey, and casein were positively correlated for SCS ($P<0.05$; $P<0.01$). Whey and SCS positively correlated for PM2 and PM3 IN SLB cows ($P<0.05$). When comparing the breeds milk from SRB cows had higher SCS compared to SLB. In previous research, SLB cows had higher SCC counts because of high exposure to diseases. Due to limited number of SLB replicates could be the reason why there was no such observation in our research.

6.2. Protein Profile

In our research, relative concentrations of β_{A2}-CN and β_{A1}-CN varied with respect to breed. SLB had a higher content of β_{A2}-CN compared to SRB, and SRB had higher relative content of β_{A1}-CN compared to SLB. This is in contrast with Wedholm (2006a), who stated that β_{A1}-CN was more common in milk from SRB cows than in SLB in Swedish dairy herds. The high frequency of A1 allele in the SRB population could be the reason for this specific observation. According to previous research, SRB had a higher concentration of β-CN compared to SLB milk. The lower concentration of β-casein in SLB milk could be due to protein degradation since they found a higher level of amino terminals in SLB compared to SRB milk. In our research higher concentrations of β-casein were not observed in SLB milk compared to SRB. The reason for this observation could be due to a limited number of SLB cows used in our research.

There was a trend that the relative concentrations of κ-CN, β_B-CN, and β_{A1}-CN decreased with higher proportions of milk from younger cows but it was not significant for any of the pooled milk samples. Total β-Casein was increased with higher proportions of milk from younger cows and it was significant only for milk from younger cows (PM5) and old cows (PM1) in the SLB breed ($P<0.05$). These results suggested that lactation number/ Pooled milk levels limited influence on milk protein profile and milk gross composition but according to a recent findings from Hallqvist, J,. (2022) Gabriella Apelthun. (2020) there was a great variety in the results for protein profile between milk from older and younger cows. Furthermore, they stated that the evidence for observed differences is not strong enough to conclude if there is a difference or not, related to lactation number.

6.3. pH and Ethanol stability

In this study average pH is similar between the two breeds. It was found that the average pH value for the two breeds was 6.6. There was no significant difference in mean pH between pooled milk samples. Most of the research found that the average pH value for normal milk is 6.6. The ethanol stability test indicates the ethanol concentration needed to cause the milk proteins to precipitate. The purpose of the ethanol test is to predict the milk heat stability and is used as a reliable indicator of raw milk stability for ultra-high temperature and milk powder production process (Boumpa et al., 2008; Omoarukhe et al., 2010). In the current study Ethanol stability of the SLB milk was higher compared to SRB. ethanol stability of milk from SLB was always above 82% but it ranged between 56% and 90 % in SRB cows. The low ethanol stability in SRB milk could be due to the content of ionized calcium but it was not estimated in our research

6.4. Curd Yield

The variances in curd yield between the breed and pooled milk levels rely on several important factors such as pH, fat percentage, and calcium ion activity (Walstra, 2006). In our research SRB cows, curd yield increased with an increased proportion of milk from younger cows. Milk from SLB cows showed greater variability in curd yield among the PM compared to SLB. There was no significance difference in curd yield between young and old cows in SRB and SLB. Casein number (Cn No %) is an important factor in cheese making (Walstra, 2006) and it is defined as total casein out of the total protein, times hundred. In our research Swedish Holstein cows had higher casein number compared to Swedish red cows. According to Lindmark-Mansson *et al*. (2013) low casein content is negative for the cheese yield and a high casein number has positive impact in cheese making (Walstra, 2006). However, In our study casein number had strong negative correlation to the curd yield (-0,776, $P<0.001$) in Swedish Holstein cows. With increasing age, casein concentration decreases and whey concentration increases, whereas with the increasing lactation, casein concentration increases (Ng-Kwai-Hang et al., 1982). In our study lowest casein number within SRB cows were observed in milk from young cows but it was not significant. In SLB cows were not observed any specific observation. According to Wickstrom lower casein number was associated with higher somatic cell count but the opposite was observed in our research. SCS was negatively correlated to curd yield. It was significant only for PM2 and PM3 in SRB cows ($P<0.05$).

6.5. Rheological Measurement

In SLB gel firmness increased in pooled milk samples with a higher proportion of milk from young cows. However, it was not significant. In SRB opposite was observed, a higher proportion of milk from young cows had lower gel firmness. According to Ng-Kwai-Hang (1998), κ-casein has a reduced coagulation time (by 10%–40%) and increased curd firmness (by 20%–140%). In this study κ-casein was higher in milk from old cows and lower in milk from young cows for both breeds. κ-casein content decreased with an increasing proportion of milk from young cows. However, according to numerous research strong gel firmness was observed in milk from young cows but it was the opposite in our research. Casein has effect on both curd firmness at cutting and firmness and composition of the cheese. Lower milk fat and casein concentrations lower the cheese yield. Total casein decreased with an increasing proportion of milk from young cows in SRB but it varied between the PM for SLB cows.(Semenova et al., 2010). Somatic cell count reflects the udder health condition and the milk quality in the herd or population. It also provides information on the inflammatory status of the mammary gland (Schukken et al., 2003).

SCS is the most important factor that affects milk coagulation properties along with the protein composition. High somatic cell score is associated with lower clotting ability of milk resulting in slower coagulation and weaker curd firmness. It negatively effect on cheese making properties as well as cheese processing (Cecchinato et al., 2016). SCS associated with pH, when SCS increased milk pH also increased simultaneously thus it reduced the enzyme activity related to milk clotting (Swaisgood., 1982). In our research total protein content was increased along with the lower SCS in SLB cows but it was not significant. SCS and total casein content positively correlation (P<0.05) in SLB but in SRB there were weak positive correlation compared to SLB. There was no significant correlation between SCS and rheological measurements. Only RCT negatively correlated with SCS in PM3 and PM4 (-0.645, -0.677) respectively in SRB cows the but level of significance was $p \leq 0.1$. Wickstrom et al. (2009) and Leitner et al. (2008) also reported that RCT was not correlated to the SCS in their studies and also no correlation was observed between SCS and gel firmness in a study by Wickstrom et al., 2009. This is in agreement with our result which there were no significance correlation between SCS and gel firmness, and SCS and RCT. It is therefore likely that the coagulation properties are affected by many other different factors.

7. CONCLUSION

When investigating the differences in raw milk quality i.e. pH, gross composition, SCS, and processability of milk i.e. ethanol stability, rennet coagulation time (RCT), gel firmness, and curd yield from cows with a different number of lactations and breeds, some differences were found but overall there was no major difference between older cows, i.e. cows who had 3≥ lactations and younger cows (2≤ lactations). Instead, as it seems, the variation in milk composition was mainly associated to breed. Swedish red and white cows (SRB) had numerically higher values of fat, total solids, SFA, UFA, MUFA, PUFA, C14:0, C16:0, C18:0, and C18:1C9, compared to Swedish Holstein (SLB). Milk from SRB cows had a numerically higher concentrations of β_{A1}-CN and lower relative concentration of β_{A2}-CN. The main aim of this study was to evaluate the effect of lactation number/Pooled milk levels on the gross composition and processability of the milk. In conclusion, in this study, there were no major differences between milk from young and old cows. In this study, only a few SLB cows were included compared to SRB which might affect the obtained statistical results since it was not possible to include both effects (i.e. lactation number and breed) at the same time. The results could therefore be considered as implications and to obtain higher reliability, a larger number of SLB cows should have been included in this study. More extensive studies would be needed in order to further support the outcomes and show further how the number of lactations of the cow affects the processability and quality of milk. Further studies may also consider modifying pooled milk combinations and the definition of old and young cows.

8. REFERENCES

Alhussien, M. N., & Dang, A. K. (2018a). Milk somatic cells, factors influencing their release, future prospects, and practical utility in dairy animals: An overview. Veterinary World, 11(5), 562. https://doi.org/10.14202/VETWORLD.2018.562-577

Alhussien, M. N., & Dang, A. K. (2018b). Milk somatic cells, factors influencing their release, future prospects, and practical utility in dairy animals: An overview. Veterinary World, 11(5), 562. https://doi.org/10.14202/VETWORLD.2018.562-577

Andrews, A. T. (1983). Proteinases in normal bovine milk and their action on caseins. Journal of Dairy Research, 50(1), 45–55. https://doi.org/10.1017/S0022029900032519

Bär, C., Mathis, D., Neuhaus, P., Dürr, D., Bisig, W., Egger, L., & Portmann, R. (2019). Protein profile of dairy products: Simultaneous quantification of twenty bovine milk proteins. International Dairy Journal, 97, 167–175. https://doi.org/10.1016/J.IDAIRYJ.2019.01.001

Bastian, E. D., & Brown, R. J. (1996). Plasmin in milk and dairy products: an update. International Dairy Journal, 6(5), 435–457. https://doi.org/10.1016/0958-6946(95)000216

Bastian, E. D., Brown, R. J., & Anthon Ernstrom, C. (n.d.). Plasmin Activity and Milk Coagulation'.Journal of Dairy Science, 74, 3677–3685. https://doi.org/10.3168/jds.S0022-0302(91)78557-3

Bijl, E., de Vries, R., van Valenberg, H., Huppertz, T., & van Hooijdonk, T. (2014). Factors influencing casein micelle size in milk of individual cows: Genetic variants and glycosylation of κ-casein. International Dairy Journal, 34(1), 135–141. https://doi.org/10.1016/J.IDAIRYJ.2013.08.001

BiopolBiopolymers in Food Colloids: Thermodynamics and Molecular Interactions - Maria Germanovna Semenova, Eric Dickinson - Google Cărți. (n.d.). Retrieved August 27, 2021, fromhttps://books.google.se/books?hl=en&lr=&id=6lPSBQAAQBAJ&oi=fnd&pg=PP1&ots=M7Y. (n.d.).

Bobbo, T., Cipolat-Gotet, C., Bittante, G., & Cecchinato, A. (2016). The nonlinear effect of somatic cell count on milk composition, coagulation properties, curd firmness modeling, cheese yield, and curd nutrient recovery. Journal of Dairy Science, 99(7), 5104–5119. https://doi.org/10.3168/JDS.2015-10512

Burgess, K. (2014). Milk and Dairy Products in Human Nutrition (2013), by E. Muehlhoff, A. Bennett and D. McMahon, Food and Agriculture Organisation of the United Nations (FAO), Rome. E-ISBN: 978-92-5-107864-8 (PDF). Available on web-site (publications-sales@fao.org). International Journal of Dairy Technology, 67(2), 303–304. https://doi.org/10.1111/1471-0307.12124

Calbindin - an overview | ScienceDirect Topics. (n.d.). Retrieved August 4, 2021, from https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/milk-lipids

Casein and Whey Proteins in Human Health. (n.d.). Retrieved August 11, 2021, from https://www.researchgate.net/publication/263378881_Casein_and_Whey_Proteins_in_Human_Health

Cederberg, C., & Flysjö, A. (n.d.). SIK-rapport Nr 728 2004 Life Cycle Inventory of 23 Dairy Farms in South-Western Sweden.

Chang, I., Im, J., Chung, M. K., & Cho, G. C. (2018). Bovine casein as a new soil strengthening binder from diary wastes. Construction and Building Materials, 160, 1–9. https://doi.org/10.1016/J.CONBUILDMAT.2017.11.009

Cinar, M., Serbester, U., Ceyhan, A., & Gorgulu, M. (2015). Effect of somatic cell count on milk yield and composition of first and second lactation dairy cows. Italian Journal of Animal Science, 14(1), 105–108. https://doi.org/10.4081/IJAS.2015.3646

Cinar, M., Serbester, U., Ceyhan, A., & Gorgulu, M. (2016). Effect of Somatic Cell Count on Milk Yield and Composition of First and Second Lactation Dairy Cows. Http://Dx.Doi.Org/10.4081/Ijas.2015.3646,14(1),105–108. https://doi.org/10.4081/IJAS.2015.3646

Čítek, J., Brzáková, M., Hanusová, L., Hanuš, O., Večerek, L., Samková, E., Křížová, Z., Hoštičková, I., Kávová, T., Straková, K., & Hasoňová, L. (2020). Technological properties of cow's milk: correlations with milk composition, effect of interactions of genes and other factors. Czech Journal of Animal Science, 65(1), 13–22. https://doi.org/10.17221/150/2019-CJAS

Composition and processability of milk from older cows - Epsilon Archive for Student Projects. (n.d.). Retrieved March 17, 2022, from https://stud.epsilon.slu.se/16140/

Cow longevity economics: The cost benefit of keeping the cow in the herd - Milkproduction.com. (n.d.). Retrieved July 27, 2021, from http://www.milkproduction.com/Library/Scientific-articles/Management/Cow-longevity-economics-The-cost-benefit-of-keeping-the-cow-in-the-herd/

COZMA, A., ANDREI, S., MIERE, D., FILIP, L., & LOGHIN, F. (2011). Proteins Profile in Milk from Three Species of Ruminants. Notulae Scientia Biologicae, 3(1), 26–29. https://doi.org/10.15835/NSB315608

Davoodi, S. H., Shahbazi, R., Esmaeili, S., Sohrabvandi, S., Mortazavian, A., Jazayeri, S., & Taslimi, A. (2016). Health-Related Aspects of Milk Proteins. Iranian Journal of Pharmaceutical Research : IJPR, 15(3), 573. /pmc/articles/PMC5149046/

De Marchi, M., Penasa, M., Tiezzi, F., Toffanin, V., & Cassandro, M. (n.d.). Prediction of milk coagulation properties by Fourier Transform Mid-Infrared Spectroscopy (FTMIR) for genetic purposes, herd management and dairy profitability.

Diary-sweden-sustainability-goal (3).pdf - Google Search. (n.d.). Retrieved July 15, 2021, from https://www.google.com/search?q=diary-sweden-sustainability-goal+(3).pdf&rlz=1C1BYYL_svSE949SE949&oq=diary-sweden-sustainability-goal+(3).pdf&aqs=chrome..69i57j69i60&sourceid=chrome&ie=UTF-8

Driessen, f., & cb, v. D. W. (1978). Inactivation of native milk proteinase by heat treatment. Inactivation of native milk proteinase by heat treatment.

Dupont, D., Croguennec, T., & Pochet, S. (2018). Milk Proteins-Analytical Methods. Milk Proteins-Analytical Methods. Reference Module in Food Science, 9780081005965. https://doi.org/10.1016/B978-0-08-100596

Ekstrand, B., Larsson-Raźnikiewicz, M., & Perlmann, C. (1980). Casein micelle size and composition related to the enzymatic coagulation process. Biochimica et Biophysica Acta (BBA) - General Subjects, 630(3), 361–366. https://doi.org/10.1016/0304-4165(80)90284-6

Emery, R. S. (1988). Milk Fat Depression and the Influence of Diet on Milk Composition. Veterinary Clinics of North America: Food Animal Practice, 4(2), 289–305. https://doi.org/10.1016/S0749-0720(15)31049-5

Fao. (2013). MILK human nutrition dairy products. www.fao.org/

Fetrow, J., Nordlund, K. V., & Norman, H. D. (2006). Invited Review: Culling: Nomenclature, Definitions, and Recommendations. Journal of Dairy Science, 89(6), 1896–1905. https://doi.org/10.3168/JDS.S0022-0302(06)72257-3

Fox, P. F. (2003). Milk Proteins: General and Historical Aspects. In Advanced Dairy Chemistry—1 Proteins (pp. 1–48). Springer US. https://doi.org/10.1007/978-1-4419-8602-3_1

Fox, P. F., Uniacke-Lowe, T., McSweeney, P. L. H., & O'Mahony, J. A. (2015). Dairy chemistry and biochemistry, second edition. Dairy Chemistry and Biochemistry, Second Edition, 1–584. https://doi.org/10.1007/978-3-319-14892-2

Gambelli, L. (2017). Milk and Its Sugar-Lactose: A Picture of Evaluation Methodologies. Beverages 2017, Vol. 3, Page 35, 3(3), 35. https://doi.org/10.3390/BEVERAGES3030035

Gänzle, M. G. (2021). Manufacture of Milk and Whey Products: Lactose. Reference Module in Food Science. https://doi.org/10.1016/B978-0-12-818766-1.00315-9

Grohn, Y., Galligan, D. T., Gröhn, Y. T., Rajala-Schultz, P. J., Allore, H. G., Delorenzo, M. A., Hertl, J. A., & Galligan, D. T. (2003). Optimizing replacement of dairy cows: Modeling the effects of diseases. Preventive Veterinary Medicine, 61, 27–43. https://doi.org/10.1016/S0167-5877(03)00158-2

Gunnarsson, S., Sonesson, U., Stenberg, M., & Kumm, K. I. (2009). Scenarios for future Swedish dairy farming in relation to sustainability of animal health, environment and economy. Sustainable Animal Production: The Challenges and Potential Developments for Professional Farming, January 2014, 445–458. https://doi.org/10.3920/978-90-8686-685-4

Guo, M. R., Wang, S., Li, Z., Qu, J., Jin, L., & Kindsted, P. S. (1998). Ethanol stability of goat's milk. International Dairy Journal, 8(1), 57–60. https://doi.org/10.1016/S0958-6946(98)00020-X

Hägglöf, P. (2004). PLASMINOGEN ACTIVATOR INHIBITOR TYPE-1: structure-function studies and its use as a reference for intramolecular distance measurements.

Hameed, A., Hussain, M., & Akhtar, S. (2017). Lactation Responses toward Milk Indigenous Enzymes. Livestock Science. https://doi.org/10.5772/66392

Hanuš, O., Samková, E., Křížová, L., Hasoňová, L., & Kala, R. (2018). Role of Fatty Acids in Milk Fat and the Influence of Selected Factors on Their Variability—A Review. Molecules : A Journal of Synthetic Chemistry and Natural Product Chemistry, 23(7). https://doi.org/10.3390/MOLECULES23071636

Hodgson, H. J. (1979). Role of the Dairy Cow in World Food Production. Journal of Dairy Science, 62(2), 343–351. https://doi.org/10.3168/jds.S0022-0302(79)83246-4

Ismail, B., & Nielsen, S. S. (2010). Invited review: Plasmin protease in milk: Current knowledge and relevance to dairy industry. Journal of Dairy Science, 93(11), 4999–5009. https://doi.org/10.3168/JDS.2010-3122

Jack, E. L., & Smith, I. ~¢~. (1956). CHEMISTRY OF MILK FAT: A REVIEW. Journal of Dairy Science, 39, 1–25. https://doi.org/10.3168/jds.S0022-0302(56)94700-2

Johansson, M., Lundh, Å., Vries, R. de, & Sjaunja, K. S. (2017). Composition and enzymatic activity in bulk milk from dairy farms with conventional or robotic milking systems. Journal of Dairy Research, 84(2), 154–158. https://doi.org/10.1017/S0022029917000140

jordbruksverk, S., & Scb, S. (2020). Statistiska meddelanden-Animalieproduktion. Års- och månadsstatistik, 2020:04.

Jõudu, I., Kaart, T., & Henno, M. (n.d.). The effect of milk proteins on milk coagulation properties in estonian dairy breeds [Pieno baltymu itaka estijos melžiamu karviu pieno koaguliacijai]. Veterinarija Ir Zootechnika. Retrieved August 11, 2021, from https://www.academia.edu/16251222/The_effect_of_milk_proteins_on_milk_coagulation_properties_in_estonian_dairy_breeds_Pieno_baltymu_itaka_estijos_melžiamu_karviu_pieno_koaguliacijai_

Jõudu, Ivi, Henno, M., Kaart, T., Püssa, T., & Kärt, O. (2008b). The effect of milk protein contents on the rennet coagulation properties of milk from individual dairy cows. International Dairy Journal, 18(9), 964–967. https://doi.org/10.1016/J.IDAIRYJ.2008.02.002

Kalyankar, S. D., Khedkar, C. D., Patil, A. M., & Deosarkar, S. S. (2016). Milk: Sources and Composition. Encyclopedia of Food and Health, 741–747. https://doi.org/10.1016/B978-0-12-384947-2.00463-3

Kilshaw, P. J., Heppell, L. M. J., & Ford, J. E. (1982). Effects of heat treatment of cows' milk and whey on the nutritional quality and antigenic properties. Archives of Disease in Childhood, 57, 842–847. https://doi.org/10.1136/adc.57.11.842

Korycha-Dahl, M., Dumas, B. R., Chene, N., & Martal, J. (1983). Plasmin Activity in Milk. Journal of Dairy Science, 66(4), 704–711. https://doi.org/10.3168/JDS.S0022-0302(83)81848-7

Kuylenstierna, J., Barraza, H. J., Benton, T., Larsen, A. F., Kurppa, S., Lipper, L., & Virgin, I. (2019). Background paper Food Security and Sustainable Food Systems Research to support a sustainable, competitive and innovative Swedish food system by 2030 Background paper.

Laben, R. C. (1963). 28) SOLBZ~G, P. Norwegian Contributions to the Development of the Amido t~lack Method Suggested by Schober & Hetzel for the Estimation of Protein in Milk and Milk Products. Journal of Dairy Science, 46(22), 1293–1301. https://doi.org/10.3168/jds.S0022-0302(63)89264-4

Larsson, M. (n.d.). Towards a Sustainable Food System Entrepreneurship, Resilience and Agriculture in the Baltic Sea Region.

Le Maréchal, C., Thiéry, R., Vautor, E., & Le Loir, Y. (2011). Mastitis impact on technological properties of milk and quality of milk products—a review. Dairy Science & Technology 2011 91:3, 91(3), 247–282. https://doi.org/10.1007/S13594-011-0009-6

Lehenbauer, T. W., & Oltjen, J. W. (1998). Dairy Cow Culling Strategies: Making Economical Culling Decisions. Journal of Dairy Science, 81(1), 264–271. https://doi.org/10.3168/jds.S0022-0302(98)75575-4

Lin, W., Dean, G. W., & Moore, C. V. (1974). An Empirical Test of Utility vs. Profit Maximization in Agricultural Production. American Journal of Agricultural Economics, 56(3), 497–508. https://doi.org/10.2307/1238602

LINN, J. G. (1988). Factors Affecting the Composition of Milk from Dairy Cows. https://www.ncbi.nlm.nih.gov/books/NBK218193/

Lisson, M., Lochnit, G., & Erhardt, G. (2013). Genetic variants of bovine β- and κ-casein result in different immunoglobulin E-binding epitopes after in vitro gastrointestinal digestion. Journal of Dairy Science, 96(9), 5532–5543. https://doi.org/10.3168/jds.2013-6684

Louis Tyasi, T., Gxasheka, M., PHILLEMON Tlabela, C., & Phillemon Tlabela, C. (2015). Assessing the effect of nutrition on milk composition of dairy cows: A review Woody Plant Encroachment in Semi-arid Savanna grasslands South Africa View project IV. INTERNATIONAL CONGRESS ON DOMESTICIC ANIMAL BREEDING GENETICS AND HUSBANDRY 2020. At: Turk. INT J CURR SCI, 17, 56–63. https://www.researchgate.net/publication/282121067

LRF Dairy Sweden - LRF. (n.d.). Retrieved July 15, 2021, from https://www.lrf.se/om-lrf/in-english/lrf-dairy-sweden/

Marziali, A. S., & Ng-Kwai-Hang, K. F. (1986). Effects of Milk Composition and Genetic Polymorphism on Coagulation Properties of Milk. Journal of Dairy Science, 69(7), 1793–1798. https://doi.org/10.3168/JDS.S0022-0302(86)80603-8

Meadows, C., Rajala-Schultz, P. J., & Frazer, G. S. (2005). A spreadsheet-based model demonstrating the nonuniform economic effects of varying reproductive performance in Ohio dairy herds. Journal of Dairy Science, 88(3), 1244–1254. https://doi.org/10.3168/JDS.S0022-0302(05)727910

Milk: Role in the Diet | Elsevier Enhanced Reader. (n.d.). Retrieved August 11, 2021, from https://reader.elsevier.com/reader/sd/pii/B9780123849472004621?token=A18AAFFEC79E03469D722B88F6CC18831323E211D14EF252E1413DDD11DC81CB55F693FAFE7809966A8811CD9349A0D9&originRegion=eu-west-1&originCreation=20210811084356

Milk composition and microbiology - Milk composition. (n.d.). Retrieved August 4, 2021, from https://www.groupe-esa.com/ladmec/bricks_modules/brick02/co/ZBO_Brick02_2.html

Milkproduction.com. (n.d.-a). Cow longevity economics: The cost benefit of keeping the cow in the herd.

Milkproduction.com. (n.d.-b). Two competing Swedish.

Mohamed, H., Johansson, M., Lundh, Å., Nagy, P., & Kamal-Eldin, A. (2020). Short communication: Caseins and α-lactalbumin content of camel milk (Camelus dromedarius) determined by capillary electrophoresis. Journal of Dairy Science, 103(12), 11094–11099. https://doi.org/10.3168/JDS.2020-19122

Näslund, J., Fikse, W. F., Pielberg, G. R., & Lundén, A. (2008). Frequency and Effect of the Bovine Acyl-CoA:Diacylglycerol Acyltransferase 1 (DGAT1) K232A Polymorphism in Swedish Dairy Cattle. Journal of Dairy Science, 91(5), 2127–2134. https://doi.org/10.3168/JDS.2007-0330

Older Cows Are More Profitable - Cornell Small Farms. (n.d.). Retrieved July 26, 2021, from https://smallfarms.cornell.edu/2021/04/older-cows-are-more-profitable/

Osorio, J. S., Lohakare, J., & Bionaz, M. (2016). Biosynthesis of milk fat, protein, and lactose: roles of transcriptional and posttranscriptional regulation. Https://Doi.Org/10.1152/Physiolgenomics.00016.2015, 48(4), 231–256. https://doi.org/10.1152/PHYSIOLGENOMICS.00016.2015

Pandalai, S. G. (2005). Recent research developments in agricultural & food chemistry : vol. (2005).

Petrotos, K., Tsakali, E., Goulas, P., & D'Alessandro, A. G. (2014). Casein and whey proteins in human health. Milk and Dairy Products as Functional Foods, 94–146. https://doi.org/10.1002/9781118635056.CH4

Poulsen, N. A., Glantz, M., Rosengaard, A. K., Paulsson, M., & Larsen, L. B. (2017). Comparison of milk protein composition and rennet coagulation properties in native Swedish dairy cow breeds and high-yielding Swedish Red cows. Journal of Dairy Science, 100(11), 8722–8734. https://doi.org/10.3168/JDS.2017-12920

Proteins Profile in Milk from Three Species of Ruminants. (n.d.). Retrieved August 11, 2021, fromhttps://www.researchgate.net/publication/50302398_Proteins_Profile_in_Milk_from_Three_Species_of_Ruminants

Schukken, Y. H., Wilson, D. J., Welcome, F., Garrison-Tikofsky, L., & Gonzalez, R. N. (2003). Monitoring udder health and milk quality using somatic cell counts. Vet. Res, 34, 579–596. https://doi.org/10.1051/vetres:2003028

Segerkvist, K. A., Hansson, H., Sonesson, U., & Gunnarsson, S. (2020). Research on environmental, economic, and social sustainability in dairy farming: A systematic mapping of current literature. Sustainability (Switzerland), 12(12), 1–14. https://doi.org/10.3390/SU12145502

Semenova, M. G., Dickinson, E., Burlakova, E., & Zaikov, G. (2010). Biopolymers in food colloids: Thermodynamics and molecular interactions. In Biopolymers in Food Colloids: Thermodynamics and Molecular Interactions. CRC Press. https://doi.org/10.1201/b12817

Sinaga, H., Bansal, N., & Bhandari, B. (2016). Effects of milk pH alteration on casein micelle size and gelation properties of milk. Http://Dx.Doi.Org/10.1080/10942912.2016.1152480, 20(1), 179–197. https://doi.org/10.1080/10942912.2016.1152480

Singh, A., Kumar, A., Saini, B., Mehrotra, A., Chaudhary, S., & Yadav, V. (2018). Effect of non-genetic factors on milk fat composition: A review. Undefined.

Startsida jordbruksverket.se - Jordbruksverket.se. (n.d.). Retrieved July 29, 2021, from https://jordbruksverket.se/

Sverige. (2007). Sweden facing climate change: threats and opportunities ; final report from the Swedish Commission on Climate and Vulnerability. In Statens offentliga utredningar (Issue 2007,60). www.fritzes.se

Sweden - agri benchmark. (n.d.). Retrieved August 17, 2021, from http://www.agribenchmark.org/dairy/country-information/sweden.html

Sweden – one of Europe's most heavily forested countries. (n.d.). Retrieved August 17, 2021, from https://www.scb.se/en/finding-statistics/statistics-by-subject-area/environment/land-use/land-use-in-sweden/pong/statistical-news/land-use-in-sweden-20102/

Swedish, T., & Swedish, T. (2020). Sustainability goals for the Swedish Dairy sector. November.

Talvela, N. ., & Bas, C. (2018). Dairy Production in Malaysia.

Tavares, T. G., & Malcata, F. X. (2013). Whey Proteins as Source of Bioactive Peptides Against Hypertension. Bioactive Food Peptides in Health and Disease. https://doi.org/10.5772/52680

Taylor, M. W., & MacGibbon, A. K. H. (2002). LIPIDS | General Characteristics. Encyclopedia of Dairy Sciences, 1544–1550. https://doi.org/10.1016/B0-12-227235-8/00259-5

The effect of milk proteins on milk coagulation properties in estonian dairy breeds [Pieno baltymu itaka estijos melžiamu karviu pieno koaguliacijai]. (n.d.). Retrieved August 11, 2021, fromhttps://www.researchgate.net/publication/279710926_The_effect_of_milk_proteins_on_

milk_coagulation_properties_in_estonian_dairy_breeds_Pieno_baltymu_itaka_estijos_melzia mu_karviu_pieno_koaguliacijai

Toledo, I. M., & Thatcher, W. W. (2022). Heat Stress: Effects on Milk Production and Composition. Encyclopedia of Dairy Sciences, 276–284. https://doi.org/10.1016/B978-0-12-818766-1.00005-2

Tong, P. S., & Berner, L. A. (2016). Dairy Processing and Products. Reference Module in Food Science. https://doi.org/10.1016/B978-0-08-100596-5.02935-8

Two competing Swedish - Milkproduction.com. (n.d.). Retrieved July 27, 2021, from http://www.milkproduction.com/Library/Scientific-articles/Reproduction/Two-competing-Swedish/

V, B., M, G., R, R., A, C., & P, C. (2013). Protein composition affects variation in coagulation properties of buffalo milk. Journal of Dairy Science, 96(7), 4182–4190. https://doi.org/10.3168/JDS.2012-6333

Van Knegsel, A. T. M., Hammon, H. M., Bernabucci, U., Bertoni, G., Bruckmaier, R. M., Goselink, R. M. A., Gross, J. J., Kuhla, B., Metges, C. C., Parmentier, H. K., Trevisi, E., Troscher, A., & Van Vuuren, A. M. (2014a). Metabolic adaptation during early lactation: Key to cow health, longevity and a sustainable dairy production chain. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources, 9(January). https://doi.org/10.1079/PAVSNNR20149002

Visentin, G., McParland, S., Marchi, M. De, McDermott, A., Fenelon, M. A., Penasa, M., & Berry, D. P. (2017). Processing characteristics of dairy cow milk are moderately heritable. Journal of Dairy Science, 100(8), 6343–6355. https://doi.org/10.3168/JDS.2017-12642

Wedholm, A., Hallén, E., Bach Larsen, L., Lindmark-Månsson, H., Hans Karlsson, A., & Allmere, T. (2007b). Comparison of milk protein composition in a Swedish and a Danish dairy herd using reversed phase HPLC. Http://Dx.Doi.Org/10.1080/09064700600836745, 56(1), 8–15. https://doi.org/10.1080/09064700600836745

Wijayanti, H. B., Bansal, N., & Deeth, H. C. (2014). Stability of Whey Proteins during Thermal Processing: A Review. Comprehensive Reviews in Food Science and Food Safety, 13(6), 1235–1251. https://doi.org/10.1111/1541-4337.12105

Wille-Sonk, S., & Lassen, B. (n.d.). Dairy production in Sweden-impressions-Dairy 1 Dairy Production in Sweden-impressions. Retrieved August 17, 2021, from www.dairyfarmer.net.

Wright, A. J., Marangoni, A. G., & Hartel, R. W. (2011). Milk Lipids: Rheological Properties and Their Modification. Encyclopedia of Dairy Sciences: Second Edition, 704–710. https://doi.org/10.1016/B978-0-12-374407-4.00340-X

Yalcin, A. (2006). Emerging Therapeutic Potential of Whey Proteins and Peptides. Current Pharmaceutical Design, 12(13), 1637–1643. https://doi.org/10.2174/138161206776843296