Journal of Dairy Research

cambridge.org/dar

Review Article

Cite this article: Vargas-Bello-Pérez E, Márquez-Hernández RI and Hernández-Castellano LE (2019). Bioactive peptides from milk: animal determinants and their implications in human health. *Journal of Dairy Research* **86**, 136–144. https://doi.org/10.1017/S0022029919000384

Received: 7 February 2019
Revised: 28 February 2019
Accepted: 18 April 2019

Keywords:
Animal nutrition; bioactive peptides; dairy cow; genetics; health; milk

Author for correspondence:
Lorenzo E. Hernández-Castellano, Email: lhc@anis.au.dk and Einar Vargas-Bello-Pérez, Email: evargasb@sund.ku.dk

Bioactive peptides from milk: animal determinants and their implications in human health

Einar Vargas-Bello-Pérez[1], Roberto I. Márquez-Hernández[2] and Lorenzo E. Hernández-Castellano[3]

[1]Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Grønnegårdsvej 3, 1870 Frederiksberg C, Denmark; [2]Programa de Maestría y Doctorado en Ciencias de la Producción y de la Salud Animal, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Ciudad Universitaria, 04510, Mexico and [3]Department of Animal Science, AU-Foulum, Aarhus University, 8830 Tjele, Denmark

Abstract

Milk is an important protein source in human diets, providing around 32 g protein/l (for bovine milk, which constitutes some 85% of global consumption). The most abundant milk proteins are α-lactalbumin, β-lactoglobulin, αs-casein, β-casein, and κ-casein. Besides their nutritional value, milk proteins play a crucial role in the processing properties of milk, such as solubility, water bonding, heat stability, renneting and foaming, among others. In addition, and most importantly for this review, these proteins are the main source of bioactive components in milk. Due to the wide range of proposed beneficial effects on human health, milk proteins are considered as potential ingredients for the production of health-promoting functional foods. However, most of the evidence for bioactive effects comes from *in vitro* studies, and there is a need for further research to fully evaluate the true potential of milk-derived bioactive factors. Animal genetics and animal nutrition play an important role in the relative proportions of milk proteins and could be used to manipulate the concentration of specific bioactive peptides in milk from ruminants. Unfortunately, only a few studies in the literature have focused on changes in milk bioactive peptides associated to animal genetics and animal nutrition. The knowledge described in the present review may set the basis for further research and for the development of new dairy products with healthy and beneficial properties for humans.

According to FAO (2013), more than 6 billion people worldwide consume milk and dairy products. Milk from dairy cows represent about 85% of the total milk produced worldwide followed by buffalo (11%), goat (2.3%), sheep (1.4%) and camel milk (0.2%) (Gerosa and Skoet, 2012). Milk is recognized as an important source of energy and contains essential nutrients including carbohydrate (lactose), lipids, minerals and vitamins. In particular though, milk is an important source of proteins in human diets, providing about 32 g protein/l (Pereira, 2014). In recent years, consumer demands for healthier foods has grown considerably (Bonanno, 2012), and milk has attracted interest across a spectrum of consumer groups, from those who focus on its undoubted nutritional benefits to those who claim adverse cardiovascular health effects of dairy fats (such claims are now largely discredited: Givens, 2018). Here we consider a third aspect, namely, the potential for peptides derived from milk proteins to have bioactive activities when consumed.

Milk proteins are classified in three different groups: caseins, whey proteins and proteins from the milk fat globule membrane (MFGM) (Hernández-Castellano *et al.*, 2014). Caseins, mainly αs-casein (αs1 and αs2), β-casein, and κ-casein, represent about 78% of the proteins in bovine milk (Heck *et al.*, 2009). Whey proteins such as β-lactoglobulin, α-lactalbumin, lactoferrin, immunoglobulins, serum albumin, glycomacropeptides, enzymes and growth factors represent another 18% or so (McGregor and Poppitt, 2013) whilst proteins from the MFGM represent less than 4% (Murgiano *et al.*, 2009). The most abundant of this latter group are mucin-1 and xanthine dehydrogenase/oxidase (Mather, 2000). In addition to their nutritional value, milk proteins contribute to define the physicochemical properties of the milk such as solubility, water bonding, viscosity and heat stability (Augustin and Udebage, 2007). Milk protein composition varies among the major dairy species. For instance, sheep milk generally contains higher concentrations of caseins, β-lactoglobulin, α-lactalbumin, serum albumin, and lactoferrin compared to cows, buffaloes or goats (Table 1).

Milk proteins and their fractions are the main sources of bioactive peptides, which are considered potential ingredients for health-promoting functional foods (Giacometti and Buretić-Tomljanović, 2017). For example, specific milk peptides have been proposed to have

Table 1. Fractions of milk protein (g/l) of different ruminants

Protein	Cow	Sheep	Goat	Buffalo
Total casein				
α_{s1}-Casein	8–10.7	15.4–22.1	0–13.0	8.9
α_{s2}-Casein	2.8–3.4	15.4–22.1	2.3–11.6	5.1
β-Casein	8.6–9.3	15.6–17.6	0–29.6	12.6–20.9
κ-Casein	2.3–3.3	3.2–4.3	2.8–13.4	4.1–5.4
γ-Casein	0.8	–	–	–
Casein micelle (nm)	150–182	180–210	260	180
Total whey proteins				
β-Lactoglobulin	3.2–3.3	6.5–8.5	1.5–5.0	3.9
α-Lactalbumin	1.2–1.3	1–1.9	0.7–2.3	1.4
Serum albumin	0.3–0.4	0.4–0.6	0.31–0.42	0.29
Proteose peptone	0.8–1.2	–	0.97–1	3.31
Lactoferrin	0.02–0.5	0.8	0.02–0.2	0.03–3.4
Lysozyme	$(70–600) \times 10^{-6}$	100×10^{-6}	250×10^{-6}	$(120–152) \times 10^{-6}$
Immunoglobulins				
IgG	0.91	0.87	0.73	0.37–1.34
IgA	0.05–0.14	–	0.03–0.08	0.01–0.04
IgM	0.58	0.38	0.41	0.04–1.91
Casein/whey ratio	4.7	3.1	3.5	4.6

Adapted from Khatoon and Josh (1987), Olaniyan *et al.* (2013), Claeys *et al.* (2014), Galán-Malo *et al.* (2014) and Hernández-Castellano *et al.* (2016*a*)

antihypertensive activity (peptides derived from αs1-casein, β-casein, α-lactalbumin, β-lactoglobulin), antilipemic activity (peptides derived from whey protein β-lactoglobulin as well as peptide IIAEK from lactostatin), antioxidant activity (peptides derived from α-lactalbumin) and antithrombotic activity (peptides derived from κ-casein, sheep κ-casein, sheep lactoferrin, α-lactalbumin) in humans. Some of these peptides are also said to enhance the immune response (peptides derived from α-β-κ-casein, whey protein and lactoferrin), reduce several inflammatory processes (peptides derived from corolase-digested casein and lactoferrin) and reduce oxidative stress (peptides derived from caseins such as YFYPEL) (Marcone *et al.*, 2017). Additional effects of milk peptides are presented in Fig. 1.

Milk protein components are well-described in the literature, however, information about ways in which animal genetics and animal nutrition can influence the concentrations of these constituents is rarely found. This review provides novel information about the use of animal genetics and animal nutrition to modulate the milk protein profile with special focus on human health and nutrition. Additionally, we compile information across different disciplines to address which milk proteins and peptides could be of interest for the development of new functional foods due to their potential beneficial effects on human health.

Milk bioactive peptides in human health

In milk, bioactive peptides are inactive within the native protein and are activated by (1) proteases present in milk, (2) digestive enzymes and enzymes produced by the gut microbiota and (3) enzymes secreted by microorganisms (i.e. starter cultures) and/or purified enzymes added to the milk during milk processing (Espejo-Carpio *et al.*, 2018). Because of their multiple putative benefits on human health, bioactive peptides from milk are commonly used in the formulation of functional foods, nutraceuticals and natural drugs (Muro *et al.*, 2011). The antimicrobial properties of some of these peptides provide a chemical barrier against bacterial growth, playing an important role in food quality and safety and extending food shelf life (Perez Espitia *et al.*, 2012). Associations between increased consumption of dairy products and improved metabolic health have been shown (McGregor and Poppitt, 2013). There is good reason for proposing that milk-derived bioactive peptides can contribute to human health, but at the same time it needs to be recognized that definitive cause and effect evidence is lacking. In the following sections, some relevant bioactive peptides from milk and their potential role in the prevention or attenuation of some human health related problems such as cardiovascular diseases, obesity, diabetes, immune system response and cancer are briefly discussed. Moreover, online Supplementary Tables S1 to S5 summarize the wide variety of bioactive peptides from milk and their proposed effects on human health.

Cardiovascular diseases

Cardiovascular diseases (CVD) have been associated with unhealthy diets rich in saturated fatty acids, trans fat (industrial hydrogenated-fats), salt and deficient consumption of fruits and vegetables (Mendis, 2017). In the past, a number of cardiovascular diseases have also been linked to full-fat dairy products (Lee *et al.*, 2018) or fat from ruminant's meat (Moreno-Indias *et al.*, 2012), however, other studies have shown how consumption of full-fat

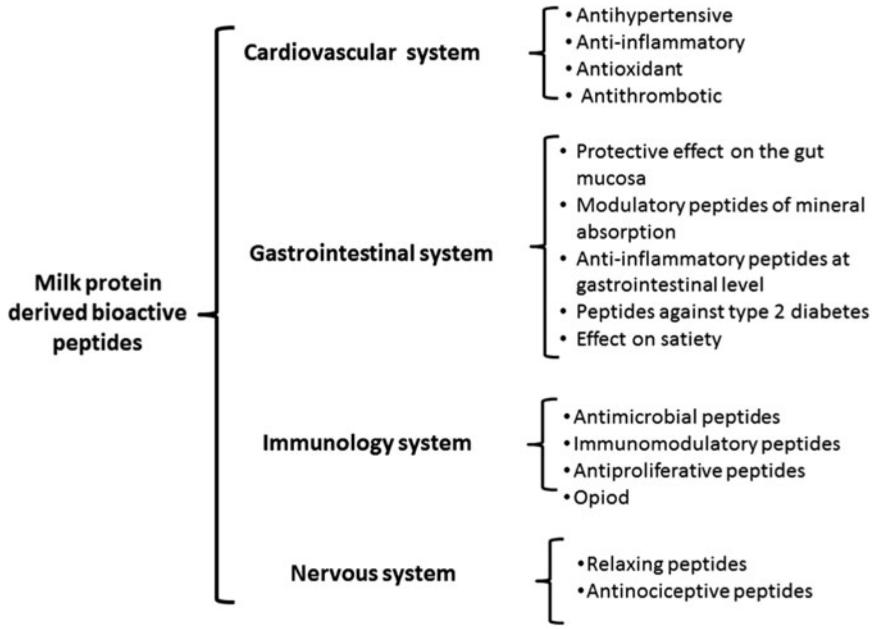

Fig. 1. Suggested impact of bioactive peptides derived from milk protein.
Note: Adapted from Korhonen (2009), Colombo *et al.* (2018), Gandini *et al.* (2017), Roman *et al.* (2017).

dairy foods, including milk, cheese and yogurt, may be inversely correlated to CVD incidence (Lee *et al.*, 2018). These studies also showed that bioactive peptides present in these foods might contribute to reduce CVD incidence. According to Ryan *et al.* (2011), antihypertensive activity is one of the most widely reported properties of milk bioactive peptides. Specifically, many milk bioactive peptides are able to reduce arterial pressure by inhibiting angiotensin-converting-enzyme (ACE). This enzyme plays a central role in the regulation of blood pressure. The enzymatic activity of renin on angiotensinogen produces angiotensin I, which is catalyzed by ACE to angiotensin II, a potent vasoconstrictor. Additionally, ACE inactivates bradykinin, an endothelium-dependent vasodilator, contributing to increased blood pressure (Marcone *et al.*, 2017). Thus, inhibition of this enzyme is considered as one of the strategies for the treatment of hypertension. Based on their antihypertensive properties, bioactive peptides from milk have gained attention in the formulation of new food products. For instance, Ong and Sha (2008) described how the addition of *Lactobacillus acidophilus* (LAFTI L®10) to cheese starter culture increases the production of κ-casein (f 96–102), αs1-casein (f 1–9), αs-casein (f 1–7), αs1-casein (f 1–7), αs1-casein (f 24–32) and β-casein (f 193–209). All of these bioactive peptides are believed to contribute to reduce arterial pressure by inhibiting ACE. Similarly, Sahingil *et al.* (2014) used *Lactobacillus helveticus* as adjunct culture in white-brined cheese to increase bioactive peptides with ACE inhibitory properties. Recently, a new bioactive peptide derived from bovine casein (YQKFPQYLQY) was reported to inhibit ACE and thereby decrease blood pleasure in rats (Xue *et al.*, 2018). In a meta-analysis performed by Cicero *et al.* (2013), isoleucine–proline–proline (IPP) and valine–proline–proline (VPP) were found to reduce blood pressure in humans. Because of this, *Lactobacillus helveticus* has been used recently to produce fermented milks rich in IPP and VPP sequences (Beltrán-Barrientos *et al.*, 2016).

Obesity control

Overweight and obesity are defined as an excessive fat accumulation that impairs health status (Bischoff *et al.*, 2016). In 2016, more than 1.9 billion adults were overweight (WHO, 2018). Satiety is one of the key factors in the prevention of obesity. Satiety is induced by nutrient ingestion and gastric distension, but especially by the release of anorexic substances such as cholecystokinin (CCK), a hormone synthesized in the small intestine (Morton *et al.*, 2006). Cholecystokinin secretion can be stimulated by glycomacropeptide (GMP) (Ricci-Cabello *et al.*, 2012), which is released when chymosin acts on κ-casein during the cheese making process (Madureira *et al.*, 2010). Glycomacropeptide constitutes 20–25% of the total proteins present in whey products such as whey powder, whey protein isolates and whey protein concentrates manufactured from cheese whey (Neelima *et al.*, 2013). Therefore, consumption of whey proteins as a natural source of GMP could be used to increase satiety, regulate food consumption and thereby reduce obesity.

Diabetes

Diabetes is a chronic disease that affects about 400 million people worldwide. This disease occurs either as a result of autoimmune destruction of the pancreatic beta cells that secrete insulin (type 1 diabetes) or as a result of insulin resistance in peripheral organs resulting in dysregulated insulin secretion and eventual failure of the beta cells (type 2 diabetes). According to Pasin and Comerford (2015) the benefits of dairy foods on insulin secretion and glycemic control are attributed to (1) the high amount of essential amino acids and bioactive peptides that stimulate insulin secretion, (2) the specific combinations of macronutrients and micronutrients, and (3) the unique probiotic strains and bioactive peptides found in cheese and yogurt. In rodents, bioactive peptides from milk (VAGTWY; Uchida *et al.*, 2011) and Gouda-type cheese (LPQNIPP; Uenishi *et al.*, 2012) can reduce plasma glucose concentrations. According to these authors specific peptides (β-CN f70–77; LPQNIPPL) can inhibit dipeptidyl-peptidase 4 (DPP-4), a key enzyme in the regulation of insulin (Ahrén *et al.*, 2004). It seems that consumption of whey products inhibits DPP-4 and in turn stimulates the secretion of insulin, which may have a beneficial effect in individuals with compromised insulin secretion. Further investigations on dairy products rich in bioactive peptides such as VAGTWY and LPQNIPP could be used to help control glycemic levels and insulin secretion in patients with diabetes.

Immune system

Milk bioactive peptides such as β-casokinins, β-casomorphin and lactoferricin B have been shown to stimulate the immune response (Clare and Swaisgood, 2000). Specifically, casein-derived peptides stimulate the proliferation of human lymphocytes and the phagocytic activity of macrophages (Korhonen and Pihlanto, 2007). Additionally, these bioactive peptides contribute to the protection against infections caused by bacteria, viruses and parasites, down-regulate autoimmune inflammatory processes and prevent rejection of transplants (Gauthier *et al*., 2006).

Lactoferrin, for instance, is a well-characterized milk bioactive protein. In addition to its ability to chelate iron, it participates in the activation of the nuclear factor kappa-light-chain-enhancer of activated B cells. In addition, lactoferrin inhibits granulopoiesis and up-regulates the activity of natural killer cells (Marcone *et al*., 2017). According to Dionysius and Milne (1997), lactoferrin has also bacteriostatic and bactericidal properties damaging the external membrane of gram-negative bacteria and causing the release of lipopolysaccharides that activates the immune response against bacterial infections.

Another milk-derived bioactive peptide, VPP, decrease monocyte adhesion to inflamed endothelia, reducing inflammation and contributing to the primary prevention of atherosclerosis (Aihara *et al*., 2009). Similarly, β-casomorphin inhibits ACE, which is the responsible enzyme for the inactivation of bradykinin, a potent vasoactive peptide that causes hypotension and in addition enhances the immune response (Clare and Swaisgood, 2000). Therefore, it seems that the consumption of dairy products rich in VPP indirectly enhances the immune response by avoiding inhibition of bradykinin by ACE.

Cancer

Several studies have suggested that bioactive peptides from milk whey might be beneficial for patients with cancer. Castro *et al.* (2009) observed that the expression of caspase-3 increased significantly in melanoma B16F10 cells when they were growing in media containing whey protein isolate. These caspases are responsible for the cleavage of key cellular proteins, such as the cytoskeletal proteins that lead to the typical morphological changes observed in cells undergoing apoptosis. Therefore, it seems that the consumption of whey products may increase the expression of caspase-3 and contribute to the protection of the organism against tumorigenesis (Zhao *et al*., 2018). However, these results are only based on *in vitro* experiments.

β-casomorphins are opioid-like peptides purified from bovine β-casein hydrolysates. The presence of proline confers high proteolytic resistance to these bioactive peptides, so they can bypass the stomach without degradation. It has been described that β-casomorphins as well as κ-casecidin have antitumoral properties in murine models as well as *in vitro* studies, due to the fact that they can induce cytotoxicity against malignant cells (leukemic cells, melanoma cells, human breast cancer cells; Sah *et al*., 2015). These opioid-like peptides can pass the gut-blood barrier and get access to their target sites. As shown by Tidona *et al.* (2009), yogurts containing β-casomorphins and κ-casecidin decreased tumor cell proliferation, associating yoghurt consumption with reduced incidence of colon cancer.

Strategies to modify the milk protein composition: animal genetics and animal nutrition management

The dairy foods industry can use diverse technological processes to modify the content of specific bioactive peptides in milk (addition of purified enzymes, protein precursors, or artificial milk proteins). However, consumer's demands are currently shifting to natural and less processed food products (Roman *et al*., 2017). In this respect, animal genetics and nutrition may become important tools to obtain natural products rich in specific bioactive peptides. Of these, animal genetics is probably the most effective and stable way to increase the content of beneficial milk bioactive peptides for human consumption. However, increasing bioactive peptides in milk through animal genetics requires time and an important economic investment (Gandini *et al*., 2017). On the other hand, nutritional strategies are a fast and practical way to improve the content of bioactive peptides in milk (Colombo *et al*., 2018).

Milk protein profile and milk protein concentration differ between animal species, and are significantly influenced by individual animal genetics and animal nutrition (Walker *et al*., 2004). Unfortunately, there are only a few studies that extend these relationships to changes in milk bioactive peptides. In the following sections, we review how animal genetics and animal nutrition are efficient tools to modify milk protein content and major milk proteins such as different caseins fractions and β-lactoglobulin concentrations. In addition, some of the studies performed in both fields in order to alter milk bioactive peptides are also described.

Animal genetics

A major objective in animal genetics is to find genes related to specific traits that can be implemented in breeding programs. For instance, milk protein genes such as *CSN1S1*, *CSN2*, *CSN3*, *LGB* can be used to either improve or modify the milk protein profile and therefore the amount of precursors for bioactive peptides in milk. Caseins, mainly αs1-casein, αs2-casein, β-casein and κ-casein, represent about 78% of the proteins present in milk and are encoded by *CSN1S1*, *CSN1S2*, *CSN2* and *CSN3*, respectively (Caroli *et al*., 2009). The abundance of polymorphisms on these genes varies among ruminant species and breeds (online supplementary table S7). Variations in the abundance of these polymorphisms are influenced by different phosphorylation and glycosylation rates in the peptide chains, which define not only those specific amino acids sequences, but also the final milk protein profile and composition (Albenzio *et al*., 2017). For instance, Heck *et al.* (2009) described that selection of cows that show both β-lactoglobulin genotype B and the β–κ-casein haplotype A2B resulted in the production of milk with higher protein yields. In Sarda goats, Vacca *et al.* (2014) showed that animals expressing the CSN1S1 BB and AB genotypes had the highest milk protein percentage (4.41 and 4.40%, respectively). However, those goats expressing the CSN1S1 EF genotype showed the highest milk yield (1.20 kg/d) and total protein content (43.6 g/d). Similar results were observed in East Friesian dairy sheep by Giambra *et al.* (2014). In this study, authors showed how sheep expressing the CSN1S1 C´´C´´ genotype had the highest milk yield (310 kg/lactation) and protein percentage in milk (5.17%). These are clear examples of how breeding programs can be used to improve milk protein yield and quality and, therefore, could be used in future studies to increase the amount of precursors for milk bioactive peptides beneficial for human health. Tacoma *et al.* (2016) reported differences in the content of milk bioactive peptides related to the immune system between Holstein and Jersey cows. Specifically, authors showed lower concentrations of ecto-nucleotide pyrophosphatase and chitinase domain-containing protein 1, and higher lactoferrin concentrations in Holstein cows than Jersey cows. By contrast, contents of osteopontin, lactoperoxidase, and growth factors including insulin-like growth factor (IGF) and transforming growth factor-β did not differ between breeds.

With the recent development of analytical tools and genomic resources, it is possible to identify Quantitative Trait Loci (QTL) and thus, study the influence of multiple genes on biological traits and milk composition (Dux *et al.*, 2018). Thus, QTL could contribute to find genes that can be used in breeding programs *via* marker-assisted selection. For example, specific QTL can affect different traits in dairy cows such as milk protein percentage (chromosomes 3, 6 and 20) and milk protein yield (chromosomes 1, 3, 6, 9, 14 and 20). QTL encoding for protein yield have been detected on BTA6 and BTA20 (*Bos taurus* autosome 6 and 20) (Khatkar *et al.*, 2004).

There are several factors to consider when using QTL information. The data are scattered in many publications, which used different statistical analysis methods. In addition, traits are defined and measured in many different ways, as there is a lack of standardized nomenclature to define similar traits for meaningful comparisons. In order to facilitate the comparison of QTL results across experiments and compile all published QTL information, the Animal QTL database, has been developed as a public repository. This database is the central source for QTL and genotype/phenotype association data for animal genetic researchers (Hu and Reecy, 2007). Additional QTL information obtained from the Animal QTL database has been described in caseins (online supplementary table S6). It is noteworthy to mention that in addition to QTL mapping, a number of other strategies for gene discovery such as genomics, proteomics and transcriptomics need to be integrated to understand the genetic architecture of specific traits (Khatkar *et al.*, 2004). In order to implement this strategy, big data analysis as well as well-characterized populations with production records will be necessary, meaning that such approaches are likely to be fruitful only in the medium- to long-term.

Animal nutrition

Feed energy is one of the most important factors to consider in the feed formulation for ruminants, having a direct impact on the milk protein profile (Walker *et al.*, 2004). According to Mackle *et al.* (1999), cows raised in extensive conditions produce milk with increased casein:whey ratio compared to cows under intensive production systems, where animals are usually fed with forages or cereal grain-based diets (5.2 *vs.* 4.8 casein:whey ratio, respectively). On the other hand, energy intake directly influences the normal function of the mammary gland in dairy ruminants, which in turn affects milk yield and milk composition (Lérias *et al.*, 2013). Particularly, low energy diets causes increased milk fat content and decreased milk yield and protein content in both large and small ruminants (Grainger *et al.*, 2009; Lérias *et al.*, 2013). In a further study, goats fed only with wheat straw for 21 d reduced about 15 to 20% of body weight compared to those fed a balanced diet. In these animals, reduced energy intake altered fatty acid and protein composition of the mammary gland (Hernández-Castellano *et al.*, 2016*b*; Palma *et al.*, 2017). Most of these changes were related to increased catabolism and fat mobilization processes in the goats receiving low energy diets, which caused an important milk yield reduction in this group compared to those fed a balance diet. Unfortunately, we have found no studies describing changes in the content of bioactive peptides in milk caused by either feed restriction or increased energy intake in dairy animals.

In addition to feed energy, protein content is another important factor to consider in ruminants diets. Dietary protein provides N for microbial protein synthesis in the rumen. Microbial protein constitutes more than 50% of the protein digested in cows (Sok *et al.*, 2017). Both microbial protein and dietary bypass supply amino acids for different animal processes such as body maintenance, growth, reproduction, and milk protein synthesis (Lee *et al.*, 2015). Consequently, manipulation of ruminal microbiota has been intensively investigated to improve the rumen metabolism efficiency and ultimately to increase milk yield and milk protein percentage (Patra and Saxena, 2011). However, further studies are necessary in order to test if the content of specific milk bioactive peptides beneficial for human health can be altered by increasing protein intake in dairy animals.

An important factor to consider in diets for ruminants is the forage:concentrate ratio. This ratio not only affects the dietary protein content of the diet, but also influences the milk protein percentage. As described by Tacoma *et al.* (2016) the forage:concentration ratio actively modifies the composition of the milk protein profile despite genetic differences between cow breeds. Specifically, these authors described that both Holstein and Jersey dairy cows fed a diet with a forage to concentrate ratio of 55:45, had similar low-abundance protein profiles. Interestingly, other immune bioactive proteins such as osteopontin, lactoperoxidase and several growth factors including insulin-like growth factor-1 and transforming growth factor-β, were also detected in similar abundances in both breeds.

Reducing the proportion of forage in favor of increased rapid fermentable carbohydrates (starch) promotes the production of propionate and microbial protein synthesis, which in turn increases milk protein percentage in dairy cows (Jenkins and McGuire, 2006). Similar results were also described by Min *et al.* (2005) in dairy goats supplemented with concentrate compared to those fed without concentrate. These authors observed how goats supplemented with either 0.66 or 0.33 kg of concentrate per extra kg of milk produced above 1.5 kg/d showed higher milk yield (3.84 and 3.50 kg of milk/d, respectively) and higher milk protein percentage (3.08 and 3.08%, respectively) than those animals fed without concentrate supplementation (2.98 kg/d and 2.90%, respectively). Similar results on specific milk bioactive peptides do not exist, however, based on the available work, it seems that diets containing high starch levels may be used to increase precursors for milk bioactive peptides. However, feeding diets high in starch and low in fiber in order to reach the dietary requirements of the high yielding dairy cows increases the risk of either acute or subacute ruminal acidosis. Therefore, the inclusion of concentrates in ruminant diets need to be precisely supervised and adjusted according to the needs and requirements of the animal. With increasing use of technologies capable of constantly monitoring feed quality, feed intake, rumen function and milk yield and composition, this precision dairy farming approach has great promise for the future.

Not only forage proportion but type of forage (legumes or grasses) used in the diet formulation influences milk protein yield and composition. In a recent meta-analysis, Johansen *et al.* (2017) analyzed differences between cows fed either legume-based or grass-based diets. These authors reported that cows fed legume-based diets reduced fat percentage compared to those fed grass-based diets (3.89 *vs.* 4.03%, respectively). Only cows fed with red clover reduced milk protein percentage (3.08%) compared to other legume-based diets such as white clover, lucerne and birds trefoil (3.18, 3.13 and 3.13%, respectively) or grass-based diets (3.16%). The presence of polyphenol oxidases in red clover may have affected the bioavailability of sulfur-containing amino acids, limiting the conversion of dietary N into milk urea N. Therefore, it seems that the type of forage can, to a limited extent, limit the amount of N available for the synthesis of milk proteins and in turn affect the concentration of milk bioactive peptides in milk. When animals are fed on high quality forages rich in N (25–35 g of N/kg DM), about 56 to 65% of the total protein content become rapidly soluble during mastication. Subsequently, a large amount of soluble protein is degraded by rumen microbes resulting in additional levels of ammonia (20–35%), which is later excreted in urine and milk (Patra and Saxena, 2011).

Milk protein composition is also affected by the ratio between rumen degradable protein (RDP) and rumen undegradable protein (RUP). Tacoma *et al.* (2017) compared the milk composition in cows fed either high RDP:RUP ratio diets (62.4:37.6) or low RDP:RUP ratio diets (48.7:51.3). Cows receiving the high RDP: RUP ratio diet showed increased milk urea N and plasma urea N concentrations (15.7 and 1.02 mmol/l, respectively) than those fed low RDP:RUP diets (14.6 and 0.98 mmol/l, respectively). However, animals fed low RDP:RUP ratio diets showed decreased β-casein, κ-casein, and total milk casein concentrations (15.8, 5.39, 36.3 mg/ml, respectively) compared to those fed high RDP:RUP ratio diets (16.3, 5.61, 37.8 mg/ml, respectively). Besides the RDP:RUP ratio, the dietary content of specific amino acids such as lysine (lys) and methionine (met) is considered essential as they limit milk protein synthesis and milk yield (NRC, 2001). In this field, Awawdeh (2016) showed how dairy cows supplemented with rumen-protected met and lys (30 g/d of met and 25 g/d of lys) increased milk yield (29.7 kg/d) and milk protein percentage (3.14%) compared to either cows only supplemented with 30 g/d of rumen-protected met (27.8 kg/d and 3.09%) or cows without supplementation (28.0 kg/d and 3.09%). According to these results, the dietary inclusion of essential amino acids such as met or lys influences not only milk yield, but also milk protein composition and could be used in future studies to increase casein, whey and bioactive peptides levels in milk.

Dietary fat content also influences milk protein percentage. De Peters and Cant (1992) showed how the dietary inclusion of either 0, 3.5 or 7% of fat (DM basis) proportionally decreased casein percentage in milk (2.50, 2.4, and 2.3%, respectively). These results are in agreement with several other studies where increased content of fat in the diet was directly correlated to decreased milk protein percentage in dairy cows (Cant *et al.*, 1993; Rabbie *et al.*, 2012). As described by Wu and Huber (1994), when part of the fermentable carbohydrates is replaced by fat, rumen microbial protein yield decreases and the use of amino acids for gluconeogenesis increases. Both effects significantly reduce the availability of amino acids for milk protein synthesis, thereby decreasing milk protein percentage. Regarding milk bioactive peptides, Scuderi (2018) reported how the dietary inclusion of grape marc, a viticulture by-product increased the abundance of apolipoprotein E, clusterin, butyrophilin subfamily 1 member A1, serum amyloid A protein, synaptic vesicle membrane protein VAT-1 homolog, and protein KRI1 homolog in Holstein dairy cows.

Other factors influencing milk protein composition

Besides animal genetics and animal nutrition, other factors such as environmental conditions or lactation stage may influence animal physiology and thereby milk yield and composition. As described by Bernabucci *et al.* (2015), season affects milk composition in Holstein cows by decreasing fat (from 3.8 to 3.2%), protein (from 3.5 to 3.3%), and total solids (from 12.6 to 11.9%) percentages from winter to summer. The decreased milk protein percentage also reduces the concentration of several milk protein fractions from winter to summer, specifically α-casein (12.8 and 9.9 g/l, respectively), β-casein (9.8 and 7.9 g/l, respectively), and κ-casein (4.1 and 3.7 g/l, respectively). On the contrary, increased temperature can cause heat stress, enhancing skeletal muscle catabolism and inducing increased blood urea N, which in turn leads to increased urea N concentration in milk (Hao *et al.*, 2016). These findings were also observed by Cowley *et al.* (2015) in Holstein cows subjected to heat stress, where milk yield, protein concentration and casein concentration all decreased (21.98 L/d, 34.1 g/l and 28.1 g/l, respectively) compared to cows under normal conditions (temperature-humidity index <70; 17.03 L/d, 33.1 g/l, 26.8 g/l, respectively).

Milk protein composition can also be influenced by the physiological state of the cow. Ho *et al.* (2010) observed an extensive caseinolysis and active generation of peptides in cows following dry-off. In this study, 202 novel β-casein-derived peptides were identified. From those, five were homologous with opioid agonists peptides (β-casein peptides 60–68), and immunomodulators peptides (β-casein peptides 191–209, 193–209, 199–208 and 193–202). These results provide useful information that can be implemented in dairy farms in order to increase the concentration of bioactive peptides in milk within short intervals following drying-off in cows.

Prospective opportunities for future

Dairy products are one of the most important sources of high biological value proteins and bioactive peptides. As described above, a considerable amount of evidence supports the notion that bioactive peptides and proteins from milk may have effects on a range of physiological processes, and by doing so potentially aid in the prevention of diverse human diseases. However, most of these results are based on *in vitro* studies and the real effect of these milk-derived peptides in human health remains unclear. Consequently, there is a need for long-term *in vivo* studies to provide stronger data supporting the positive effects of bioactive peptides from milk on human health. Many studies have been performed using bioactive peptides isolated from milk, without considering the impact of other milk components in the absorption of these peptides. Future studies should also focus on the bioavailability of specific beneficial bioactive peptides in consumed milk or dairy products.

It is clear that genetics and nutrition can be harnessed to modify diverse milk components, including protein content and yield. Unfortunately, very few studies have examined more specific effects on individual bioactive peptides. Therefore, future studies should combine knowledge from both food science and animal science fields in order to improve the healthiness of milk protein and milk bioactive peptides. The present review may set the basis for optimizing yields of bioactive peptides in milk and developing new functional foods incorporating these bioactive dairy peptides.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0022029919000384

References

Ahrén B, Gomis R, Standl E, Mills D and Schweizer A (2004) Twelve- and 52-week efficacy of the dipeptidyl peptidase IV inhibitor LAF237 in metformin treated patients with type 2 diabetes. *Diabetes Care* **27**, 2874–2880.

Aihara K, Ishii H and Yoshida M (2009) Casein-derived tripeptide, Val-Pro-Pro (VPP), modulates monocyte adhesion to vascular endothelium. *Journal of Atherosclerosis and Thrombosis* **16**, 594–603.

Albenzio M, Santillo A, Caroprese M, della Malva A and Marino R (2017) Bioactive peptides in animal food products. *Foods (Basel, Switzerland)* **6**, 35–49.

Augustin M and Udabage P (2007) Influence of processing on functionality of milk and dairy proteins. *Advances in Food and Nutrition Research* **53**, 1–38.

Awawdeh M (2016) Rumen-protected methionine and lysine: effects on milk production and plasma amino acids of dairy cows with reference to metabolisable protein status. *Journal of Dairy Research* **83**, 151–155.

Beltrán-Barrientos LM, Hernández-Mendoza A, Torres Llanez MJ, González-Córdova AF and Vallejo-Córdoba B (2016) Fermented milk as antihypertensive functional food. *Journal of Dairy Science* **99**, 4099–4110.

Bernabucci U, Basiricò L, Morera P, Dipasquale D, Vitali A, Cappelli FP and Calamari LUIGI (2015) Effect of summer season on milk protein fractions in Holstein cows. *Journal of Dairy Science* **98**, 1815–1827.

Bischoff SC, Boirie Y, Cederholm T, Chourdakis M, Cuerda C, Delzenne NM and Koletzko B (2016) Towards a multidisciplinary approach to understand and manage obesity and related diseases. *Clinical Nutrition* **36**, 917–938.

Bonanno A (2012) Some like it healthy: demand for functional and conventional yogurts in the Italian market. *Agribusiness* **28**, 67–85.

Cant JP, DePeters EJ and Baldwin RL (1993) Mammary amino acid utilization in dairy cows fed fat and its relationship to milk protein depression. *Journal of Dairy Science* **76**, 762–774.

Caroli A, Chessa S and Erhardt G (2009) Invited review: milk protein polymorphisms in cattle: effect on animal breeding and human nutrition. *Journal of Dairy Science* **92**, 5335–5352.

Castro G, Maria D, Bouhallab S and Sgarbieri V (2009) *In vitro* impact of a whey protein isolate (WPI) and collagen hydrolysates (CHs) on B16F10 melanoma cells proliferation. *Journal of Dermatological Science* **56**, 51–57.

Cicero A, Aubin F, Azais-Braesco V and Borghi C (2013) Do the lactotripeptides isoleucine—proline—proline and valine—proline—proline reduce systolic blood pressure in European subjects? A meta-analysis of randomized controlled trials. *American Journal of Hypertension* **26**, 442–449.

Claeys WL, Verraes C, Cardoen S, De Block J, Huyghebaert A, Raes K, Dewettinck K and Herman L (2014) Consumption of raw or heated milk from different species: an evaluation of the nutritional and potential health benefits. *Food Control* **42**, 88–201.

Clare D and Swaisgood H (2000) Bioactive milk peptides: a prospectus. *Journal of Dairy Science* **83**, 1187–1195.

Colombo M, Todorov SD, Eller M and Nero LA (2018) The potential use of probiotic and beneficial bacteria in the Brazilian dairy industry. *Journal of Dairy Research* **85**, 487–496.

Cowley FC, Barber DG, Houlihan AV and Poppi DP (2015) Immediate and residual effects of heat stress and restricted intake on milk protein and casein composition and energy metabolism. *Journal of Dairy Science* **98**, 2356–2368.

De Peters E and Cant JP (1992) Nutritional factors influencing the nitrogen composition of bovine milk: a review. Methionine and lysine on casein in milk when diets high in fat or concentrate are fed. *Journal of Dairy Science* **75**, 2043–2070.

Dionysius D and Milne J (1997) Antibacterial peptides of bovine lactoferrin: purification and characterization. *Journal of Dairy Science* **80**, 667–674.

Dux M, Muranowicz M, Siadkowska E, Robakowska-Hyżorek D, Flisikowski K, Bagnicka E and Zwierzchowski L (2018) Association of SNP and STR polymorphisms of insulin-like growth factor 2 receptor (IGF2R) gene with milk traits in Holstein-Friesian cows. *Journal of Dairy Research* **85**, 138–141.

Espejo-Carpio F, Pérez-Gálvez R, Guadix A and Guadix E (2018) Artificial neuronal networks (ANN) to model the hydrolysis of goat milk protein by subtilisin and trypsin. *Journal of Dairy Research* **85**, 339–346.

Food and Agriculture Organization of the United Nations (FAO) (2013) Milk and dairy product composition. In Milk and dairy products on human nutrition. Rome, Italy.

Galán-Malo P, Valares JA, Langa V, Razquin P and Mata L (2014) Determination of IgG levels in bulk ewe's milk. *Small Ruminant Research* **119**, 156–160.

Gandini G, Turri F, Rizzi R, Crotta M, Minozzi G and Pizzi F (2017) Economic evaluation of genetic improvement in local breeds: the case of the Verzaschese goat. *Italian Journal of Animal Science* **16**, 199–207.

Gauthier SF, Pouliot Y and Saint-Sauveur D (2006) Immunomodulatory peptides obtained by the enzymatic hydrolysis of whey proteins. *International Dairy Journal* **16**, 1315–1323.

Gerosa S and Skoet J (2012) Milk availability trends in production and demand and medium-term outlook. Food and Agriculture Organization of the United Nations FAO. Rome, Italy.

Giacometti J and Buretić-Tomljanović A (2017) Peptidomics as a tool for characterizing bioactive milk peptides. *Food Chemistry* **230**, 91–98.

Giambra IJ, Brandt G and Erhardt G (2014) Milk protein variants are highly associated with milk performance traits in East Friesian Dairy and Lacaune sheep. *Small Ruminant Research* **121**, 382–394.

Givens I (2018) Milk and dairy foods: implications for cardiometabolic health. *Cardiovascular Endocrinology and Metabolism* **7**, 56–57.

Grainger C, Auldist MJ, O'Brien G, Macmillan KL and Culley C (2009) Effect of type of diet and energy intake on milk production of Holstein-Friesian cows with extended lactations. *Journal of Dairy Science* **92**, 1479–1492.

Hao LY, Wang J, Sun P and Bu DP (2016) The effect of heat stress on the metabolism of dairy cows: updates and review. *Austin Journal of Nutrition and Metabolism* **3**, 10363–11041.

Heck J, Schennink A, van Valenberg H, Bovenhuis H, Visker M, van Arendonk J and van Hooijdonk A (2009) Effects of milk protein variants on the protein composition of bovine milk. *Journal of Dairy Science* **92**, 1192–1202.

Hernández-Castellano LE, Almeida AM, Castro N and Argüello A (2014) The colostrum proteome, ruminant nutrition and immunity: a review. *Current Protein and Peptide Science* **15**, 64–74.

Hernández-Castellano LE, Ferreira AM, Nanni P, Grossmann J, Argüello A, Capote J, Cai G, Lippolis J, Castro N and de Almeida AM (2016*a*) The goat (*Capra hircus*) mammary gland secretory tissue proteome as influenced by weight loss: a study using label free proteomics. *Journal of Proteomics* **145**, 60–69.

Hernández-Castellano LE, Almeida A, Renaut J, Argüello A and Castro N (2016*b*) A proteomics study of colostrum and milk from the two major small ruminant dairy breeds from the Canary Islands: a bovine milk comparison perspective. *Journal of Dairy Research* **83**, 366–374.

Ho CH, Chang CJ, Liu WB, Peh HC, Chen SE, Chen HY, Ho TH, Chen MT and Nagahata H (2010) *In situ* generation of milk protein-derived peptides in drying-off cows. *Journal of Dairy Research* **7**, 487–497.

Hu Z-L and Reecy J (2007) Animal QTLdb: beyond a repository. A public platform for QTL comparisons and integration with diverse types of structural genomic information. *Mammalian Genome* **18**, 1–4.

Jenkins TC and McGuire MA (2006) Major advances in nutrition: impact on milk composition. *Journal of Dairy Science* **89**, 1302–1310.

Johansen M, Lund P and Weisbjerg MR (2017) Feed intake and milk production in dairy cows fed different grass and legume species: a meta-analysis. *Animal: An International Journal of Animal Bioscience* **12**, 66–75.

Khatkar MS, Thomson PC, Tammen I and Raadsma HW (2004) Quantitative trait loci mapping in dairy cattle: review and meta-analysis. *Genetics, Selection, Evolution* **36**, 163–190.

Khatoon JA and Josh VK (1987) Effect of different heat treatments on protease-peptone and non protein nitrogen contents of goat milk. *Indian Journal of Dairy Science* **40**, 243–245.

Korhonen H (2009) Milk-derived bioactive peptides: from science to applications. *Journal of Functional Foods* **1**, 177–187.

Korhonen H and Pihlanto A (2007) Technological options for the production of health-promoting proteins derived from milk and colostrum. *Current Pharmaceutical Design* **13**, 829–843.

Lee C, Giallongo F, Hristov AN, Lapierre H, Cassidy TW, Heyler KS and Parys C (2015) Effect of dietary protein level and rumen-protected amino acid supplementation on amino acid utilization for milk protein in lactating dairy cows. *Journal of Dairy Science* **98**, 1885–1902.

Lee M, Lee H and Kim J (2018) Dairy food consumption is associated with a lower risk of the metabolic syndrome and its components: a systematic review and meta-analysis. *British Journal of Nutrition* **120**, 373–384.

Lérias JR, Hernández-Castellano LE, Morales-delaNuez A, Araújo SS, Castro N, Argüello A and Almeida AM (2013) Body live weight and milk production parameters in the Majorera and Palmera goat breeds from the Canary Islands: influence of weight loss. *Tropical Animal Health and Production* **45**, 1731–1736.

Mackle TR, Bryant AM, Petch SF, Hooper RJ and Auldist MJ (1999) Variation in the composition of milk protein from pasture-fed dairy cows in late lactation and the effect of grain and silage supplementation. *New Zealand Journal of Agricultural Research* **42**, 147–154.

Madureira AR, Tavares T, Gomes AMP, Pintado ME and Malcata FX (2010) Invited review: physiological properties of bioactive peptides obtained from whey proteins. *Journal of Dairy Science* **93**, 437–455.

Marcone S, Belton O and Fitzgerald D (2017) Milk derived bioactive peptides and their health promoting effects: a potential role in atherosclerosis. *British Journal of Clinical Pharmacology* **83**, 152–162.

Mather IH (2000) A review and proposed nomenclature for major proteins of the milk-fat globule membrane. *Journal of Dairy Science* **83**, 203–247.

McGregor RA and Poppitt SD (2013) Milk protein for improved metabolic health: a review of the evidence. *Nutrition and Metabolism* **10**, 46–59.

Mendis S (2017) Global progress in prevention of cardiovascular disease. *Cardiovascular Diagnosis and Therapy* **7**, 32–38.

Min B, Hart S, Sahlu T and Satter L (2005) The effect of diets on milk production and composition and on lactation curves in pastured dairy goats. *Journal of Dairy Science* **88**, 2604–2615.

Moreno-Indias I, Morales-delaNuez A, Hernández-Castellano LE, Sánchez-Macías D, Capote J, Castro N and Argüello A (2012) Docosahexaenoic acid in the goat kid diet: effects on immune system and meat quality. *Journal of Animal Science* **90**, 3729–3738.

Morton G, Cummings D, Baskin D, Barsh G and Schwartz M (2006) Central nervous system control of food intake and body weight. *Nature* **443**, 289–295.

Murgiano L, Timperio AM, Zolla L, Bongiorni S, Valentini A and Pariset L (2009) Comparison of milk fat globule membrane (MFGM) proteins of Chianina and Holstein cattle breed milk samples through proteomics methods. *Nutrients* **1**, 302–315.

Muro U, Álvarez F, Rodriguez R, Cuenca A and Jurado T (2011) Review: production and functionality of active peptides from milk. *Food Science and Technology International* **17**, 293–317.

National Research Council (NRC) (2001) *Nutrient Requirements of Dairy Cattle*, 7th Edn. Washington, DC: National Academy Press.

Neelima SR, Rajput Y and Mann B (2013) Chemical and functional properties of glycomacropeptide (GMP) and its role in the detection of cheese whey adulteration in milk: a review. *Dairy Science and Technology* **93**, 21–43.

Olaniyan B, Zhao X and Mustafa A (2013) Determination of caprine serum albumin in milk using bromocresol green dye. *Open Journal of Animal Sciences* **3**, 261–267.

Ong L and Shah NP (2008) Release and identification of angiotensin-converting enzyme-inhibitory peptides as influenced by ripening temperatures and probiotic adjuncts in Cheddar cheeses. *Journal of Food Science and Technology* **41**, 1555–1566.

Palma M, Alves SP, Hernández-Castellano LE, Capote J, Castro N, Argüello A, Matzapetakis M, Bessa RJB and de Almeida AM (2017) Mammary gland and milk fatty acid composition of two dairy goat breeds under feed-restriction. *Journal of Dairy Research* **84**, 264–271.

Pasin G and Comerford KB (2015) Dairy foods and dairy proteins in the management of type 2 diabetes: a systematic review of the clinical evidence. *Advances in Nutrition* **6**, 245–259.

Patra AK and Saxena J (2011) Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. *Journal of the Science of Food and Agriculture* **91**, 24–37.

Pereira PC (2014) Milk nutritional composition and its role in human health. *Nutrition* **30**, 619–627.

Perez Espitia PJ, Soares NFF, Coimbra JSR, Andrade NJ, Cruz RS and Medeiros EAA (2012) Bioactive peptides: synthesis, properties, and applications in the packaging and preservation of food. *Comprehensive Reviews in Food Science and Food Safety* **11**, 187–204.

Rabiee AR, Breinhild K, Scott W, Golder HM, Block E and Lean IJ (2012) Effect of fat additions to diets of dairy cattle on milk production and components: a meta-analysis and meta-regression. *Journal of Dairy Science* **95**, 3225–3247.

Ricci-Cabello I, Olalla Herrera M and Artacho R (2012) Possible role of milk-derived bioactive peptides in the treatment and prevention of metabolic syndrome. *Nutrition Reviews* **70**, 241–255.

Roman S, Sanchez-Siles LM and Siegrist M (2017) The importance of food naturalness for consumers: results of a systematic review. *Trends in Food Science and Technology* **67**, 44–57.

Ryan J, Ross R, Bolton D, Fitzgerald G and Stanton C (2011) Bioactive peptides from muscle sources: meat and fish. *Nutrients* **3**, 765–791.

Sah BNP, Vasiljevic T, Mckechnie S and Donkor ON (2015) Identification of anticancer peptides from bovine milk proteins and their potential roles in management of cancer: a critical review. *Comprehensive Reviews in Food Science and Food Safety* **14**, 123–138.

Sahingil D, Hayaloglu A, Kirmaci H, Özer B and Simsek O (2014) Changes of proteolysis and Angiotensin-I converting enzyme-inhibitory activity in white-brined cheese as affected by adjunct culture and ripening temperature. *Journal of Dairy Research* **81**, 394–402.

Scuderi RH (2018) The effects of diet on the bovine milk proteome. Graduate College Dissertations and Theses. 846. Available at https://scholarworks.uvm.edu/graddis/846.

Sok M, Ouellet DR, Firkins JL, Pellerin D and Lapierre H (2017) Amino acid composition of rumen bacteria and protozoa in cattle. *Journal of Dairy Science* **100**, 5241–5249.

Tacoma R, Fields J, Ebenstein DB, Lam YW and Greenwood SL (2016) Characterization of the bovine milk proteome in early-lactation Holstein and Jersey breeds of dairy cows. *Journal of Proteomics* **130**, 200–210.

Tacoma R, Fields J, Ebenstein DB, Lam YW and Greenwood SL (2017) Ratio of dietary rumen degradable protein to rumen undegradable protein affects nitrogen partitioning but does not affect the bovine milk proteome produced by mid-lactation Holstein dairy cows. *Journal of Dairy Science* **100**, 7246–7261.

Tidona F, Criscione A, Guastella AM, Zuccaro A, Bordonaro S and Marletta D (2009) Bioactive peptides in dairy products. *Italian Journal of Animal Science* **8**, 315–340.

Uchida M, Oshiba Y and Mogami O (2011) Novel dipeptidyl peptidase-4-inhibiting peptide derived from β-lactoglobulin. *Journal of Pharmacological Sciences* **117**, 63–66.

Uenishi H, Kabuki T, Seto Y, Serizawa Y and Nakajima D (2012) Isolation and identification of casein-derived dipeptidyl-peptidase 4 (DPP-4)-inhibitory peptide LPQNIPPL from gouda-type cheese and its effect on plasma glucose in rats. *International Dairy Journal* **22**, 24–30.

Vacca G, Dettori M, Piras G, Manca F, Paschino P and Pazzola M (2014) Goat casein genotypes are associated with milk production traits in the Sarda breed. *Animal Genetics* **45**, 723–731.

Walker G, Dunshea B and Doyle A (2004) Effects of nutrition and management on the production and composition of milk fat and protein: a review. *Australian Journal of Agricultural Research* **55**, 1009–1028.

World Health Organization (2018) Available at https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight.

Wu Z and Huber JT (1994) Relationship between dietary fat supplementation and milk protein concentration in lactating cows: a review. *Livestock Production Science* **39**, 141–155.

Xue L, Wang X, Hu Z, Wu Z, Wang L, Wang H and Yang M (2018) Identification and characterization of an angiotensin-converting enzyme inhibitory peptide derived from bovine casein. *Peptides* **99**, 161–168.

Zhao R, Kaakati R, Lee AK, Liu X, Li F and Li CY (2018) Novel roles of apoptotic caspases in tumor repopulation, epigenetic reprogramming, carcinogenesis, and beyond. *Cancer and Metastasis Reviews* **37**, 227–236.