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# Genetic factors affecting the composition and quality of cow's milk

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## 1 Introduction

Milk secures the cognitive development, health and growth of mammalian neonates by the mother, offering a tailored cocktail of fat, protein and lactose together with essential vitamins and minerals, as well as an overwhelming number of minor bioactive components (German et al., 2002). Since the domestication of cattle, dairying has played a pivotal role for humans, which the strong selective sweep for lactase persistence (lactose tolerance) also documents.

So why does one cow produce milk with, for example, a high casein number and superior coagulation properties, compared to other cows? It is well known that variances in milk composition result not only from feeding and management, but also from genetic factors, as has been established, for example, for the milk protein fraction (Fig. 1). These variances may to a large extent relate to the genetic predisposition of specific cows, given that the genome has a major impact on the specific milk quality trait of interest. Hereditary traits can be improved through selective breeding, where the goal is to improve traits like cow health, fertility, milk yield and milk quality. Bulls

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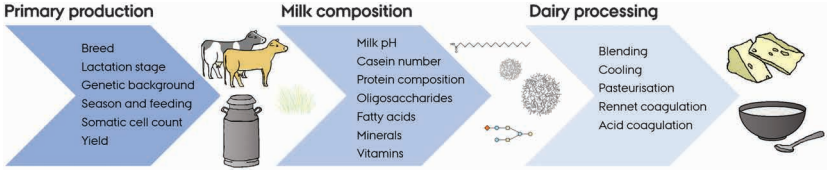


**Figure 1** Factors which may affect milk composition, and thus milk functionality, during dairy processing.

with premium genetic merit are selected to increase the genetic gain for traits of interest. For a long time, effects of genetic variants of milk proteins on milk composition and quality have been documented. However, with the current use of more sensitive methods for milk phenotyping, combined with the reduced cost and optimised bioinformatics for genotyping array, numerous genes and genomic regions affecting both lactation performance, specific components of milk and even functional properties have been identified.

Performing genome-wide association studies (GWAS) can identify single-nucleotide polymorphisms (SNPs) contributing to the phenotypic trait of interest. This leads to the identification of genomic regions that may contain candidate genes associated with the trait of interest or specific pathways, which may influence the trait. Many of these traits are polygenic, complex traits, and therefore it is often hard to identify causal genes underlying these. However, GWAS provide valuable insight into the molecular basis of the traits and enhance our understanding of underlying factors, but in terms of dairy breeding programs, the estimation of genetic merit is more important (Goddard and Hayes, 2009). Heritability provides an estimate of how much of the phenotypic variance can be ascribed to additive genetic variance, which in other words, defines the part of the variation that can be changed through selective breeding or genomic selection. Apart from heritability, the estimation of genetic correlations among traits may also be of interest in order to pinpoint potential correlated effects on other traits, especially when these are also included in the breeding goal.

The phenotypic variance is the sum of genetic variance and environmental variance. Heritability ($h^2$) is defined as the proportion of phenotypic variance, which can be explained by additive genetic variance. Heritability estimates envisage the potential for genetic progress, and thus the possibility of changing a specific trait through selective breeding. Within breed, variance components are estimated to calculate heritability. In the past, especially economically valuable traits such as protein, fat and milk yield have been the targets for selection, which over the years have resulted in large genetic gain achieved as a result of selection for these productivity traits. Both milk yield and concentration of fat, protein or even lactose exhibit significant heritabilities,

whereas urea and somatic cell count (SCC), for example, which are more closely related to environmental factors, are less heritable (Stoop et al., 2008).

Ogorevc et al. (2009) developed a quantitative trait loci database based on literature, identifying cattle candidate genes and genetic markers for milk production and mastitis. Over the past 20 years, milk genomics studies have identified a number of major QTL regions, including, to a profound extent, the regions around *DGAT1* on BTA14, *SCD1* on BTA26, *FASN* on BTA19, as well genes for major milk proteins such as the casein gene cluster on BTA6, *PAEP* (encoding for β-lactoglobulin (β-LG)) on BTA 11 and *ALBA* (encoding α-lactalbumin (α-LA)) on BTA5. These specific genes or regions seem to influence numerous milk traits, including total yields of milk, protein and fat (Ogorevc et al., 2009).

The genomic organisation of the casein loci on BTA6 spans 250 kb (Threadgill and Womack, 1990). As 80% of the proteins in bovine milk are caseins, this region is expected to have a large influence on milk protein and milk protein–related traits, and is found to play a significant role for the caseins (Schopen et al., 2009; Buitenhuis et al., 2016). Likewise, this region has been identified to have a strong association with rennet- and acid-induced coagulations (Gregersen et al., 2014; Gregersen et al., 2015). The genomic region on BTA11, which harbours the *PAEP* gene encoding β-LG, has been associated with milk compositional traits in a number of studies (Gregersen et al., 2014; Poulsen et al., 2018), and affects β-LG in milk (Schopen et al., 2009).

*DGAT1* codes for acyl-CoA:diacylglycerol acyltransferase 1, an enzyme that plays an important role in triacylglycerol synthesis by catalysing the esterification of a fatty acyl-CoA to the sn-3 position of a diacylglycerol in the synthesis of triglycerides (Grisart et al., 2002). The effect of the *DGAT* K232A polymorphism has been intensively studied (Grisart et al., 2002; Bovenhuis et al., 2016), but the gene, or the chromosomal region(s) around *DGAT1,* also affects a range of economically important milk compositional traits like milk yield, concentrations of fat and protein, as well as casein number, milk metabolites, vitamins and fatty acid composition (Schennink et al., 2007; 2009; Stoop et al., 2009; Schopen et al., 2009; Buitenhuis et al., 2013; 2014; 2016; Melzer et al., 2013; Poulsen et al., 2015b). The enzyme stearoyl-coenzyme A desaturase 1 (SCD1) catalyses the conversion of the group of C10:0–C18:0 saturated fatty acids into their mono-unsaturated counterparts. In a number of studies, the *SCD1* gene is associated with milk fatty acid composition and desaturase indexes (Mele et al., 2007; Schennink et al., 2008; Stoop et al., 2009; Conte et al., 2010; Buitenhuis et al., 2014).

## 2 Breed

Today, high-yielding Holstein–Friesians are widespread, and in many areas, the breed of choice for modern dairy production. However, since domestication

around 11 000 years ago, diversification of animal genetic resources has resulted in over 1000 cattle breeds worldwide, that through selection and isolation represent unique genetic make-up (FAO, 2015). Due to low census population sizes, many indigenous breeds are classified as endangered and at risk of extinction (FAO, 2015). International commitments and guided breeding programs are pivotal to conserve these genetic resources for the future, but the documentation of phenotypic and performance characteristics of the breeds is also central (FAO, 2015). Studies have documented genetic distinctiveness and phylogenetic relationship among local and modern breeds (Kantanen et al., 2000; Tapio et al., 2006; Iso-Touru et al., 2016), but in many areas, local breeds are mainly used for either low-input farming or maintenance of grasslands and open landscapes. Thus, the characterisation of milk composition from local indigenous breeds in a global perspective is lacking. However, given their unique genetic variation, these breeds may possess distinct milk composition with possibilities for exploitation into niche dairy products, in order to promote awareness, economic incitement and thus sustainable use of these genetic resources. Apart from local breeds, commercial modern dairy breeds such as Jersey, Ayrshire and Simmental also possess well-documented differences in milk composition utilised by dairies worldwide. This relates, for example, to higher protein and fat concentrations in milk from the overall lower-yielding Jersey cows (Poulsen et al., 2012), but differences in genetic casein variants that may be manifested into the milk quality are also documented for both modern and local breeds, as discussed in Section 3.

## 3 Milk proteins and genetic variants

Milk from cattle is characterised by the six major milk proteins, including the four caseins ($\alpha_{S1}$-CN, $\alpha_{S2}$-CN, β-CN, and κ-CN), and the two major whey proteins, α-LA and β-LG. At the DNA level, synonymous mutations are silent mutations, which do not change the protein sequence, whereas non-synonymous mutations lead to amino acid changes. Functional polymorphisms leading to amino acid changes in the peptide backbone, or even deletions, result in heterogeneity of the major milk proteins, summarising into $\alpha_{S1}$-CN (8), $\alpha_{S2}$-CN (4), β-CN (12), κ-CN (11), β-LG (11) and α-LA (3) variants (Farrell et al., 2004). Due to linkage disequilibrium (LD) at the casein cluster, the casein variants are often presented as haplotypes or composite genotypes instead of individual variants (Bonfatti et al., 2010; Vallas et al., 2012; Poulsen et al., 2013). Furthermore, variations in the non-coding regions, for example, in regulatory areas, may affect the expression of protein genetic variants, and thereby the level or proportion of specific proteins found in milk. Thereby, the genetic imprint of protein genetic variants into milk phenotypes includes both differences directly related to the

individual variants due to amino acid changes, and varying expression of the proteins characteristic for different protein variants.

Milk protein variants have been characterised in many breeds and population variant frequencies estimated, identifying a number of common and rare variants (Caroli et al., 2009). This results in high variant richness and genetic distinctiveness, for example, as observed for 70 breeds across Europe (Beja-Pereira et al., 2003). For $\alpha_{S1}$-CN, the most common and widespread variant is variant B; for $\alpha_{S2}$-CN it is variant A; whereas for β-CN both $A^1$, $A^2$ and B are widespread and common in most breeds; while for the κ-CN, variants A and B are most common (Caroli et al., 2009). For β-LG, variants A and B are common, whereas for α-LA, variant B is predominant in *Bos taurus* (Caroli et al., 2009). As exemplified across common and indigenous Danish and Swedish breeds, large variation exists in terms of both the number of variants present as well as their frequencies (Table 1). It should be noted, however, that given the low sample set of the indigenous breeds, these frequencies may not present true population averages but give a good indication.

The protein genetic variants are strongly associated with the compositional traits of milk and the relative protein profile is particularly affected (Heck et al., 2008; Gustavsson et al., 2014a). Higher κ-CN concentration is associated with κ-CN B (Hallén et al., 2008; Bonfatti et al., 2010), whereas lower β-LG concentration is associated with β-LG B (Hallén et al., 2008). This means that a higher casein number (casein/total protein) is associated both with β-LG B and k-CN B (Hallén et al., 2008; Bonfatti et al., 2010). Devold et al. (2000) found $\alpha_{S1}$-CN BB genotypes to have significantly larger micelles than BC genotypes and k-CN AB genotypes to have significantly smaller micelles than AA and AE genotypes, whereas no significant effect was found from β-LG variants. Glantz et al. (2011) confirmed that k-CN B was associated with smaller casein micelles. Apart from the specific effects on milk proteins, the genetic variants also affect milk yield, as well as fat and protein concentration and yield (Ikonen et al., 1999). In relation to protein composition, Buitenhuis et al. (2016) identified three major QTL regions common to the Holstein and Jersey breeds. These included BTA6 (for k-CN) covering the casein gene complex, BTA14 (for protein percent and CN percent) covering the *DGAT* gene, and BTA11 (for β-LG) covering the *PAEP* gene. This is in line with the findings of Schopen et al. (2011) identifying the same major regions of importance for bovine milk protein composition. The heritabilities of major milk proteins are generally moderate to high (Schopen et al., 2009; Bonfatti et al., 2014; Buitenhuis et al., 2016). The strong negative genetic correlation between β-LG and casein number (Schopen et al., 2009) suggests that selection for increasing casein content will decrease β-LG in milk.

In addition to the allelic genetic protein variants, a number of splice variants have also been identified in bovine milk, especially for $\alpha_S$-CNs (Martin et al., 2013). Documentation includes, among others, minor non-allelic isoforms of

**Table 1** Variant frequencies of $\alpha_{S1}$-CN, β-CN, κ-CN and β-LG in 455 Danish Holstein (DH) and 433 Danish Jersey (DJ), 12 Jutland, 28 Danish Red anno 1970 (RDM70), 367 Swedish Red (SR), 23 Swedish Mountain cattle (SM) and 8 Swedish Red Polled (SRP) cows. Results are summarised from Poulsen et al. (2013), Poulsen et al. (2016b), Poulsen et al. (2017a) and unpublished data

| Protein | Variant | DH ($n$ = 455) | DJ ($n$ = 433) | Jutland ($n$ = 12) | RDM70 ($n$ = 28) | SR ($n$ = 367) | SM ($n$ = 23) | SRP ($n$ = 8) |
|---|---|---|---|---|---|---|---|---|
| $\alpha_{S1}$-CN | B | 0.995 | 0.57 | 1.000 | 0.982 | 0.996 | 0.848 | 0.938 |
| | C | 0.005 | 0.43 | - | 0.018 | 0.004 | 0.152 | 0.062 |
| β-CN | $A^1$ | 0.254 | 0.081 | 0.500 | 0.286 | 0.474 | 0.305 | 0.250 |
| | $A^2$ | 0.621 | 0.629 | 0.292 | 0.482 | 0.526 | 0.565 | 0.750 |
| | $A^3$ | 0.004 | - | - | - | - | - | - |
| | B | 0.044 | 0.22 | - | - | - | 0.130 | - |
| | F | 0.008 | - | 0.208 | 0.232 | NA | - | - |
| | I | 0.069 | 0.07 | - | - | - | - | - |
| κ-CN | A | 0.697 | 0.203 | 0.833 | 0.643 | 0.696 | 0.304 | 0.625 |
| | B | 0.235 | 0.797 | 0.167 | 0.321 | 0.240 | 0.696 | 0.188 |
| | E | 0.068 | - | - | 0.036 | 0.064 | - | 0.188 |
| β-LG | A | 0.538 | 0.546 | 0.583 | 0.107 | NA | 0.283 | 0.188 |
| | B | 0.462 | 0.422 | 0.417 | 0.893 | NA | 0.717 | 0.813 |
| | C | - | 0.032 | - | - | NA | - | - |

- = not observed in the dataset.
NA, Not available.

$\alpha_{S1}$-CN, where specific exons or Gln have been skipped (Miranda et al., 2020). The effect of such splice variants on milk composition and functionality is, however, scarcely explored.

The different structural features embedded in the sequences of the protein genetic variants can give rise to differences in digestibility and, for example, the release of bioactive peptides. In relation to the gastro-intestinal digestion of genetic variants of β-CN, there has been a lot of controversy due to the potential health effects of the β-CN $A^1$ variant vs. $A^2$ (Truswell, 2005; He et al., 2017; Ramakrishnan et al., 2020). The difference between the $A^1$ and $A^2$ variants of β-CN is one amino acid exchange at position 67, where the $A^2$ variant holds a Proline (Pro), while the $A^1$ variant holds a Histidine (His) in the protein sequence. The hypothesis around the $A^1/A^2$ milk case is that the amino acid exchange from Pro to His enables enzymatic release of the peptide called BCM-7 (Y-P-F-P-G-P-I) upon digestion of $A^1$ protein but not of $A^2$. Based on the variation at position 67, the β-CN variants can be grouped into 'families', with, for example, $A^1$, B and F variants belonging to the $A^1$ family and the $A^2$, $A^3$ and I belonging to the $A^2$ family, a fact that is often overlooked in the debate and interpretation of the frequencies and the impact of β-CN variants on the population level.

The BCM-7 peptide is claimed to be linked with stomach discomfort in some persons (Jianqin et al., 2016), but it is still very much debated. The stomach discomfort discussed resembles the discomfort experienced by individuals with lactose intolerance, but recent studies may indicate that some of these individuals may benefit from drinking $A^2$ milk instead of milk containing $A^1$, even when the lactose has been hydrolysed (He et al., 2017). The release of the BCM-7 peptide by digestion of either purified β-CN or of β-CN in milk from homozygous cows has been studied in two recent studies (Asledottir et al., 2017, 2018). Using mass spectrometry for BCM-7 detection, it was found that BCM-7 was released from the $A^1$ family milk as expected, but also from the $A^2$ family milk, though at lower levels (around 4–5 times lower). It is therefore still a matter of consideration, whether this release of BCM-7 has an impact on gastro-intestinal discomfort in some persons, and whether there is a significant difference between the $A^1$ and $A^2$ milks. Future controlled studies are needed to settle this.

Petrat-Melin et al. (2015) studied the *in vitro* gastro-intestinal digestion and generated bioactivities via isolated β-CN B, $A^1$, $A^2$ and I from homozygous milk and found that, in particular, one ≈4 kDa peptide with the N-terminal sequence 106H-K-E-M-P-F-P-K- was absent from digest of β-CN variant B, but present in the digests of the other variants. This is likely a result of the 122Ser to Arg substitution in β-CN B, introducing a novel trypsin cleavage site, leading to the changed digestion pattern. Further, both antioxidant and angiotensin-converting enzyme (ACE) inhibitory capacities of the hydrolysates increased significantly for all variants, with the B variant reaching the highest ACE inhibitory

activity (Petrat-Melin et al., 2015). In another study, *in vitro* digests of the same purified β-CN genetic variants were studied and BCM-7 precursor peptides identified, representing V-Y-P-F-P-G-P-I-H-N from β-CN $A^1$/B, and V-Y-P-F-P-G-P-I-P-N from β-CN $A^2$/I (Petrat-Melin et al., 2017). The bioactivities of these peptides were assessed in an *in vitro* system of intestinal CaCo-2 cells, and it was found that the BCM-7 precursor peptide from β-CN $A^1$/ B exhibited strong ACE inhibitory activity, both before and after exposure to the Caco-2 cellular layer. In contrast, the equivalent peptide from β-CN $A^2$/I had lower initial ACE inhibitory activity but doubled its activity after exposure to the CaCo-2 intestinal cells after trimming off the C-terminal N residue, possibly due to the activity of brush-border enzyme carboxypeptidases into a BCM-9 peptide.

Petrat-Melin et al. (2016) further studied the digestion and bioactivities of isolated κ-CN A, B and E from homozygous milk. It was found that the variants displayed different *in vitro* gastro-intestinal digestion patterns and the antioxidant capacity of *in vitro*-digested κ-CN E was significantly lower than for κ-CN A or B. Lisson et al. (2013) also found that the genetic polymorphisms of both β-CN ($A^1$, $A^2$, B) and κ-CN (A, B, E) affected the *in vitro* digestion pattern of the different purified genetic variants and that this was further reflected in the IgE-binding epitopes of the digests. Combined, these results document that genetic polymorphisms affect the digestion of these proteins, but also that variants of β- and κ-CN could affect the allergenicity and bioactivity of the proteins.

## 4 Milk proteins and post-translational modifications

Apart from genetic variation, the heterogeneity of the milk proteins is high due to post-translational modifications (PTM), including disulphide bonds, phosphorylations and glycosylations (Fig. 2). Phosphate groups are esterified to the casein molecules during synthesis, via hydroxyl groups of mainly serine (Ser) residues, making P-Ser. Even though the structure of casein micelles in milk is not fully solved, there is a consensus that the P-Ser molecules of the caseins are anchor points for the micellar-bound calcium (colloidal calcium phosphate). The number of phosphorylations commonly found are in the ranges of: $\alpha_{S1}$-CN (8-9P), $\alpha_{S2}$-CN (10-13P), β-CN (5P) and κ-CN (1-2P). However, by the use of more sensitive mass spectrometry instruments, additional low-abundance isoforms have been identified (e.g. Miranda et al., 2020). In addition to phosphorylations, O-glycosylation of κ-CN results in glycan attachment at threonine (Thr) residues, resulting in attachment of 1–6 glycans at one or more of the specific sites: Thr121, Thr131, Thr133, Thr136 (only in A and E variants), Thr142, Thr145 and Thr165. In addition, there are two cysteine residues (Cys11 and Cys88) in κ-CN and two cysteines in $\alpha_{S2}$-CN (Cys36 and Cys40). These are engaged in disulphide bonds in native bovine

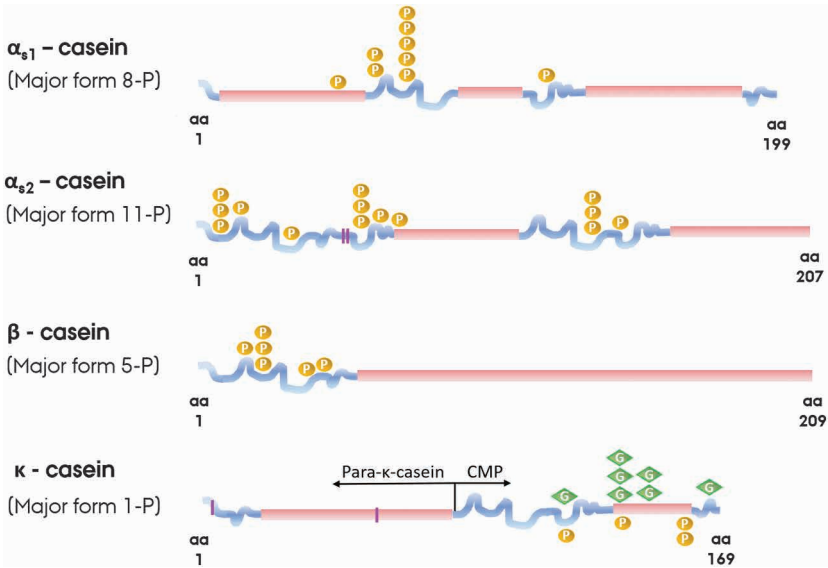


**Figure 2** Post-translational modifications of caseins based on the review by Le et al. (2020). Hydrophilic and hydrophobic regions of the mature protein are indicated. Blue stretches: hydrophilic regions, red stretches: hydrophobic regions. Purple lines indicate positions of Cys. All possible phosphorylation and glycosylation sites are highlighted. P, phosphorylation; G, glycosylation. Note that stacked phosphorylations and glycosylation are due to PTM sites in close proximity within the protein sequence, which appear to be at similar positions due to scale.

CN micelles, in the form of κ-CN homo-multimers with little or no content of free SH-groups, and in the case of $\alpha_{S2}$-CN, in the form of dimers which are connected in either parallel or antiparallel pattern of the S-S bonds (Rasmussen et al., 1992a,b).

The PTMs of the CNs affect CN micelle stability, and especially the highly glycosylated, hydrophilic part of κ-CN, the casein-macropeptide (CMP) part, ensures electrostatic and steric repulsion between micelles in bovine milk (Dziuba and Minkiewicz, 1996). Improved proteomic separation in later years, combined with quantitative determinations of the PTM isoforms, have shed light on their specific importance and the underlying genetic influence of PTMs on milk proteins. The two major phosphorylation isoforms of $\alpha_{S1}$-CN ($\alpha_{S1}$-CN 8P and 9P) were recently reported to be regulated by different sets of genes (Bijl et al., 2014b). It was shown that both isoforms were regulated by a specific region on BTA6. Furthermore, the $\alpha_{S1}$-CN 8P isoform was associated by a region harbouring *PAEP* on BTA11, while the $\alpha_{S1}$-CN 9P isoform was additionally influenced by a region harbouring *DGAT1* on BTA14 (Bijl et al., 2014b). In Buitenhuis et al. (2016), a significant region on BTA12 was identified to influence $\alpha_{S1}$-CN 8P percentage in Holstein, but regions on BTA6 and BTA11

were also identified; however, these were not significant. Interestingly, the phenotypic and genetic correlations between $\alpha_{S1}$-CN 8P and 9P isoforms were low (Bijl et al., 2014b), which supports somewhat of an independency between these isoforms. Heritability estimates for $\alpha_{S2}$-CN phosphorylation isoforms (10P-14P) were low to moderate, whereas heritabilities for $\alpha_{S1}$-CN 8P and 9P isoforms were high (Fang et al., 2017).

For glycosylation of κ-CN, BB k-CN genotypes have higher content (g/L) of both glycosylated (G) and unglycosylated (UG) κ-CN than AA genotypes (Bonfatti et al., 2014), and high heritabilities have been found for G κ-CN, for UG κ-CN and for the glycosylation degree (GD, measured as the ratio of G κ-CN to total κ-CN) (Bonfatti et al., 2014). For relative proportions of κ-CN, κ-CN BB had higher proportions of both UG κ-CN and G κ-CN relative to total protein, compared with κ-CN AA, whereas κ-CN AB showed intermediate results (Poulsen et al., 2016a). From a gel-based study, it was found that 95% of the κ-CN molecules in a pooled milk sample based on milk from individual cows were phosphorylated (1 or 2 P), while 36 % were glycosylated (identified with 1, 2, or 3 O-glycosylations) in Holstein. For Jersey, the numbers were 96% and 34 %, respectively (Jensen et al., 2012a). Using liquid chromatography electrospray ionisation mass spectrometry, quadrupole time of flight (LC-ESI/MS Q-TOF) on a small subset of samples with distinct κ-CN genotypes, the proportion of κ-CN relative to total protein was higher in milk from κ-CN BB cows, as compared with either κ-CN AA, AB, EE or AE genotypes in Holstein and with AB or AA genotypes in Jersey (Jensen et al., 2015). This further documents that a single cow produces a variety of κ-CN molecular isoforms, comprising variation in levels of both the number of phosphorylations and glycosylations, as well as the specific glycan structures of O-glycosylations, which relates to five different glycan structures (Saito and Itoh, 1992).

Several studies have documented that variation in the κ-CN content is associated with casein micelle size (Frederiksen et al., 2011; Day et al., 2015), and especially the glycosylated part seems to play a role (Bijl et al., 2014a). Thus, casein micelle size was strongly negatively correlated with G κ-CN, but not with the content of UG κ-CN or total κ-CN, in milk from Montbéliarde cows (Bijl et al., 2014a). In a study by Buitenhuis et al. (2016), there was a profound difference in the heritabilities between the Danish Holstein and the Danish Jersey (Holstein > Jersey,) for k-CN percentage-related traits. These heritability differences could potentially explain differences in the number of QTL detected and reported for G k-CN and UG k-CN between the breeds. A comparison of GWAS results for k-CN%, UG k-CN% and G k-CN% relative to total protein revealed a number of especially G k-CN percentage-specific QTL peaks, indicating that there is a potential to genetically differentiate between the proportions of G k-CN and UG k-CN relative to total protein in bovine milk. Among the genes assigned to the significant SNP markers for G k-CN, two genes were found to be related to

the PTMs of caseins, including casein kinase 1 gamma 3 (*CSNK1G3*) on BTA7 and protein kinase C theta (*PRKCQ*) on BTA13 , both potentially involved in the PTMs of caseins (Buitenhuis et al., 2016).

## 5 Milk coagulation and other functional properties

Milk coagulation is a major economic trait in cheese manufacturing and important for both the quality and yield of cheese (Wedholm et al., 2006), as well as for process control at the dairies. Therefore, the possibilities for genetic improvement of this trait have received substantial attention, especially after finding a relatively high prevalence of non-coagulating milk at the individual cow level in several breeds. Rennet-induced milk coagulation, which is the first step in cheese manufacturing, is a two-phase process initiated by the enzymatic cleavage of k-CN by the addition of chymosin (rennet), which hydrolyses k-CN at Phe105-Met106 into CMP and para-k-CN resulting in the loss of the electrostatic and steric hindrance between casein micelles promoting aggregation and curd formation. The rennet coagulation time (RCT) defines the onset of milk coagulation, followed by changes in firmness, as the protein network develops and elasticity increases, defined by curd-firming rate and curd firmness at specific time points. From a dairy perspective, a relatively short clot time and a high gel firmness are desired. Bittante et al. (2012) identified a number of direct and indirect genetic effects affecting milk coagulation properties. These included breeds, genetic variants (especially k-CN) of the major proteins and other genes. In general, comparison among studies can be hard due to the use of different analytical methodologies and trait definitions. However, as summarised by Bittante et al., (2012), favourable milk coagulation properties have consistently been reported in milk from, for example, Brown Swiss, Simmental, Montbeliarde and several Alpine breeds (De Marchi et al., 2007), together with Jersey (Poulsen et al., 2013), compared to Holstein–Friesian. In contrast, Finnish Ayrshire (Tyrisevä et al., 2004), Swedish Red (Gustavsson et al., 2014b) and local Scandinavian breeds (Poulsen et al., 2016b) generally possess impaired milk coagulation properties with longer RCT and lower curd firmness, as measured at individual cow level on fresh, unpasteurised and non-standardised milk.

Given that milk protein content and composition play a significant role in milk coagulation, the influence of genetic protein variants on overall cheese-making capacity and milk coagulation properties has strong support (see reviews by Jakob and Puhan, 1992; Caroli et al., 2009). The influence of additive genetic variation is evident from β-LG, $\alpha_{S1}$-CN, k-CN, β-CN variants or by casein haplotypes or composite genotypes in various breeds (Comin et al., 2008; Bonfatti et al., 2010; Poulsen et al., 2013). Especially, the positive association of k-CN B with milk coagulation properties is well-documented (Hallén et al., 2007;

Joudu et al., 2007; Glantz et al., 2011; Ikonen et al., 1999; Vallas et al., 2012), as well as the positive association of $\alpha_{S1}$-CN C and β-CN B and the negative association of β-CN A[2] (Hallén et al., 2007; Poulsen et al., 2013). For β-LG, Glantz et al. (2011) documented favorable effects of β-LG A on rennet-induced gel strength, gelation time and yield stress, whereas others have documented the positive effect of β-LG B on cheese yield (Wedholm et al., 2006) and casein number (Hallén et al., 2008). In indigenous Danish Red anno 1970 and Jutland breeds, high frequencies of the otherwise rare β-CN variant F (Table 1) were associated with impaired milk coagulation properties (Poulsen et al., 2016b).

The results from Bonfatti et al. (2014) suggest that variation in RCT is mainly attributed to G k-CN and not to UG k-CN. Jensen et al. (2015) documented that chymosin-induced hydrolysis of k-CN was lower for glycosylated isoforms compared to non-glycosylated isoforms, suggesting that the carbohydrate attachments to some extent hinder the access of chymosin. Higher proportions of total k-CN to total protein, G k-CN to total k-CN and $\alpha_{S1}$-CN 8P to total $\alpha_{S1}$-CN are all related to good milk coagulation, that is, milk with low RCT and high CFR (Jensen et al., 2012b; Poulsen et al., 2016a).

Gregersen et al. (2014) used a GWAS to identify genomic regions affecting RCT and CFR in Holstein. In total, 19 genomic regions on 10 different chromosomes affected RCT or CFR. Apart from the major regions already described, the study identified *CTSD* on BTA29, coding for cathepsin D, as well as potential novel candidate genes such as *CWC15*, *MAP2K5* and *LMAN1*. In Swedish Red, a GWAS for rennet-induced coagulation also showed a major QTL around the casein gene cluster on BTA6, as well as a region encompassing *GALNT1* on BTA24, coding for UDP-N-acetyl-α-D-galactosamine:polypeptide N-acetylgalactosaminyl-transferase 1 (Gregersen et al., 2015). This enzyme plays a role in the O-glycosylation of κ-CN. Furthermore, regions containing *CTSZ* on BTA13 and *CTSC* on BTA29 were identified, which support that cathepsins may play a role in milk coagulation, probably through proteolysis (Gregersen et al., 2015). Poulsen et al. (2017b) confirmed the association between haplotypes identified from the GWAS study by Gregersen et al. (2014) and rennet-induced milk coagulation. The identified haplotypes were located within a major QTL on BTA6, where three significant SNPs located in intron regions or downstream *CSN3* (encoding k-CN) were identified, suggesting the genetic variation to be related to the regulation of k-CN.

With the identification of non-coagulating milk among samples from different breeds, including several Scandinavian breeds, and also Holstein (Ikonen et al., 2004; Tyrisevä et al., 2004; Cecchinato et al., 2011; Gustavsson et al., 2014b), genomic evaluation of non-coagulation has been a target. While no universal definition exists for non-coagulation, it is generally defined as milk with no recordable firmness after 30–60 min, but variation in temperature, enzyme concentration and analytical methodologies may affect the

rheological evaluation, and thereby, definition. Furthermore, the evaluation and inclusion of non-coagulating milk samples in genetic parameter estimation and GWAS will affect the outcome, as discussed by Bittante et al. (2012), and should always be considered. Tyrisevä et al. (2008) used Finnish Ayrshire to identify marker genes for non-coagulation and identified two potential candidate genes, *LOC538897* (BTA 2), which encodes a nonspecific serine/threonine kinase, and *SIAT4B* (BTA18), which encodes a sialyltranferase involved in the last step of k-CN glycosylation, by catalysing the addition of sialic acid to galactose. This suggests that PTMs of caseins play a role in the ability of milk to coagulate. In Swedish Red cows, where the prevalence of non-coagulation was estimated to be as high as 18%, Duchemin et al. (2016) found a significant QTL region on BTA18, where *VPS35* was identified as the candidate gene of interest for non-coagulation. This gene relates to a mammary gene set and is in close proximity to the BTA18 region identified by Tyrisevä et al. (2008).

Milk coagulation properties are heritable, with moderate to high heritabilities estimated and reported (Ikonen et al., 2004; Cassandro et al., 2008; Vallas et al., 2010). In Jersey, the heritability for curd-firming rate (CFR) was found to be relatively low (0.15) compared with the high heritability in Holstein cows (0.75). In contrast, the heritability estimates were more similar for RCT, ranging from 0.28 in Holstein to 0.45 in Jersey (Poulsen et al., 2015a). The very high heritability for CFR in Holstein compared to Jersey and other studies may relate to study population, trait variability and underlying design. Generally, gel firmness and clot time are highly phenotypically and genetically correlated, and milk coagulation traits have further been documented to be genetically correlated to protein and casein contents, as well as to SCC, pH and acidity (Cassandro et al., 2008; Vallas et al., 2010; Gustavsson et al., 2014b; Poulsen et al., 2015a). In the Swedish Red, heritability for non-coagulation has been estimated to 0.28 (Duchemin et al., 2020), and is thought to be a multifactorial trait, but despite this, may be changed through selective breeding.

For acid-induced coagulation, genetic polymorphism especially of β-LG plays a role, which relates to casein number and β-LG concentration, where a higher β-LG concentration (associated with β-LG A) is preferable for fermented products (Allmere et al., 1998; Hallén et al., 2009). For acid-induced milk gels, Glantz et al. (2015) found QTLs containing *CSN3* (κ-CN) and *PAEP* (β-LG) as well as regions containing other potential candidate genes.

## 6 Fatty acids and minor milk components

The genetic influence on milk proteins is strong and the variations are less affected by, for example, feeding. For other milk traits, the influence of the genome may be smaller and less direct with many QTLs, each explaining a small proportion of the genetic variation. Important pathways/genes can,

however, still be identified. For fatty acids, increasing the proportion of unsaturated fatty acids may be the target for selective breeding, even though the discussion of beneficial over unfavourable fatty acids relative to human health is complex (German and Dillard, 2006). Furthermore, when it comes to functional properties, an increasing proportion of unsaturated fatty acids may not be desired, as it relates to softer fat/higher spreadability and risk of increased lipid oxidation. Palmitic acid (C16:0), which is the main fatty acid in milk, derived partly from feed and partly from the mammary *de novo* synthesis, has been suggested to be the main objective for genetic selection due to its suggested negative effect on human health (Givens, 2010).

Heritability estimates for individual fatty acids are generally found to be low to moderate, but with the *de novo* fatty acids, which are synthesised in the mammary gland, exhibiting higher estimates than feed-derived fatty acids (Schennink et al., 2007; Krag et al., 2013). In contrast, desaturase indices, which reflect a proxy for $\Delta^9$-desaturase activity in the mammary gland, generally exhibit high heritabilities (Krag et al., 2013). The heritabilities for specific fatty acids indicate that it may be possible to alter the composition of fatty acids in bovine milk through selective breeding. Current selection for fat percent will mainly increase C16:0 (Stoop et al., 2008). Generally the genetic correlations between C4:0 to C14:0 fatty acids are positive and high (Stoop et al., 2008). Due to the negative correlation between groups of saturated fatty acids and unsaturated fatty acids (Krag et al., 2013), it may be possible to reduce the concentration of the less healthy saturated fatty acids and increase the concentration of unsaturated fatty acids. For unsaturated fatty acids, the results from Krag et al. (2013) suggest that the largest improvements can be achieved through changes in feeding. However, the heritability results also suggest that some achievement can be reached through selective breeding.

As stated earlier, major effects on milk fatty acid composition relate to genomic regions harbouring especially *FASN*, *DGAT1* and *SCD1*, though other regions also play a significant role (Schennink et al., 2009). When comparing QTLs for C6:0, C8:0, C10:0, C12:0, C14:0, C16:0, C18:0, C14:1, C16:1 and CLA, there was little overlap between the QTL found in the Dutch Holstein (Bouwman et al., 2011) and those found in Danish Holstein (Buitenhuis et al., 2014). For the Danish Holstein, significant SNP markers were detected for C14:1, C16:1 and CLA on the same chromosomes as those detected by Bouwman et al. (2011).

Undoubtedly, bovine milk is one of the best sources of several vitamins and minerals in human nutrition, including riboflavin (vitamin B2), cobalamin (vitamin B12), phosphorus and calcium. The primary origin of riboflavin and other B vitamins is through microbial biosynthesis in the rumen (Schwab et al., 2006). Poulsen, et al. (2015b) found substantial interbreed differences in milk riboflavin content. Milk from Jersey cows contained significantly higher levels of riboflavin (1.93 mg/L milk) than milk from Holstein cows (1.40 mg/L milk)

and reported high heritabilities for riboflavin in both breeds, suggesting that the riboflavin content in milk is under significant genetic influence, and could be changed through selective breeding. Like riboflavin, vitamin B12 in milk is of microbial origin, and in Dutch Holstein Friesian cows vitamin B12 had an estimated heritability of 0.37, which also suggests that vitamin B12 content in milk can be improved by genetic selection (Rutten et al., 2013). A GWAS conducted on milk riboflavin content identified significant SNPs associated with *DGAT1* (Poulsen et al., 2015b). The most significant SNP for Holstein was located on BTA13 and assigned to *SLC52A3*, which is a riboflavin transporter gene. This gene could be a good candidate gene responsible for the genetic regulation of riboflavin content in milk (Poulsen et al., 2015a). For B12, none of the significant SNPs could be assigned to known candidate genes (Rutten et al., 2013).

The mineral fraction in milk constitutes approximately 7.1–7.4 g/L and comprises cations, including, among others, calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), and anions, including, for example, phosphorus (P) and chloride (Cl) (Lindmark-Månsson et al., 2003; Hermansen et al., 2005). Bovine milk minerals and trace elements are essential for both human nutrition and dairy product quality. Several minerals occur in both colloidal and soluble states, which are important for heat stability of milk and milk coagulation properties, mainly related to their role in the structure and stability of casein micelles and levels of ionic $Ca^{2+}$.

Previous results documented considerable variation in milk mineral content from Swedish and Danish herds, especially due to season, but also to breed (Lindmark-Månsson et al., 2003; Hermansen et al., 2005). Results by van Hulzen et al. (2009) and Buitenhuis et al. (2015) also showed considerable variation for mineral content in milk and moderate to high heritabilities for Ca, P, Mg, K and Zn in Dutch Holstein (van Hulzen et al., 2009), for Ca, Cu, P and Zn in both Danish Holstein and Danish Jersey and for Mg, K and Mn in Danish Jersey (Buitenhuis et al., 2015). In both studies, non-existent to low heritabilities were found for Se, suggesting that Se is mainly affected by management and feeding and dietary manipulation. GWAS performed on milk minerals identified several significant SNPs for minerals, including BTA6 and BTA14 (Buitenhuis et al., 2015).

Apart from lactose, which is the main sugar component in bovine milk, milk also contains free milk oligosaccharides (OS) in small quantities. In human milk, OS are bioactive molecules that act as prebiotics and provide numerous health benefits to developing infants by stimulating the growth of beneficial intestinal bacteria, preventing the binding of pathogens and contributing to brain development (Pacheco et al., 2015; Jacobi et al., 2016). Bovine milk contains several OS structures in common with human milk (Aldredge et al., 2013; Kirmiz et al., 2018), and OS from dairy streams may thus act as value-added ingredients for infant formula or nutraceutical applications (Barile et al., 2009; Mehra et al., 2014). Heritability estimates for 15 individual OS in Danish

Holstein and Danish Jersey were generally high, documenting a solid breeding potential for bovine milk oligosaccharides, which ultimately could facilitate large-scale extraction of bovine milk OS. By GWAS, genomic regions with a major impact on milk OS were identified including specific glycosyltransferases as candidate loci for the synthesis of milk OS (Poulsen et al., 2019).

Milk metabolites reflect metabolic processes of the cow and may thus be important as indicator traits for cow health or play a role due to association with other traits important for the technological properties of milk. Genetic parameter estimation of milk metabolites has revealed moderate to high heritabilites for a number of milk metabolites including choline, citrate, glycerophosphocholine and orotate (Buitenhuis et al., 2013). Furthermore, urea, lactose and citrate display significant heritabilities (Stoop et al., 2007; Miglior et al., 2007; Mucha and Strandberg, 2011) and genetic improvement of these traits may be of interest.

## 7 Mid-infrared spectroscopy as large-scale phenotyping for genetic parameter estimation

In order to implement specific milk compositional traits in the breeding programmes, large-scale phenotyping and indirect ways to predict milk compositional traits or functional properties are needed. Thus, low-cost, accurate and reliable routine phenotyping is required, and as mid-infrared spectroscopy (MIRS) is widely used in milk recordings, there has been a great interest for population-level phenotyping of detailed milk composition by MIRS, as reviewed by De Marchi et al. (2014). Especially, MIRS-based prediction of fatty acids and groups of fatty acids have been of interest (Soyeurt et al., 2006; Rutten et al., 2009; Eskildsen et al., 2014) despite the somewhat indirect measurement (Eskildsen et al., 2014). In the Danish dairy cattle population, MIRS is used for routine screening of individual fatty acids (C14:0, C16:0, C18:0 and C18:1) and groups of fatty acids, which opens for the possibility of genetic selection as drivers for milk fatty acid changes (Hein et al., 2018). Also prediction of other important traits, including protein profile and genetic variants (Bonfatti et al., 2011; Rutten et al., 2011; Eskildsen et al., 2016), minerals (Soyeurt et al., 2009) and specific metabolites (Zaalberg et al., 2020) has been evaluated with varying degrees of predictabilty. MIRS-based prediction of milk coagulation has also received a lot of attention, both to be used in the milk recordings with the aim of breeding against non- and poor milk coagulation, and at the dairies for process control (Dal Zotto et al., 2008; De Marchi et al., 2009; Cecchinato et al., 2009), but to our knowledge, this has not been implemented. The majority of the MIR wavenumbers have moderate to high heritabilities (Wang et al., 2016), and Wang and Bovenhuis (2018) identified genomic regions associated with individual milk MIR wavenumbers. Apart from identifying known regions

affecting the fat, protein and lactose percent, new regions possibly associated with milk phosphorus, citrate and orotic acid were also identified.

## 8 Possibilities for genetic improvement in relation to dairy milk

As outlined, the genetic influence on milk compositional traits is large, and for most traits, there is a good possibility for improvement through selective breeding. Different breeds differ phenotypically in their milk compositions, as well as in their genetic make-up, as these breeds have been separated for many generations (Kantanen et al., 2000), and have undergone strong selective breeding. This may also be reflected in the difference in the LD structure, where, for example, the Danish Jersey has higher bin-wise LD across the genome than the Danish Holstein (Buitenhuis et al., 2016). Across studies and across different breeds, differences in results may reflect the underlying design, the actual study population, or the analytical methods applied. However, for the more closely examined traits like milk proteins and fatty acids, strong and consistent evidence of the genetic influence is clear, both within and among breeds, and GWAS has confirmed major QTL regions and identified novel candidate genes that contribute to the variation of a high number of milk compositional traits.

From a dairy perspective, the desired improvement of different traits may relate to increased quality and output for specific valuable products and/or ingredients and the best use of natural genetic variation (Fig. 3). Such breeding programmes can go hand in hand with feeding and management to secure high-quality milk that meets future challenges in terms of sustainable production and consumer's needs.

Consumer milk holds a potential for differentiation with regard to, for example, production system or management or special local breeds. Differentiation of drinking milk based on genetic $A^1$ and $A^2$ variants of β-CN is significant in some parts of the world. Despite being still controversial, this relates to potential claimed health-related differences between $A^1$ and $A^2$ genetic variants, as have been discussed (see e.g. Truswell, 2005; Jianqin et al., 2016; He et al., 2017). For UHT milk, quality and stability are mainly related to enzymatic load and probably less to the raw milk protein composition as such. However, milk calcium, and especially ionic $Ca^{2+}$ influences stability of long-shelf-life UHT milk negatively (Lewis, 2011; Lewis et al., 2011). Overall, the total calcium level closely follows the total protein (Bijl et al., 2013), as a tight linkage between total protein to casein and further on to micellar calcium exists. The role of calcium in the casein micelle structure and in the milk coagulation process is fundamental (Holt, 1992). Impaired clotting properties have, among others, been linked to a lower content of the main cations in milk, calcium, and magnesium, along with the main anion, inorganic phosphate (van Hooydonk

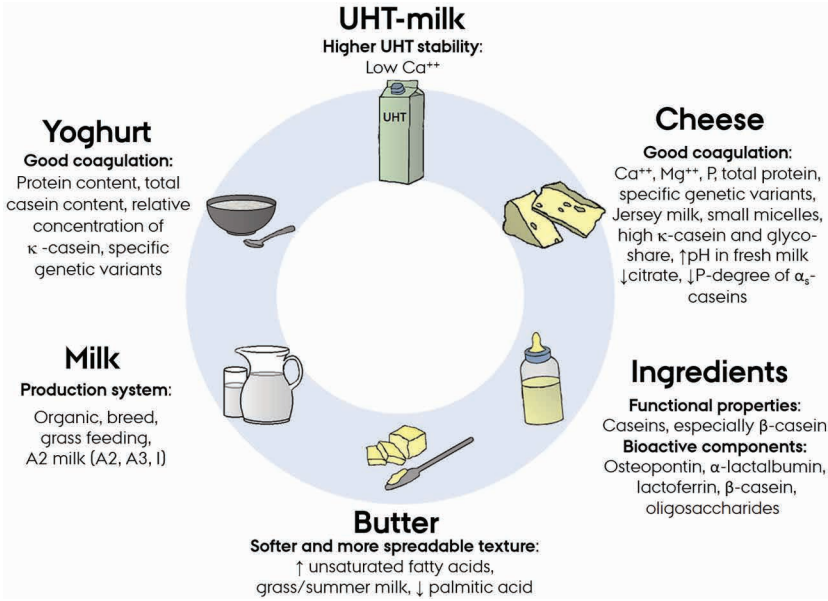


**Figure 3** Potentials of utilising variances in raw milk composition for specific dairy products, as based on the traits covered in the present chapter.

et al., 1986; Tsioulpas et al., 2007; Frederiksen et al., 2011), and a higher citrate content (Sundekilde et al., 2011). The minerals in milk exist in dynamic equilibrium between a soluble serum phase and a colloidal micellar phase. This mineral distribution is influenced by both cooling, lower milk pH and organic acids like citrate, which all lead to the dissolution of calcium phosphate from the micellar phase and may influence the integrity of the casein micelles and thereby potentially negatively influence the coagulation properties (Gaucheron, 2005). In milk, a strong positive correlation between nonsedimentable calcium and citrate has been documented (Bijl et al., 2013). However, whether natural variation in citrate, which is expected to be related to the *de novo* synthesis of fatty acids, is enough to affect milk quality during processing remains unclear. Overall, it seems that a certain level of calcium is needed to saturate and stabilise the casein micelles, and make them optimal for the cheese-making process, while on the other hand, a certain level of ionic calcium is needed in order to diminish the repulsion between the casein micelles in the second phase of the rennet induced milk coagulation. However, the mechanisms governing the calcium distribution in raw milk are less understood.

Changes in the proportions of specific proteins and of their specific PTMs may also be a strategy to improve milk technological properties, as a higher fraction of the least phosphorylated forms of both $\alpha_{S1}$- CN and of $\alpha_{S2}$- CN, and a higher fraction of glycosylated κ-CN are all positively associated with good

rennet coagulation properties (Frederiksen et al., 2011; Jensen et al., 2012b). The implications of these well-documented variations in the coagulation properties of raw bovine milk point towards possibilities for improvement of raw milk composition for cheese production. However, the extent to which these variances in the coagulation properties of the raw milk are manifested onto properties of cheese milk after mixing and cooling in silo, heat-treatment and the addition of starter cultures, remains to be established. This will have to be the focus of future studies. Furthermore, non-coagulating milk is clearly important to monitor, but also a trait that is still not fully understood, despite the identification of a number of risk factors and significant trait heritability. Hallén et al. (2010) documented that the addition of $CaCl_2$ within the approved range for dairy milk could somewhat restore milk and eliminate the variation between poorly/non-coagulating and well-coagulating milk.

As with rennet-induced coagulation, several traits related to milk composition (e.g. major milk proteins, fat and lactose content) have all been linked to properties resulting from gels formed by acid-induced coagulation (Lucey and Singh, 1998; Hallén et al., 2009). Gustavsson et al. (2014b) found a positive phenotypic correlation between acid-induced coagulation and protein content (including both total CN as well as relative κ-CN concentration), whereas negative correlations were found for gel strength or yield stress with milk yield, in milk from Swedish Red cows. However, a general pattern was observed, where the observed genetic correlations were stronger than the phenotypic correlations, and findings have suggested only a weak genetic association between rennet- and acid-induced coagulation properties (Gustavsson et al., 2014b). Using this knowledge on the genetic parameters associated with poor acid-induced coagulation characteristics might ultimately benefit the production of yoghurt and fermented dairy products.

The rapidly growing milk ingredient industry exploits the vast nutritional and biological potential of milk. The main drivers are globalisation, demand for high-quality nutrients and stagnation at the European market for traditional yellow cheese. Thus, it is mandatory to explore the possibility of tailoring the content of high-value proteins in bovine milk to ensure the most optimal value proposition of milk at the global ingredient market. High-value proteins, phospholipids, and OS exert nutritional and health-promoting effects for consumers. Such value has traditionally been exploited through more traditional dairy products, but a growing market for specialised milk additives and ingredients with special functionalities (physiologically and technologically) adds great value to raw milk. An example of this is α-LA, one of the main proteins in human milk, which is added to infant formula in order to balance the composition from bovine towards that of human milk. Other functional important proteins include one of the major milk proteins, β-CN, as well as the less abundant but bioactive proteins, like osteopontin and lactoferrin. For β-CN and α-LA, large biological

variations in their contents in individual cow's milk were revealed, showing potentials for exploiting this to elevate the levels of these valuable components through natural means (Le et al., 2020). Production of β-CN $A^2$ infant formulae has also gained interest, which may lead to an increase of β-CN $A^2$ cows/farms. For many breeds, β-CN $A^2$ is the predominant genetic variant. With regard to OS, milk from Danish Jersey cows contained higher relative amounts of both sialylated and the more complex neutral fucosylated OS, whereas milk from Danish Holstein had a higher abundance of smaller and simpler neutral OS (Sundekilde et al., 2012, Robinson et al., 2018a,b). The results point at the possibility of exploiting Jersey milk for the enrichment of products with fucosylated oligosaccharides, which bear more resemblance to those in human milk.

As discussed, the composition of milk fat from dairy cows is governed by both genetic and environmental factors. From a nutritional point of view, it is desirable to increase the level of unsaturated fatty acids in milk, which depends on the interplay between the feed composition, rumen metabolism and mammary synthesis. The nutritional value of milk is related to essential fatty acids (linoleic and α-linolenic acid), as well as specific fatty acids showing beneficial health effects, such as conjugated linoleic acid (CLA) and omega-3 fatty acids (German and Dillard, 2006). An increase in healthy fatty acids in human food sources and, likewise, a decrease in fatty acids with a negative impact on human health (in particular palmitic acid) are thus desirable (Mensink et al., 2003; Givens, 2010).

Generally, for butter production and quality, the more unsaturated fat that is present, the softer and more spreadable the butter appears. Apart from the fatty acid profile and the share of unsaturated to saturated fatty acids, the fat globule size and crystallisation are also important for the texture of butter products. It should also be noted that a higher level of unsaturated fatty acids generally can also lead to increased oxidation and potential development of off-flavour. As vitamin E can have antioxidant activities, variances in vitamin E can potentially counteract this effect to some degree, by combining high vitamin E levels with high-unsaturated fatty acid milk for butter production. Elevated vitamin E has been shown to be correlated with feeding of grass products (Poulsen et al., 2012).

As also outlined by Miglior et al. (2017), it is of importance that there is 1) an economic benefit for the farmer, 2) an economic interest for the dairy industry and 3) a reliable method to easily measure the milk phenotypes at a large scale in order to implement heritable milk compositional traits, either in the national breeding goal or for milk differentiation at specific farms. Furthermore, it is preferable that large-scale phenotyping can be implemented in the current infrastructure of national milk recording systems, by, for example, MIRS or similar easy and reliable fast methods. This will ensure that breeding

values genetic correlations to current breeding goal traits can be established, increasing the potential economic benefit for both farmer and dairy.

## 9 Where to look for further information

Reports from the Food and Agriculture Organization of the United Nations (FAO) are important for global assessment of the current livestock genetic resources and state. A good overview of especially milk proteins and associated genetic variants and their effect on milk composition and quality can be found in McSweeney, P. L. H. and Fox, P. F. (Eds.) Advanced Dairy Chemistry: Volume 1A: Proteins: Basic Aspects, (4th ed., 2003). Springer Science and Boland, M. and Singh, H. (Eds) Milk Proteins - From Expression to Food (3rd ed., 2020). Elsevier. Important conferences include World Congress on Genetics Applied to Livestock Production (WCGALP), which is held every fourth year as well as dairy conferences, for example, from the American Dairy Science Association. Further, the International Milk Genomics Consortium (www.milkgenomics.org) and their annual symposium also cover lactation biology, comparative genetics and the effect of genetic parameters on milk quality.

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