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HEALTH ISSUES OF WHEY PROTEINS: 3. GUT HEALTH PROMOTION

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ABSTRACT: ***This paper reviews the potential of whey protein to promote gut health. The high digestibility and specific amino acid composition of whey protein, as present in whey powder, whey protein concentrate and whey protein isolate, explain why ingestion of whey protein will exert this beneficial effect. The high true digestibility will reduce the flow of nitrogen from the ileum into the colon and limit the formation by the intestinal flora of nitrogenous compounds that are toxic to the colonic epithelial cells. Whey protein is an excellent source of threonine, S-containing- and branched-chain- essential amino acids (BCAA). Threonine is largely and directly incorporated into intestinal mucins, which protect the intestinal cells and strengthen the barrier function of the gut. Sulfur-containing amino acids serve as precursors of the anti-oxidant compounds glutathione and taurine, which display anti-inflammatory properties. This is important in the prevention and/or reduction of inflammatory bowel disease. Glutamine in whey and glutamine derived from the precursor BCAA serve as substrate for immune competent cells, which are largely present in the gut-associated lymphoid tissue. Other issues relating whey protein to gut health are antimicrobial and anti-viral proteins in whey and peptides with anti-microbial activity that are formed during whey protein digestion.***

KEY WORDS: Colonic Health, Prebiotics, Probiotics and Taurine

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INTRODUCTION

There is a considerable market for products designed to promote gut health via improvement of the intestinal flora. In this area fermented dairy products with probiotics have a strong position (Makkinga et al., 2003; Menrad, 2003;). Also a lot of attention in this field is given to prebiotics, like fructo- and galacto-oligosaccharides (Macfarlane et al., 2006). Furthermore recent recommendations on the Adequate Intake of dietary fiber (3.4 g/MJ) stress the importance of fiber to improve intestinal functions and reduce nutrition related chronic diseases, e.g. cardiovascular disease (Institute of Medicine, 2002). So far, relatively little attention has been given to the significance of dietary proteins, peptides and amino acids in relation to gut health. Although it is well known that certain specific bioactive proteins and/or peptides displaying anti-microbial and anti-viral activities, (e.g. immunoglobulins, lactoperoxidase, lactoferrin and lactoferricin) are positioned in the market as being beneficial for gut health for special target groups (Florisa et al., 2003), no other proteins or protein fractions are positioned in the market of gut health promotion and no position paper is available on the role that proteins, peptides and amino acids may have in this field. It is the aim of this comprehensive paper to enter into this emerging area by reviewing existing knowledge on whey protein consumption in relation to gut health promotion and to discuss the potential nutritional benefits. Special emphasis is put on the significance of the complex mixture of whey proteins rather than on individual whey protein fractions. Protein digestibility and the specific essential amino composition of whey protein with its high concentrations of branched chain-, S-containing amino acids and threonine (Schaafsma, 2006) appear to provide interesting links to gut health promotion. The paper addresses the following issues:

- Protein digestibility and colonic health
- Mucosal quality, mucous formation and prebiotic effects
- Anti-oxidant properties
- Effects on the immune system
- Antimicrobial effects

Protein digestibility and colonic health

According to Hughes et al. (2000), products of colonic bacterial protein degradation and metabolism, including ammonia, phenols, indols, sulphides and N-nitroso compounds, are toxic to the colonic mucosa and it has been shown that diets rich in meat and low in fiber increase cytotoxicity of the faecal water. Toxicity of phenol has been implicated as factor causing chronic bowel inflammation (Pedersen et al., 2002). A high ileal digestibility of dietary proteins will limit the input of nitrogen from the ileum into the colon and reduce toxicity, caused by nitrogenous compounds. Milk proteins are known to be highly digestible and it has been shown that ileal digestibility of milk proteins is higher than that of plant proteins, like soy proteins, reducing the amount of amino acids released into the colon (Gaudichon et al., 2002): with soy protein digestibility of valine, threonine, histidine, tyrosine, alanine and proline were significantly lower than from milk proteins.

Various studies in rodents have shown that chemically-induced tumor incidence or number of aberrant crypt foci (ACF) is reduced by the ingestion of whey proteins as compared to other proteins, e.g. casein and red meat (Belobrajdic et al., 2003; Hakkak et al., 2001; Bonous et al., 1988). The underlying mechanistic explanation is not exactly known, but it could be related to improvement of the glutathione (GSH) status of the whey protein-fed animals, enhancing antioxidant capacity and decreasing mutagenicity (Bonous and Molson, 2003). It should however be stressed that non-protein components of whey protein concentrates, that were used in the animal studies, may be implicated. Such substances with potential anti cancer properties are lactose, sphingolipids and conjugated linoleic acid (Berra et al., 2002; Belobrajdic et al., 2003). The epidemiological association between consumption of red meat and colon cancer is attributable to cytotoxic effects of haem (Freeman et al., 2004).

Mucosal quality, mucous formation and prebiotic effects

Mucins are large molecules, composed of a peptide core (10% of the mass) and oligosaccharide side chains (90% of the mass), which are linked with O-glycosidic bonds to serine- and threonine residues in the peptide core. 22% of the peptide core is made up of threonine, the essential AA, which is often low in plant proteins. Mucins have an important role in the barrier function of the intestine, protecting against toxins, bacteria, self-digestion and physical abrasions. Threonine is highly directly utilized within the gut for mucin synthesis and it has been shown in pigs that threonine-deficient animals have a decreased mucosal weight, villus height and mucin content (Law et al., 2000). The gut uses about 60% of total dietary threonine intake, primarily for the synthesis of intestinal mucins (Myrie et al., 2001). Whey proteins are remarkably rich in threonine (Schaafsma, 2006) and theoretically whey proteins benefit mucin synthesis. A study in growing rats (Faure et al., 2005) showed that mucin synthesis was highly sensitive to dietary threonine restriction, but when dietary threonine requirements were adequately met, a higher intake did not stimulate it further. Under certain clinical conditions, however, including inflammatory bowel disease, cystic fibrosis, intestinal infections and intestinal damage caused by anti-inflammatory drugs, mucine secretion is enhanced and threonine requirement is likely to be increased (Ballevre et al., 2004).

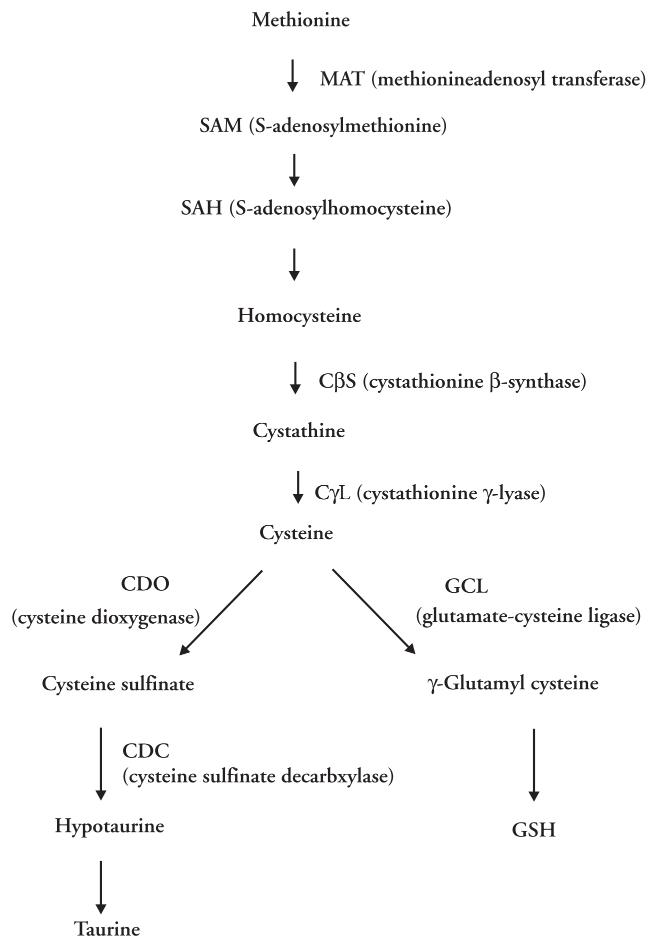
Mucins form a substrate for bacterial fermentation in the colon and mucin production will favour saccharolytic fermentation, leading to formation of short-chain volatile fatty acids and reduction of the pH of colonic contents. Such effects are considered to be beneficial for the colonic mucosa and may even enhance colonization resistance against pathogenic bacteria (Cummings et al., 2004).

So far no or very little attention has been given to dietary proteins as prebiotic substances. Prebiotic effects (selective stimulation of beneficial bacteria in the gut) are almost entirely linked to non-digestible oligosaccharides (Macfarlane et al., 2006). Whey favors the growth of bifidobacteria (Romond et al., 1998), but this could be due to other whey components than proteins.

Anti-oxidant properties

The high content of sulfur containing amino acids in whey protein is relevant to its potential to increase the anti-oxidant capacity of the body. The amino acids methionine and cysteine are precursors of glutathione and taurine. Both compounds serve as 'body's own' anti-oxidants.

FIGURE 1. Synthesis of glutathione (GSH) and taurine from S-amino acids



Glutathione (GSH) synthesis

GSH is a tripeptide synthesized from glutamate, cysteine, and glycine (see figure 1). It is the major cellular antioxidant. Depletion of GSH results in increased vulnerability of the cell to oxidative stress consequent to the accumulation of intracellular reactive oxygen species (ROS). Maintenance of a high intracellular concentration of GSH is critical for cellular defense against oxidative stress. GSH can donate its sulfhydryl proton to quench ROS. Thereby GSH is converted to GSSG. Maintaining a high intracellular GSH/GSSG ratio provides optimal protection against oxidant induced cell damage. The availability of S-containing amino acids (cysteine and methionine) is a determinant of glutathione synthesis. Especially cysteine is the rate limiting amino acid for GSH synthesis. Ingestion of whey protein enhances intracellular levels of glutathione in a variety of tissues, including gut mucosa, lymphocytes, as well as in plasma (Kent et al., 2003; Nkabyo et al., 2006; Grey et al., 2003; Micke et al., 2002; Sido et al., 1998). Particularly in inflammatory bowel disease (IBD) an increased need exist for GSH to counterbalance the inflammatory process and to reduce tissue damage. Sido et al. (1998) argued that GSH deficiency, as observed in IBD patients, might be a target for therapeutic intervention.

Taurine synthesis

Taurine (2-aminoethanesulfonic acid) is an S-containing beta amino acid that is not incorporated in body proteins, but which is present in free form in many tissues, including the intestine. Taurine is taken into cells by a taurine transporter protein. Taurine is synthesized in the human body from cysteine (see figure 1) and the availability of cysteine is a limiting factor for taurine synthesis. Taurine is also readily taken up from the diet (meat, fish, milk). It is the most abundant S-amino acid in the body and has an important role in many biological processes. These include osmoregulation, detoxification (including conjugation of bile acids), stimulation of glycolysis and glycogenesis and protection against oxidant-induced injuries, and regulation of intracellular calcium concentrations (Birdsall, 1998; Stapleton et al., 1999; Atmaca, 2004). By binding to pro-oxidative compounds produced by neutrophils and macrophages, taurine may inhibit the pro inflammatory mediators and reduce inflammation (Ito and Azuma, 2004). In in-vitro experiments with Caco-2 cells Mochizuki et al. (2004) showed indeed that the pro-inflammatory cytokine TNF-alpha increased the amounts of the taurine transporter protein and the corresponding mRNA, indicating upregulation of the responsible gene. Taurine appeared to attenuate cell damage caused by the inflammation. It was suggested that taurine could be a useful substance for the therapy of intestinal problems, such as inflammatory bowel disease. In rat studies (Son et al., 1998a and 1998b) it was found that taurine administration decreased the severity of inflammatory bowel disease, which was induced by trinitrobenzene sulfonic acid. This supports the view that taurine reduces IBD, but as yet there are no data from direct studies in humans to confirm this.

Of particular interest is the question to what extent the taurine production in the body is dependent on the supply of S-containing amino acids with the diet. There are no data from studies in humans to answer this question. In rats it has been shown that taurine production is increased when a low protein diet is supplemented with these amino acids (Bagley and Stipanuk, 1995).

Only a small part of the body pool of taurine is used for the conjugation of bile acids. Solubilization of the secondary bile acids deoxycholic acid and litocholic acid requires the peptide linkage to either glycine or taurine. Conjugation reduces the cytotoxicity of bile acids to intestinal cells.

Effects on the immune system

Glutamine is known to be an important metabolic fuel for intestinal cells and immune competent cells and has a major impact on the functionality of the immune system (Reeds and Burrin, 2001). A large part of the immune competent cells (approx. 70%) is present in the gut associated lymphoid tissue, which plays an important role in the barrier function of the intestine. During catabolic conditions there is a progressive depletion of glutamine in the body as a consequence of increased requirements of the GI-tract, the kidney and the lymphocytes and a reduction of the endogenous production of glutamine (Reeds and Burrin, 2001). A recent meta analysis of clinical trials (Melis et al., 2004), indicated that parenteral glutamine supplementation had beneficial effects on infectious complications and hospital stay. Since BCAA may serve as a precursor of glutamine and since whey protein has a high content of these amino acids, whey protein will indirectly increase the availability of glutamine. This will support the immune competent cells in the gut and contribute to the enhancement of the gut barrier function.

TABLE 1. Antimicrobial proteins in whey proteins (g/100 g protein)[1]

Lactoferrin	1 - 2
Immunoglobulins (mainly IgG)	10 - 15
Lactoperoxidase	0.5

[1] Schaafsma (2006)

Anti microbial effects

Whey contains antimicrobial proteins, including immunoglobulins, lactoferrin, and lactoperoxidase (see Table 1), which may strengthen the barrier function of the intestine. It is well known that these bioactive proteins from milk may display anti-viral and anti-bacterial properties (Florisa et al., 2003) but without isolation and purification the concentration of lactoferrin and lacto-peroxidase in whey from mature milk is probably too low to have substantial biological significance in humans. Moreover thermal processing and the high proteolytic activity in the proximal part of the small intestine will further reduce any significant activity. Whereas these considerations are also applicable to the immunoglobulins, it must be stressed that normally these bioactive molecules are not raised by the immune system of the cow to antigens, which originate from organisms that are pathogenic to humans and thus will not offer specific protection in humans. It is possible to enhance the concentration in milk and whey of specific immunoglobulins that can be used in the treatment of human disease. This can be achieved by treating cows with specific antigens, derived from organisms that are pathogenic to humans, during pregnancy and lactation (Korhonen et al., 1995; www.mucovax.nl; www.lactive.nl). In this way whey protein preparations can be obtained with specific antibacterial properties and that could be used in the treatment of intestinal infections. It has been shown further that during whey protein digestion peptides are formed that display anti bacterial properties in vitro (Florisa et al., 2003). Although the significance of such peptides in the prevention of infection remains obscure, they may further contribute to the barrier function of the intestine.

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