Quality parameters and protein profile in raw milk from individual cows with different lactation number and breed

Kvalitetsparametrar och proteinprofil i mjölk från individuella kor med olika laktationsnummer och ras

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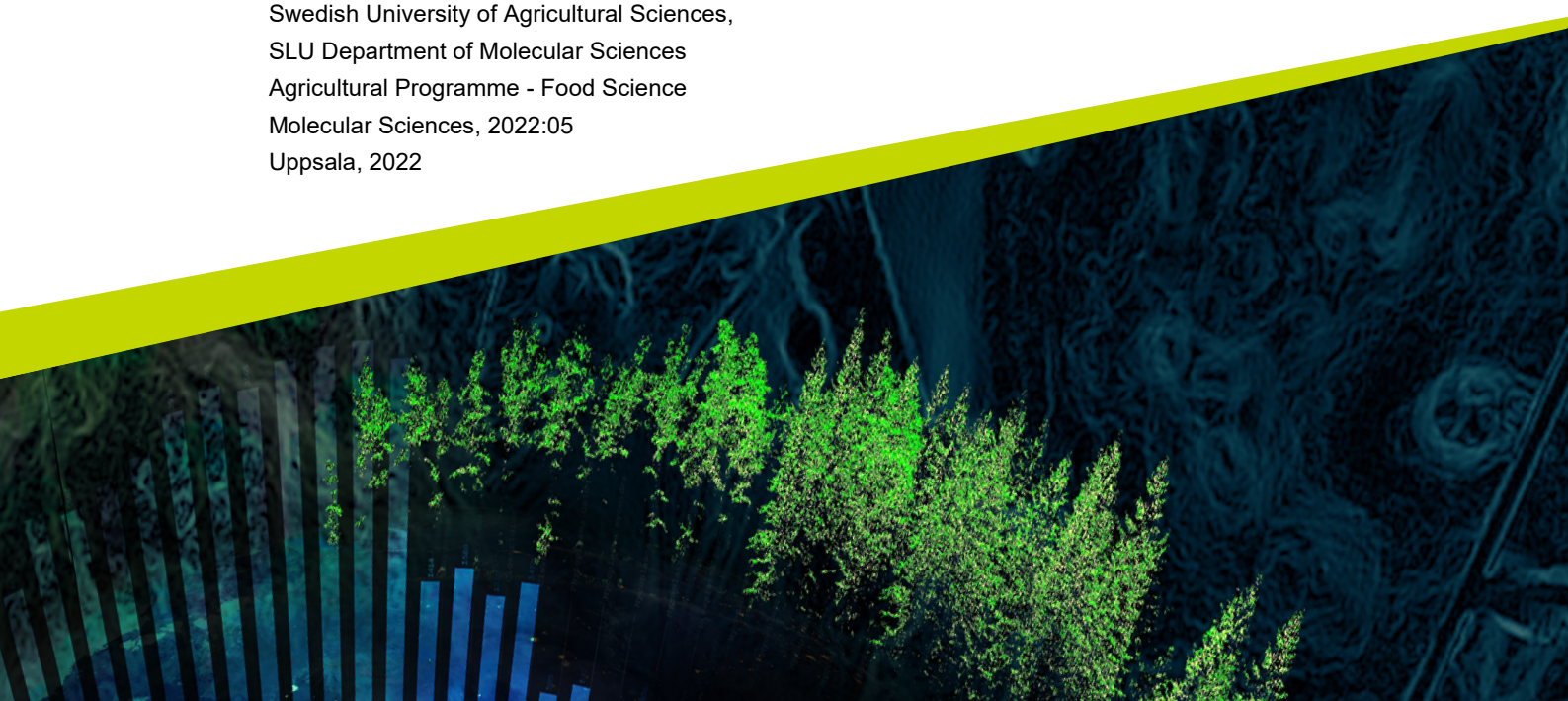

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Abstract

Swedish dairy cows have an average life expectancy of 5 years, which means that each cow undergoes about 2.5 lactation cycles through her life. Reasons for culling dairy cows are mainly impaired fertility, udder health (mastitis) and low milk yield. Animal welfare is related to increased longevity, which in turn leads to lower greenhouse gas emissions per unit of milk, thus reduced climate impact. The aim of this study was to investigate if the number of lactations or breed, affect milk quality paramters, i.e. protein profile, gross composition, pH and somatic cell count for the two major breeds of Swedish dairy cows. For this study, 110 milk samples from SRB and SLB cows were collected in herds partcipating in an on-going research project. Results from multivariate analysis did not show any major differences in milk composition between cows with different lactation numbers. Instead, milk composition seemed to differ between cows of different breeds and between cows from different farms. Milk from SRB cows had 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. Additionally, milk from SRB cows had higher relative concentrations of β-CN A1and lower relative concentrations of β-CN A2, compared to milk from SLB cows. Further reasearch, including larger numbers of individual cows, is required to be able to conclude whether the number of lactations has an affect on raw milk composition.

Keywords: Raw milk quality, protein profile, lactation number, Swedish Red, Swedish Holstein

Sammanfattning

Svenska mjölkkor lever i genomsnitt 5 år, och genomgår under sitt liv ungefär 2,5 laktationer. Anledningar till varför man slaktar mjölkkor är framförallt nedsatt fertilitet, juversjukdom (mastit) och låg mjölkavkastning. God djurvälfärd är förknippat med att kornas levnadslängd ökar, vilket i sin tur skulle leda till lägre utsläpp av växthusgaser per mjölkenhet och därmed minskad klimatpåverkan. Syftet med denna studie var att undersöka huruvida laktationsnummer och ras påverkar kvalitetsparametrar i mjölk, dvs. proteinprofil, mjölksammansättning, pH och celltal (SCC). I denna studie ingick 110 mjölkprover från SRB och SLB kor, som ingick i besättningar i ett redan pågående forskningsprojekt. Resultat från multivariat analys visade inte på några tydliga skillnader i sammansättningen av mjölk från kor i olika laktationsnummer. Däremot fanns en tendens till liknande sammansättning för mjölkprover insamlade från kor av samma ras eller från samma gård. Mjölk från SRB-kor visade sig ha numeriskt högre halter av fett och torrsubstans, SFA, UFA, MUFA, PUFA, C14:0, C16:0, C18:0 and C18:1C9 (relativa koncentrationer), jämfört med mjölk från SLB-kor. Vidare hade mjölk från SRB-kor högre relativ koncentration β-CN A1och lägre relativ koncentration β-CN A2, jämfört med mjölk från SLB-kor. Ytterligare forskning, med fler individuella mjölkprover, krävs för att kunna dra slutsatser om huruvida laktationsnummer påverkar mjölkens sammansättning.

Nyckelord: Mjölkkvalitét, proteinprofil, laktationsnummer, svensk röd och vit boskap, svensk låglands boskap

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Abbreviations

AA	Amino Acid
α-LA	alfa-lactalbumin
α_{s1}-CN	$alfa_{s1}$-casein
α_{s2}-CN	$alfa_{s2}$-casein
β-CN	beta-casein
β_B-CN	beta-casein B
β-LG	beta-lactoglobulin
β-CN A1	$beta_{A1}$-casein
β-CN A2	$beta_{A2}$-casein
CE	Capillary Electrophoreses
FA	Fatty Acid
γ-CN	gamma-casein
κ-CN	κappa-casein
LN	Lactation Number
SCC	Somatic cell count
SLB	Swedish Holstein
SJB	Swedish Jersey
SLF	Svensk Lantbruksforskning
SKB	Swedish Polled
SRB	Swedish Red

1. Introduction

Cattle were domesticated around 8000 years ago, and have since then been selectively bred, especially during the last 200 years. The practices of breeding have selected for different characteristics, such as health, fertility and milk and meat production. Of the global total milk production, 84% is estimated to be from cows. Dairy products are in particular of great importance in Europe, United States, Canada, Argentina, India, Australia and New Zeeland (O'Mahony & Fox 2014).

1.1. Sustainable dairy production

Sustainable dairy production is associated with economic, environmental and social domains, as well as with animal welfare and consumer values. The interest of farmers focuses mainly on the aspects of production, as a source of income. However, there is a positive effect on production with good animal welfare and management practices, which strengthens the reason for taking good care of the animals. The interest of European consumers focuses mainly on the quality of food products and animal welfare (Lovarelli et al. 2020).

1.1.1. Climate impact

The production of meat and milk generates greenhouse gas emissions, partly as methane from ruminants feed digestion and fertilizer management, partly as carbon dioxide and nitrous dioxide from feed production. Although, constant methane emission does not further increase the global temperature, since the gas does not stay in the atmosphere in the same way as carbondioxide does. However, with increasing methane emissions, temperature will increase. In the short term, methane emission has a larger effect on the climate, compared to carbon dioxide. Over a 100 years period, the effect of methane on climate will be up to 34 times larger than the effect of carbondioxide (Röös 2019).

Under certain circumstances, greenhouse gas emission can be, partially or completely, compensated by carbon sequestration by soil, e.g. soil used for grazing or growing feed. Soil with low carbon content, such as overgrazed areas, has the largest potential to bind and store carbon (Röös 2019).

The productive lifespan of a dairy cow is often much shorter than their natural life expectancy, which is approximately 20 years. The climate impact caused by milk production may be reduced with increased longevity, thus increased productive lifespan, of dairy cows. With an increased productive lifespan in dairy herds, there will be fewer replacement heifers that produce no milk. A herd with a higher proportion of multiparous dairy cows also excretes less phosphorus and emits less methane per unit of milk and meat, compared to a herd with a high number of heifers (De Vries & Marcondes 2020).

1.1.2. Animal welfare and profitability

Animal welfare is strongly related to increased animal longevity, i.e. life length of the cow. Injury, poor health, infertility or bad temperament can be reasons for involuntary culling of the animal (Schneider et al. 2007; Langford & Stott 2012). Infertility, mastitis and lameness are main reasons for early culling. The conditions are costly to treat, with negative effects on the value of the cow at the market and reduce the welfare of the cow in different ways. Nevertheless, the decision of culling must not only be involuntary, it can also be a decision based on economic factors, e.g., replacing a cow because of low productivity. Other factors to consider when deciding to cull a cow are pregnancy status, stage of lactation and age (Schneider et al. 2007). The farmer has to balance the risk of future losses from the cow in question, against the net cost of a replacement (Langford & Stott 2012).

Culling a dairy cow in a herd introduces costs, if the herd is to remain at unchanged number of animals. Mostly, this is because replacement heifers need to be reared (Schneider et al. 2007). In fact, many farmers are not aware of the true cost of rearing dairy heifers. According to Boulton et al. (2017), it takes dairy farmers an average of 1.5 lactations, or 530 days, to repay the cost of rearing a heifer to calving. Therefore, it is of interest to investigate whether older cows can be retained in production for a longer period of time, thus increase longevity and productive life of cows, without having milk quality parameters being negatively affected.

1.2. Dairy production in Sweden

In Sweden, the number of dairy farms is steadily declining. The number of dairy farms in year 2000 were 12 700, compared to 3000 in 2020. Moreover, in June 2020, the number of cows for milk production amounted to 303 400 heads, which is a decrease by 8.3% since 2016. At the same time, the amount of delivered to Swedish dairies has changed only marginally, due to an increased average milk yield per dairy cow (Jordbruksverket 2020).

In 2020, the average Swedish dairy herd had 98 cows, an increase of four cows per herd since 2019, and 66% of Swedish dairy cows were found in herds with more than 100 dairy cows. Additionally, the trend of loose housing management systems continues to increase (Jirskog 2021). In Swedish agriculture, the milk production sector has the largest economic value of the agricultural products produced for further trade (Jirskog 2021).

Swedish dairy cows have an average life expectancy of 5 years, which means that each cow undergoes about 2.5 lactation cycles through her life (Växa Sverige 2020). A large proportion of dairy cows are culled involuntary before their full potential is reached, mainly because of impaired fertility (18.2%), udder health (mastitis) (15.4%) or low milk yield (3.1%). Moreover, animal welfare issues can be another reason for early culling, e.g., illness (3.4%) and injuries from accidents (2.6%) (Schneider et al. 2007; Växa Sverige 2020).

1.2.1. Major dairy breeds

Today, the two by far most common dairy breeds in Sweden are the Swedish Holstein (SLB) and the Swedish Red (SRB) (Wedholm et al. 2006a; Svenskt kött 2021). The two breeds differ in various ways, for instance in appearance, where SLB are black and white while SRB are reddish brown with white elements (Svenskt kött 2021). Until 2001 SRB was the most common breed in Sweden, but this has subsequently changed. In 2019, the number of registered SLB cows was close to 119 000 heads, and just over 70 000 heads of SRB (Växa Sverige 2020).

The average milk yield for SLB is 10 551 kg/year, compared to 9 245 kg/year for SRB. However, the average concentration of fat is higher in SRB milk compared to SLB milk (Växa Sverige 2020), as well as average concentration of total protein (Wedholm et al. 2006a; Växa Sverige 2020). SLB milk contains on average 4.11% fat and 3.52% protein, in contrast to SRB milk of which 4.40 % is fat and 3.70 % is protein (Table 1) (Växa Sverige 2020). Moreover, the protein profile has been shown to differ between these two breeds (Wedholm et al. 2006a). In addition, SRB has a better general health compared to SLB, such as a lower incidence of udder diseases, leg and hoof problems and other diseases (Växa Sverige 2020).

Swedish Jersey (SJB) and Swedish Polled (SKB) are two other dairy breeds in Sweden, yet not near as common as SLB and SRB. The small SJB dairy cow has a lower milk yield compared to SLB and SRB, however, the milk has a higher fat and protein content. For SKB, the fat and protein content are similar to SLB and SRB, yet the yield is lower reaching an average 5 700 kg/year (Table 1) (Växa Sverige 2020).

Table 1 Mean values for milk production of Swedish Holstein (SLB), Swedish Red (SRB), Swedish Polled (SKB) and Swedish Jersey (SJB) cows *(Växa Sverige 2020)*

	SLB	**SRB**	**SKB**	**SJB**
Fat (g/100g)	4.11	4.40	4.35	4.92
Protein (g/100g)	3.52	3.40	3.58	4.19
Kg milk/year	10 551	9 245	5 700	7 144
Kg ECM/year	10 790	9 910	6 024	9 248

Abbreviations: EMC=Energy corrected milk

1.3. Milk composition

The natural function of milk is to nourish the young mammal and to contribute with all the essential nutrients needed for optimal growth (Walstra et al. 2005; Willett & Ludwig 2020). The raw milk composition and its properties has an important impact on dairy processing (Lindmark-Mansson et al. 2003; Chandan et al. 2015), such as the milk clotting properties, cheese yield and the final product quality (Wedholm et al. 2006b).

In average, raw cow's milk contains 87.1% water, 4.6% lactose, 4.0% fat, 3.3% protein and 0.7% mineral substances (Walstra et al. 2005). Factors known to cause variation in milk composition (i.e. protein, fat, lactose and mineral content) are breed, stages of lactation, feed and health status of the cow (Walstra et al. 2005; Wedholm et al. 2006b; Chandan et al. 2015), as well as milking intervals, milking system, lactation period, season, age, different quarters of udder, nutritional level, environmental temperature, weather, estrus cycle, gestation period and exercise (Lindmark-Mansson et al. 2003; Chandan et al. 2015).

Lactose

Lactose, a disaccharide composed of glucose and galactose, is the distinguishing carbohydrate of milk (Walstra et al. 2005; Rangel et al. 2016). Lactose is synthesised in the mammary epithelial cells and, by osmotic pressure, regulates transportation of fluid from blood to the alveoli. This osmotic activity of lactose is therefore important for milk secretion and yield. Because of lactose being the major osmole of milk, it is difficult to vary its concentration by e.g. feeding schemes (McManaman et al. 2006).

Fat

Nearly all lipids in milk are present in fat globules, which makes it possible to concentrate milk fat with means of creaming due to gravity, such as centrifugation (Walstra et al. 2005). Milk fat is a complex mixture composed of mainly triglycerides (~98%). These fat molecules concist of a large variety of different

fatty acids (FA) with various length (2-20 carbon atoms) and different degree of unsaturation (number of double bonds), determining chemical reactivity such as autooxidation (Walstra et al. 2005; van Valenberg et al. 2013). Over 400 FA of various types have been identified in cow's milk (van Valenberg et al. 2013) Additionally, other lipids, e.g., phospholipids, free FA, cholesterol, monoglycerides and diglycerides are present in milk (Walstra et al. 2005). The fat content in milk can be controlled with dietary supplementation. Feed rich in polyunsaturated fatty acids (PUFA) can increase the proportion of monounsaturated fatty acids (MUFA) and PUFAs (such as omega-3 fatty acids) in milk, which can be beneficial for human health (Beauchemin et al. 2009).

Protein

Milk protein and its properties are of great significance for manufacturing of dairy products, not least for cheese production (Coulon et al. 2001; Caroli et al. 2009). The synthesis of milk proteins in the mammary gland requires available amino acids and high energy feed. Therefore, the need of energy and protein increases drastically during lactation (Bionaz et al. 2012). The milk protein yield and protein composition are to a large extent determined by the animal's genetics, and is therefore difficult to influence through nutrition in feed (Wedholm et al. 2006a; Bionaz et al. 2012). However, as mentioned, since protein synthesis requieres large amount of energy, the milk protein content can be negatively affected by low energy in feed (Reynolds et al. 1994; Coulon et al. 2001). In Swedish bulk milk, the concentration of total protein and casein vary because of differences in feed quality and feeding practices in different geographical regions (Lindmark-Mansson et al. 2003).

1.4. Milk protein profile

The concentrations of different milk proteins plays a key role of for the technological properties of the milk, yield and quality of some dairy products, e.g., cheese (Hallén 2008). Cow's milk consists primarily of four caseins, i.e., alfa$_{s1}$-casein (α_{s1}-CN), alfa$_{s2}$-casein (α_{s2}-CN), beta-casein (β-CN) and kappa-casein (κ-CN) and two major whey proteins, i.e., alfa-lactalbumin (α-LA) and beta-lactoglobulin (β-LG) (Threadgill & Womack 1990; Hallén 2008; Visker et al. 2011). Milk protein can be defined by their solubility at pH 4.6, in which the caseins precipitate and the whey proteins remains in solution (Hallén 2008). Additionally, small amounts of bovine serum albumin (BSA), immunoglobulins (Ig), degradation products of casein and enzymes, (table 2) exist in the milk serum fraction (Wedholm 2008).

Table 2 Approximate protein composition in cow´s milk *(Ng-Kwai-Hang 2011)*

Protein	Concentration of milk protein (g/L)	Concentration of milk protein (% of total protein)
α_{s1}-CN	10.0	32
α_{s2}-CN	2.6	8
β-CN	9.3	30
κ-CN	3.3	10
α-LA	1.2	4
β-LG	3.2	10
BSA	0.4	1
Ig	0.7	2
Others	0.8	3

Abbreviations: α_{s1}-CN=alfa$_{s1}$-casein; α_{s2}-CN=alfa$_{s2}$-casein; κ-CN=kappa-casein; β-CN=beta-casein; α-LA=alfa-lactalbumin; β-LG=beta-lactoglobulin; BSA=bovine serum albumin; Ig=immunoglobulins

1.4.1. Casein

About 80% of the total protein in cow's milk are caseins (Hallén 2008; Wedholm 2008; Caroli et al. 2009; Fang et al. 2016). In cow's milk, α_{s1}-CN, α_{s2}-CN, β-CN and κ-CN are present at a ratio of 4:1:4:1, respectively (Hallén 2008; Fang et al. 2016). These four caseins differ with respect to degree of phosphorylation, glycosylation, proteolysis, disulphide bonding and genetic polymorphism (Hallén 2008; Wedholm 2008; Ng-Kwai-Hang 2011). For example, proteolytic enzyme (plasmin) split part of β-CN into gamma-casein (γ-CN) and proteose-peptones (Walstra et al. 2005). The degree of casein phosphorylation is one of the most important factors responsible for construction and stabilization of the casein micelle (Fang et al. 2016).

Due to the relatively high proline content in caseins, they show very little tertiary or organized secondary structures. With this property of having little structure to fold, caseins are stable against heat denaturation (Hallén 2008; Wedholm 2008). However, at temperatures > 140º C, the caseins will coagulate (Singh 1995), either because of chemical cross linking or by colloidal aggregation (Walstra et al. 2005; Wedholm 2008). Caseins have both polar and non-polar regions, forming an amphiphilic structure. The proline and phosphate content, together with the amphiphilic properties, allow the formation of a colloidal calcium-protein complex (Farrell et al. 2006; Hallén 2008).

Casein micelle

The majority of the casein proteins in cow's milk (~95%) are organised in colloidal structures, known as casein micelles. The major function of the micelle is to fluidise the casein molecules, as well as solubilise and transport phosphate and calcium to the young (Farrell et al. 2006). The constituents of casein micelles are water,

proteins (caseins) and salts. The caseins (as caseinate) binds cations, foremost calcium and magnesium. The other salts occur as colloidal calcium phosphate (CCP) with a small amount of citrate. At lower pH, and low temperature, the CCP together with β-CN, leaves the micelle (Walstra et al. 2005; Schiffer et al. 2021).

The three hydrophobic caseins αs1-CN, αs2-CN and β-CN initiates the formation of micelle structures with calcium binding to their phosphoserine residues. κ-CN interacts with and stabilizes the calcium insoluble caseins, which in turn forms a stable micelle structure (Farrell et al. 2006). However, κ-CN is easily attacked by chymosin. This rennet enzyme cleaves the protruding, glycosylated moiety of the κ-CN molecule, which thereby loses its stabilising ability in form of steric and electrostatic repulsion (Walstra et al. 2005). In nature, this takes place in the calf stomach; κ-CN is cleaved by chymosin to initiate aggregation of casein for efficient digestion of the milk (Farrell et al. 2006).

The detailed arrangement of caseins in the micelle is not yet fully understood (Holt & Carver 2022). Although, in all models explaining the casein micelle structure, κ-CN is believed to predominate on the structure surface.

α_{s1}-CN and α_{s2}-CN

40% of the casein fraction in milk are α_{s1}-CN, whereas α_{s2}-CN constitutes 10% of the entire fraction (Farrell et al. 2004). α_{s1}-CN has a high phosphate content and a high negative net charge (Walstra et al. 2005). In cow's milk α_{s1}-CN has mainly two phosphorylation isoforms (Fang et al. 2016), of which both are single chain polypeptides composed of the same amino acid (AA) sequence. The degree of phosphorylation is the only difference between these two proteins.

α_{s2}-CN contains two cysteine residues which form a disulphide bridge (Walstra et al. 2005). The family of α_{s2}-CN consists of two major- and several minor components, showing minor degrees of intermolecular disulphide bonding and varying levels of phosphorylation.

β-CN and γ-CN

The family of β-CN constitutes up to 45% of the caseins in cow´s milk (Farrell et al. 2004). β-CN is the most hydrophobic of the caseins and contains a large number of proline residues. Because of that, part of the β-CN goes into solution at low temperature, since the hydrophobic bonds get weaker. The β-CN family is affected by the milk protease plasmin, which cleaves the molecule at different positions resulting in the γ-CN fragments and proteose-peptones (Farrell et al. 2004; Walstra et al. 2005). Protein degradation, and therefore the amount of γ-CN, varies depending on temperature, age of the milk and levels of plasmin (Walstra et al. 2005).

Mutations in the bovine β-CN gene have given rise to 12 genetic variants, of which A1 (β-CN A1) and A2 (β-CN A2) are the two most common. The difference

between these two variants is that in β-CN A1 there is histidine at AA position 67, while in β-CN A2 there is proline at this position. This single AA variation leads to a conformational change in the proteins' secondary structure (Sodhi et al. 2012).

κ-CN

κ-CN differs from the other caseins. It has a net negative charge because it contains carbohydrate groups, making parts of κ-CN hydrophilic (Skeie 2010). The two cysteine residues of κ-CN forms intermolecular disulphide bonds. Therefore, κ-CN in milk appear as oligomers that contain 5-11 monomers. κ-CN molecules vary with respect to carbohydrate content, charge, number of ester phosphate groups and other minor configurations (Walstra et al. 2005).

1.4.2. Whey proteins

In contrast to casesins, serum proteins, often referred to as whey proteins, are soluble at pH 4.6. The whey protein fraction consists mainly of β-LG, α-LA and BSA, making up approximately 20% of the total milk proteins. The whey fraction also consists of Ig, enzymes and trace amounts of several other proteins. Because the whey proteins are mostly organised in secondary and tertiary structures, they are sensitive to heat denaturation at temperatures above 60˚C (Hallén 2008). Most of the whey proteins contain a high amount of α-helical structures, and they are more hydrophobic compared to caseins (Wedholm 2008).

β-LG

β-LG is the major protein of the whey fraction. The properties of β-LG therefore dominate the properties of the total whey protein, not least the reactions that occur upon heat treatment. The solubility of β-LG depends mainly on ionic strength and pH. However, it will not precipitate when milk is acidified. The same pattern holds true for the other whey proteins (Walstra et al. 2005).

β-LG has two disulphide bonds and one free sulfhydryl-group. The secondary and tertiary structure of this protein is well known. With changes in pH or temperature, the structure is subjected to changes. In milk, under physiological conditions, β-LG is present as a dimer, where both molecules are bound tightly to each other. This dimer is dispersed when exposed to high temperatures. Because of a hydrophobic "cavity", β-LG has the ability to bind and transport small hydrophobic molecules, such as retinol (vitamin A) (Walstra et al. 2005; Edwards & Jameson 2014). There are 10 known genetic variants of β-LG in cow's milk, of which the β-LG A and β-LG B are the most abundant. The difference between these two, is an interchange by only two AA (Edwards & Jameson 2014).

α-LA

α-LA is a compactly folded, small, almost spherical molecule. It has a specific binding site for a calcium ion, which is strongly bound and act to stabilize the conformation of the protein. When pH is lowered to about 4, the calcium ion dissociates and partial unfolding of α-LA occurs. At this state, even at relatively low temperatures, the protein is exposed to irreversible heat denaturation (Walstra et al. 2005; Edwards & Jameson 2014). As it seems, α-LA is unfolded already at 63-67 ˚C (Wedholm 2008). The biological function of α-LA is to act as coenzyme in lactose synthesis. It modifies the action of the enzyme galactosyl-transferase to catalyse lactose formation from glucose and uridine-diphosphate-galactose (UDP-galactose) (Threadgill & Womack 1990; Walstra et al. 2005; Wedholm 2008; Edwards & Jameson 2014). The concentration of α-LA in milk is therefore directly related to the concentration of lactose (Wedholm 2008).

BSA and Ig

BSA is a minor protein that occurs in milk as a result of leakage from the blood (Walstra et al. 2005; Hallén 2008). BSA is a large, elongated shaped molecule consisting of three globular domains. It has 17 disulphide-bonds and one sulfhydryl-group (Walstra et al. 2005). BSA tends to function as a discursive transporter of hydrophobic molecules (Edwards & Jameson 2014).

Ig proteins are antibodies synthesised in response to specific antigens (Walstra et al. 2005; Hallén 2008), and in milk they protect the gut mucosa against pathogenic microorganisms (Edwards & Jameson 2014). Ig are large glycoprotein molecules formed and secreted by different secretory cells, i.e., B lymphocytes (Walstra et al. 2005). There are various classes of Ig in milk, including IgG (gammaglobulins), IgA and IgM (macroglobulins) (Walstra et al. 2005; Edwards & Jameson 2014). In cow´s milk, the most predominant Ig proteins are members of IgG subfamily. In colostrum, the concentration of IgG is 40-300 times greater than that of milk (Edwards & Jameson 2014). The concentration of Ig in colostrum can be up to 100 g/L, with a fast decrease to about 1 g/L just within a week of time after calving (Hallén 2008). This is because the role of colostrum is to transmit immunity to the neonate, during the time of which its own immune system is still developing (Edwards & Jameson 2014).

1.4.3. Variation of milk components

Variation in milk composition is caused by many different factors. Those factors include breed, stage of lactation, feed and health status (Walstra et al. 2005; Wedholm et al. 2006b; Chandan et al. 2015), milking intervals, milking systems, lactation period, season, age, udder quarters, nutritional level, environmental temperature, weather, estrus cycle, gestation period and exercise (Lindmark-Mansson et al. 2003; Chandan et al. 2015).

Seasonal variation

The seasonal variation in cow´s milk characteristics has been shown to be significant. According to Bertocchi et al. (2014), in a study conducted in north Italy, the summer season seems to be the most critical of all seasons. In the study, July presented the most critical proportions of total bacterial count, and low fat and protein content. August presented higher values of somatic cellcount (SCC), which might be because of high temperatures and humidity during summer months. The cows will also be exposed to higher numbers of pathogens and vectors during this time, because of being outside on pasture (Bertocchi et al. 2014).

Udder health

The udder health status of dairy cows may affect milk protein composition (Ng-Kwai-Hang et al. 1987). Udder health is indicated by the number of somatic cells e.g. white blood cells in the milk. Cows suffering from mastitis, i.e. an udder inflammation which in most cases is due to bacterial infection have higher SCC than normal. This condition is associated with milk quality detoriation, such as increased proteolytic degradation of casein proteins (Verdi et al. 1987).

Lactation cycle

Concentrations of different milk constituents vary during lactation (Wedholm 2008; Skeie 2010). In particular fat and protein, which are both much higher in colostrum milk than in later lactation. Around lactation week 5, the fat and protein content are at minimum concentration, with a steady increase further on in the lactation cycle. During late lactation, milk normally contains more whey protein than caseins. The lactose content in milk decreases slowly during the time of lactation (Skeie 2010).

Breed and milk protein polymorphism

Mostly, because of genetics, proteins do occur in two or more variants of its primary structure. The frequency occurrence of a specific variant tends to be dependent on breed (Walstra et al. 2005). Milk composition from the two dominating breeds in Sweden, SRB and SLB, differ to some extent. Milk from SRB is expected to contain higher concentration of total fat and protein, compared to milk from SLB. Most probably, the protein composition is related to the frequency of certain genetic variants of milk proteins in individual cows (Wedholm 2008).

Cow's milk protein composition is dependent mostly on genetic polymorphism of milk proteins (Wedholm et al. 2006a). About 95% of cow's milk proteins are encoded by six highly polymorphic genes, with around 47 different protein variants identified (Martin et al. 2002; Caroli et al. 2009).

The four casein proteins arise from four genes which codes for four polypeptide chains (i.e., α_{s1}-CN, α_{s2}-CN, β-CN and κ-CN) (Wedholm 2008; Fang et al. 2016). These four casein genes have been mapped on chromosome 6 in cattle, while α-LA

and β-LG are coded by genes mapped on chromosomes 5 and 11 (Martin et al. 2002). The concentration of β-LG has been shown to be higher in milk with genotype AA, than with AB or BB. Therefore, AA milk has proportional lower CN content.

1.5. Milk quality parameters for payment basis

Obtaining a high fat and protein content in milk became especially important for Swedish farmers after year 2000. At this time, the economic values of fat and protein were re-evaluated in the milk payment systems. As a result of breeding, the protein content in Swedish milk increased from year 1980, until stabilising at around 3.5% during recent years. Between 1980 and 2019, the milk fat content in Swedish milk increased from 4.14% to 4.19% (Växa Sverige 2020). Currently, milk is graded according to total protein and fat concentrations. However, if a specific marker could be used to identify milk especially suitable for cheese production, i.e. milk with high casein content and good clotting properties, this would provide an economical advantage for the dairy industry (Wedholm et al. 2006b).

At each occasion of milk collection on the farm, a milk sample is taken for further analysis. Analysis of fat and protein content and SCC is done upon each collection, while other quality attributes. e.g. bacterial count, spores and antibiotics are analysed with lower frequency. In Sweden, milk producers are paid less when the SCC exceeds 300 000/mL (Arlagården 2019) This regulation is not only because of cow health concerns, such as mastitis, it is also associated with deteriorated milk quality, such as increased proteolytic degradation of caseins. The degradation of milk proteins is a continuous process that can be reduced and controlled by pasteurisation (72 ˚C for 15 sec), because some of the proteolytic enzymes are partly inactivated with this heat treatment (Wedholm 2008).

1.6. Objective and aims

The objective of this study was to investigate if milk composition is affected by lactation number. For this purpose, milk from individual cows from different farms, with different number of lactations, and of two breeds were characterized with respect to protein profile, gross composition (fat, protein, lactose, total solids, density and fatty acids), pH and SCC. The aim was also to evaluate if the milk composition differed between two breeds, SRB and SLB.

2. Materials and methods

2.1. Selection of animals and milk sampling

Milk samples for this study were collected in herds participating in an on-going research project funded by SLF. The milk samples were collected from individual cows at 11 different farms in Uppland, Södermanland, Västmanland and Västerbotten, Sweden. At 10 of the farms, samples were collected during the period 22 September – 10 November 2020, and at one farm in January 2021. For practical reasons, farms using AMS were not included in the study. The selection of cows in the participating herds was based on breed, lactation stage, milking system and number of lactations. Milk samples were collected from 110 dairy cows, of which 20 individuals were Swedish Holstein (SLB) and 90 individuals were Swedish Red (SRB). In each herd the group of cows selected (n=10) consisted of five younger i.e., ≤ 2 lactations and five older i.e., ≥ 3 lactations, cows (Table 3). Two samples were excluded during the process, of which both were milk samples from SRB cows with lactation numbers 3 and 4, respectively.

Criterias in this study were that the cows should be in mid lactation, i.e. at least eight weeks after last calving and no later than twelve weeks before next calving. The cows participating in the study were milked either in milking parlour or tied system. Milk sampling took place during evening milking. After sampling, samples were transported at 4˚C to SLU and stored at -20˚C until analysis. This study was conducted in the research facilities at the department of Molecular Sciences, Swedish University of Agricultural Sciences (SLU) in Uppsala, Sweden.

Table 3 Lactation numbers of young (1-2 lactations) and old (≥3 lactations) SLB and SRB cows (n=110) that were selected for the study

		SLB (n=20)	SRB (n=90)
	Lactation number	**Number of animals**	**Number of animals**
Young	1	6	26
	2	4	19
Old	3	2	12
	4	6	14
	5	2	9
	6	0	5
	7	0	3
	8	0	2

2.2. Milk sample preparation

Milk samples were stored at -20˚C until use. 50 ml of milk sample from each individual cow was placed in a 39˚C water bath for thawing for about 20 minutes, until the fat in the milk sample was homogenous. The milk samples were then cooled to room temperature and analysed for pH, SCC, density, and gross composition (total fat, fat composition, contents of protein, lactose and total solids), The detailed milk protein profile was measured with capillary electrophoresis (CE).

2.2.1. pH measurement

Milk samples of 50 ml from each individual cow were analysed for pH. The pH was determined using Mettler Toledo, SevenCompact pH meter S210.

2.2.2. Milk gross composition analysis

Milk samples from each individual cow were analysed for gross composition. Concentrations of total fat, protein, lactose, total solids, density, saturated FA (SFA), unsaturated FA (UFA), mono unsaturated FA (MUFA), poly unsaturated 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).

2.2.3. SCC analysis

SCC was analysed by electronic fluorescence-based cell counting (Fossomatic Foss FT 120, Hilleröd, Denmark).

2.2.4. Analysis of milk protein profile

Preparation of buffers

For the CE analysis, sample- and run buffer were prepared according to a standard operating procedure. Shortly, 0.3 L of urea stock was first prepared by mixing 108.1 g of 6M urea (Mw 60.06), 0.15 g hydroxypropylmethyl cellulose (MHEC) (0.05%) and 5.4 g ion exchange resin (AG 501-X8 Resin, Bio-Rad Laboratories, Inc, CA). After dissolution (overnight), the urea solution was filtered through a 0.45µm membrane.

Run buffer (RB) was composed of 0.02M trisodium citrate dehydrate (Mw 294.14) and 0.19M citric acid monohydrate (Mw 210.14 in 6M urea solution to reach a total volume of 50 ml. Run buffer was divided into aliquots of 2 ml and stored at -20˚C until analysis. Sample buffer (SB) consisted of 0.167M tris[hydroxymethyl]aminomethane (Triss; Mw 121.14), 0.067M ethylenediaminetetraacetic acid (EDTA; Mw 372.24) and 0.042M 4-morpholinopropanesulfonic acid (MOPS; Mw 209.26), in 6M urea solution to reach a total volume of 200 ml. 10 ml of sample buffer was placed in 15 ml falcon tubes and stored at -20 ˚C together with the RB aliquots until used for analysis. On the day of sample preparations and analysis, 0.0017M D, L-dithiothreitol (DTT; Mw 154.25) was added to SB to disrupt the disulphide bridges of the milk whey proteins. Chemicals used were obtained from Sigma-Aldrich (Sigma-Aldrich, USA) unless else stated.

Preparation of milk samples

From each milk sample, 2 ml of milk was placed in an Eppendorf tube and defatted in a centrifuge (Sorvall, Super T21, Sorvall Products L.P., Newton, Connecticut, USA); rotator (ST-H750) at 10 000 RPM at 4˚C for 10 minutes. After centrifugation, the layer of fat on the surface of the milk sample was removed with help of a cotton stick. From each sample, 200µl of milk were pipetted into an Eppendorf tube and mixed with 400µl of SB, to which DTT was freshly added. The sample solution was vortexed and incubated at room temperature for one hour. Thereafter the samples where again defatted in a centrifuge at 10 000 RPM and 4˚C for 10 minutes (Hitachi T15A61-0606), and the lipid layer on the surface was removed with a cotton stick. Samples were then filtered through a 13mm 0.45µl econofilter nylon membrane (Agilent Technologies, Agilent Captiva Econofilter) into a new Eppendorf tube. Before the CE analyses, 30µl of each filtrated sample was transferred to a conic vial.

Capillary electrophoresis analysis

Conic vials including milk samples prepared as stated above were loaded in the CE machine (Aglient Technologies 7100, Capilary electrophoresis), and analysed for milk protein profile. The method was performed according to Johansson et al. (2013). The online UV-VIS detector was used to measure the absorbance at wave length 214 nm. The relative concentration of the individual proteins α_{s1}-CN, α_{s2}-CN, β_B-CN, β-CN A1, β-CN A2, κ-CN, α-LA and β-LG, were calculated in percent of total protein based on the electropherogram, consisting of peak areas for the individual proteins. Total casein was defined and calculated as the sum of the peak areas identified as α_{s1}-CN, α_{s2}-CN, β_B-CN, β_{A1}-CN, β-CN A2and κ-CN, respectively. Total whey protein was defined and calculated as the sum of peak areas identified as α-LA and β-LG, respectively. Total β-CN was defined as and calculated as the sum of peak areas identified as β_B-CN, i.e. β-CN A1 and β-CN A2. Each milk sample, from each individual cow, was analysed once by CE.

2.2.5. Capillary electrophoresis method

Principals behind the electrophoresis method is to separate ions in a solution under influence of an applied electric field. Solutes, in our case milk proteins, have different mobility under the influence of an electric field, depending on their size, shape and net charge. The CE system includes a power supply and a computer, by which the system is controlled and data is collected. The CE instrument contains two electrodes in which the electric field is applied (up to 500 V/cm). Modern CE systems include an online detector measuring UV-vis absorbance, electrochemical detection, mass spectrometry or laser-induced fluorescence (Hage 2019) In most cases, detection is in-line, i.e., through a small window in the polyamide coating of the silica capillary (Perrett 1999).

CE is faster, more efficient and easier to perform compared to traditional electrophoresis methods. Moreover, CE requires just a very small amount of sample and can be used with various detectors and detection formats (Xu 1995; Perrett 1999; Hage 2019). Compared to high-performance liquid chromatography (HPLC), CE often provides shorter analysis time, a higher resolving power and has a lower operational cost (Xu 1995; Perrett 1999). HPLC is considered to be a precise method useful for small molecular weights, such as drugs. Although, application of HPLC method requires skilled operators (Perrett 1999). A wide range of biologically active molecules can be separated by CE, i.e., proteins, AA, peptides, hormones, steroids, vitamins, carbohydrates etc.

2.3. Statistical Analysis

Mean values and standard deviations (SD) were calculated for each parameter. One-way analysis of variance (ANOVA) was performed to investigate if there was a significant effect of lactation number on the different milk quality parameters (95% confidence interval). Tukey pairwise comparisons were used for pairwise investigation of differences between lactation numbers (LN). ANOVA was performed using Minitab 19.0 software (Minitab Inc., State College, PA, USA). Graphical illustrations were made using Microsoft® Excel® version 2110.

Principal component analysis (PCA), a multivariate analysis method, is often used to reduce the number of variables, the dimensionality, of a large data set, while at the same time, preserving as much statistical information as possible (Jolliffe & Cadima 2016). PCA was performed using SIMCA 17.0 software (Sartorius Stedim Data Analytics AB, Västerbotten, Sweden), to observe correlations between the 26 variables that were analysed in milk samples from 108 individual cows.

One milk sample from a SRB cow with lactation number 2, was judged as outlier and excluded from ANOVA and multivariate analysis, since the total casein content was unexpetedly low compared to that in the other samples. In total 10 samples were excluded from the multivariate analysis because of a large number of missing data for those samples. For PUFA, 7 values were excluded from ANOVA because of negative values, meaning that the concentrations were below the detection limit.

3. Result

3.1. Overall variation in milk composition

PCA showed that the total variation in milk composition was mainly explained by the variables total solids, total fat, density, SFA, MUFA, UFA, PUFA, C16:0, C18:0, C18:1C9 and C14:0, distributed along the first principal component (PC1), contributing to 35.6% of the variation (figure 1 D). Total whey protein, total casein, α-LA, β-LG, total β-CN, α_{s1}-CN, β-CN A1 and β-CN A2 were distributed along PC2, contributing to 17.2% of the total variation. There was no evident effect on milk composition of LN (figure 1 A), but trends for effects of breed (figure 1 B) and farm (figure 1 C). Background data for the PCA can be found in appendix II .

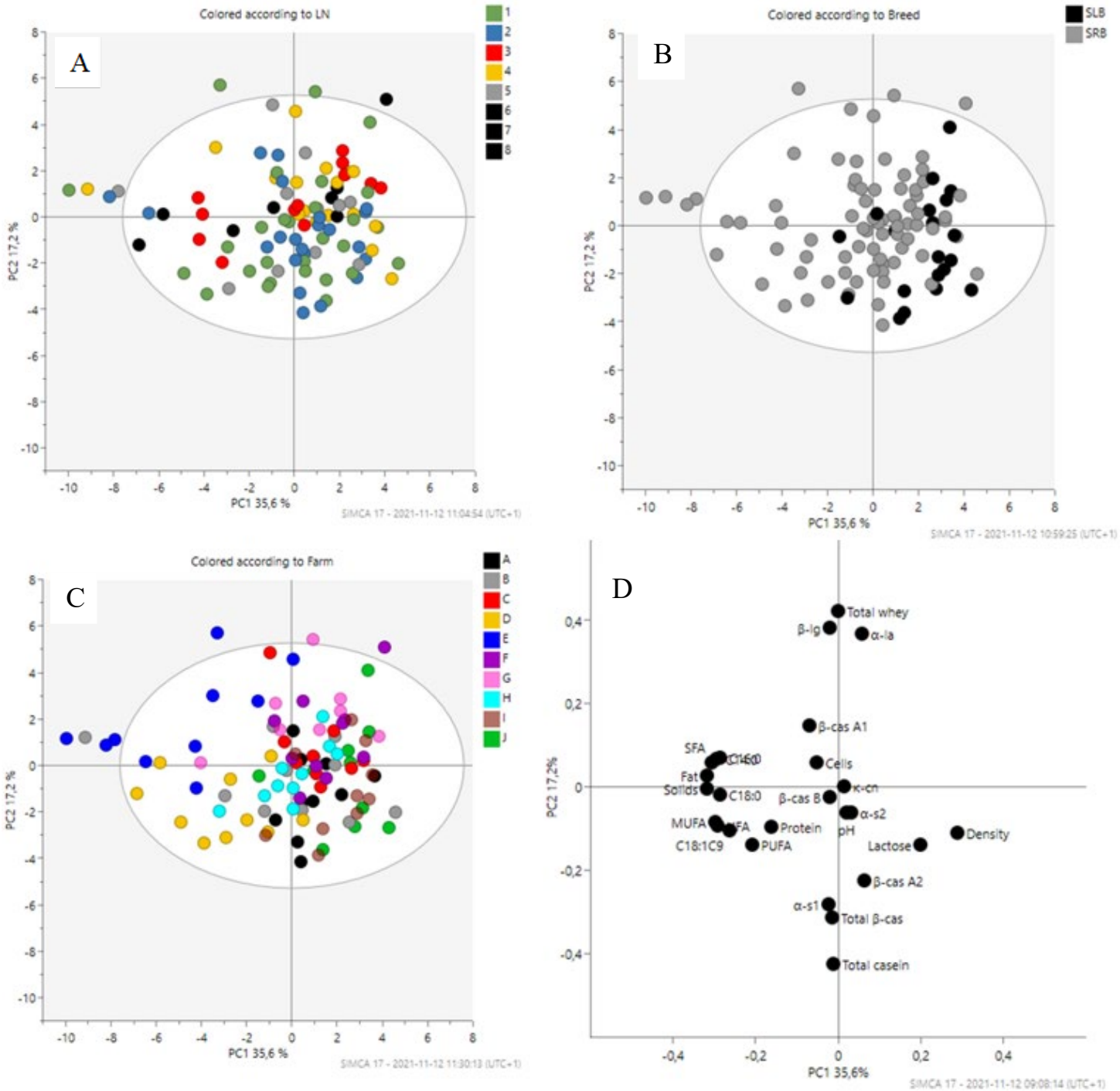

Figure 1 A, B and C show the same PCA score plot, with each observation representing one individual cow milk sample. Figure A shows the observations coloured according to lactation number, figure B according to breed and figure C according to participating farm. Figure D is the PCA loading plot, illustrating how the 26 analysed milk components contributed to the variation. Of the total variation, the first and second principal components (PC1 and PC2) explained 35.6% and 17.2% of the observed variation, respectively. The six first principal components altogether explained 81% of the variation in the dataset.

Abbreviations: PCA=principal component analysis; LN=lactation number; α_{s1}-CN=alfa$_{s1}$-casein; α_{s2}-CN=alfa$_{s2}$-casein; κ-CN=kappa-casein; β-CN B=beta-casein B; β-CN A1=beta$_{A1}$-casein; β-CN A2=beta$_{A2}$-casein; α-LA=alfa-lactalbumin; β-LG=beta-lactoglobulin; SCC=somatic cell count; SFA=saturated fatty acids; UFA= unsaturated fatty acids; MUFA=mono unsaturated fatty acids; PUFA=poly unsaturated fatty acids; C16:0=palmitic acid; C18:0=stearic acid; C18:1C9=oleic acid; C14:0=myristic acid.

3.2. Protein profiles in milk from cows with different lactation numbers

Milk protein profile, expressed in percent, was calculated as the proportion of integrated peak area of a specific protein, divided by the total integrated area of all peaks detected (Figure 2).

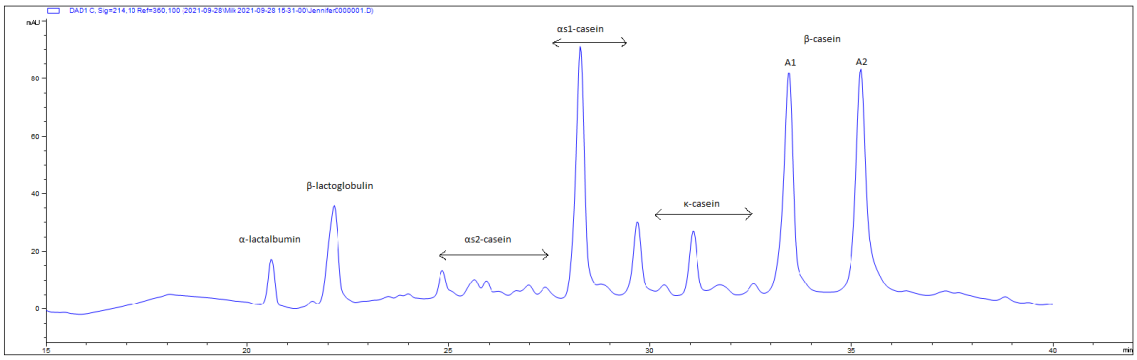

Figure 2 Representative capillary electrophoresis electropherogram, showing the protein peak distribution.

The average protein composition in milk samples from cows with different lactation numbers is presented in tables 4 and 5. In milk from SRB cows, the relative concentrations of five variables, i.e. α-LA, α_{s2}-CN, β-CN B, total β-CN and total casein, were shown to differ significantly when comparing between LN (table 4). Moreover, pairwise differences were observed for 'α-LA', 'α_{s2}-CN', 'total β-cas' and 'total casein' between different LN, as shown in detail in section 3.3.1 (table 7). In milk from SLB cows, none of the proteins differed significantly between different LN (table 5).

Table 4 Average protein composition (% of total protein) of milk from SRB cows (n=88) with lactation numbers 1-8. Values presented are mean and standard deviation, minimum and maximum

LN	1 (n=26 cows)			2 (n=19 cows)			3 (n=11 cows)			4 (n=13 cows)			5 (n=9 cows)			6 (n=5 cows)			7 & 8 (n=5 cows)		
	Mean	SD	Min-Max	Mean	SD	Min-Max	Mean	SD	Min-Max	Mean	SD	Min-Max	Mean	SD	Min-Max	Mean	SD	Min-Max	Mean	SD	Min-Max
α-la	2.06	1.00	0.86-4.73	1.37	0.85	0.20-3.40	2.21	0.77	0.85-3.28	2.86	0.84	1.86-4.72	2.02	0.80	0.863.57	2.10	0.61	1.47-2.96	2.40	0.75	1.68-3.20
β-lg	7.53	3.50	0.29-15.85	7.54	2.15	3.67-11.45	9.04	1.96	4.91-12.64	8.74	2.27	5.30-12.65	8.30	2.46	5.32-11.99	7.81	4.30	0.76-12.37	9.75	1.50	8.41-12.07
α-s2	6.67	0.88	3.86-7.92	6.42	0.91	4.34-8.05	7.28	0.80	6.22-8.88	6.67	0.78	4.97-8.00	7.58	0.78	6.40-8.48	7.17	1.57	4.49-8.60	7.51	1.07	6.45-8.91
α-s1	29.10	2.00	26.01-33.67	29.40	1.69	25.94-33.07	29.51	1.47	27.15-31.56	29.61	1.95	24.52-31.99	30.20	1.85	27.62-33.13	30.38	1.18	28.52-31.57	29.49	1.26	27.50-30.73
κ-cn	6.54	1.59	3.86-9.54	6.97	1.25	4.14-8.94	6.75	1.17	4.47-8.61	6.52	1.13	4.99-8.72	7.56	0.97	5.47-8.64	7.34	1.51	5.04-8.52	8.36	1.28	6.86-9.98
β-cas B	0.41	0.48	0.00-1.39	0.28	0.60	0.00-1.80	0.38	0.18	0.00-0.61	1.05	0.52	0.47-2.22	0.82	0.47	0.42-1.73	0.60	0.14	0.41-0.78	0.98	0.25	0.56-1.16
β-cas A1	15.44	14.43	0.00-45.38	11.34	13.95	0.00-44.50	13.31	13.81	0.00-39.71	28.67	12.67	0.0-42.62	10.27	12.86	0.00-33.18	10.95	15.60	0.00-33.47	18.38	13.11	0.00-37.00
β-cas A2	28.89	14.36	0.00-47.57	33.35	13.14	0.00-44.47	28.56	13.14	0.00-42.06	11.50	13.58	0.0-37.92	29.36	14.37	0.00-41.53	24.41	22.30	0.00-42.00	19.89	13.91	0.00-39.35
Total β-cas	44.74	2.87	38.57-50.80	44.97	2.41	39.53-48.74	42.26	2.21	39.02-46.49	41.22	2.74	36.41-45.24	40.46	2.88	33.90-43.16	35.97	8.49	21.89-42.55	39.25	1.06	38.05-40.13
Total whey protein	9.59	3.84	2.79-19.86	8.91	2.83	4.20-14.84	11.24	2.64	5.76-15.92	11.59	2.95	8.36-17.38	10.32	3.12	6.39-15.56	9.91	4.36	2.91-14.70	12.15	2.08	10.16-15.22
Total casein	87.05	3.68	76.86-92.01	87.76	2.62	81.93-92.76	85.79	2.86	82.03-91.98	84.02	2.29	80.95-87.56	85.80	4.77	74.89-90.86	80.86	9.38	64.63-87.65	84.61	1.98	82.74-87.44

Abbreviations: LN=lactation number; SD=standard deviation; α_{s1}-CN=alfa$_{s1}$-casein; α_{s2}-CN=alfa$_{s2}$-casein; κ-CN=kappa-casein; β-CN B=beta-casein B; β-CN A1=beta$_{A1}$-casein; β-CN A2=beta$_{A2}$-casein; α-LA=alfa-lactalbumin; β-LG=beta-lactoglobulin

Table 5 Average protein composition (% of total protein) of milk from SLB cows (n=20) with lactation numbers 1-5. Values presented are mean and standard deviation, minimum and maximum

LN	1 (n=6 cows)			2 (n=4 cows)			3 (n=2 cows)			4 (n=6 cows)			5 (n=2 cows)		
	Mean	SD	Min-Max	Mean	SD	Min-Max	Mean	SD	Min-Max	Mean	SD	Min-Max	Mean	SD	Min-Max
α-la	1.53	1.41	0.34-3.95	0.79	0.46	0.44-1.44	2.08	0.39	1.81-2.35	1.81	0.49	1.26-2.69	2.19	0.36	1.94-2.44
β-lg	7.64	3.68	3.22-13.57	4.94	1.66	3.16-7.15	9.72	2.08	8.25-11.19	8.04	2.95	3.65-12.66	6.50	1.61	5.36-7.64
α-s2	7.07	0.84	5.88-8.04	6.30	0.31	5.86-6.56	6.42	0.57	6.02-6.82	6.71	1.27	4.96-8.85	7.98	0.33	7.75-8.22
α-s1	29.28	1.95	26.20- 31.68	30.71	1.41	28.62-31.54	30.59	2.28	28.98-32.21	31.13	1.28	29.85-33.13	30.74	3.11	28.54-32.94
κ-cn	7.14	1.78	4.93-9.57	6.83	1.37	5.64-8.78	7.00	3.72	4.37-9.36	7.07	1.79	4.48-9.95	7.29	0.28	7.10-7.49
β-cas B	0.29	0.48	0.00-1.25	0.63	0.73	0.00-1.40	0.18	0.25	0.00-0.35	0.60	0.32	0.19-1.01	0.72	0.12	0.64-0.81
β-cas A1	7.23	11.26	0.00-23.41	5.87	11.74	0.00-23.47	19.36	1.50	18.30-20.42	7.12	11.03	0.00-21.53	0.00	0.00	0.00-0.00
β-cas A2	37.25	10.83	21.88-45.95	40.99	11.52	24.26-50.42	22.31	0.75	21.78-22.84	35.13	10.13	22.66-46.05	42.67	0.56	42.27-43.07
Total β-cas	44.77	2.97	40.57-48.68	47.48	2.96	44.79-51.53	41.85	1.00	41.14-42.56	42.85	3.97	35.63-46.60	43.39	0.68	42.91-43.88
Total whey protein	9.18	5.07	3.56-17.52	5.73	2.11	3.56-8.59	11.80	1.70	10.60-13.00	9.85	3.42	4.91-15.35	8.69	1.97	7.29-10.08
Total casein	88.27	4.97	80.08-93.85	91.32	1.39	89.32-92.31	85.86	1.01	85.15-86.57	87.76	3.92	81.04-93.09	89.41	1.81	88.13-90.69

Abbreviations: LN= lactation number; SD=standard deviation; α_{s1}-CN=alfa$_{s1}$-casein; α_{s2}-CN=alfa$_{s2}$-casein; κ-CN=kappa-casein; β-CN B=beta-casein B; β-CN A1=beta$_{A1}$-casein; β-CN A2=beta$_{A2}$-casein; α-LA=alfa-lactalbumin; β-LG=beta-lactoglobulin

3.3. Effect of lactation number on milk composition

Using ANOVA, significant differences were observed for protein composition in the analysis of milk from individual SRB cows with different lactation numbers (Table 6). Significant variables were α-LA, α-s2, β-CN B, total β-CN and total casein. There was a trend for κ-CN also being significant (p-value 0.076) for SRB cows (Appendix II). No significant differences were observed in protein composition in milk from individual SLB cows with different LN (Appendix II). Likewise, no significant effect of LN on milk gross composition was observed, neither in milk from SRB nor in milk from SLB cows (Appendix II). There was a trend for an effect of LN on pH in milk from SRB and SLB cows, respectively, with p-values 0.067 and 0.069 (Appendix I).

Table 6 Variables which were significantly affected by lactation number in the analysis of milk samples from individual SRB cows (n=88) with different lactation numbers. Using ANOVA, the statistical significance of the effect of lactation number on each variable is indicated by p-value. P<0.05 is considered significant

SRB	p-value
α-LA	0.002
α_{s2}-CN	0.014
β-CN B	0.014
Total β-casein	0.000
Total casein	0.007

Abbreviations: α-LA=alfa-lactalbumin; α_{s2}-CN=alfa$_{s2}$-casein; β-CN B=beta-casein B

3.3.1. Pairwise differences within lactation numbers

The Tukey method was used to analyse for pairwise differences in the milk variables that were significantly affected by LN in individual SRB cows (Table 7). Although there were significant differences in α-LA between lactations 2 and 4, there was no trend related to LN. In contrast, the relative concentration of total β-casein showed a clear trend with decreasing values with increasing LN. No significant pairwise difference was observed in milk protein composition amongst different LN in SLB cows (Appendix II). Likewise, no significant pairwise differences was observed in milk gross composition amongst different LN in neither SRB nor SLB cows (Appendix II).

Table 7 Differences in protein composition (% of total protein) between milk samples from individual SRB cows (n=88) with different lactation numbers. Tukey's test for post-hoc analysis was used to test for significances ($p<0.05$). Values presented are mean and standard deviation. Means that do not share the same letter are significantly different

SRB

Lactation number

	1 (n=26 cows)	**2 (n=19 cows)**	**3 (n=11 cows)**	**4 (n=13 cows)**	**5 (n=9 cows)**	**6 (n=5 cows)**	**7&8 (n=5 cows)**
α-LA (%)	2.06±1.0	1.38±0.86[a]	2.21±0.77	2.86±0.84[b]	2.02±0.80	2.10±0.61	2.40±0.75
α_{s2}-CN (%)	6.67±0.88	6.42±0.91[a]	7.28±0.80	6.67±0.78	7.59±0.78[b]	7.17±1.57	7.51±1.07
Total β-CN (%)	44.74±2.88[a]	44.97±2.41[a]	42.26±2.21	41.23±2.74[b]	40.46±2.88	35.97±8.49[c]	39.25±1.06
Total casein (%)	87.05±3.68[a]	87.76±2.62[a]	85.79±2.86	84.03±2.29	85.8±4.77	80.86±9.38[b]	84.61±1.98

Abbreviations: α_{s2}-CN=alfa$_{s2}$-casein; β-CN=beta-casein; α-LA=alfa-lactalbumin

3.4. Composition of milk from SRB and SLB cows

Milk from SRB had numerically higher concentrations of fat, total solids, SFA, UFA, MUFA, PUFA, C14:0, C16:0, C18:0 and C18:1C9, and higher SCC compared to SLB. There was also a numerically higher concentrations of total protein in milk from SRB (table 8). A tendensy of higher total casein was observed in milk from SLB compared to SRB, as well as a tendensy of higher total whey proteins in SRB compared to SLB (table 7). Moreover, there were numerical differences in relative concentrations of β-CN A1 and β-CN A2 between the two breeds. SRB had higher relative concentration of β-CN A1 compared to SLB, while SLB had higher relative concentration of β-CN A2 compared to SRB (table 8).

Table 8 Average composition (% of total composition) of milk from SRB (n=78-88) and SLB (n=20) cows. Values presented are mean and standard deviation

	SRB		SLB	
	Mean	SD	Mean	SD
α-LA (%)	2.07	0.95	1.59	0.92
β-LG (%)	8.12	2.78	7.31	2.95
α_{s2}-CN (%)	6.86	0.97	6.84	0.94
α_{s1}-CN (%)	29.49	1.77	30.40	1.77
κ-CN (%)	6.90	1.38	7.06	1.65
β-CN B (%)	0.56	0.53	0.48	0.46
β-CN A1(%)	15.67	14.60	7.42	10.42
β-CN A2 (%)	26.48	15.51	36.41	10.45
Total β-CN (%)	42.71	3.98	44.31	3.39
Total whey protein (%)	10.19	3.32	8.90	3.77
Total casein (%)	85.97	4.07	88.60	3.71
pH	6.46	0.10	6.39	0.14
Fat content (%)	5.31	1.73	4.22	0.91
Protein content (%)	3.73	0.39	3.57	0.40
Lactose content (%)	4.66	0.18	4.83	0.12
Total solids (%)	14.34	1.81	13.05	0.95
Density (g/mL)	1.03	0.00	1.03	0.00
SCC (10^3/mL)	181.81	252.73	111.20	96.03
SFA (%)	3.81	1.34	2.93	0.68
UFA (%)	1.25	0.36	0.94	0.25
MUFA (%)	0.97	0.31	0.71	0.20
PUFA (%)	0.08	0.05	0.03	0.05
C14:0 (%)	0.67	0.27	0.52	0.12
C16:0 (%)	1.73	0.64	1.40	0.42
C18:0 (%)	0.69	0.22	0.52	0.13
C18:1C9 (%)	0.76	0.23	0.56	0.17

Abbreviations: LN= lactation number; SD=standard deviation; α_{s1}-CN=alfa$_{s1}$-casein; α_{s2}-CN=alfa$_{s2}$-casein; κ-CN=kappa-casein; β-CN B=beta-casein B; β-CN A1=beta$_{A1}$-casein; β-CN A2=beta$_{A2}$-casein; α-LA=alfa-lactalbumin; β-LG=beta-lactoglobulin; SCC=somatic cell count; SFA=saturated fatty acids; UFA= unsaturated fatty acids; MUFA=mono unsaturated fatty acids; PUFA=poly unsaturated fatty acids; C14:0=myristic acid; C16:0=palmitic acid; C18:0=stearic acid; C18:1C9=oleic acid

4. Discussion

4.1. Effect of lactation number on milk composition

In our study, the results suggested that LN only had a limited influence on milk composition and milk protein profile in SRB cows. LN had a significant effect on the relative concentrations of α-LA, α_{s2}-CN, β-CN B, total β-casein and total casein, in milk from SRB cows. There was a trend that the relative concentrations of α-LA and α_{s2}-CN increased with higher LN, whereas the relative concentrations β-CN B, total β-CN and total casein instead deccreased with higher LN. For SRB cows, there were no significant effect of LN on any of the investigated variables. With only 20 individual SLB cows, there is reason to doubt the representativeness of the results. To obtain higher reliability, a larger number of SLB cows should have been included in this study.

In agreement with results from ANOVA, multivariate analysis (PCA) did not show any evident association between LN and milk quality parameters. This, since the PCA score plot with observations coloured according to LN (figure 1 A) revealed no clusters of observations related to the same LN or clusters of observations related to different LN.

4.2. Effect of breed on milk composition

In this study, milk from SRB had numerically higher values of fat, total solids, SFA, UFA, MUFA, PUFA, C14:0, C16:0, C18:0 and C18:1C9, compared to SLB. However, no tests were performed for significant differences between the two breeds. Concidering the results by Wedholm (2008), the concentration of total fat and protein was expected to be higher in milk from SRB compared to SLB. This is in agreement with our results, where SRB had 5.31 g/100g of fat and 3.73 g/100g of protein and SLB had 4.22 g/100g of fat and 3.57 g/100g of protein. However, recent data from Växa Sverige (2020) showed conciderably lower values for fat and to some extent also for protein. According to Växa Sverige (2020) average values in Sweden for total fat was 4.40 g/100g for SRB and 4.11 g/100g for SLB, and total

protein was 3.40 g/100g for SRB and 3.52 g/100g for SLB. One explanation for the differences in fat and protein contents could be that in our study, data is based on 110 milk samples, whereas, many thousand samples form the basis for the data reported from Växa Sverige (2020). Växa Sverige's data must therefore be concidered more representative for all milk in Sweden. Seemingly, the total protein content is fairly close between the two breeds, which was also the case in our study.

In our study, the higher mean SCC in SRB can be explained by very the large SD, illustrating that SCC differed largely between individual SRB cows included in this study. Higher SCC is associated with milk quality detoriation, such as increased proteolytic degradation of casein proteins (Verdi et al. 1987; Skeie 2010). In our study there is a possible correlation between high SCC and lower concentration of caseins in milk from SRB, that could possibly be because of protein degradation. Generally, milk from SRB had lower relative concentration of total casein and higher relative concentration of total whey proteins and, reasonably, higher relative concentration of α-LA and β-LG. In contrast, SLB had higher relative concentrations of several individual caseins and somewhat higher relative concentration of total casein. In a study from Ng-Kwai-Hang (1987), increased SCC was associated with lower β-CN. Wedholm (2006a) showed significantly higher concentrations of total protein, total casein, β-CN and κ-CN in milk from SRB compared to SLB. Given this information, there is an even stronger reason to believe that, in our study, the caseins in milk from SRB had been partially degraded.

The relative concentrations of α_{s1}-CN, α_{s2}-CN, κ-CN, α-LA and β-LG were numerically lower in milk from both SRB and SLB (table 4) compared to the values reported by Ng-Kwai-Hang (2011) in table 2. The relative concentration of total β-CN in our study was higher in milk from both SRB and SLB cows, 42.71% and 44.31% respectively, compared to 30% previously reported by Hallén (2008). Selection of milk samples in our study can not be considered representative for all milk in Sweden. Results in our study may be affected by the fact that cows sampled at the same farm, may share extensive genetic material (mother, daughters, sisters). Thus, cows that are related might have a higher content of, for example, β-CN. Additionally, the observed differences in protein composition can also be a result of the more recent breeding programs focusing on caseins since year 2000, when the protein was included as a payment parameter in Sweden.

In our study, relative concentrations of β-CN A1 and β-CN A2 differed with respect to breed. SRB had higher relative concentration of β-CN A1 compared to SLB, and SLB had higher relative concentration of β-CN A2 compared to SRB. This is in contrast with Wedholm (2006a), who reported that β-CN A2 was more common in milk from SRB cows than in SLB in Swedish dairy herds.

In our study, average concentration of lactose was numerically higher in milk from SLB cows, compared to SRB (table 8). Wedholm (2006a) reported significant

higher concentrations of lactose in milk from SLB than in milk from SRB, thus in agreement with our results. α-LA has a biological function to act as coenzyme in lactose synthesis (Walstra et al. 2005). Therefore, concentration of α-LA in milk is directly related to concentration of lactose (Wedholm 2008). However, there was no positive correlation between 'α-LA' and 'lactose' in our study. Moreover, PCA showed that SCC did not vary much between cows in our study, with this parameter being close to the middle in the PCA plot. The PCA does not compare numerical values, it compares quantity of variation, meaning that other parameters explained more of the overall variation in milk composition. The mean SCC for all cows was 178,11 x 10^3, with a SD of 242 x 10^3 (appendix I). A high SD tells us that there was a large variation in SCC between milk samples and no significant difference in SCC between breeds. Consequently, the variation in SCC was "masked" by other variation in the PCA.

Using PCA, a trend was clear when observations were coloured according to breed (figure 1 B). In SRB cows, the milk composition variation seemed, to a larger extent, to be caused by parameters on the x-axis (PC1), with observations from SRB cows being spread widely in this direction . That is in agreement with numerical values of milk composition in SRB and SLB cows reported in this study. However, for both breeds, observations were spread similarly over the y-axis (PC2) e.g. the variation of protein composition seemes to be fairly equal for both breeds.

In the loading plot the largest variation in data is showed along PC1 and the second largest variation in data along PC2. Mainly, PC1 in figure 1D consisted of parameters from anlysis of gross composition, whereas PC2 consisted of different proteins. As expected, 'total casein' and 'total whey protein' were observed on opposite sides of PC2, explained by the fact that milk with higher casein content has lower concentration of whey protein, and vice versa. In our study, results from CE showed that milk with higher casein content, in our case from SLB cows, also had lower content of whey proteins. Also, milk from SRB with higher whey protein content, had lower casein content. 'α-LA' and 'β-LG', constitutents of total whey fraction, where observed close to 'total whey' (figure 1 D), as expected.

4.3. Effect of farm on milk composition

Amongst all observations, regardless LN and breed, there was a trend for milk samples collected on the same farm to form clusters in the PCA (figure 1 C). This is in particular the case for farms E (blue) and D (yellow), separating from the other observations. As explained by Lindmark-Mansson et al. (2003), concentrations of caseins and total protein in milk from Swedish dairy cows, can be a result of geographical variations. Those variations can be explained by differences in feeding practices and feed quality in those different regions. In the case of our study, milk composition varied less with respect to milk from the same farm, but there

was a clear variation between the farms. As previously mentioned, one potential explanation could be that cows on the same farm are related and share genetic material. Other explanations for the variation between farms include differences in management, not least in feed and feeding strategies. Also, there is a geographical variation amongst the different farms, which can affect the milk composition.

4.4. Milk composition relevance for dairy production

In Sweden, dairy cows are often culled already at the age of 5 years or younger, and undergoes about 2.5 lactation cycles (Växa Sverige 2020). Culling a dairy cow is costly because of the need of rearing new heifers for milk production. Moreover, increased life length of the cow is directly related to animal welfare (Schneider et al. 2007), and reduced climate impact (De Vries & Marcondes 2020). In the case of our study, older cows with higher LNs did not seem to have different milk composition compared to younger cows with lower LNs. This result suggests that Swedish farmers would not be paid a lower price for their milk if they kept cows in milk production for a longer time, since the milk quality parameters are not clearly affected by the age of the cow. Keeping older cows in production, would reasonably be better for the climate, for the economy of the farmer and for the animal welfare, thus leading to increased consumer trust.

Many consumers focuse mainly on animal welfare and product quality (Lovarelli et al. 2020). The quality of dairy products is affected by the raw milk quality parameters analysed in this study. This means that, most probably, the quality of dairy products would not be imapired if the milk used in dairy processes was obtained from older cows. Milk is currently paid according to total protein and fat concentrations. For cheese production, milk with high casein content is especially important (Wedholm et al 2006b). Our results showed that relative concentration of total casein and total β-CN where significantly higher in lower LNs. However, it is hard to draw any conclusions whether these results are credible, because of the low number of cows with high LNs in this study. Yet, our study showed that relative concentration of casein was higher in SLB cows compared to SRB cows, indicating that milk from SLB is more valuable for the industry of cheese production. However, the breeds were not compared at a significant level, thus results were not proven significant. Moreover, the number of milk samples in our study is relatively low and can not be considered representative for all milk in Sweden. Nevertheless, this study gives an important indication about the effect of lactation number of cows on quality parameters of their milk.

5. Conclusion

The aim of this study was to investigate whether different LNs in cows influenced on milk quality paramters, i.e. protein profile, gross composition, pH and SCC. Results from multivariate analysis showed that the milk composition was not affected by lactation number. Instead, as it seems, the variation in milk composition was mainly associated to breed and farm where the cows were sampled. Relative concentrations of α-LA, α_{s2}-CN, β-CN B, total β-cas and total casein, were shown to be the only parameters that were significantly affected by LN and this was only the case with milk from SRB cows. For α-LA and α_{s2}-CN the relative concentrations were higher with higher LN, whereas for β-CN B, total β-cas and total casein the relative concentrations were higher with lower LN. For SLB cows, there was no significant effect of LN on protein profile, gross composition, pH or SCC.

Another aim of this study was to evaluate the effect of breed on milk composition, comparing milk from SRB and SLB cows. In conclusion, in this study there were no significiant differences in milk composition between the two breeds. However, milk from SRB had numerically higher values of fat, total solids, SFA, UFA, MUFA, PUFA, C14:0, C16:0, C18:0 and C18:1C9, compared to SLB. Milk from SRB cows had numerically higher concentration of β-CN A1 and lower relative concentration of β-CN A2, compared to SLB. However, with only 20 individual SLB cows, results are not fully trustworthy.

Results in this study indicate no major differences in milk from young cows with LN 1-2, compared to milk from old cows with LN 3-8. The observed differences are not strong enough to make a conclusion whether LN affects the investigated parameters in cows milk. However, results indicate that milk quality parameters differ between cows of different breeds and from different farms. Further research, including more individuals is required to be able to draw firmer conclusions regarding the effect of number of lactations on raw milk composition.

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Appendix I – Popular scientific summary

Cattle have been domesticated by humans for about 8000 years, and have since then been selectively bred for different attributes. For example, we want cows that stay healthy, puts on weight efficently and produce large volumes of high quality milk. All around the world, dairy products are an important part of our diet. For many people it has become increasingly important to buy food produced in a sustainable way. Sustainable dairy production embraces many different obsticals, such as environmental conciderations, the farmers profitability and animal welfare.

Cows release the greenhouse gas methane when they digest feed, which contributes to increased global temperature with effect on our climate. Therefore, it is important for industries, such as dairy production, to work towards a lower emission of greenhouse gases. Worth to mention is that in grazing areas, and where grass for production of cow feed is grown, carbon will be stored in the soil. This compensates for some of the gas emission from cows. One suggestion for farmers to further reduce methane emission is to increase the life length of their cows, so that every cow will be kept for a longer time and produce more milk during its life. That would decrease the methane emission per cow and per unit of total milk solid, meaning the nutritive value of the milk. In addition to that, increased life length of cows, would also increase the animal welfare, which in turn leads to a more valuable product for consumers.

In Sweden, dairy cows are often culled already at the age of 5 years or younger, because of infertility problems, udder diseases or because of low milk yield. However, many farmers are not aware of the true cost of culling dairy cows, thus introducing new heifers at a high rate into production. On average, it takes approximately 1.5 lactations, or 530 days, to repay the cost of rearing a heifer until she starts producing milk, i.e. in connection to having her first calf. This, since during the time of rearing, the heifer eats feed but produce no milk. The question is if it is possible to save money, reduce gas emissions and improve animal welfare by keeping cows in milk production for longer time than we do today? And, if we do, will it affect the milk quality?

In this study, we wanted to investigate if there is any differences in milk quality when comparing milk from younger and older cows. If we want to keep cows in production when they get older, the milk has to keep the same quality standard. Otherwise, the milk can not be sold with the same profit, and farmers will not be convinced of any other benefits. Milk from two dominant breeds in Sweden, i.e. SRB and SLB, was collected from 11 Swedish farms. In total, milk samples from 110 cows with different lactation numbers, i.e. number of calfs born, ranging from 1-8, were collected. Each milk sample was analyzed to investigate differences amongst them. Milk gross composition (fat, protein, lactose, total solids, density and fatty acids), pH and SCC was measured and compared. Protein profile, i.e. the relative concentrations of different caseins and whey proteins, was analyzed with a method called capillary electrophoresis. This method separates the milk proteins depending on their size, shape and charge.

Results from our study showed no major differences in milk from young cows with lactation number 1-2, compared to milk from old cows with lactation number 3-8. However, cows from the same farm tended to have more similar milk composition, as well as cows of the same breed. At farms, cows are typically mothers and daughters, meaning that they share genetic material. This probably explains why there is a larger diversity between milk from different farms. Milk from SRB cows had numerically higher values of fat, total solids and fatty acids, compared to SLB. Also, two caseins called beta$_{A1}$-casein and beta$_{A2}$-casein, differed numerically between the two breeds. Milk from SRB cows had higher beta$_{A1}$-casein and lower beta$_{A2}$-casein, compared to SLB.

Our study suggests that there are no evident differences in milk composition between young and old cows. However, this study can only serve as guidance and further research is needed within this topic. In future studies it would be important to include an even larger number of individual cows, specifically from the SLB breed. A larger data set would provide more reliable conclusions on wheter lactation number, or age of cows, has an effect on raw milk composition.

Appendix II - Statistics

Table 9 Non-significant effects of lactation number on the composition and properties of milk from individual SRB (n=88) and SLB cows (n=20). Effects of lactation number on pH, fat content, protein content and SCC (n=88), and on the remaining milk components (n=78) in milk from individual SRB cows. Effects of lactation number on gross composition (fat, protein, lactose, total solids, density and fatty acids), pH, SCC and protein profile in milk from individual SLB cows (n=20). The statistical significance of the effect of lactation number on the variables is indicated by the p-value. P>0.05 is considered non-significant

	Non-significant variables	P-value
SRB	β-lg (%)	0.468
	α-s1 (%)	0.666
	κ-cn (%)	0.076
	β-cas A1 (%)	0.503
	β-cas A2 (%)	0.164
	Total whey protein (%)	0.185
	pH	0.067
	Fat content (%)	0.976
	Protein content (%)	0.886
	SCC (10^3/mL)	0.376
	Lactose content (%)	0.247
	Total solids (%)	0.999
	Density (g/mL)	0.827
	SFA (%)	0.984
	UFA (%)	0.703
	MUFA (%)	0.731
	PUFA (%)	0.155
	C16:0 (%)	0.943
	C18:0 (%)	0.968
	C18:1C9 (%)	0.430
	C14:0 (%)	0.939

SLB	α-la (%)	0.328
	β-lg (%)	0.365
	α-s2 (%)	0.289
	α-s1 (%)	0.494
	κ-cn (%)	0.998
	β-cas B (%)	0.222
	β-cas A1 (%)	0.367
	β-cas A2 (%)	0.270
	Total β-cas (%)	0.203
	Total whey protein (%)	0.376
	Total casein (%)	0.485
	pH	0.069
	Fat content (%)	0.505
	Protein content (%)	0.348
	Lactose content (%)	0.913
	Total solids (%)	0.323
	Density (g/mL)	0.931
	SCC (10^3/mL)	0.406
	SFA (%)	0.651
	UFA (%)	0.391
	MUFA (%)	0.362
	PUFA (%)	0.793
	C16:0 (%)	0.801
	C18:0 (%)	0.611
	C18:1C9 (%)	0.467
	C14:0 (%)	0.599

Abbreviations: α_{s1}-CN=alfa$_{s1}$-casein; α_{s2}-CN=alfa$_{s2}$-casein; κ-CN=kappa-casein; β-CN B=beta-casein B; β-CN A1=beta$_{A1}$-casein; β-CN A2=beta$_{A2}$-casein; α-LA=alfa-lactalbumin; β-LG=beta-lactoglobulin; *SCC=somatic cell count; SFA=saturated fatty acids; UFA= unsaturated fatty acids; MUFA=mono unsaturated fatty acids; PUFA=poly unsaturated fatty acids; C16:0=palmitic acid; C18:0=stearic acid; C18:1C9=oleic acid; C14:0=myristic acid*

Table 10 Differences in protein composition (% of total protein) between milk samples from individual SLB cows (n=20) with different lactation numbers. Tukey's test for post-hoc analysis was used to test for significances ($p<0.05$). Values presented are mean and standard deviation. Means that do not share the same letter are significantly different

SLB

	Lactation number				
	1 (n=6 cows)	**2 (n=4 cows)**	**3 (n=2 cows)**	**4 (n=6 cows)**	**5 (n=2 cows)**
α-LA	1.53±1.41	0.80±0.46	2.08±0.39	1.81±0.49	2.19±0.36
β-LG	7.64±3.68	4.94±1.66	9.72±2.08	8.04±2.95	6.5±1.61
α_{s2}-CN	7.07±0.84	6.3±0.31	6.42±0.57	6.72±1.27	7.98±0.33
α_{s1}-CN	29.28±1.95	30.71±1.41	30.59±2.28	31.13±1.28	30.74±3.11
κ-CN	7.14±1.78	6.83±1.37	7±3.72	7.07±1.79	7.29±0.28
β-CN B	0.44±0.55	1.25±0.21	0.35±X	0.60±0.33	0.73±0.12
β-CN A1	21.7±2.41	23.47±X	19.36±1.5	21.36±0.24	
β-CN A2	37.25±10.83	40.99±11.52	22.31±0.75	35.13±10.13	42.67±0.56
Total β-CN	44.77±2.97	47.48±2.96	41.85±1.00	42.85±3.97	43.39±0.69
Total whey	9.18±5.07	5.73±2.11	11.8±1.7	9.85±3.42	8.69±1.97
Total casein	88.27±4.97	91.32±1.39	85.86±1.01	87.76±3.92	89.41±1.81

X indicates that no standard deviation was observed (n=1) for that variable

Abbreviations: α_{s1}-CN=alfa$_{s1}$-casein; α_{s2}-CN=alfa$_{s2}$-casein; κ-CN=kappa-casein; β-CN B=beta-casein B; β-CN A1=beta$_{A1}$-casein; β-CN A2=beta$_{A2}$-casein; α-LA=alfa-lactalbumin; β-LG=beta-lactoglobulin

Table 11 Differences in milk composition (% of total composition) between milk samples from individual SRB cows (n=78-88) with different lactation numbers. Tukey's test for post-hoc analysis was used to test for significances ($p<0.05$). Values presented are mean and standard deviation. Means that do not share the same letter are significantly different

SRB	Lactation number						
	1 (n=26 cows)	**2 (n=19 cows)**	**3 (n=11 cows)**	**4 (n=13 cows)**	**5 (n=9 cows)**	**6 (n=5 cows)**	**7&8 (n=5 cows)**
pH	6.47±0.08	6.40±0.08[a]	6.42±0.07	6.51±0.13[b]	6.47±0.10	6.47±0.05	6.47±0.16
Fat content (%)	5.11±1.65	5.54±1.85	5.40±1.88	5.24±1.73	5.69±1.87	5.19±2.34	5.1±1.34
Protein content (%)	3.73±0.37	3.77±0.24	3.85±0.45	3.61±0.57	3.73±0.42	3.69±0.46	3.72±0.23
Lactose content (%)	4.74±0.2	4.63±0.17	4.65±0.11	4.62±0.18	4.61±0.19	4.69±0.18	4.52±0.15
Total solids (%)	14.23±1.76	14.36±1.67	14.56±2.03	14.22±2.02	14.53±2.01	14.17±2.52	14.37±1.13
Density (g/mL)	1.03±0.00	1.03±0.00	1.03±0.00	1.03±0.00	1.03±0.00	1.03±0.00	1.03±0.00
SCC (10^3/mL)	149.1±230.2	116.2±83.7	144.1±167.5	234.3±303.1	307±473	164.2±109.7	334±325
SFA (%)	3.61±1.30	3.93±1.49	3.92±1.39	3.88±1.29	4±1.51	3.54±1.60	3.79±0.80
UFA (%)	1.31±0.42	1.16±0.23	1.19±0.34	1.19±0.34	1.26±0.28	1.32±0.55	1.51±0.67
MUFA (%)	1.02±0.37	0.90±0.19	0.93±0.31	0.92±0.30	0.99±0.25	1.04±0.47	1.20±0.57
PUFA (%)	0.09±0.06	0.07±0.03	0.07±0.03	0.07±0.04	0.08±0.05	0.10±0.07	0.17±0.06
C16:0 (%)	1.61±0.59	1.81±0.69	1.78±0.69	1.78±0.62	1.81±0.76	1.53±0.70	1.72±0.28
C18:0 (%)	0.72±0.25	0.68±0.23	0.66±0.18	0.65±0.21	0.70±0.16	0.71±0.29	0.74±0.35
C18:1C9 (%)	0.82±0.28	0.69±0.11	0.73±0.20	0.72±0.21	0.77±0.19	0.84±0.34	0.96±0.47
C14:0 (%)	0.64±0.24	0.70±0.27	0.72±0.26	0.71±0.22	0.73±0.29	0.63±0.27	0.67±0.10

Abbreviations: SCC=somatic cell count; SFA=saturated fatty acids; UFA= unsaturated fatty acids; MUFA=mono unsaturated fatty acids; PUFA=poly unsaturated fatty acids; C16:0=palmitic acid; C18:0=stearic acid; C18:1C9=oleic acid; C14:0=myristic acid

Table 12 Differences in milk composition (% of total composition) between milk samples from individual SLB cows (n=20) with different lactation numbers. Tukey's test for post-hoc analysis was used to test for significances ($p<0.05$). Values presented are mean and standard deviation. Means that do not share the same letter are significantly different

SLB

	Lactation number				
	1 (n=6 cows)	**1 (n=6 cows)**	**1 (n=6 cows)**	**1 (n=6 cows)**	**1 (n=6 cows)**
pH	6.33±0.15	6.35±0.11	6.44±0.10	6.52±0.12	6.26±0.01
Fat content (%)	4.70±1.21	4.00±0.40	4.50±0.71	3.80±0.86	4.36±0.91
Protein content (%)	3.82±0.45	3.34±0.5	3.51±0.23	3.58±0.22	3.31±0.46
Lactose content (%)	4.84±0.16	4.81±0.12	4.81±0.08	4.81±0.1	4.91±0.2
Total solids (%)	13.71±1.21	12.59±0.69	13.21±0.83	12.68±0.75	12.97±0.71
Density (g/mL)	1.03±0.00	1.03±0.00	1.03±0.00	1.03±0.00	1.03±0.00
SCC (10^3/mL)	94.8±91.5	69.3±38	227±212	103.8±60.7	151±163
SFA (%)	3.2±0.89	2.75±0.26	3.15±0.28	2.65±0.69	3.15±0.93
UFA (%)	1.1±0.23	0.93±0.22	0.96±0.52	0.83±0.16	0.81±0.3
MUFA (%)	0.85±0.2	0.71±0.17	0.74±0.42	0.61±0.13	0.61±0.21
PUFA (%)	0.08±0.04	0.07±0.04	0.08±X	0.05±0.03	0.07±X
C16:0 (%)	1.5±0.56	1.31±0.2	1.54±0.08	1.25±0.42	1.57±0.66
C18:0 (%)	0.57±0.16	0.53±0.14	0.56±0.26	0.45±0.07	0.54±0.01
C18:1C9 (%)	0.65±0.16	0.56±0.13	0.57±0.37	0.49±0.11	0.45±0.25
C14:0 (%)	0.59±0.17	0.46±0.06	0.53±0.04	0.49±0.12	0.54±0.11

X indicates that no standard deviation was observed (n=1) for that variable

Abbreviations: SCC=somatic cell count; SFA=saturated fatty acids; UFA= unsaturated fatty acids; MUFA=mono unsaturated fatty acids; PUFA=poly unsaturated fatty acids; C16:0=palmitic acid; C18:0=stearic acid; C18:1C9=oleic acid; C14:0=myristic acid

Table 13 Milk composition (% of total composition) of milk samples from individual cows. Cows were either SRB (n=88) or SLB (n=20) and had different lactation numbers, ranging from 1-8. Samples were obtained from 11 different farms in Uppland, Södermanland, Västmanland and Västerbotten, Sweden. Values presented are mean and standard deviation

	Mean	SD
α-LA (%)	1.91	0.96
β-LG (%)	7.79	2.83
α_{s2}-CN (%)	6.87	0.99
α_{s1}-CN (%)	29.84	1.72
κ-CN (%)	6.93	1.41
β-CN B (%)	0.56	0.53
β-CN A1(%)	13.46	14.22
β-CN A2 (%)	28.97	15.17
Total β-CN (%)	42.99	4.04
Total whey protein (%)	9.69	3.42
Total casein (%)	86.63	4.25
pH	6.43	0.11
Fat content (%)	5.21	1.71
Protein content (%)	3.70	0.41
Lactose content (%)	4.69	0.18
Total solids (%)	14.08	1.74
Density (g/mL)	1.03	0.00
SCC (10^3/mL)	178.11	242.00
SFA (%)	3.63	1.28
UFA (%)	1.18	0.36
MUFA (%)	0.92	0.31
PUFA (%)	0.07	0.06
C16:0 (%)	1.66	0.61
C18:0 (%)	0.66	0.22
C18:1C9 (%)	0.72	0.24
C14:0 (%)	0.65	0.24

Abbreviations: α_{s1}-CN=alfa$_{s1}$-casein; α_{s2}-CN=alfa$_{s2}$-casein; κ-CN=kappa-casein; β-CN B=beta-casein B; β-CN A1=beta$_{A1}$-casein; β-CN A2=beta$_{A2}$-casein; α-LA=alfa-lactalbumin; β-LG=beta-lactoglobulin; *SCC=somatic cell count; SFA=saturated fatty acids; UFA= unsaturated fatty acids; MUFA=mono unsaturated fatty acids; PUFA=poly unsaturated fatty acids; C16:0=palmitic acid; C18:0=stearic acid; C18:1C9=oleic acid; C14:0=myristic acid*

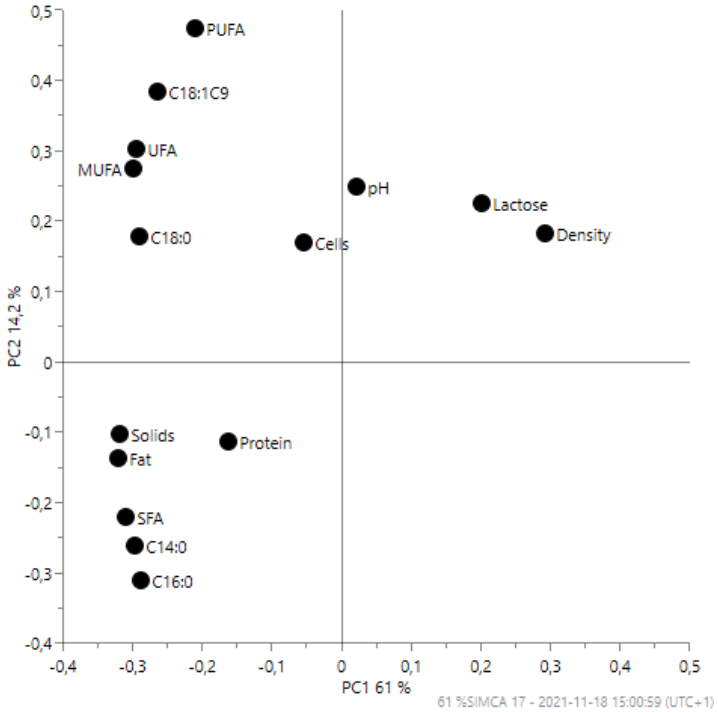

Figure 3 Principal component analysis loading plot, showing the cause of variation with respect to milk gross composition, pH and SCC. Of the total variation, the first and second principal components (PC1 and PC2) explained 61% and 14.2%, respectively.

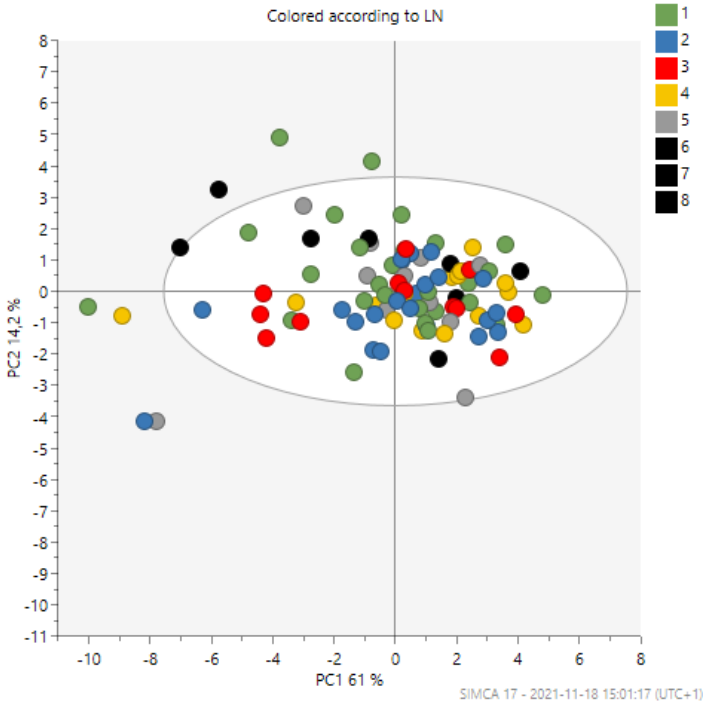

Figure 4 Principal component analysis score plot, showing the overall variation in milk gross composition, pH and SCC of individual cow milk samples (n=110). Each observation represents an individual cow milk sample, coloured according to the lactation number of the cow. Of the total variation, the first and second principal components (PC1 and PC2) explained 61% and 14.2%, respectively.

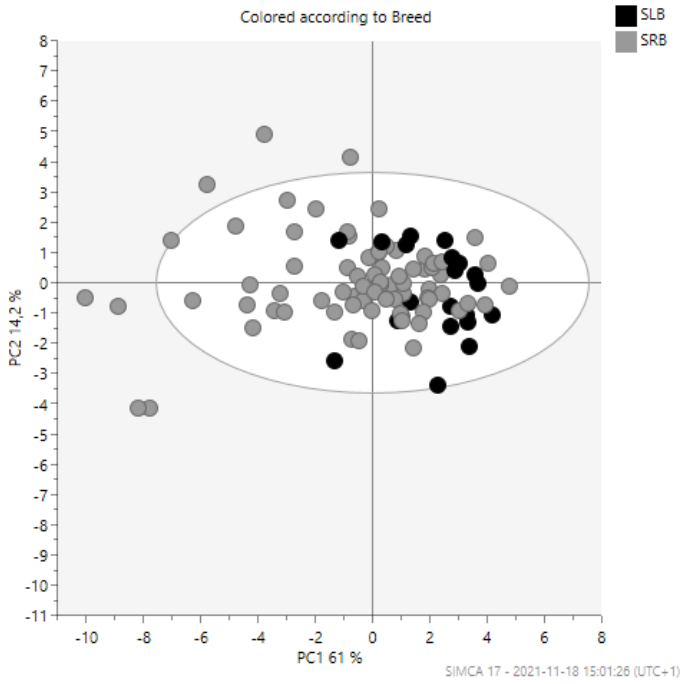

Figure 5 Principal component analysis score plot, showing the overall variation in milk gross composition, pH and SCC of individual cow milk samples (n=110). Each observation represents an individual cow milk sample, coloured according to the breed of the cow. Of the total variation, the first and second principal components (PC1 and PC2) explained 61% and 14.2%, respectively.

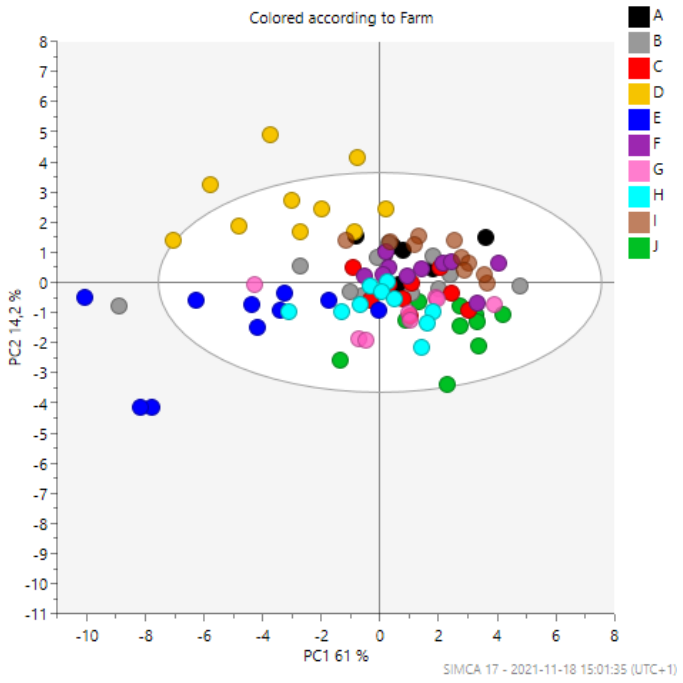

Figure 6 Principal component analysis score plot, showing the overall variation in milk gross composition, pH and SCC of individual cow milk samples (n=110). Each observation represents an individual cow milk sample, coloured according to on which farm the cow was sampled. Of the total variation, the first and second principal components (PC1 and PC2) explained 61% and 14.2%, respectively.

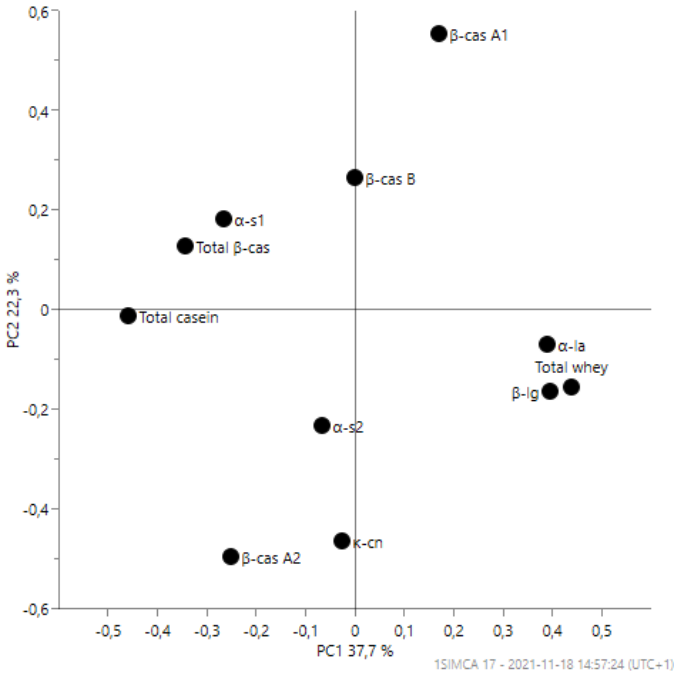

Figure 7 Principal component analysis loading plot, showing the cause of variation with respect to milk proteins. Of the total variation, the first and second principal components (PC1 and PC2) explained 37.7% and 22.3%, respectively.

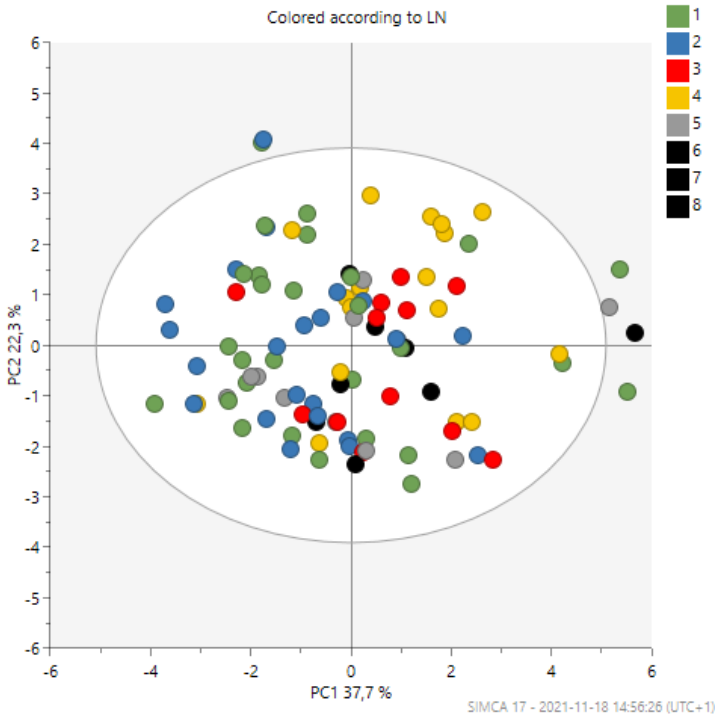

Figure 8 Principal component analysis score plot, showing the overall variation in milk protein composition of individual cow milk samples (n=110). Each observation represents an individual cow milk sample, coloured according to the lactation number of the cow. Of the total variation, the first and second principal components (PC1 and PC2) explained 37.7% and 22.3% respectively.

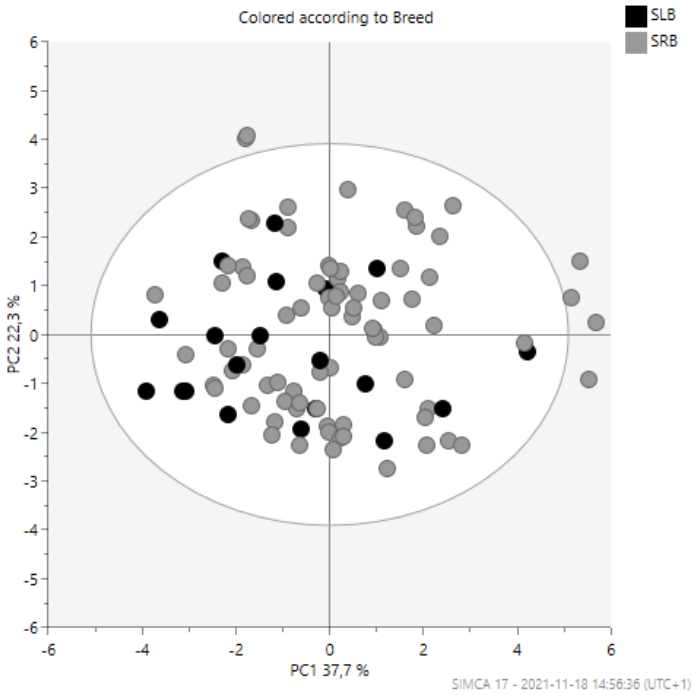

Figure 9 Principal component analysis score plot, showing the overall variation in milk protein composition of individual cow milk samples (n=110). Each observation represents an individual cow milk sample, coloured according to the breed of the cow. Of the total variation, the first and second principal components (PC1 and PC2) explained 37.7% and 22.3% respectively.

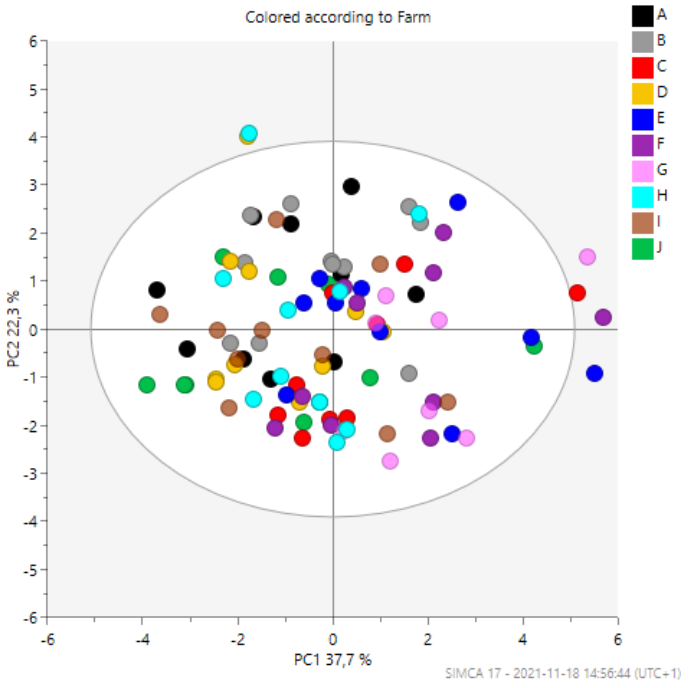

Figure 10 Principal component analysis score plot, showing the overall variation in milk protein composition of individual cow milk samples (n=110). Each observation represents an individual cow milk sample, coloured according to on which farm the cow was sampled. Of the total variation, the first and second principal components (PC1 and PC2) explained 37.7% and 22.3% respectively.