REVIEW 5 MARCH 2014

Milk protein: new insights into quality and function

The quality of milk protein is higher than previously estimated.
Milk protein has more functions than previously known.

SUMMARY

Milk protein consists of the major whey and casein fractions, numerous minor bioactive peptides, and essential (indispensable) and non-essential (dispensable) amino acid building blocks. The traditional anabolic functions of protein remain important, yet recent research has identified additional metabolic and neutraceutical roles of the biologically active peptides of milk protein. At present the amino acids determine the quality of the milk protein – something largely underestimated for animal-source foods by common assessment methods. The quality of milk protein is higher than previously believed.

THE COMPOSITION OF MILK PROTEIN

Milk is an important source of protein in the human diet. The protein in milk has a soluble whey and a less insoluble casein fraction. Whey makes up about 20% (w/w) of milk protein and is rich in branched chain amino acids (leucine, iso-leucine and valine). Casein has'n higher proportion of histidine, methionine and phenylalanine and constitutes about 80% (w/w) of milk protein (Boye et al, 2012). Milk also contains bioactive peptide components with unique functions. Some of these are listed in Table 1.

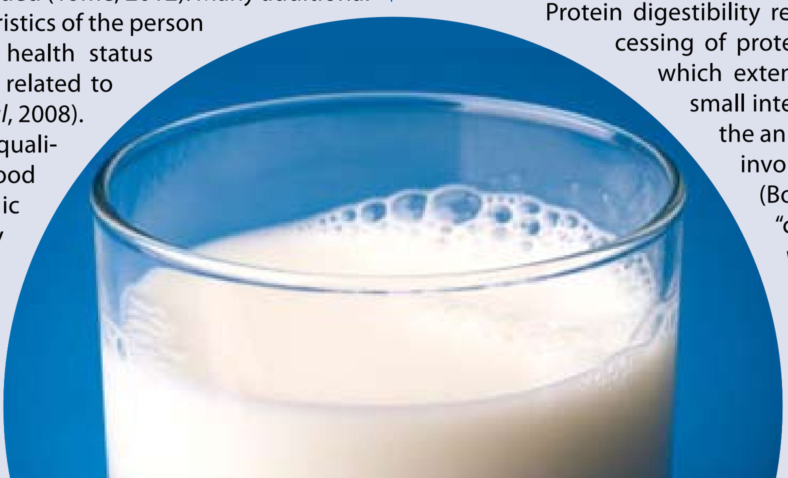
Amino acids are the building blocks of protein. Traditionally amino acids are classified as essential or non-essential. The essential or indispensable amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine and valine) must be provided by the diet, whilst the others (cysteine, tyrosine, glycine, argenine, glutamine, proline, asparagine, glutamic acid, alanine and serine) can be produced in the body and are hence called dispensable. The first six in this latter list plus taurine may become essential under special conditions (Boye 2013). The biological value of a food protein is determined by how well the profile of the essential amino acids matches that required by the human body.

Table 1: Concentration and function of selected cows milk proteins (Pereira 2013; Severin & Wenshiu, 2005)

Protein	Concentration (g/L)	Functions
Total caseins	26.0	Mineral transport (Ca, PO4, Fe, Zn, Cu) and precursor of bioactive peptides
α-Casein	13.0	
β-Casein	9.3	
k-Casein	3.3	
Total whey proteins	6.3	
β -Lactoglobulin	3.2	Retinol carrier and fatty acids binding; possible antioxidant
α - Lactoalbumin	1.2	Lactose production, calcium transport, immunomodulator; anticarcinogen
Immunoglobulin (A, M and G)	0.7	Immune protection
Alanine	0.4	Nitrogen transport in blood
Lactoferrin	0.1	Antimicrobial, antioxidant, immunomodulator, iron absorption, anticarcinogen
Lactoperoxidase	0.03	Antimicrobial
Lysozyme	0.0004	Antimicrobial, synergy actions with immunoglobulins and lactoferrin
Miscellaneous others	0.8	
Proteose-peptone	1.2	
Glycomacropeptides	1.2	Antiviral, bifidogen

MILK PROTEIN QUALITY

Proteins differ in their in composition, indispensable amino acid content and physico-chemical properties of the food matrix in which they are embedded (Tome, 2012). Many additional factors, such as the characteristics of the person consuming the food (age, health status and energy intake) are also related to protein quality (Millward *et al*, 2008). Generally speaking, protein quality refers to the ability of a food protein to meet the metabolic demand of the (human) body for amino acids and nitrogen (N) (Boye *et al*, 2012). However, different criteria and markers can be used to define dietary protein requirements. In addition to the composition of a food protein, physiological criteria such as digestibility and bioavailability are core concepts that should be used when describing protein quality (Boye *et al*, 2012).



Protein digestibility refers to the proteolytic processing of proteins to release amino acids, which extends from the mouth to the small intestine ("ileal digestibility") or the anus ("faecal digestibility") and involves many progressive steps (Boye *et al*, 2012). "Apparent", "corrected", "*in vitro*", and "*in vivo*" protein digestibility are distinguished.

Bioavailability of an amino acid is the proportion of

Table 2: Methods to evaluate protein quality (Boye *et al*, 2012; Bos *et al*, 2000; Tome, 2012)

Method	Description and/or comments
Nitrogen balance	Nitrogen balance uses the measurement of the difference between N intake and N losses. Nitrogen retention efficiency and nitrogen efficiency for growth can also be included
Biological value ("true" and "relative")	Utilization of absorbed dietary essential and non-essential amino acids
Protein efficiency ratio (PER) ("estimated" and "maximum")	PER compares the growth response of young rats, fed a marginal amount of test protein, with that of control rats, fed a similar amount of casein (Schaafsma, 2012).
Net protein utilization (NPU)	NPU is the product of digestibility (digestion and absorption) and biological value (the amount of utilized N divided by the amount of absorbed N in a rat model (Schaafsma, 2012).
Amino acid score (AAS)	Content of first limiting amino acid in a test protein (mg/g) divided by the content of the corresponding amino acid in a reference protein (mg/g). An amino acid scoring pattern, according to the 2007 Report of the WHO/FAO/UNU is age-specific. It is obtained by dividing essential amino acid requirements by minimum requirements of high quality proteins. Three amino acid scoring patterns have been published: 0.5 y, 1-2 y, and >18y. It is clear that minimum requirements do not necessarily represent optimum nutrition, beyond N balance and growth (Schaafsma, 2012). Millward (2012) published an adapted amino acid scoring pattern for infants, children, adolescents and adults, taking amino acids requirements and safe protein intakes into consideration.
Protein digestibility-corrected amino acid score (PDCAAS)	The PDCAAS is derived from a comparison of the first limiting amino acid in the protein under investigation to the corresponding amino acid concentration in a reference amino acid pattern, corrected for faecal N digestibility (Schaafsma, 2000; 2012). Formula: AAS x true N digestibility (%)
Digestible indispensable amino acid score (DIAAS)	DIAAS% =[(mg of digestible indispensable amino acid in 1 g of dietary protein)/(mg of the same indispensable amino acid in 1 g of reference protein)] x 100 (Tome, 2013).

consumed protein that is absorbed in a chemical form for it to be used by the human body. This utilization can be influenced by food processing (e.g. Maillard reactions due to heat treatment affecting lysine bioavailability; spray drying, extrusion, irradiation, fermentation) or by antinutritional factors (e.g. enzyme inhibitors, lectins, tannins), as well as the interaction between these compounds and processing (Boye *et al*, 2012; Gilani, 2012; Schaafsma, 2012).

Digestibility and bioavailability may be inter-related; sometimes specific processes applied during processing may affect the various protein fractions differently. A recent example includes the gelation process through acidification or through renneting. The resultant gels affected the kinetics of milk protein digestion and amino acid availability differently (Barbe et al, 2014).

In Table 2 some methods used in the assessment of protein quality are summarized.

Because of its relative simplicity and direct relationship to the human requirements related to growth and tissue repair, the PDCAAS is widely used and it is the score still recommended by the WHO/FAO/UNU in spite of some limitations. These limitations include the following:

- The so-called truncation rule of PDCAAS states that the biological value may not exceed 1. This means that proteins with extra essential amino acids (i.e. beyond those in the reference amino acid pattern) do not get due credit (Gilani, 2012b; Schaafsma, 2012). To correct this, so-called "SP values" (supplementation power values) have been published. These values are based on the power of the protein to balance diets which are deficient in limiting amino acids (i.e. LYS in cereals, sulphur amino acids in legumes, THR in some cereals, and TRY in maize). In the case of milk powder the SP values for lysine, sulphur amino acids, threonine and tryptophane are respectively 1.46, 1.22, 1.30 and 1.54 (Schaafsma, 2012). These values indicate that milk protein has the power to supply the limiting amino acids from plant based proteins.
- Amino acid availability is not accounted for (Schaafsma, 2012; Gilani, 2012b).
- Anti-nutritional factors in plant food protein sources are not taken into account (Schaafsma, 2012; Gilani, 2012a).

In practice this means that proteins of high biological value, for example milk protein, are of even better quality than the original (uncorrected) PDCAAS method suggests. It has been recommended that a new expert consultation re-examines protein quality assessment methods. The DIAAS may address some of the limitations, but international consensus is as yet outstanding.

FUNCTIONS OF MILK PROTEIN

Milk protein has numerous functions. All classical functions ascribed to proteins as a group also apply to dairy protein. This refers to the provision of amino acids for protein turnover (i.e. anabolism and catabolism in health and disease throughout the life cycle, and as part of biological catalysts, plasma and membrane transport, movement, structure, protein folding, immunity, growth and differentiation [Brosnan & Young, 2003]).

Some unique functions of milk protein fractions have already been listed in table 1. In addition, McGregor and Poppitt (2013) have reviewed numerous roles of milk protein in metabolic health. Decreased prevalences of hypertension, dyslipidaemia and mild hyperglycemia have been associated with milk protein intakes. In the case of the effect of dairy on cardiometabolic risk factors, Pal and Radavelli-Bagatini (2013) narrowed down the dairy fraction to the whey component, and especially highlighted the potential role of leucine in this regard. Dairy protein may indirectly aid weight management through its effect on satiety and body composition, and seems to function synergistically with physical activity (McGregor & Poppitt, 2013; Boye, 2012; Pal & Radavelli-Bagatini, 2013). Among the non-communicable diseases, osteoporosis and bone health are also related to dairy protein intakes (Heaney & Layman, 2008).

The biologically active components of dairy have been labelled "neutraceuticals" (Severin & Wenshiu, 2005), resulting in milk and dairy been classified as functional foods (Bhat & Bhat, 2011).

Tome (2012) has suggested that the unique response of specific target tissues (e.g. bone, muscle) and hormones (e.g. insulin, IGF1) to a particular protein be taken into account when determining protein quality. As new research reveals more and more functions for protein and amino acids in the regulation of body composition and bone health, gastrointestinal function and bacterial flora, glucose homeostasis, cell signalling and satiety, researchers and practitioners need to integrate this in the interpretation and planning of diets (Millward *et al*, 2008). It follows that protein quality and protein functions have become intricately interlinked. This has direct implications for certain groups or situations such as infants, athletes, pregnancy and older adults.

The discovery of more functions of protein necessitates new assessment methods for protein quality and a rethinking of intake recommendations.

IMPLICATIONS FOR PROTEIN REQUIREMENTS

The official definition of protein requirement still refers to being "the lowest level of dietary protein intake that will balance the losses of nitrogen from the body and thus maintain the body's protein mass in persons at energy balance with modest levels of physical activity" (WHO/FAO/UNU, 2007; Uauy, 2013a:228). New evidence challenges scientists to rethink this definition. The requirements for protein depend on numerous factors, and practical intake recommendations for populations and individuals are influenced by additional considerations, such as affordability and acceptability of specific foods. The following points are pertinent food for thought:

- As protein quality decreases, the percentage of energy from protein needed to meet the requirements increases (Uauy, 2013a:230). This implies that more protein must be consumed to meet amino acid requirements when the bioavailability is lower (Pencharz, 2013:238). Similarly, quality-adjusted protein-energy ratios have been proposed for the recommended protein and amino acid intakes (Millward & Jackson, 2003).
- Essential amino acid requirements per gram of protein (a measure of protein quality) for children are now considered to be higher than indicated in previous international (WHO/FAO/UNU) recommendations (Uauy, 2013a:229). However, current protein intake recommendations still reflect the minimum, and are based on ideal conditions, whereas the reality is that children (in developing countries) live under conditions of repeated infections, chronic energy deficiency, poor sanitation and psychological stress (Uauy, 2013a:231; Kurpad, 2013:235).
- Cows' milk protein is key in the treatment of severe acute malnutrition (SAM), because adding dairy protein improves protein quality, which makes it possible to reduce total protein content of a product or diet used in the treatment of SAM (Michaelsen, 2013a:249). Dairy protein in the treatment of SAM has metabolic advantages (Michaelsen, 2013a; Tome, 2013), including improving linear growth without excess body fat gain (Uauy, 2013b:259; Michaelsen, 2013b:268), muscle mass and functional test scores (Allen, 2013:265). By using less soy and cereal in the treatment of SAM, the antinutritional effects of the plant-proteins are reduced (Gilani *et al*, 2012a). The increased cost of using dairy, should, however, not limit the number of potential beneficiaries, and the ability to tolerate lactose may require consideration in this vulnerable group. (Michaelsen, 2013a; Hoppe *et al*, 2009).
- Current recommendations do not yet consider the "new" metabolic and neutraceutical functions of protein related to proteogenic and non-proteogenic pathways (McGregor & Poppitt, 2013; Tome, 2013).

CONCLUSIONS

The importance of the protein in milk has been known for centuries. This is appreciated now more than ever before, as current evidence shows that the quality of milk protein is higher than previously acknowledged; functions of dairy protein beyond the provision of amino acids and organic nitrogen are discovered. The new South African food-based dietary guideline "Have milk, maas or yoghurt every day" (Vorster *et al*, 20130 is a step in this direction.

"Milk protein is even better than we thought"
"Milk protein can do even more than we thought"

REFERENCES

Allen L. Comparing the value of protein sources for maternal and child nutrition. *Food Nutr Bull* 2013;34(2):263-266.

Barbe F, Menard O, Le Gouar Y et al. Acid and rennet gels exhibit strong differences in the kinetics of milk protein digestion and amino acid bioavailability. *Food Chem* 2014;143;1-8.

Bhat ZF, Bhat H. Milk and dairy products as functional foods: a review. *Int J Dairy Sci* 2011;6(1):1-12.

Bos C, Gaudichon C, Tome D. Nutritional and physiological criteria in the assessment of milk protein quality for humans. *J Am Coll Nutr* 2000;19(2):191S-205S.

Boye J, Wijesinha-Bettoni R, Burlingame B. Protein quality evaluation twenty years after the introduction of the protein digestibility-corrected amino acid score method. *Br J Nutr* 2012; 108:S183-S211.

Brosnan JT, Young VR. Integration of metabolism 2: protein and amino acids. In: Gibney MJ, Macdonald IA, Roche HM (Ed). *Nutr Metab. Blackwell Publishing.* 2003. Chapter 4 (pp43-73).

Gilani GS, Xiao CW, Cockness KA. Impact of antinutritional factors in food proteins on the digestibility of protein and the bioavailability of amino acids and on protein quality. *Br J Nutr* 2012a;108:S315-S332.

Gilani SG. Background on international activities on protein quality assessment of foods. *Br J Nutr* 2012b;108:S168-S182.

Heaney RP, Layman DK. Amount and type of protein influences bone health. *Am J Clin Nutr* 2008; 87(suppl):1567S-1570S.

Hoppe C, Andersen GS, Jacobsen S et al. The use of whey or skimmed milk powder in fortified blended foods for vulnerable groups. *J Nutr* 2008;138:145S-161S.

Kurpad A. Overview of changing protein and amino acid requirements and application to pregnancy requirements. *Food Nutr Bull* 2013;34(2):234-236.

Mc Gregor RA, Poppitt SD. Milk protein for improved metabolic health: a review of the evidence. *Nutr Metab* 2013;10:46 (http://www.nutritionandmetabolism.com/content/10/1/46).

Michaelsen KF. Cow's milk in the prevention and treatment of stunting and wasting. *Food Nutr Bull* 2013a;34(2):249-251.

Michaelsen. KF. Effect of protein intake from 6-24 months on insulin-like growth factor levels, body composition, linear growth velocity, and linear growth acceleration: What are the implications for stunting and wasting? *Food Nutr Bull* 2013b;34(2):268-271.

Millward JD, Jackson AA. Protein/energy ratios for current diets in developed and developing countries compared with a safe protein/energy ratio: implications for recommended protein and amino acid intakes. *Publ Health Nutr* 2003;7(3):387-405.

Millward DJ. Amino acid scoring patterns for protein quality assessment. *Br J Nutr* 2012; 108:S31-S43.

Millward DJ, Layman DK, Tome D, Schaafsma G. Protein quality assessment: impact of expanding understanding of protein and amino acid needs for optimal health. *Am J Clin Nutr* 2008;879(suppl): 1576S-1581S.

Pal S, Radavelli-Bagatini S. The effects of whey protein on cardiometabolic risk factors. *Obesity Reviews* 2013;14:324-343.

Pencharz P. Methodology for evaluating protein quality and implications of requirements for product fortification. *Food Nutr Bull* 2013;34(2):237-239.

Perreira PC. Milk nutritional composition and its role in human health. *Nutr* 2013. doi: 10.1016/j.nutr.2013.10.011.

Schaafsma G. Advantages and limitations of the protein digestibility-corrected amino acid score (PDCAAS) as a method for evaluating protein quality in human diets. *Br J Nutr* 2012;108: S333-S336.

Schaafsma G. The protein digestibility-corrected amino acid score/ *J Nutr* 2000;130:1865S-1865S.

Severin S, Wenshiu X. Milk biologically active components as neutraceutical: review. *Crit Rev Food Sci Nutr* 2005;45:645-656.

Tome D. Digestibility issues of vegetable versus animal proteins: protein and amino acid requirements – functional aspects. *Food Nutr Bull* 2013;34(2):272-274.

Tome D. Criteria and markers for protein quality assessment – a review. *Br J Nutr* 2012; 108: S222-S229.

Uauy R. Keynote: Rethinking protein. *Food Nutr Bull* 2013a; 34(2):228-231.

Uauy R. Improving linear growth without excess body fat gain in women and children. *Food Nutr Bull* 2013b;34(2):259-262.

Vorster HH et al. "Have milk, maas or yoghurt every day": a food-based dietary guideline for South Africa. *S Afr J Clin Nutr* 2013;26(3)Suppl:S57-S65.

WHO/FAO/UNU. Protein and amino acid requirements in human nutrition: report of a joint WHO/FAO/UNU expert consultation. WHO Technical Report Series 935. Geneva. FAO/WHO/UNU, 276. 2007.

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