# Omics and Systems Biology: Integration of Production and Omics Data in Systems Biology

**Kasper Hettinga and Lina Zhang**

**Abstract** Omics technologies have become of mainstream use in the study of farm animals, to better understand the physiology of the animal and the quality of the products produced by those animals. Such studies can be done at the level of genes, transcripts, proteins and/or metabolites. An important aspect of doing such omics studies is understanding of variation. For example, in relation to parity, lactation, feeding status and animal health, variation can happen in transcripts, proteins or metabolites found in farm animals and the products produced. This variation can help in better understanding the physiology of the animal. Also variation between individual animals exists, which may assist in better understanding of the animal's physiology. One limitation of the majority of the studies in this area is that they are performed using one specific omics technology. Integrating omics data captured using multiple omics technologies, using a systems biology approach, can shed more light on the biochemistry of the farm animal's physiology. At the end of this chapter, the outlook on such studies and the (software) developments that would be needed for optimal integration of omics data is discussed.

**Keywords** Genomics • Transcriptomics • Proteomics • Metabolomics • Interactomics • Systems biology • Computation biology • Farm animal • Milk • Biochemistry

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## Abbreviations

| | |
|---|---|
| CN | Casein |
| DGAT1 | Diglyceride acyltransferase 1 |
| DNA | Deoxyribonucleic acid |
| GWAS | Genome-wide association study |
| IgA | Immunoglobulin A |
| miRNA | MicroRNA |
| mRNA | Messenger RNA |
| RNA | Ribonucleic acid |
| RNA-seq | RNA sequencing |
| SNP | Single nucleotide polymorphisms |

## 1 Introduction to Omics Technologies

In the past decades, many omics technologies have been developed for studying biology on different levels, from genes to metabolites. Figure 1 gives an overview of the different levels of biology with the associated omics technologies available. On the metabolite level, different omics technologies are applied, depending on the target metabolites of interest, with the term metabolomics mainly used for small molecules that are part of the core metabolism of organisms.

Due to continuous technological improvements, more comprehensive technology has been, and is, developed on all these levels. These improvements are leading to a higher resolution of analysis, a higher throughput and all that at a lower cost of analysis. These techniques have become more widely used by scientists around the world in many disciplines, including scientists in animal and food science. In this chapter, the application of the different omics technologies will be explained, to indicate how these can be used to better understand the physiology of farm animals. Most of the omics research focus on better understanding the production characteristics of farm animals (e.g. how much product is produced, what is the quality of the product produced) or animal physiology (e.g. understanding the mammary

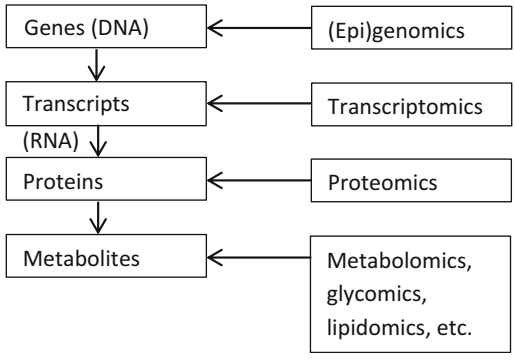


**Fig. 1** Overview of biology from gene to metabolite, with the associated omics technologies that can be used for studying the different levels

gland physiology, health of the animal). In this first section, the use of different omics technologies is briefly explained.

However, as already indicated in Fig. 1, biology does not happen on a single level but is an interaction between different levels, from genes, through transcripts and proteins, to metabolites. To perform integrative research on all these levels, combining multiple omics technologies aids in obtaining a better insight in the biology compared to studying the biology on a single level. Such combined approaches can range from studies combining two levels of biology, all the way up to full integration over all these levels. This integrative approach is the basis of systems biology research. In this chapter, the application of omics technologies will be described starting from single omics technologies up till full integration from a system's biology perspective. The chapter will finish with challenges and future developments that are envisaged for further research in this area.

## *1.1 Genomics*

The effect of genes of farm animals on production characteristics and animal physiology has mostly been studied by linking these outcome parameters to variation in gene sequences. Most research on farm animals is done by looking either at a large number of single nucleotide polymorphisms (SNPs, single mutations at specific positions in the genome) or by sequencing target genes that are expected to be involved in the outcome of interest. More recently, whole genome sequencing has become more popular, partly due to a reduction in the cost of performing such analyses. This allows using information about the whole genome sequence, making the translation of findings to the underlying mechanism easier.

When determining many SNPs in the animal's genome, association studies can be done to link these SNPs to specific traits (e.g. milk yield). This is usually conducted in the form of a genome-wide association study (GWAS). Many of such studies have been performed on a large scale in farm animals (Schennink et al. 2009).

One disadvantage of studies based on GWAS is that it is relatively difficult to determine what the causative gene/causative mutation is underlying the found relation. A GWAS will only indicate genomic regions associated with the trait, in which many genes may still be present. Based on screening the genes in the genomic region, and trying to find genes that may be causally associated with the trait, a hypothesis on the role of a specific gene could be made. Once these potentially causative genes are found based on a GWAS, the genes can be sequenced to search for specific mutations (Duchemin et al. 2014; Schennink et al. 2009). This is where whole genome sequencing, as mentioned above, has an advantage, as the full genomic information is collected, so mutations in any gene can be searched for.

A more recent development in the field of genomics is looking at chemical modifications of DNA instead of variations in the sequence, which is called epigenomics. Chemical modifications, such as methylation, of the DNA strand

may alter the propensity of the gene to transcription (Singh et al. 2012). Epigenomics thereby provide a link between the genomics studies, as mentioned above, to transcriptomics as will be discussed in the next section.

## *1.2 Transcriptomics*

Whereas genomics studies the genetic information as such, transcriptomics studies the transcription of genes into mRNA. Before proteins/enzymes are formed, transcription of the genes is the first step towards expressing the activity originating from the genes. In transcriptomics studies, the mRNA molecules present in cells are isolated and characterized. In the past, this was usually done using hybridization-based microarrays. The main disadvantage of such a microarray approach is that it is required that the target sequences are known before the experiment is done. To solve this, RNA-seq has been developed, in which nontargeted RNA sequencing is performed (Kukurba and Montgomery 2015). For studying farm animals, both approaches (microarrays and RNA sequencing) are currently being used.

Transcriptomics is extensively used in farm animal studies. Studies have, for example, focused on differences in the transcriptome during illness (Moran et al. 2016; Younis et al. 2016) or when performing experimental, for example, pharmaceutical, interventions (McCoard et al. 2016). Another area where transcriptomics is frequently used is to compare animals that differ in production characteristics (Bai et al. 2016; Shen et al. 2016; Wall et al. 2013). In all these studies, the objective is usually to determine how animals that differ in specific output parameters differ in the transcription of their genes. This can then be translated back to the known function of genes, to be able to study the underlying mechanism. The data from such experiments can be used to determine whether specific pathways are up- or downregulated in response to the studied contrast in output parameter.

Besides mRNA-based transcriptomics, other types of RNA transcripts may also be studied. One example is microRNA, which are short pieces of non-coding RNA that can influence transcriptional activity, often being involved in reduced transcription. The transcriptional regulation of a specific miRNA can be targeted at many genes simultaneously, thus giving a broad range of possible functions to individual miRNA molecules. Levels of miRNA have been related to animal physiology (Salilew-Wondim et al. 2016; Ioannidis and Donadeu 2016). These miRNAs may not only have local effect in the cell of synthesis but have also been shown to be transported through serum, with exosomes being plausible vectors in which miRNAs are transported (Zhao et al. 2016). Because of the potential broad range of effects on transcription of many genes, as well as their ability to be transported across cells in the organism, changes in miRNA levels can have many consequences, both in local and systemic physiology. These miRNAs can have influences within an individual but can also be transported across individuals, for example, through milk. For milk, transport of miRNA through exosomes has been shown to occur. Such milk-borne miRNAs may function as transcriptional regulators in the newborn (Perge et al. 2016). In addition miRNAs may also play a

role across species; the presence of these components in farm animal (e.g. dairy) products may also be relevant for product quality, as these food-derived miRNAs may have specific transcriptional consequences in the consumer (Benmoussa et al. 2016; Kirchner et al. 2016).

### *1.3 Proteomics*

The next step after transcription of genes into mRNA is the translation of the resulting mRNA into proteins. As mentioned above, this process can be influenced by factors such as miRNA. In this step, highly abundant (food) proteins may be produced, as well as low-abundant proteins which have a wide range of functions across the whole physiology of the farm animal.

Of the highly abundant food productions, much research has been done on the major milk proteins, such as caseins and the major whey proteins (α-lactalbumin and β-lactoglobulin). The genes associated with these proteins have been studied in detail in several dairy animals, showing large genetic variation. The variations have been linked to differences in milk and protein composition (Heck et al. 2009; Buitenhuis et al. 2016). This variation not only exists on the level of protein abundance but also exists on the level of post-translational modifications (phosphorylation and glycosylation). This information is actively used, both directly through genomic selection as well as indirectly through traditional breeding programmes (Hayes et al. 2009) to improve the quality of the farm animal products produced. Besides using it for improving quality through animal breeding, proteomics can also be used to study product quality in more detail. An example of this is meat, in which proteomics has been used for monitoring meat quality throughout the whole production chain (Paredi et al. 2013). It has also been used for cheese, in which proteomics was used to monitor the progress of ripening by looking at the degradation of the major milk proteins (Hinz et al. 2012).

For the low-abundant proteins in farm animals, and their products, these have been studied in relation to many physiological disturbances in such animals (Bendixen et al. 2011; Almeida et al. 2015). Many diseases may occur in farm animals, although most interest has traditionally gone to production-related diseases (Nir Markusfeld 2003), because these occur most frequently and usually have large economic consequences for the farm animal sector. Proteomics has many applications in this area. First, it is often used in relation to studying the physiological effects of problems with animal health. In dairy animals, it has often been used to study the physiological response to mastitis, which also may include the response to specific pathogens, as reviewed by (Boehmer 2011). These researches have shown the up- and downregulation of many proteins, indicating a decrease in milk protein synthesis with a concomitant increase in proteins that are known to support the host defence system.

But proteomics is not limited to the traditional farm animals, it has also been applied in, for example, aquaculture (Rodrigues et al. 2012). As with the traditional farm animals, in aquaculture the focus is on both product quality and animal health.

In aquaculture, diseases can have a large impact on the amount of product that is produced (in other words, the growth rate of the fish). In addition, studying the response of fish to pathogens (as just before described for the case of mastitis in dairy animals) can help unravel the physiology of diseases and thereby lead to a better understanding of the host defence (Zhou et al. 2011). Another topic that has been studied in fish, as with farm animals living on land, is the response to stress, being one of the main determinants of animal welfare but also having an impact on the quality of the product produced (Morzel et al. 2006).

## *1.4 Metabolomics*

As described above, the components of the proteome (protein/enzymes) are involved in the animal's metabolism. Besides studying such enzymes directly using proteomics technology, research can also focus on the metabolites that are produced by the enzymes of interest. Although the metabolites are several steps away from the genes of the cow, research has shown that there are many correlations that can be detected across all levels, from genes to metabolites (Wittenburg et al. 2013).

Many different categories of metabolites exist that can be studied, including lipids, water-soluble metabolites and volatile metabolites. These different categories of metabolites all require their own analytical approach for detection (Wang et al. 2010).

The research areas, in which metabolomics is applied, are for a large part similar to those of transcriptomics and proteomics. The underlying research themes are thus often the same but aiming at components on a different level of the animal's physiology. An example of a specific area of research in metabolomics that is less studied than the other levels is the rumen. Many farm animals are ruminants, and in their rumen a wide range of microorganisms are present to breakdown plant material eaten by the animals. These microorganisms produce a whole range of metabolites that can be detected with metabolomics technologies (Zhao et al. 2014). Although these metabolites are thus not produced by the ruminant itself, they can end up in the body of the animal and thereby in animal food products such as meat and milk. Some of these metabolites in the animal (or its products) can thus be used as a reflection of the metabolic state of the rumen (Antunes-Fernandes et al. 2016). Another area of research in which metabolomics (but also transcriptomics and proteomics) has frequently been used is bovine mastitis. The aim is again to understand the physiology but now on the level of metabolites present (Sundekilde et al. 2013). In poultry research, metabolomics is also used for improving physiological understanding. An example of such research is the production disease ascites syndrome, for which the metabolomic response by chicken has been described (Shen et al. 2014). Although the above examples focus on physiological understanding, the differences in metabolites cannot only be used to explain such metabolic perturbations but also be frequently used for biomarker research. Detection of individual metabolites as biomarker of diseases is often used, and

metabolomics research can contribute to finding such biomarkers in complex samples from farm animal (products).

One specific category of metabolites that is often studied in all different farm animals is the class of lipids. This class includes different categories of lipids and lipid-soluble components [fatty acids, triglycerides, phospholipids, sterols (Sokol et al. 2015)]. Lipids in farm animal products such as milk is an obvious area in which lipid metabolites are studied (Lu et al. 2013, 2015; Sokol et al. 2015; Li et al. 2017), but also blood lipids have been studied in relation to health (Li et al. 2017; Gerspach et al. 2017). In both cases, the lipids are relevant as part of product quality as well as the physiology of the farm animal.

## 2 How Omics Technologies Can Help in Better Understanding Production Characteristics and Animal Physiology

In the first section of this chapter, many omics technologies are mentioned that have been applied in the study of farm animals. This second section focuses more in detail on how the omics technologies can be applied to better understand the farm animal. This will be done based on three different research directions. First, the combination of variation in the omics data between individual farm animals studied can be used to better understand the animal as such. Capturing the variation requires quantitative omics technologies. Especially in products of farm animals, like milk, there are many examples of capturing variation to better understand the animals producing the product. A second option to better understand the animal and its physiology is the comparison between species. Different farm animal species producing similar products also have similar underlying physiology. Omics technologies applied to study differences among species can help understand these animals better. Finally, omics technologies are often applied to better understand the functioning of specific organs. In this section, especially the morphology of the mammary is used as an example of such research approaches.

### *2.1 Importance of Capturing Variation*

As mentioned above in the introduction of this section, capturing variation is a tool to better understand farm animals and their products. A clear example of such an approach is the study of milk. Milk is a complete and complex food suited to the requirements for the growth and development of the neonate. Milk and dairy products are also central elements in the human diet. The principal function of milk is providing energy and nutrients. For understanding of how farm animal are able to produce these necessary milk components, all levels of physiology can be studied using omics technologies, focusing on capturing variation at all these levels.

Milk yield and milk composition, including milk lactose, protein and fat concentration, for example, have been shown to have large variation with changes in environmental temperature (Alstrup et al. 2016). All casein fractions, except for γ-casein, were present at lower concentration in summer than in winter, whereas immunoglobulins, serum albumin were present at higher concentrations in summer than winter. A consequent worsening of milk coagulation properties was observed in summer season, which may influence cheese production from such milk. In addition, a mild effect of season was observed for milk somatic cell count, with higher values in summer than in the winter and spring (Bernabucci et al. 2015). This data suggests that the risk of mastitis is higher in summer than in other seasons. Linking the variation in milk composition to the genes of the dairy cow, it was shown that the centromeric region of bovine chromosome 14 was strongly associated with test day fat percentage. Several SNPs were associated with eicosapentaenoic acid, docosapentaenoic acid, arachidonic acid, rumenic acid and linolenic acid (Ibeagha-Awemu et al. 2016b). This study also reported some novel potential candidate genes, such as ERCC6, TONSL, NPAS2, ACER3, ITGB4, GGT6, ACOX3, MECR, ADAM12, ACHE, LRRC14, FUK, NPRL3, EVL, SLCO3A1, PSMA4, FTO, ADCK5, PP1R16A and TEP1, which may be involved in complex dairy traits, including milk traits and mammary gland functions (Ibeagha-Awemu et al. 2016b). Another example is that DGAT1 gene mutation is related to milk with changes in saturated, unsaturated and omega-3 fatty acid concentrations. Moreover, milk fat composition also differs between seasons. Summer bovine milk contains higher amounts of unsaturated fatty acids and lower amounts of saturated fatty acids compared with winter bovine milk (Duchemin et al. 2013). In addition, milk fat composition changed with the increase of lactation, for example, C18:1 fatty acid in bovine milk (Samková et al. 2012). This variation in milk fatty acid composition is mainly linked to feed composition. However, part of this variation is also linked to gene x environment interaction (Duchemin et al. 2013). These studies together show that genomics studies are important to better understand the variation in milk metabolites. By this improved understanding, a larger part of the captured variation can be explained.

Proteins in farm animal products not only provide nutrition through the presence of essential amino acids, but many proteins in the farm animal and its products are also involved in the development of the immune system. The investigation of the changes of the proteome provides information on the frequency, onset and progression of different markers (e.g. proteins) due to exogenous (e.g. season, disease) and endogenous (age, lactation) factors, as mentioned in the first section of this chapter. To achieve these objectives, the variation in these proteins needs to be determined using quantitative proteomics technologies. Information from such studies may help farmers to better manage their animals, improving milk yield and providing high-quality milk and milk products for human consumption.

The composition of farm animal (products) shows large variations depending on animal age and for dairy animals especially also over lactation stages. Looking specifically at milk over lactation, the milk host defence-related proteins, such as immunoglobulins, decreased remarkably from colostrum to mature milk in both human and bovine milk (Zhang et al. 2015a, b, 2016b). In addition to these

well-known proteins, many other low-abundant immune-related proteins also decreased from early lactation stage to middle lactation stage, such as complement proteins, lactoferrin, osteopontin, glycosylation-dependent cell adhesion molecule 1, alpha-1-acid glycoprotein 1 and protease inhibitors (Zhang et al. 2013, 2015b, 2016b; Korhonen 2009). On the other hand, lipid transport proteins, including apolipoprotein A-I, A-IV and C-III, were shown to increase from early to middle lactation (Korhonen 2009). In late lactation, proteins related to milk fat synthesis (e.g. adipophilin, fatty acid-binding protein, butyrophilin) and proteins related to lactose synthesis (e.g. α-lactalbumin and β-1,4-galactosyltransferase) were shown to decline (Zhang et al. 2015b, 2016b; Lu et al. 2014), whereas the immune-related proteins increased at this late lactation stage, suggesting the decrease in milk synthesis and a concomitant increase in the protection by immune-related proteins of the mammary gland during involution (Boggs et al. 2015, 2016).

The milk proteins not only show variation over lactation but also show variation depending on breed and genotype (Lu et al. 2015), with also unexplained variation between individual animals (Zhang et al. 2015a, b). Milk from Danish Holstein cows was mainly characterized by higher relative contents of β-casein, α-lactalbumin and β-lactoglobulin and a higher fraction of glycosylated κ-casein, whereas milk from Danish Jersey cows was characterized by higher relative contents of κ-casein, $\alpha_{S2}$-casein and the less phosphorylated forms of $\alpha_{S1}$-casein and $\alpha_{S2}$-casein (Poulsen et al. 2016).

Some of the genetic variability that is known to impact milk composition has been studied from a proteomics perspective. Genotypic variation in the DGAT1 gene (K232A polymorphism) was shown to induce changes in expression of the lipid synthesis-related protein stomatin (Lu et al. 2015). Moreover, genotype variation resulted in the differences in post-translational modifications of milk proteins, which could be related to milk coagulation properties. Poulsen et al. (2016) found that milk from cows with κ-CN BB genotype had relative higher contents of both unglycosylated κ-CN and glycosylated κ-CN compared with that of κ-CN AA (Poulsen et al. 2016).

With respect to the variation between individual animals, this has been studied, for example, in the milk protein of dairy cows. To study this individual variation, we collected proteomics data according to Zhang et al. (2015a) of 17 individual healthy cows in mid-lactation. This unpublished data shows that there was a relative high overlap (80%) in the qualitative milk proteome; however, at the quantitative level, there was a large variation in relative protein concentrations among individual cows (Fig. 2). The variation of relative protein concentration between individual cows in mid-lactation was discussed by Zhang (2015). This quantitative variation in the milk proteins between individual animals is probably due to a multiple factors. Parity/age of cows may result in changes of milk serum proteins. For example, in bovine milk, β-lactoglobulin and immunoglobulins were positively correlated with cow's age, and bovine serum albumin increased from the first to fourth parity followed by a decline as cows became older (Ng-Kwai-Hang et al. 1987).

The variation in the concentration of milk metabolites has also been reported. A metabolomic study in the milk between heat-stress-free and heat-stressed dairy

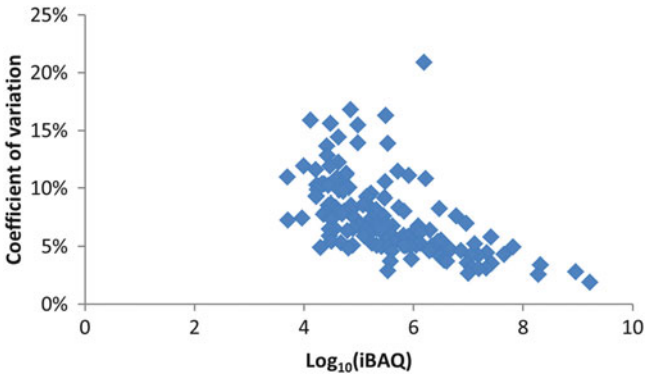


**Fig. 2** Quantitative variation of milk proteins among 17 healthy (SCC <250,000) individual cows in mid-lactation (lactation stage from day 112 to day 247). Proteomics analysis was performed according to Zhang et al. (2015a)

cows showed a total of 53 discriminating metabolites that were significantly up- or downregulated in the heat-stressed group compared with the heat-stress-free group, respectively. These metabolic biomarkers were involved in pathways of carbohydrate, amino acid, lipid and gut microbiome-derived metabolism (Tian et al. 2016).

All in all, these previous researches show that by studying the variation in farm animals from the different omics approaches can help better understand the farm animal physiology, as well as how this relates to the products produced from or by the animal.

## *2.2 Comparison Between Species*

Apart from variation within species, as discussed above, also differences between species can be used to better understand the farm animal physiology.

When it comes to genetic differences between species, these are relatively broad. Genetic similarity between species depends on the evolutionary origin of these species and how far back in time a common ancestor existed. At the moment, as far as we are aware, these genetic differences between species have not related to physiological differences between species in scientific research. The same is for transcriptomic differences between species.

Similar to capturing variation, comparison between species has also been comprehensively investigated in milk. It was previously shown that milk composition differs between mammalian species (Yangilar 2013). Of the mammals studied, human milk contained the highest amount of lactose and the lowest amount of proteins, especially casein. Sheep milk, on the other hand, is very high in both fat and casein content (Yangilar 2013). Differences between species not only exist on the level of macronutrients but also exist when comparing different omics dataset between species.

The differences in the milk proteome between species have frequently been reported. For example, in milk serum, β-lactoglobulin is the most abundant protein in bovine and caprine milk serum (Tsiplakou and Zervas 2013), whereas it is absent in human and camel milk serum (Zhang et al. 2016a, b). Of the low-abundant host defence proteins, IgG is the predominant antibody in bovine, caprine and camel milk, while it is IgA in human milk (Zhang et al. 2016b; Stelwagen et al. 2009; Sanchez-Macias et al. 2014). IgG can be transferred to the foetus prior to birth in humans but not in several animals, for example, ruminants such as cattle and sheep (Stelwagen et al. 2009). Furthermore, even the lower abundant proteins, such as complement proteins, antibacterial proteins, acute phase proteins, blood coagulation proteins and protease inhibitors, also are present in very different concentrations between species (D'Auria et al. 2005; Hettinga et al. 2011).

In addition to the qualitative and quantitative proteome differences between species, the changes of milk proteins over lactation stages also differ between species. Transport proteins, enzymes and immunologically active proteins are three dominant protein groups which were shown to change differently over lactation between bovine milk and human milk (Zhang 2015). In particular the immunologically active proteins decreased more rapidly in bovine milk than in human milk in early lactation (Fig. 3). The differences in the changes of these three groups of proteins over lactation can be related to the differences in the needs

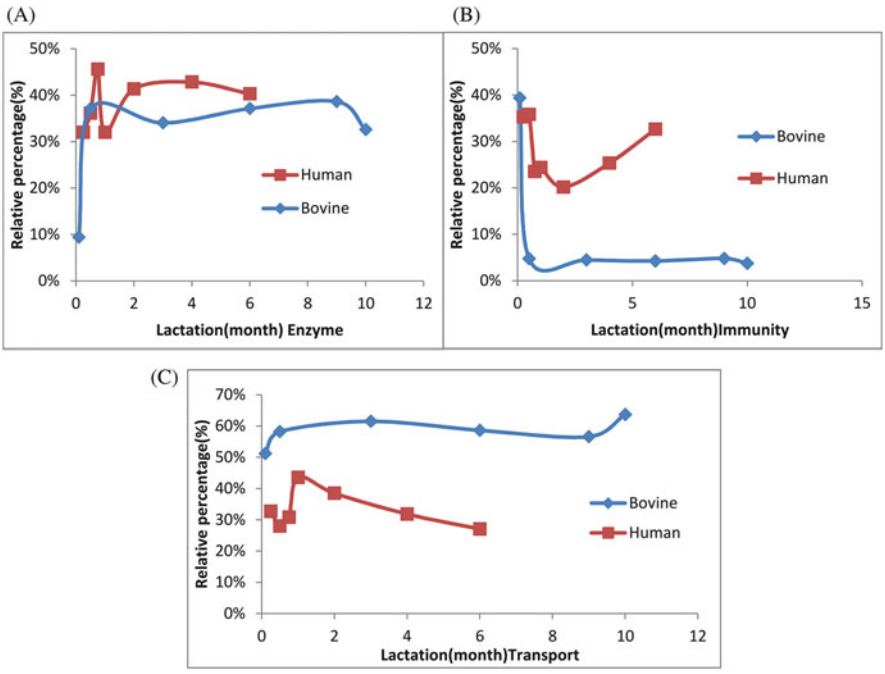


**Fig. 3** The changes of enzymes (**a**), immune proteins (**b**) and transport proteins (**c**) in both human and bovine milk over lactation (Zhang et al. 2017)

between infants and calves (Hettinga et al. 2011). This knowledge on variation between species can be used to better understand the role of milk in the development of the newborn mammal. The rapid decrease in immunologically active proteins, for example, is related to the quick production of these proteins by the calf, requiring less support from milk. When designing feed for young animals or infant formula for newborn babies, this knowledge is useful in deciding on the required quality of these milk-based products.

Of the metabolites in milk, the lipids were also shown to differ between species. A comparison of milk fat composition between cow, buffalo, donkey, sheep and camel showed that the total fatty acid composition were quite similar between species. However, the sn-2 fatty acid, triacylglycerol (TAG), phospholipid and phospholipid fatty acid compositions and melting and crystallization profiles were very different between species (Zou et al. 2013).

In addition, other milk metabolites were also shown to differ between species (Qian et al. 2016). These differences in the milk metabolites were mainly clustered into four groups: (1) nonesterified fatty acids, (2) free amino acids, (3) tricarboxylic acid intermediates and (4) free carbohydrates. Metabolic differences between species have been used to distinguish milk from different dairy animals. For example, choline and succinic acid were only identified in milk from Holstein cows but not in milk of Jersey cows, yak, buffalo, goat, camel and horse. Glycerophospholipid metabolism as well as valine, leucine and isoleucine biosynthesis were similar among ruminant animals (Holstein, Jersey, buffalo, yak and goat), and biosynthesis of unsaturated fatty acids was similar among the non-ruminant animals (camel and horse), as shown by Yang et al. (Yang et al. 2016). This indicates that the metabolism of different dairy animals differs, which may be due to differences in the level of milk synthesis and differences in the milk that is produced.

The above studies show how identifying differences between species helps in better understanding different farm animals. Also, the need of the newborn when it comes to nutrition and host defence can be established through such omics studies. These studies also indicate that different dairy animals differ in their metabolic activity. A special case is the study of human milk. Although human milk is not a commercial product, knowledge on its composition can help in designing optimal replacer for human milk (infant formula), which are commonly produced based on farm animal milks (mainly of bovine and caprine origin).

## *2.3 Omics Studies in the Morphology, Development and Regulation of the Mammary Gland in Health, Disease and Production*

Innovative and high-throughput technologies such as genomics, transcriptomics, proteomics and metabolomics can be used to better understand the functioning of

organs in general. One area where this has been applied is in getting much broader and more detailed knowledge on the morphology, development and regulation of the mammary gland. Previous research has studied the mammary gland in a healthy situation, and when it is in a diseased state, giving more information that aids in optimal production for adequate management of dairy farming.

One of the omics technologies applied to study the mammary gland is epigenomics. It was previously shown that epigenetic regulation, by, for instance, DNA methylation or histone modifications (methylation and acetylation), has been addressed as a non-genetic mechanism of regulating mammary function, as explained in the first section of this chapter. A substantial proportion of unexplained phenotypical variation in the dairy cattle has been claimed to be involved in epigenetic regulation, which should also be considered when studying milk production management practices to optimize production (Singh et al. 2010).

Stress and disease of the mammary gland are important factors that can influence milk production and milk quality. Connor et al. (2008) investigated the physiological changes occurring within the mammary gland during stress induced by more frequent milking. Changes in gene expression related to cell proliferation and differentiation, extracellular matrix (ECM) remodelling, metabolism, nutrient transport and immune function were found (Connor et al. 2008). Besides stress, disease is also often studied. Mastitis is the most devastating disease causing staggering economic losses worldwide to the dairy industry (Kaneene and Scott Hurd 1990). It can be caused by a wide range of organisms, including bacteria, fungi and algae. Transcriptomics studies have provided growing evidence that *E. coli*-induced mastitis causes a far higher expression/regulation of TLR genes, especially TLR2 and TLR4 genes, when compared to *S. aureus* (Yang et al. 2008). Proteomics enabled the detection of the increase of immune-related proteins, immunoglobulins, cathelicidins, lactoferrin, lactadherin, alpha-1-acid glycoprotein and serpin A3-8 in the milk from cows with mastitis (Zhang et al. 2015c; Yang et al. 2009). Monitoring the differences in the milk proteome between healthy animals and animals with disease may help to identify disease-related biomarkers. PTGDS was hypothesized to be a biomarker in bulk milk for mastitis, due to its high correlation with the principally accepted indicator for mastitis (somatic cell count) (Zhang et al. 2015c). Recently, metabolomics was also applied in the investigation of the mammary gland's response to infection. Components of bile acid metabolism, linked to the FXR pathway-regulating inflammation, were found to be increased during mammary gland infections. Furthermore, metabolites mapped to carbohydrate and nucleotide metabolism showed a decreasing trend in concentration up to 81 h post-challenge, whereas an increasing trend was found in lipid metabolites and di-, tri- and tetrapeptides up to the same time point, suggesting the degradation of milk proteins during mastitis (Thomas et al. 2016).

## 3 The Benefit of Combining Data from Different Omics Technologies into a Systems Biology Approach

In research, omics techniques are widely used as single approaches to study changes in animals on the level of genes, transcripts, proteins or metabolites, as described in the first two sections of this chapter. This has led to a better understanding of the physiology of the animal. Combining insights acquired from these different studies have given a better insight in the physiology of farm animals. However, to further our understanding of the physiology of farm animals, integration of these different fields is required based on a more integrated approach. This section will describe the integration of omics technologies, starting from the combination of multiple omics technologies to the full integration from a systems biology approach, all aimed at better understanding of farm animals.

### *3.1 Interactions Between the Different Omics Techniques*

As explained in the first section, biology in general and farm animal physiology in particular can be studied through omics techniques on different levels (Fig. 1). Most studies using omics technologies apply single techniques, because differences are expected on a specific level. However, in real life, biology is often not that easy, and effects on one level will also have influences on other levels. Therefore, studying farm animals on multiple levels using multiple omics technologies simultaneously can be very helpful to better understand the underlying physiology.

Starting at the genetic level, one gene studied extensively in dairy cows is DGAT1. As mentioned in the second section of this chapter, a polymorphism in DGAT1 has been shown to have a wide range of effects on milk synthesis and milk composition (Schennink et al. 2007). To mechanistically study why a single gene polymorphism can have such broad effects, the effect of this polymorphism has been studied on different levels, linking this genetic variation to transcriptomics, proteomics and metabolomics. Transcriptomic studies of cows differing in the DGAT1 K232A polymorphism have been performed to obtain an overview of the gene transcription changes related to this polymorphism. Thereby, that study aimed at better understanding the mechanism underlying the effects this polymorphism has on milk compositional parameters. This microarray-based transcriptomics analysis showed that cows differing in DGAT1 polymorphism had many genes that were differentially regulated, with the largest effects on transcripts related to energy metabolism, although no difference in transcription of the DGAT1 gene itself was found (Mach et al. 2012). This study showed that genes do not necessarily affect the transcript of the gene they encode, but there may be many correlated effects on completely different genes, which may lead to a much broader range of effects. In relation to the same genetic polymorphism, research has also been performed on the proteome and metabolome level (Lu et al. 2015). The results

showed a single protein, stomatin, was differentially regulated. Stomatin is involved in membrane structures in general and of milk fat globule membrane in particular. Simultaneously, lipid metabolites that are also involved in membrane structure were also differentially regulated in the milk fat globule and its membrane. This combination of omics dataset on different levels led to a hypothesis for an underlying mechanism related to differences in membrane structure between cows with different DGAT1 polymorphisms. This shows the benefit of simultaneously collecting omics data on different levels.

The same authors also studied the effect on proteome and metabolome level of another important factor in cow physiology, the negative energy balance in the periparturient period (Lu et al. 2013). In this study, changes in the same protein (stomatin) that is involved in membrane structuring were found. Also enzymes involved in cholesterol synthesis as well as cholesterol itself which is a lipid that is also important in membrane structure itself were changed. In addition, leakage of intracellular water-soluble metabolites was found. This study, by integrating proteomics and water- and lipid-soluble metabolomics, gave rise to the hypothesis that cell integrity and membrane structure were altered in cows in severe negative energy balance in early lactation. The studies mentioned above all linked different omics levels together and thereby reached new hypotheses on mammary gland physiology that could not have been reached if studies would have been limited to only a single level omics research.

To further study hypotheses that have been reached by analysing multiple omics datasets within the same sample, research could also look at multiple samples from the same animal. For example, samples can be taken of both the animal and its products or from different parts of the animal. Studying multiple samples from the same animal may lead to a better understanding of the underlying physiology. One example of such research aimed at better understanding milk synthesis by taking samples from both milk and the mammary gland itself into account. This was done in a study following a feeding intervention consisting of an increased intake of unsaturated fat by dairy cows (Ibeagha-Awemu et al. 2016a). This study showed how lipids in milk that changed after a feeding intervention were correlated with transcriptome alterations in the mammary gland. Such a study gives a more direct indication how interventions in cow management can influence milk synthesis in the mammary gland and thereby the composition of the milk produced. This approach has not only been used to better understand the mammary gland and milk synthesis but also for a better understanding of meat quality, as reviewed elsewhere (Mullen et al. 2006). An important benefit of studying the quality of farm animal products, like milk and meat, on different levels, from genes to metabolites and from animals to products, is that it leads to a better understanding of the underlying mechanisms. And once the mechanism is known, this can lead to better interventions to improve aspects such as animal health and product quality.

### 3.2 Full Integration of Omics Datasets into Systems Biology

The examples discussed above provide insight in the benefits of combining omics dataset collected on different levels. This can be further extended to looking from a network perspective: genes do not work in isolation but sets of genes encode, through sets of transcripts, sets of proteins that are involved in specific metabolic pathways. Interpretation of omics data can use information on such metabolic pathways, also called network analysis, for its interpretation. Once the genome of an animal is sequenced and annotated, this information can be used to construct metabolic pathways, which describe the integrated picture how different processes in a specie work together. The construction of overviews of metabolic pathways is a first necessary step towards research aiming at a better understanding of the metabolism of an animal. This construction of metabolic pathways can be done using knowledge of known enzymatic reactions and pathways to which the annotated genes can be linked. Different software-assisted approaches exist to perform this task automatically. These pathways by themselves do not directly explain the underlying biology; however, they provide the basic required information for understanding the metabolism, and its regulation, of animals (Seo et al. 2013). Of the farm animals, this has been done almost exclusively for the cow. This approach has been used to study the gene networks involved in lipid (Bionaz and Loor 2008) and protein (Bionaz and Loor 2011) synthesis of dairy cows. More recently, it was also shown that these gene networks are not uniquely associated with either lipid or fat synthesis but also interact (Li et al. 2016). These findings show that milk synthesis pathways for different milk components interact with each other and should not be studied in isolation. This also may explain the earlier mentioned pleiotropic effects of the DGAT1 polymorphism influencing many milk-related parameters, although an analysis of this effect from this perspective has not yet been performed. Furthermore illnesses of the mammary gland have been studied from such a pathways perspective. It was, for example, shown that mastitis was associated with an upregulation of the immune system pathway and a downregulation of the lipid metabolism pathway (Buitenhuis et al. 2011). On the level of proteins, it was also shown than an upregulation of host defence proteins occurred (Boehmer 2011; Boehmer et al. 2010).

This is not only true for the mammary gland, but also for the bovine liver (Khan et al. 2015), where multiple gene networks were shown to be differentially regulated during the periparturient period of negative energy balance. From the perspective of the homeostasis of an organism, balancing many metabolic pathways through many interactions makes sense, but it does complicate research in which often single cause-effects relations are searched for.

Such integrated insights are not only helpful for understanding the physiology of the cow, as discussed above, but also can help us to get a better understanding of the quality of the product. For example, meat has been extensively discussed previously (D'Alessandro and Zolla 2013; Paredi et al. 2012, 2013). By using multiple omics technologies, the development of muscle tissue in the growing animal, also

its degradation post-mortem, as well as its quality (for example, tenderness) can be better understood. Studying this from a systems biology approach by taking into account all relevant metabolic pathways (e.g., pathways related to apoptosis and autophagy) is required for a full understanding of the conversion from muscle into meat (Hollung et al. 2014).

In the end, all these integrated approaches aim at better understanding of the underlying physiology (Davidsen et al. 2016) but, as can also be seen from the relatively small number of published studies in this field, such analyses are not easy to perform. The challenges and outlooks for the systems biology approach to farm animals will be discussed next in the final section of this chapter.

## 4 Outlook on Future Developments

In the previous sections of this chapter, the current state of development in the area of omics technologies for systems biology application in farm animals is described. In this final section, a discussion is given of the different future developments that could aid the further development in this research area.

### *4.1 What Are the Challenges of Applying Systems Biology for Farm Animals*

One of the main challenges remaining in the computational approaches to systems biology is the software that can be used for combining different omics datasets. Integration of multiple omics dataset, with integrated computer analyses, could be a very powerful tool for understanding the systems biology of healthy animals but is currently limited by software options as discussed previously (Suravajhala et al. 2016). Such studies will as a minimum require an in-depth understanding of all the interactions, both within each level, as well as between each level, as shown in Fig. 1. For bovine this knowledge is to a larger extent available than for other farm animals. This will require more effort in the future to get a better integrated picture of the metabolism of different farm animals. As discussed in Sect. 2, there are many differences between farm animals. However, the basic underlying genes, enzymes and metabolites involved in muscle development and milk synthesis are rather similar between species. The development of these metabolic pathways in other farm animals can thus be based on information already available for the cow.

Another challenge is the availability and format of collected data. Most omics dataset are collected on wide variety of platforms, all with their own structure and encoding of the data. A lot of available software for integrative analysis of omics data is therefore developed for specific types of data. In collaborations, where multiple types of instruments are used, this poses specific challenges in being

able to compare and analyse all data using an integrative perspective. On top of that, discrepancies between datasets (transcripts/proteins/metabolites obtained on one system and not on the other) pose an additional challenge. And finally, mismatches between gene-transcript-protein-metabolites can further complicate systems biology approaches. Due to different underlying detection principles between different analytical approaches, it will not be easy to solve these problems.

Multivariate approaches to analyse integrated omics datasets using, for example, pattern recognition are also not been broadly developed yet. One successful example of such an approach in which support vector machines were applied for biomarker discovery in large omics datasets has been published (Kim et al. 2017). However, the application of such tools in the analysis of very different types of datasets was not shown in practice yet.

### *4.2 What Can We Learn from Other Fields of Biology*

The field of systems biology is of course not exclusive to farm animals. It is frequently, and extensively, used in humans to better understand the physiology of health and disease. For example, in cancer research, multiple omics techniques have previously been combined for diagnosis, prognosis and monitoring treatment (Seeree et al. 2015; Dimitrieva et al. 2016). Also for other human diseases, the combination of multiple omics field data for biomarker discovery has frequently been applied (Kussmann and Blum 2007; Castro-Santos et al. 2015; Mehta et al. 2015).

More generally, knowledge on interaction between different levels of omics, also called interactomics, can be used for better understanding illnesses (Bellay et al. 2012). Specifically on the level of proteins, such an interactomic overview of milk has been previously done (D'Alessandro et al. 2011; Zhang et al. 2017). These studies show how milk proteins are involved in different specific protein-protein interaction networks, leading to a better understanding of the role of milk proteins in the growth and development of the neonate. Further extending such studies to all levels of biology from gene to metabolite could further our understanding of milk and more in general of the farm animal.

## References

Almeida AM, Bassols A, Bendixen E, Bhide M, Ceciliani F, Cristobal S, Eckersall PD, Hollung K, Lisacek F, Mazzucchelli G, McLaughlin M, Miller I, Nally JE, Plowman J, Renaut J, Rodrigues P, Roncada P, Staric J, Turk R (2015) Animal board invited review: advances in proteomics for animal and food sciences. Animal 9(1):1–17

Alstrup L, Soegaard K, Weisbjerg MR (2016) Effects of maturity and harvest season of grass-clover silage and of forage-to-concentrate ratio on milk production of dairy cows. J Dairy Sci 99(1):328–340

Antunes-Fernandes EC, van Gastelen S, Dijkstra J, Hettinga KA, Vervoort J (2016) Milk metabolome relates enteric methane emission to milk synthesis and energy metabolism pathways. J Dairy Sci 99(8):6251–6262

Bai X, Zheng Z, Liu B, Ji X, Bai Y, Zhang W (2016) Whole blood transcriptional profiling comparison between different milk yield of Chinese Holstein cows using RNA-seq data. BMC Genomics 17(Suppl 7):512

Bellay J, Michaut M, Kim T, Han S, Colak R, Myers CL, Kim PM (2012) An omics perspective of protein disorder. Mol BioSyst 8(1):185–193

Bendixen E, Danielsen M, Hollung K, Gianazza E, Miller I (2011) Farm animal proteomics—a review. J Proteomics 74(3):282–293

Benmoussa A, Lee CH, Laffont B, Savard P, Laugier J, Boilard E, Gilbert C, Fliss I, Provost P (2016) Commercial dairy cow milk microRNAs resist digestion under simulated gastrointestinal tract conditions. J Nutr 146(11):2206–2215

Bernabucci U, Basirico L, Morera P, Dipasquale D, Vitali A, Piccioli Cappelli F, Calamari L (2015) Effect of summer season on milk protein fractions in Holstein cows. J Dairy Sci 98 (3):1815–1827

Bionaz M, Loor JJ (2008) Gene networks driving bovine milk fat synthesis during the lactation cycle. BMC Genomics 9:366

Bionaz M, Loor JJ (2011) Gene networks driving bovine mammary protein synthesis during the lactation cycle. Bioinform Biol Insights 5:83–98

Boehmer JL (2011) Proteomic analyses of host and pathogen responses during bovine mastitis. J Mammary Gland Biol Neoplasia 16(4):323–338

Boehmer JL, Ward JL, Peters RR, Shefcheck KJ, McFarland MA, Bannerman DD (2010) Proteomic analysis of the temporal expression of bovine milk proteins during coliform mastitis and label-free relative quantification. J Dairy Sci 93(2):593–603

Boggs I, Hine B, Smolenksi G, Hettinga K, Zhang L, Wheeler TT (2016) Proteomics data in support of the quantification of the changes of bovine milk proteins during mammary gland involution. Data Brief 8:52–55

Boggs I, Hine B, Smolenski G, Hettinga K, Zhang L, Wheeler TT (2015) Changes in the repertoire of bovine milk proteins during mammary involution. EuPA Open Proteom 9:65–75

Buitenhuis B, Poulsen NA, Gebreyesus G, Larsen LB (2016) Estimation of genetic parameters and detection of chromosomal regions affecting the major milk proteins and their post translational modifications in Danish Holstein and Danish Jersey cattle. BMC Genet 17:114

Buitenhuis B, Røntved CM, Edwards SM, Ingvartsen KL, Sørensen P (2011) In depth analysis of genes and pathways of the mammary gland involved in the pathogenesis of bovine Escherichia coli-mastitis. BMC Genomics 12(1):130

Castro-Santos P, Laborde CM, Diaz-Pena R (2015) Genomics, proteomics and metabolomics: their emerging roles in the discovery and validation of rheumatoid arthritis biomarkers. Clin Exp Rheumatol 33(2):279–286

Connor EE, Siferd S, Elsasser TH, Evock-Clover CM, Van Tassell CP, Sonstegard TS, Fernandes VM, Capuco AV (2008) Effects of increased milking frequency on gene expression in the bovine mammary gland. BMC Genomics 9:362. https://doi.org/10.1186/1471–2164–9-362

D'Alessandro A, Zolla L (2013) Meat science: from proteomics to integrated omics towards system biology. J Proteomics 78:558–577

D'Alessandro A, Zolla L, Scaloni A (2011) The bovine milk proteome: cherishing, nourishing and fostering molecular complexity. An interactomics and functional overview. Mol BioSyst 7 (3):579–597

D'Auria E, Agostoni C, Giovannini M, Riva E, Zetterstrom R, Fortin R, Greppi GF, Bonizzi L, Roncada P (2005) Proteomic evaluation of milk from different mammalian species as a substitute for breast milk. Acta Paediatrica (Oslo, Norway: 1992) 94(12):1708–1713

Davidsen PK, Turan N, Egginton S, Falciani F (2016) Multilevel functional genomics data integration as a tool for understanding physiology: a network biology perspective. J Appl Physiol (Bethesda, Md: 1985) 120(3):297–309

Dimitrieva S, Schlapbach R, Rehrauer H (2016) Prognostic value of cross-omics screening for kidney clear cell renal cancer survival. Biol Direct 11(1):68. https://doi.org/10.1186/s13062-016-0170-1

Duchemin S, Bovenhuis H, Stoop WM, Bouwman AC, van Arendonk JAM, Visker MHPW (2013) Genetic correlation between composition of bovine milk fat in winter and summer, and DGAT1 and SCD1 by season interactions. J Dairy Sci 96(1):592–604

Duchemin SI, Visker MH, Van Arendonk JA, Bovenhuis H (2014) A quantitative trait locus on Bos taurus autosome 17 explains a large proportion of the genetic variation in de novo synthesized milk fatty acids. J Dairy Sci 97(11):7276–7285

Gerspach C, Imhasly S, Gubler M, Naegeli H, Ruetten M, Laczko E (2017) Altered plasma lipidome profile of dairy cows with fatty liver disease. Res Vet Sci 110:47–59

Hayes BJ, Bowman PJ, Chamberlain AJ, Goddard ME (2009) Invited review: genomic selection in dairy cattle: progress and challenges. J Dairy Sci 92(2):433–443

Heck JM, Schennink A, van Valenberg HJ, Bovenhuis H, Visker MH, van Arendonk JA, van Hooijdonk AC (2009) Effects of milk protein variants on the protein composition of bovine milk. J Dairy Sci 92(3):1192–1202

Hettinga K, van Valenberg H, de Vries S, Boeren S, van Hooijdonk T, van Arendonk J, Vervoort J (2011) The host defense proteome of human and bovine milk. PLoS ONE 6(4):e19433

Hinz K, O'Connor PM, O'Brien B, Huppertz T, Ross RP, Kelly AL (2012) Proteomic study of proteolysis during ripening of Cheddar cheese made from milk over a lactation cycle. J Dairy Res 79(2):176–184

Hollung K, Timperio AM, Olivan M, Kemp C, Coto-Montes A, Sierra V, Zolla L (2014) Systems biology: a new tool for farm animal science. Curr Protein Pept Sci 15(2):100–117

Ibeagha-Awemu EM, Li R, Ammah AA, Dudemaine PL, Bissonnette N, Benchaar C, Zhao X (2016a) Transcriptome adaptation of the bovine mammary gland to diets rich in unsaturated fatty acids shows greater impact of linseed oil over safflower oil on gene expression and metabolic pathways. BMC Genomics 17:104

Ibeagha-Awemu EM, Peters SO, Akwanji KA, Imumorin IG, Zhao X (2016b) High density genome wide genotyping-by-sequencing and association identifies common and low frequency SNPs, and novel candidate genes influencing cow milk traits. Sci Rep 6:31109

Ioannidis J, Donadeu FX (2016) Circulating microRNA Profiles during the Bovine Oestrous Cycle. PLoS ONE 11(6):e0158160

Kaneene JB, Scott Hurd H (1990) The national animal health monitoring system in Michigan. III. Cost estimates of selected dairy cattle diseases. Prev Vet Med 8(2):127–140

Khan MJ, Jacometo CB, Riboni MV, Trevisi E, Graugnard DE, Correa MN, Loor JJ (2015) Stress and inflammatory gene networks in bovine liver are altered by plane of dietary energy during late pregnancy. Funct Integr Genomics 15(5):563–576

Kim S, Jhong JH, Lee J, Koo JY (2017) Meta-analytic support vector machine for integrating multiple omics data. BioData Mining 10:2

Kirchner B, Pfaffl MW, Dumpler J, von Mutius E, Ege MJ (2016) microRNA in native and processed cow's milk and its implication for the farm milk effect on asthma. J Allergy Clin Immunol 137(6):1893–1895.e1813

Korhonen HJ (2009) Bioactive components in bovine milk. In: Bioactive components in milk and dairy products. Wiley-Blackwell, Ames, IA, pp 13–42

Kukurba KR, Montgomery SB (2015) RNA sequencing and analysis. Cold Spring Harb Protoc 2015(11):951–969

Kussmann M, Blum S (2007) OMICS-derived targets for inflammatory gut disorders: opportunities for the development of nutrition related biomarkers. Endocr Metab Immune Disord Drug Targets 7(4):271–287

Li Q, Zhao Y, Zhu D, Pang X, Liu Y, Frew R, Chen G (2017) Lipidomics profiling of goat milk, soymilk and bovine milk by UPLC-Q-exactive orbitrap mass spectrometry. Food Chem 224:302–309

Li S, Hosseini A, Danes M, Jacometo C, Liu J, Loor JJ (2016) Essential amino acid ratios and mTOR affect lipogenic gene networks and miRNA expression in bovine mammary epithelial cells. J Animal Sci Biotechnol 7:44

Lu J, Antunes Fernandes E, Paez Cano AE, Vinitwatanakhun J, Boeren S, van Hooijdonk T, van Knegsel A, Vervoort J, Hettinga KA (2013) Changes in milk proteome and metabolome associated with dry period length, energy balance, and lactation stage in postparturient dairy cows. J Proteome Res 12(7):3288–3296

Lu J, Boeren S, van Hooijdonk T, Vervoort J, Hettinga K (2015) Effect of the DGAT1 K232A genotype of dairy cows on the milk metabolome and proteome. J Dairy Sci 98(5):3460–3469

Lu J, van Hooijdonk T, Boeren S, Vervoort J, Hettinga K (2014) Identification of lipid synthesis and secretion proteins in bovine milk. J Dairy Res 81(1):65–72

Mach N, Blum Y, Bannink A, Causeur D, Houee-Bigot M, Lagarrigue S, Smits MA (2012) Pleiotropic effects of polymorphism of the gene diacylglycerol-O-transferase 1 (DGAT1) in the mammary gland tissue of dairy cows. J Dairy Sci 95(9):4989–5000

McCoard SA, Hayashi AA, Sciascia Q, Rounce J, Sinclair B, McNabb WC, Roy NC (2016) Mammary transcriptome analysis of lactating dairy cows following administration of bovine growth hormone. Animal 10(12):2008–2017

Mehta SM, Banerjee SM, Chowdhary AS (2015) Postgenomics biomarkers for rabies-the next decade of proteomics. OMICS 19(2):67–79

Moran B, Cummins SB, Creevey CJ, Butler ST (2016) Transcriptomics of liver and muscle in Holstein cows genetically divergent for fertility highlight differences in nutrient partitioning and inflammation processes. BMC Genomics 17(1):603

Morzel M, Chambon C, Lefevre F, Paboeuf G, Laville E (2006) Modifications of trout (Oncorhynchus mykiss) muscle proteins by preslaughter activity. J Agric Food Chem 54 (8):2997–3001

Mullen AM, Stapleton PC, Corcoran D, Hamill RM, White A (2006) Understanding meat quality through the application of genomic and proteomic approaches. Meat Sci 74(1):3–16

Ng-Kwai-Hang KF, Hayes JF, Moxley JE, Monardes HG (1987) Variation in milk protein concentrations associated with genetic polymorphism and environmental factors. J Dairy Sci 70(3):563–570

Nir Markusfeld O (2003) What are production diseases, and how do we manage them? Acta Vet Scand Suppl 98:21–32

Paredi G, Raboni S, Bendixen E, de Almeida AM, Mozzarelli A (2012) "Muscle to meat" molecular events and technological transformations: the proteomics insight. J Proteomics 75 (14):4275–4289

Paredi G, Sentandreu MA, Mozzarelli A, Fadda S, Hollung K, de Almeida AM (2013) Muscle and meat: new horizons and applications for proteomics on a farm to fork perspective. J Proteomics 88:58–82

Perge P, Nagy Z, Decmann A, Igaz I, Igaz P (2016) Potential relevance of microRNAs in inter-species epigenetic communication, and implications for disease pathogenesis. RNA Biol 14:1–11

Poulsen NA, Jensen HB, Larsen LB (2016) Factors influencing degree of glycosylation and phosphorylation of caseins in individual cow milk samples. J Dairy Sci 99(5):3325–3333

Qian L, Zhao A, Zhang Y, Chen T, Zeisel SH, Jia W, Cai W (2016) Metabolomic approaches to explore chemical diversity of human breast-milk, formula milk and bovine milk. Int J Mol Sci 17(12):2128

Rodrigues PM, Silva TS, Dias J, Jessen F (2012) PROTEOMICS in aquaculture: applications and trends. J Proteomics 75(14):4325–4345

Salilew-Wondim D, Ibrahim S, Gebremedhn S, Tesfaye D, Heppelmann M, Bollwein H, Pfarrer C, Tholen E, Neuhoff C, Schellander K, Hoelker M (2016) Clinical and subclinical endometritis induced alterations in bovine endometrial transcriptome and miRNome profile. BMC Genomics 17:218

Samková E, Spicka J, Pesek M, Pelikánová T, Hanus O (2012) Animal factors affecting fatty acid composition of cow milk fat: a review. S Afr J Anim Sci 42:83–100

Sanchez-Macias D, Moreno-Indias I, Castro N, Morales-Delanuez A, Arguello A (2014) From goat colostrum to milk: physical, chemical, and immune evolution from partum to 90 days postpartum. J Dairy Sci 97(1):10–16

Schennink A, Bovenhuis H, Leon-Kloosterziel KM, van Arendonk JA, Visker MH (2009) Effect of polymorphisms in the FASN, OLR1, PPARGC1A, PRL and STAT5A genes on bovine milk-fat composition. Anim Genet 40(6):909–916

Schennink A, Stoop WM, Visker MH, Heck JM, Bovenhuis H, van der Poel JJ, van Valenberg HJ, van Arendonk JA (2007) DGAT1 underlies large genetic variation in milk-fat composition of dairy cows. Anim Genet 38(5):467–473

Seeree P, Pearngam P, Kumkate S, Janvilisri T (2015) An omics perspective on molecular biomarkers for diagnosis, prognosis, and therapeutics of cholangiocarcinoma. Int J Genomics 2015:179528

Seo S, Larkin DM, Loor JJ (2013) Cattle genomics and its implications for future nutritional strategies for dairy cattle. Animal 7(Suppl 1):172–183

Shen B, Zhang L, Lian C, Lu C, Zhang Y, Pan Q, Yang R, Zhao Z (2016) Deep sequencing and screening of differentially expressed MicroRNAs related to milk fat metabolism in bovine primary mammary epithelial cells. Int J Mol Sci 17(2):200

Shen Y, Shi S, Tong H, Guo Y, Zou J (2014) Metabolomics analysis reveals that bile acids and phospholipids contribute to variable responses to low-temperature-induced ascites syndrome. Mol BioSyst 10(6):1557–1567

Singh K, Erdman RA, Swanson KM, Molenaar AJ, Maqbool NJ, Wheeler TT, Arias JA, Quinn-Walsh EC, Stelwagen K (2010) Epigenetic regulation of milk production in dairy cows. J Mammary Gland Biol Neoplasia 15(1):101–112

Singh K, Molenaar AJ, Swanson KM, Gudex B, Arias JA, Erdman RA, Stelwagen K (2012) Epigenetics: a possible role in acute and transgenerational regulation of dairy cow milk production. Animal 6(3):375–381

Sokol E, Ulven T, Færgeman NJ, Ejsing CS (2015) Comprehensive and quantitative profiling of lipid species in human milk, cow milk and a phospholipid-enriched milk formula by GC and MS/MSALL. Eur J Lipid Sci Technol 117(6):751–759

Stelwagen K, Carpenter E, Haigh B, Hodgkinson A, Wheeler TT (2009) Immune components of bovine colostrum and milk. J Anim Sci 87(13 Suppl):3–9

Sundekilde UK, Larsen LB, Bertram HC (2013) NMR-based milk metabolomics. Metabolites 3 (2):204–222

Suravajhala P, Kogelman LJA, Kadarmideen HN (2016) Multi-omic data integration and analysis using systems genomics approaches: methods and applications in animal production, health and welfare. Genet Sel Evol 48(1):38

Thomas FC, Mudaliar M, Tassi R, McNeilly TN, Burchmore R, Burgess K, Herzyk P, Zadoks RN, Eckersall PD (2016) Mastitomics, the integrated omics of bovine milk in an experimental model of Streptococcus uberis mastitis: 3. Untargeted metabolomics. Mol BioSyst 12 (9):2762–2769

Tian H, Zheng N, Wang W, Cheng J, Li S, Zhang Y, Wang J (2016) Integrated metabolomics study of the milk of heat-stressed lactating dairy cows. Sci Rep 6:24208

Tsiplakou E, Zervas G (2013) Changes in milk and plasma fatty acid profile in response to fish and soybean oil supplementation in dairy sheep. J Dairy Res 80(2):205–213

Wall EH, Bond JP, McFadden TB (2013) Milk yield responses to changes in milking frequency during early lactation are associated with coordinated and persistent changes in mammary gene expression. BMC Genomics 14:296

Wang JH, Byun J, Pennathur S (2010) Analytical approaches to metabolomics and applications to systems biology. Semin Nephrol 30(5):500–511

Wittenburg D, Melzer N, Willmitzer L, Lisec J, Kesting U, Reinsch N, Repsilber D (2013) Milk metabolites and their genetic variability. J Dairy Sci 96(4):2557–2569

Yang W, Zerbe H, Petzl W, Brunner RM, Gunther J, Draing C, von Aulock S, Schuberth HJ, Seyfert HM (2008) Bovine TLR2 and TLR4 properly transduce signals from Staphylococcus aureus and E. coli, but S. aureus fails to both activate NF-kappaB in mammary epithelial cells and to quickly induce TNFalpha and interleukin-8 (CXCL8) expression in the udder. Mol Immunol 45(5):1385–1397

Yang Y, Zheng N, Zhao X, Zhang Y, Han R, Yang J, Zhao S, Li S, Guo T, Zang C, Wang J (2016) Metabolomic biomarkers identify differences in milk produced by Holstein cows and other minor dairy animals. J Proteomics 136:174–182

Yang YX, Zhao XX, Zhang Y (2009) Proteomic analysis of mammary tissues from healthy cows and clinical mastitic cows for identification of disease-related proteins. Vet Res Commun 33 (4):295–303

Yangilar F (2013) As a potentially functional food: goats' milk and products. J Food Nutr Res 1 (4):68–81

Younis S, Javed Q, Blumenberg M (2016) Meta-analysis of transcriptional responses to mastitis-causing Escherichia coli. PLoS ONE 11(3):e0148562. https://doi.org/10.1371/journal.pone.0148562

Zhang L (2015) Dynamics of the proteome in human and farm animal milk. Wageningen University, Wageningen

Zhang L, Boeren S, Hageman JA, van Hooijdonk T, Vervoort J, Hettinga K (2015b) Bovine milk proteome in the first 9 days: protein interactions in maturation of the immune and digestive system of the newborn. PLoS ONE 10(2):e0116710

Zhang L, Boeren S, Hageman JA, van Hooijdonk T, Vervoort J, Hettinga K (2015c) Perspective on calf and mammary gland development through changes in the bovine milk proteome over a complete lactation. J Dairy Sci 98(8):5362–5373

Zhang L, Boeren S, Smits M, van Hooijdonk T, Vervoort J, Hettinga K (2016a) Proteomic study on the stability of proteins in bovine, camel, and caprine milk sera after processing. Food Res Int 82:104–111

Zhang L, Boeren S, van Hooijdonk AC, Vervoort JM, Hettinga KA (2015a) A proteomic perspective on the changes in milk proteins due to high somatic cell count. J Dairy Sci 98 (8):5339–5351

Zhang L, de Waard M, Verheijen H, Boeren S, Hageman JA, van Hooijdonk T, Vervoort J, van Goudoever JB, Hettinga K (2016b) Changes over lactation in breast milk serum proteins involved in the maturation of immune and digestive system of the infant. J Proteomics 147:40–47

Zhang L, van Dijk AD, Hettinga K (2017) An interactomics overview of the human and bovine milk proteome over lactation. Proteome Sci 15:1

Zhang Q, Cundiff J, Maria S, McMahon R, Woo J, Davidson B, Morrow A (2013) Quantitative analysis of the human milk whey proteome reveals developing milk and mammary-gland functions across the first year of lactation. Proteomes 1(2):128

Zhao K, Liang G, Sun X, Guan le L (2016) Comparative miRNAome analysis revealed different miRNA expression profiles in bovine sera and exosomes. BMC Genomics 17(1):630

Zhao S, Zhao J, Bu D, Sun P, Wang J, Dong Z (2014) Metabolomics analysis reveals large effect of roughage types on rumen microbial metabolic profile in dairy cows. Lett Appl Microbiol 59 (1):79–85

Zhou S, Wan Q, Huang Y, Huang X, Cao J, Ye L, Lim TK, Lin Q, Qin Q (2011) Proteomic analysis of Singapore grouper iridovirus envelope proteins and characterization of a novel envelope protein VP088. Proteomics 11(11):2236–2248

Zou X, Huang J, Jin Q, Guo Z, Liu Y, Cheong L, Xu X, Wang X (2013) Lipid composition analysis of milk fats from different mammalian species: potential for use as human milk fat substitutes. J Agric Food Chem 61(29):7070–7080