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Effect of Diet on Mucin Kinetics and Composition: Nutrition and Health Implications

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The mucus gel covering the gut epithelium is in dynamic balance between synthesis and secretion of mucin from goblet cells and proteolytic and physical erosion that releases mucin into the lumen. In the lumen, mucin is partially protected from proteolysis by carbohydrate chains, and it contributes to endogenous protein reaching the ileum. Dietary components modulate the contribution of mucin to endogenous protein components and their qualitative composition. In addition, mucin plays a key role in gastrointestinal protection in association with the microflora. In this review, we will attempt to evaluate the consequences of dietary manipulation of mucin on gut health.

Key words: mucus, mucin, endogenous losses, digestion, protein

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Introduction

Mucins are polymeric glycoproteins that comprise the main component of the mucus layer that covers the epithelium of the gastrointestinal tract (GIT), as well as all epithelia of mammals. This mucus layer exists at the interface between the external environment corresponding to the gut lumen and the gut epithelium. The main function of the mucus is to protect the epithelium from chemical, enzymatic, physical, and bacterial aggressors that may be present in the gut lumen. In healthy animals, the mucus gel is in a dynamic balance. Erosion on the luminal face is countered by synthesis and secretion from specialized differentiated cells named goblet cells distributed throughout the epithelium. Proteolytic breakdown of mucus gel and physical abrasion are suggested to be the main factors that cause the release of mucin into the lumen and thereby in the chyme.[1] In the lumen, mucin is partially protected from further proteolysis by the coat of oligosaccharides that cover up to 80% (by weight) of the protein backbone. Mucin is therefore poorly digested in the small intestine and could represent an important proportion of the endogenous protein that reaches the large intestine and is thereby lost for the animal.[2]

During the last decades, the objectives of animal nutrition research were to improve body weight gain and feed conversion efficiency by enhancing the nutritional quality of feed. This made it possible to reduce nutritional costs, which currently represent up to 60% of total costs for animal production. This research also reduced the release of undigested material into the environment. More recently, the relationship between nutrition and animal health has been addressed because of the ban on in-feed antibiotics as growth promoters in various European countries. Because the underlying mechanisms of gut pathophysiology are not fully understood, the scientific community needs more information about the effect of feed and nutrients on GIT and physiologic process. Because mucus plays a key role in mucosal protection, studies on mucin might contribute to the understanding key aspects of health maintenance.

In this review, we discuss the contribution of mucin to endogenous protein, the effects of dietary factors on this recovery, and the qualitative composition of mucin. We will also evaluate the impact of nutritional and metabolic costs. Finally, we will discuss the consequences of modifying mucus properties by feed and feeding strategies on the protection of the GIT mucosa.

Structure and Function of Mucin

The family of mucin (*MUC*) genes has 13 members, ten of which are found in the GIT. For details about the structural features, functions, and physiology of digestive mucin, comprehensive reviews by Gum,[3] Strous and Dekker,[4] Forstner and Forstner,[5] Perez-Villar and Hill,[6] and Lien et al.[7] are available.

Broadly, mucins can be divided into two groups: secreted and membrane-associated. The secreted mucins

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are characterized by a very high molecular weight (up to 2×10^6 Da) and size, a high proportion of O-linked carbohydrates (50–80% of dry weight), and an ability to form viscoelastic gels. Membrane-associated mucins share many of these structural properties but they have additional properties such as being active membrane components. Each subunit of a mucin protein backbone contains a central domain that is rich in serine, threonine, proline, alanine, and glycine, and two extending peptides (N and C terminal) that contain cysteine. Threonine and serine residues are numerous and they provide attachment sites for the oligosaccharide chains. Five different monosaccharides are commonly found on mucins, namely N-acetylgalactosamine, N-acetylglucosamine, galactose, fucose, and sialic acids. Depending on the monosaccharide composition, mucins are classified into neutral and acidic subtypes. The latter are further subdivided into sulfated (sulfomucin) and nonsulfated (sialomucin) mucins. The proportions of these three classes vary spatially along the GIT and temporally during postnatal development of mammals. They are also influenced by diet.

Mucin monomers are bound together, end to end, by disulfide bridges to form large, flexible, hydrated polymers. These components form a viscous solution corresponding to the mucus layer, which is the functional form of mucin. The mucus layer lubricates the gastrointestinal epithelium, protecting it from mechanical damage by dietary constituents (Table 1). It also protects the epithelium from corrosive action of the acidic gastric juice and from proteolysis by digestive enzymes. Mucus prevents infection by binding viruses or bacteria through specific interactions with the carbohydrate chains; it also plays an important role in digestive processes by creating a digestion zone in which enzymes are immobilized near the epithelium surface, which prevents their rapid removal by peristalsis and places them in a more favorable position for hydrolysis and absorption. Finally, the mucus layer acts as a selective diffusion barrier that filters the nutrients able to be absorbed and prevents larger compounds from reaching the epithelium. Owing to all these properties, mucus plays a central role in animal nutrition and health, and an intact mucous layer at the surface of the gut epithelium is required for optimal protection and functioning.

Table 1. Functional Properties of Digestive Mucins

Roles in relation with gut physiology
Lubrication of the gut epithelium
Protection of the epithelium against acidic environment (stomach and duodenum)
Protection against endogenous and bacterial proteases
Selective diffusion barrier permeable to nutrients but not to macromolecules
Roles in relation with gut health
Fixation of commensal bacteria permitting colonization resistance
Fixation of pathogen bacteria, viruses, and parasites
Component of the gut-associated lymphoid tissue (GALT)
Epithelium reparation (synergic action with trefoil peptides)
Substrate for bacterial fermentation

Adapted from Forstner and Forstner.[5]

Mucin Contribution to Endogenous Protein

Definitions and Hypothesis

Endogenous protein flowing along the GIT has been arbitrarily fractionated into a basal, or nonspecific, component and a fraction specific of the diet.[8] The basal endogenous protein is considered to be associated with normal functioning of the GIT. Its importance is related to body size and could be considered part of the obligatory endogenous losses to be compensated for by dietary intake for maintenance requirements. Basal endogenous losses are classically quantified as nitrogen or amino acids found in ileal digesta of animals fed nitrogen- or protein-free diets. Total endogenous protein increases with the inclusion of various dietary constituents. The difference between total and basal losses is called "specific losses."

Various observations suggest that the recovery of mucin in ileal digesta could represent a considerable proportion of endogenous protein and carbohydrate. First, the small intestinal mucosa has a high metabolic activity; endogenous nitrogen derived from it represents between 50 and 65% of total endogenous nitrogen entering the gut lumen.[9,10] Second, little digestion of mucin occurs prior to the large intestine.[2] By contrast, 70 to 80% of endogenous nitrogen is estimated to be reabsorbed before the end of the small intestine.[10] Third, the predominance of threonine, serine, and proline in endogenous protein is consistent with the hypothesis that mucin, because of its high content in these amino acids, is an important component of endogenous protein.

Mucin Contribution to Basal Losses of Endogenous Protein

To our knowledge, Lien et al.[11] were the first to quantify the daily output of mucin in ileal digesta of pigs by using galactosamine and glucosamine as markers. This would amount to 3.9 g/kg dry matter intake (DMI) in 55-kg pigs that were fed a protein-free diet and given either a complete amino acid mixture or saline intravenously. Mucin accounted for 5 to 11% of endogenous protein, depending on the infusion treatment. Although mucin represented a relatively small proportion of endogenous

amino acids in ileal digesta, the contribution of threonine, serine, and proline were much higher: 28 to 35%, 13 to 16%, and 7 to 24%, respectively. In that study, mucin flowing at the terminal ileum was estimated to originate mostly from the small intestine (73%); the rest came from the stomach.

In pigs fed a protein-free diet, when the DMI level was increased by threefold (1–3 kg/day), the flow of galactosamine in ileal digesta did not significantly increase, whereas the flow of nitrogen doubled from 3.6 to 7.3 g/day ($P < 0.05$).[12] Consequently, the galactosamine-to-nitrogen ratio in ileal digesta fell from 0.44 to 0.28. Several reasons have been suggested for these changes. Mucins from different sections of the GIT vary in amino-sugar composition. It may be also that mucin secretion was not stimulated to the same extent in different parts of the GIT when the DMI level increased. Moreover, components other than mucin were undoubtedly stimulated to a greater extent when DMI increased.

Using a specific enzyme-linked immunosorbent assay (ELISA), investigators in our laboratory measured the flow of mucin along the small intestine of preruminant calves that were fed a protein-free diet.[13,14] The basal flow of mucin protein at the duodenum, jejunum, and ileum was 1.1, 1.8, and 4.0 g/kg DMI, respectively (i.e., 2.1, 3.4, and 7.5 g mucin/kg DMI). The latter value at the ileum was twice the value obtained in pigs (3.9 g/kg DMI).[11] However, the authors recognized that their value was probably underestimated. In the calf, mucin protein accounted for 19% of the total basal endogenous losses of crude protein ($N \times 6.25$) at the ileum. This was again much higher than the value estimated in the pig (11%). The difference could be partially explained by the fact that intestinal mucin is 2.5 times richer in protein in calves (53%)[13] than in pigs (21%).[1] Nevertheless, in these calves, the contribution of mucin amino acids to the total flow of amino acids was quite high for lysine (40%), and to a lesser extent for glutamic acid (29%), and threonine, serine, and aspartic acid (25% each).[14] We also measured the flow of mucin protein at the duodenum, which originate from the upper GIT and the stomach. This would represent 25% of the ileal loss of mucin protein,[14] which concurs with the value in pigs provided by Lien et al.[11]

Mucin Contribution to Specific Endogenous Protein Losses

Secretion of mucin, erosion of the mucus, and consequently the recovery of mucin in endogenous ileal losses, could be influenced by many dietary factors including fiber, protein, and anti-nutritional factors.

Dietary fiber. Several studies have reported that dietary fiber increased endogenous protein synthesis and losses, as well as excretion of mucin at the terminal ileum, in the guts of monogastric farm animals.[15,16] When wheat bran (150 g/kg) was added to a protein-free diet and fed at 3 kg DM/day to pigs, the output of both galactosamine and N at the terminal ileum doubled. This was not observed at a low level of DMI (1 kg/day). Moreover, when DMI increased from 1 to 3 kg/day, the galactosamine flow increased by more than fourfold (0.8 to 4.1 g/day) with a similar increase for N flow (3.1 to 14.7 g/day). In these cases, the galactosamine-to-nitrogen ratio was little affected.[12] When increasing amounts of pea fiber (0, 80, 160, and 240 g/day) were fed to pigs in addition to 1.6 kg/day of a wheat-based diet, the ileal output of mucin tended to increase linearly from 6.1 to 7.3 g/day for the supplemented diets.[7] Increased ileal excretion of mucin was also observed in pigs fed a protein-free diet supplemented at three levels (17, 34, and 102 g/kg) with a mixture of dietary fiber from wheat straw, corn cobs, and wood cellulose.[17] Glucosamine and galactosamine excretion increased linearly with fiber intake, whereas endogenous nitrogen losses increased between 17 and 34 g/kg to reach a plateau at higher fiber levels.

In pigs fed a protein-free diet containing different levels of pea inner fiber (from 5 to 200 g/kg diet), a linear relationship was established between the water-holding capacity of the diet (in g water retained/kg diet dry matter [DM]) and the ileal flow of crude mucus in g/kg DMI.[18] The ileal flow of nitrogen from mucin ranged from 4.5 to 6.5% of the total digesta nitrogen. In rats, daily excretion of the four mucin-derived sugars—fucose, galactose, glucosamine and galactosamine, which account for approximately 85% of mucin by weight in this animal species—was increased threefold in feces of germ-free rats fed a diet containing psyllium seed husk when compared with a fiber-free diet (223 vs. 70 μmol/g dry feces).[19] Increased mucin sugars from 17 to 37 $\mu mol \cdot day^{-1} \cdot g^{-1}$ of food intake was also measured in excreta of colectomized rats fed either a fiber-free diet or a diet containing 44 g/kg gum arabic.[20] By contrast, in humans with ileostomy, soy fiber consumption affected neither the daily mucin output nor the N digestibility, suggesting that soy fiber did not compromise digestion in this study.[21]

The effect of fiber on the recovery of mucin in ileal digesta seems to depend on fiber solubility. Erosion and enzymatic proteolysis of the mucus layer are the determining factors that lead to a release of mucin in the GIT lumen.[1] Insoluble fiber has a more abrasive action, scraping mucin from mucosa as it passes down the gut. In work by Leterme et al.,[18] the pea inner fiber behaved rather as insoluble fiber. It was therefore suggested that the effect of fiber on the recovery of mucin in ileal digesta resulted from the effect of swollen fiber on the intestinal wall. In addition to this physical aspect, indirect effects may also play a role in mucus erosion.

Indeed, the type of fiber has been shown to affect the activities and distribution of proteolytic enzymes in the intestinal lumen.[22] This may also contribute to changes in the degradation of mucus gel (Figure 1).

As explained above, the erosion of mucus must be counter-balanced by its synthesis and secretion by goblet cells. Several studies provide evidence for such modifications. Changes in intestinal morphology and cytokinetics at the jejunum were observed in rats fed bulky diets supplemented with insoluble dietary fiber (100 g/kg cellulose or wheat bran).[23] The percentage of goblet cells was lower with cellulose than with bran or the fiber-free control. However, greater percentages of ^{3}H-glucose (twofold) and sodium ^{35}S-sulfate (2.5-fold) were incorporated into mucin in the case of wheat bran and cellulose compared with the control. Also, germ-free rats fed a commercial bulky diet (37% fiber), when compared with a standard diet (4.5%), had increased capacity for mucin secretion as indicated by the density and length of crypts, crypt cells, and mature goblet cells in the proximal and distal colon.[24,25] The level of mucin (assayed by ELISA) increased by 390 and 210%, respectively, at the surface of the stomach and small intestine of rats fed a diet containing 50 g/kg of citrus fiber when compared with a fiber-free control.[26]

The improved capacity of mucin secretion can be regarded as an adaptation to a chronic mechanical irritation. A complex mechanism was proposed by Schmidt-Wittig et al.[24] First, the low caloric yield of a diet containing fiber would induce an increase in daily food intake. That might result in more bulky chyme and thereby more abrasion of the mucus layer. Second, insufficient epithelial protection would be followed by cellular injury allowing luminal antigen access. Third, superficial and recruited cells should release cytokines and metabolites of the arachidonic pathways. Some cytokines are potent mediators of colonic inflammation, goblet cell proliferation, and mucous secretion from goblet cells.

Dietary fiber may induce changes in mucin content or composition through another indirect mechanism. This would involve short-chain fatty acids (SCFAs), which are formed in the colon following fermentation of dietary fiber by bacteria.[27] Barcelo et al.[28] first demonstrated, using an isolated vascularly perfused rat colon model, that acetate (5–100 mM) induced a dose-dependant release of mucus in the lumen. Physiologic concentration of butyrate (5 mM) caused an increase in mucus secretion, but increasing its concentration to 100 mM provoked a gradual decrease in mucus discharge. Inversely, propionate (5–100 mM) did not induce mucin release.[28] Moreover, sodium butyrate induced a striking increase in mucin synthesis by human colonic biopsy specimens.[29]

The composition of mucin secreted with a high-fiber diet may also be quite different than that observed with standard European diet diets.[30] In germ-free rats, mucin released after such stimulation exhibited an increase of acidic constituents,[25] corresponding to a higher goblet cell content in sialic acids and sulfate ester.[31,32] In the same way, after adaptation to chronic mechanical irritation by dietary fiber, goblet cells released more mucins with a higher negative net charge, which cause mucus to become more viscous.[24] This shift may be caused by enhanced cell turnover and stimulated synthesis, which lead to more immature mucin.[33] Ichikawa et al.[34] suggested that enhanced oligosaccharide synthesis does not allow all transferases to develop their complete activity.

Dietary protein. Although the effect of dietary protein on mucin has been less often studied than dietary fiber, protein has also been shown to modify the recovery of mucin in endogenous protein. In pigs fed a diet

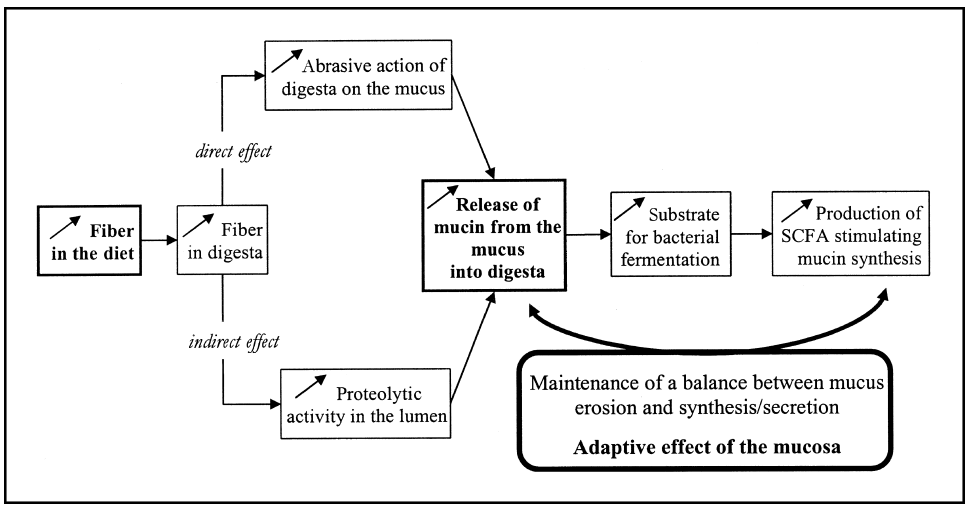

Figure 1. Hypothetical effects of dietary fiber on the balance between mucus erosion releasing mucin into the gut lumen and synthesis and secretion of mucin from the goblet cells. SCFA = short-chain fatty acids.

containing isolated soybean protein, the hexosamine ileal excretion increased when the dietary crude protein (CP) content exceeded 55 g/kg DM,[17] suggesting increased mucin loss at the terminal ileum. In calves fed milk substitutes provided with 14 to 278 g of CP/kg DMI, the flow of mucin protein at the duodenum significantly increased with the dietary CP level, from 1.06 to 2.43 g/kg DMI; the maximum flow of mucin protein was 4.21 g/kg DMI for a 20% CP diet.[14] The presence of dietary proteins or peptides in the abomasum enhances the secretion of enzymes such as pepsin and chymosin.[35] These enzymes are able to hydrolyze the mucus layer, thus releasing mucin into abomasal digesta. In the same study, however, increasing the dietary CP content had no effect on the flow of mucin protein at the jejunum and ileum.[14] This observation corroborates the fact that the loss of endogenous protein at the terminal ileum did not vary with the dietary protein content.[36]

Several studies reported that the flow of threonine in ileal digesta differs widely depending on the nature of dietary protein. Apart from leading to a low apparent digestibility for this amino acid, this variability suggests an impact of dietary protein on the flow of mucin. In the calf, when skim milk protein was partially replaced by plant protein, the flow of mucin protein increased along the small intestine. At the ileum, it increased from 3.9 g · kg DMI^{-1} · day^{-1} with the control diet based on skim-milk powder to 9.1, 7.1, and 4.9 g · kg DMI^{-1} · day^{-1} with a soybean protein concentrate, a partially hydrolyzed soybean protein isolate, or a potato-protein concentrate provided at one-half of the CP content in the milk replacer.[14] Montagne[37] also showed that the flow of endogenous animal protein at the jejunum and ileum of calves fed with milk substitutes containing plant protein was positively correlated with the flow of mucin at the corresponding sites [Spearman coefficient $\rho = 0.721$, $P < 0.05$ and $\rho = 0.885$, $P < 0.01$, respectively]. Such an increase in mucin flow (mean increase was threefold) was also observed at the ileum of calves fed a milk substitute containing raw pea flour or colostrum.[13]

The actual reasons for such an increase are not fully understood. Dietary peptides might interact with the gut and might be responsible for the increase of mucin excretion observed with plant protein. This hypothesis is supported by observations in rats fed pure phaseolin, the storage globulin of kidney bean *Phaseolus vulgaris*.[38] Native phaseolin and/or related fragments that escaped digestion were bound to the small intestinal epithelium. These peptides could act as natural secretagogues that stimulate secretion of endogenous protein, especially mucin, from the mucosa.[38] Resistant dietary protein fractions have been observed in ileal digesta of calves fed soybean or potato protein sources,[39–41] in pigs fed kidney bean,[42] and in chickens fed pea protein.[43] Clearly, more work is needed in this area.

The impact of dietary protein on the composition of mucin has not been studied extensively. Only Turck et al.[44] reported that the composition of colonic mucin of 21-day-old piglets differed between breast-fed and artificial milk–fed piglets. Mucin from colons of sow-fed pigs contained more fucose and glucosamine and less sulfate, and was therefore considered as more mature than mucin from artificially fed piglets.

Anti-nutritional factors. Tannins, lectins, and protease inhibitors have been shown to increase the recovery of endogenous protein in ileal digesta and feces[15,45]; this leads to decreased apparent digestibility of feedstuffs and amino acids, especially of threonine. The extra losses of endogenous protein may consist of digestive enzymes, protein sloughed off mucosa cells, and mucin.[46] In rats, fecal glucosamine excretion was 0.99 mg/g intake with a commercial diet based on low-tannins sorghum and increased to 1.28 mg/g intake with high-tannins sorghum (Savanna variety).[47] The mechanism by which tannins induce hypersecretion of mucin is unknown. In particular, it is not clear whether this response was mediated via a direct effect of tannin per se, or rather via an indirect effect involving reduced digestibility with increased fecal matter.[47]

The ability of dietary lectins to bind glycoproteins has important consequences on the output of mucin in the GIT.[7] Such interactions could lead to decreased mucin hydrolysis by digestive enzymes in the small intestine and by increased mucin fermentation in the cecum and colon. Another mode of action of lectins could be through histamine; lectins can induce the release of histamine, which is a known mucus secretagogue.[48]

Implications in Animal Nutrition

Quantitative and qualitative changes in mucin induced by dietary ingredients might have nutritional consequences. The body of evidence of these changes must be taken into account when formulating the diets.

Nutrient Absorption

As a molecule is actively or passively absorbed from the intestinal lumen into the cytoplasm of the enterocyte, it must cross two major diffusion barriers: the unstirred layer composed mainly of hydrated mucin[49] and the protein lipid membrane of the microvilli. Consequently, an increase in mucin content at the mucosal surface could impair the rate of nutrient absorption. This was observed in rats and humans fed a diet containing 0 to 15 g/L of citrus pectin.[50] The thickness of the intestinal unstirred water layer increased linearly from 520 to 850 μm in rats and from 270 to 365 μm in humans. This enlargement was closely associated with the reduction of absorption of linoleic acid (34% and 47% between 0 and

10 g/L of pectin for rat and human, respectively) and glucose (28% and 10% for rat and human, respectively). The increase in the unstirred water layer thickness was also observed in the jejunum of rabbits fed guar gum.[51] Under these feeding conditions, the unstirred water layer can become a limiting factor to the uptake of highly permeant nutrient molecules and can significantly reduce their uptake. Similarly, removal of mucin by rinsing the intestine has been reported to reverse the inhibitory effects of dietary fiber on nutrient transport from the GIT lumen into enterocytes.[52,53]

Amino Acid and Energy Metabolism

The GIT as a whole, and the small intestine mucosa in particular, have a high rate of protein synthesis and energy expenditure. Although the gut contributes only between 3 and 6% of the total body weight, therefore, it accounts for 20 to 35% of whole-body protein turnover and energy expenditure.[54,55] The small intestine mucosal tissue exhibits high synthesis and secretory activity. The in vivo mucin synthesis rate was first assessed by Faure et al.[56] using a specific purification method based on gel filtration in tandem with stable isotope techniques. The fractional synthesis rate of mucin was highest in ileum and jejunum (>130%/day) and lowest in duodenum (116%/day) and colon (112%/day). The synthesis rate of mucin was always higher than that of other mucosal proteins, except in the duodenum.[56] The secretion of mucin probably has a substantial impact on the requirements for some specific amino acids and may be a significant contributor to the energy needs of an organism.[57] Factors that increase the production of mucin would increase maintenance requirements and, therefore, decrease the availability of amino acids and energy for growth and production.

Amino acids from endogenously secreted proteins that reach the large intestine are lost by the animal. As already mentioned, mucin represents 19% of the total basal endogenous losses of crude protein at the ileum for calves[14] and 11% for pigs.[11] The contribution of threonine to mucin protein is 25% in calves and between 28 and 35% in pigs; this value varies with the diet. Mucin secretion should have a measurable influence on the organism's requirements for these amino acids. Indeed, in man, endogenous losses of threonine consist of up to 60% of the daily maintenance requirement and between 14 and 33% of other essential amino acids.[58]

Amino acids used for mucosal protein synthesis originate from both luminal and arterial sources.[59] The portal appearance of dietary threonine, when expressed as a proportion of intake, is lower than the portal appearance of other essential amino acids.[57] Moreover, Zhao et al.,[60] who studied the threonine balance of adult humans, came to the conclusion that there was an additional non-oxidative pathway of threonine loss from the body. They suggested that it consisted of the continual net loss of threonine in mucin secretions. This first-pass utilization of threonine for mucin synthesis has a nutritionally significant impact on the net availability of portal amino acids.[57] The pool of amino acids that reaches the portal flow, and that is thereby available for peripheral organs, will be modified compositionally.

Oligosaccharide chains account for up to 80% of mucin by weight. Because glucose is a precursor for these sugars, mucin synthesis may also have a significant effect on glucose utilization by the GIT. Stoll et al.[61] reported that the intestinal tissues in piglets utilized less than 2% of the luminal glucose in first pass. However, the portal-drained viscera extracted 6% of the arterial glucose flux that accounted for 25% of the whole-body glucose flux of the animal. Mucin synthesis was probably substantially responsible for this use.

Implications on Gut Sanitary Status

Mucin and the Gut Ecosystem

As proposed by Conway,[62] the gut ecosystem could be divided into three major components, namely the diet, the gut mucosa, and the commensal flora. The mucosa is composed of the digestive epithelium, the gut-associated lymphoid tissue (GALT), and the mucus overlying the epithelium. These components interact with each other to form a delicate and dynamic equilibrium within the GIT that ensures efficient functioning of the digestive system. In addition, an increasing number of protective proteins including immunoglobulins A, trefoil peptides, various growth factors, and cytokines, interact with the adherent mucus layer at the mucosal surface.[63]

Interactions between mucin and bacteria are particularly important in term of gut health; these interactions can be classified into two types. First, many commensal and pathogenic bacteria specifically adhere to complex carbohydrates of mucin. An optimal protection of the epithelium against bacterial infection requires an intact mucus layer at the surface, depending on both its quantitative (thickness) and qualitative (ability to fix bacteria) properties. Second, mucin is a potential substrate for bacterial fermentation. The end products of fermentation, especially SCFAs and ammonium, have different influences on gut health.[64] Quantitative or qualitative changes in mucin secretion, composition, and removal from the GIT lumen, as described below, may modify the mucosal defense barrier, may influence the fermentation capacity of the hindgut, and so may have important physiologic implications.[65,66]

Mucin and Bacterial Fixation

The fixation of commensal bacteria on the mucus layer prevents colonization by opportunistic pathogens (Fig-

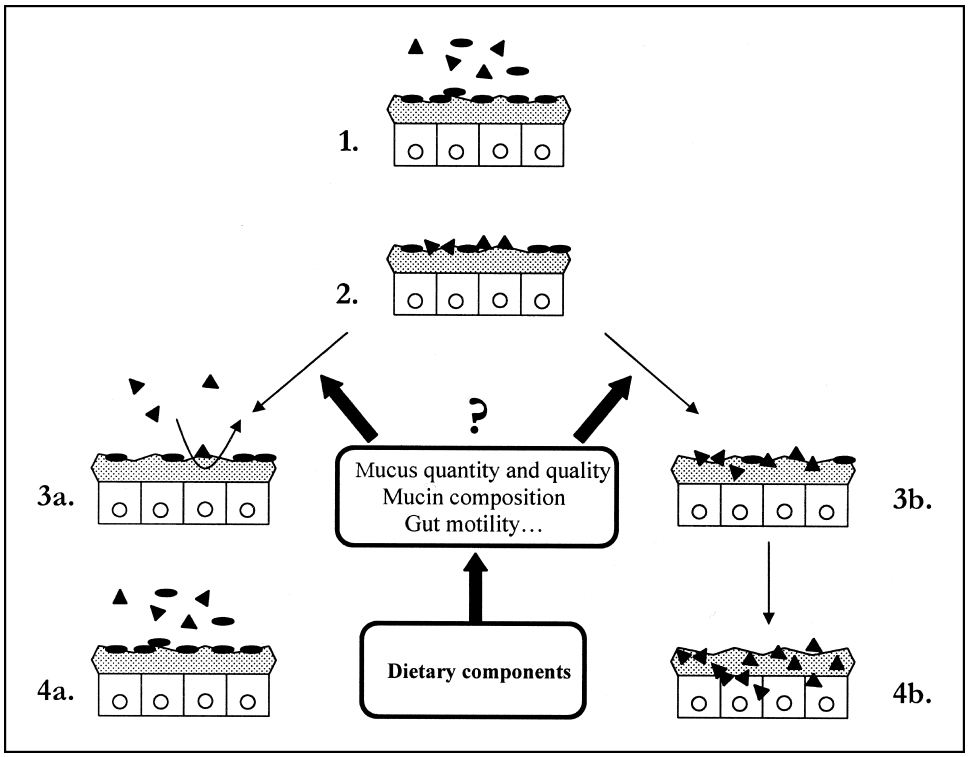

Figure 2. Fate of pathogenic bacteria in the gastrointestinal tract. (1) Commensal bacteria (●) are fixed on the luminal face of the mucus layer preventing fixation by pathogenic bacteria (▲), a phenomenon called the "barrier effect" or "colonization resistance." (2) If the colonization resistance is not complete, pathogenic bacteria can adhere to the mucus layer. This fixation can be beneficial (3a) or detrimental (3b) for the animal. (3a) Fixation of pathogenic bacteria on the mucus restricts their access to the underlying epithelium. Pathogenic bacteria are removed with mucus erosion and infection does not occur (4a). (3b) The rate of bacterial growth exceeds the natural turnover rate of the mucus. Bacterial colonization and proliferation occur leading to intestinal infection (4b). Factors relevant to mucin composition, quality and quantity of mucus, and gut motility influence the beneficial or deleterious outcome. Adapted from Forstner and Forstner.[5]

ure 2). This so-called colonization resistance may be a consequence of competitive exclusion of pathogens by the indigenous flora.[67] In other situations, the control exerted by commensal flora is more subtle and may involve the synthesis of molecules that non-specifically stimulate the immune system or chemically interfere with the interaction between pathogens and intestinal epithelia. Fixation of pathogenic bacteria in the mucus can be beneficial or detrimental to the host.[5] For many pathogens, such a fixation restricts free access to the underlying mucosa, causing mucus to act as an impermeable barrier or retention zone. Conversely, the ability of pathogenic bacteria to interact with mucin can be an important step in facilitating colonization of the GIT. If the bacteria are able to bind strongly to the mucus layer, their clearance through motility and abrasive forces of digestion may be delayed and colonization of the GIT may be favored. In addition, the rate of bacterial growth and penetration in the mucus can exceed the natural turnover rate of this layer and, therefore, favor bacterial colonization further.[68]

The state of GIT protection against bacterial infection seems to be linked to the degree of mucin maturation. Mature mucins are mainly sulfated.[69] The presence of sulfate and sialic acids on the carbohydrate chains confers to the intestinal acidic mucins physicochemical properties that are different from those of neutral mucins, resulting in higher viscosity and acidity.[70] These acidic mucins increase the ability of mucus to resist attacks by bacterial enzymes.[65]

Turck et al.[44] observed that fucose, glucosamine, and sulfate content of colonic mucin were increased in sow-fed piglets, when compared with piglets fed an artificial milk substitute. At 21 days of age, the protein content of mucin was significantly lower than at birth for both diet groups, but it was significantly higher than in the mucins of mature pigs. Moreover, the composition of colonic mucins in pigs fed sow's milk was more mature than that in artificially fed littermates, with respect to fucose, glucosamine, and sulfate. If a mature mucin composition is a more effective defense against intestinal infection, young breast-fed animals may have advantage over young artificially fed animals.[44] In the same way, it is conceivable that particular glycosyl structures appearing transiently represent "windows of opportunity" for infection of enteropathogens, whose adhesins exhibit the

appropriate specificity; such a rationale may explain in part why diarrhea attributable to some bacterial species prevails at certain stages of development.[71]

The state of health of the GIT seems to also be linked with changes in the amounts of mucus produced. For example, in growing pigs, feeding different prebiotics led to increases in the number of goblet cells in intestinal villi, which might have induced an increased epithelial mucin activity.[72] In sections of the proximal colon, the thickness of the mucus layer was significantly higher when the pigs were fed with these prebiotics than in control pigs.

Mucin and Bacterial Fermentation

Carbohydrates and proteins from mucin are fermented in the large intestine.[2] The end products of carbohydrate fermentation are SCFAs, mainly acetate, propionate, and butyrate. SCFAs are rapidly absorbed by the mucosa. Such preservation of energy has major implications for the maintenance of the colonic bacterial population and the metabolic needs of the epithelium.[64] SCFAs have a potentially positive role on the GIT because of their trophic effect on the epithelium and their antimicrobial effect. However, if the carbohydrate-to-nitrogen ratio in the digesta reaching the large intestine decreases, the fermentation becomes more and more proteolytic.[73] Fermentation of amino acids lead to branched-chain SCFAs (i.e., isobutyrate, valerate, isovalerate) but also to the formation of potentially toxic metabolites such as ammonia, amines, volatile phenols, and indoles.[74] Ammonia can disturb the development of the mucosa. The end products of mucin fermentation in the large intestine are, to our knowledge, not clearly known. They are probably largely dependent on the composition of mucin, particularly the carbohydrate-to-nitrogen ratio that is influenced by the diet.

In addition, several studies suggested that the incidence and severity of colibacillosis in piglets can be reduced by decreasing the amount of digesta reaching the large intestine.[75] Therefore, all dietary factors increasing the mucin release in the GIT lumen might favor the development of colibacillosis. By contrast, some digesta components might favor the development of a stable microflora in the cecum and colon. Clearly, more work is needed in this area.

Conclusions

Mucins are substantial constituants of basal endogenous protein flowing along the GIT. Dietary factors (fiber, protein, and anti-nutritional factors) might affect both the synthesis and secretion of mucin from the goblet cells, and the recovery of mucin in digesta. Dietary factors that increase the mucin synthesis and erosion would increase the maintenance requirements for amino acids (mainly threonine) and energy and, therefore, decrease their availability for animal growth and production. Changes in mucin secretion and synthesis following interaction with dietary components might be accompanied by changes in the composition of mucin and in mucus properties, including mucus thickness. In association with commensal bacteria and the GALT, mucin plays a key role in the maintenance of an optimal physiologic status that prevents GIT from pathology. More work is need to demonstrate whether manipulating mucin, through feed or feeding strategies, prevents or enhances GIT pathologies. This may represent a new interesting possibility for enhancing GIT function. The challenge is to find nutritional strategies that maximize the protective effect of mucus and minimize the metabolic costs associated with mucin production.

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