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Review Article

Endogenous mucin conveyed to the mucosa with microbes can assure lumen fermentation and large intestinal security—swine versus fowl

Edwin T. Moran [a, 1], Michael R. Bedford [b, *, 1]

[a] Poultry Science Department, Auburn University, AL 36849-5416, USA
[b] AB Vista, Woodstock Court, Blenheim Road, Marlborough Road, Wiltshire, SN8 4NA, UK

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ABSTRACT

Endogenous protein leaving the ileum largely consists of accrued mucins from the upper gastrointestinal tract (GIT) that had resisted digestion. The amounts released rely on their mucosal generation during enteral feeding which vary with age as well as diet. These digestion resistant proteins of endogenous origin continue to be unavailable in the large intestine, whereas those of dietary origin provide amino acids that largely support the existing microbial population while denying limited amounts for absorption. Other mucins pre-exist within the large intestine as two layers at the lumen surface. A loose layer harboring a diverse microbial population is superimposed on the unstirred water layer (USWL) which simultaneously acts as an obstacle to microbes at the loose layer while performing as a molecular sieve for nutrients. The USWL is formed through interplay between enterocyte and goblet cells; however, the basis for presence of the loose layer is elusive. Large intestinal fermentation predominates within the colon of swine, whereas fowl employ their ceca. Motility within the colon of swine segregates fine materials into haustrae out-pocketings that parallel their placement within the ceca of fowl. Viscous mucins from small intestinal endogenous losses may envelop microbes within the large intestinal lumen to present successive adherents on the USWL that assemble its loose layer. The loose layer continually functions as a microbial reservoir in support of lumen fermentation. Microbial catabolism of mucin within the loose layer is known to be slow, but its proximity to the enterocyte is of advantage to enterocyte absorption with by-product amino acids fostering the USWL.

1. Introduction

Endogenous proteins entering from the upper gastrointestinal tract (GIT) that proceed to the large intestine are overwhelmingly dominated by mucins that vary in amount with diet composition and age of the animal. Although pancreatic enzymes, bile, and other contributors are also of endogenous origin, autolysis enables the vast majority of their associated nutrients to be recovered before leaving the ileum. On the other hand, mucins from the oral cavity and gastric system together with those from the small intestine combine contribute to represent ileal loss; again, the large intestine's microbial population also has difficulty with their dismemberment (Lien et al., 2001). Measurements of amino acids associated with endogenous loss are typically employed to allow for the determination of the true amino acid digestibility of an ingredient or diet. The mucin component of endogenous losses is not constant, but continuously changes with the conditions and terms of enteral feeding while parenteral nutrition bypasses mucosal modification of mucin regeneration (Leterme et al., 1996; Rérat, 1995). The many aspects of endogenous loss have been reviewed for swine (Boisen and Moughan, 1996; Nyachoi et al., 1997) as well as fowl (Soomro et al., 2018).

Gastric and small intestinal secretions of mucin can be significant, the entire contents of goblet cells being released and replaced in approximately 12 h in an almost continuous process, particularly

* Corresponding author.
E-mail address: mike.bedford@abvista.com (M.R. Bedford).
[1] Both authors contributed equivalently to this work.
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those goblet cells located in the villi (Schneider et al., 2018). Goblet cells in the crypts tend to accumulate and release mucin in a more pulsatile manner and all goblet cells release almost all their mucin in an explosive exocytosis process in response to acetylcholine release but release also responds to many signals, diet, host and bacterially derived (Sittipo et al., 2019), some of which are listed below. Estimates of basal mucin output from 55 kg pigs suggested as much as 3.9 kg of mucin is normally produced per kilogram of intake. In protein-free diets there is relatively limited production compared with wheat or bean-based diets which not only increase mucin production markedly but favour proportionately more gastric as opposed to small intestinal loose mucin production (Lien et al., 2001). Supply of threonine, proline and serine are critical for the synthesis of the backbone of mucins and limitations in their supply can restrict mucin production (Ravindran et al., 2004). Fermentable dietary fibre can significantly increase mucin production as a result of accumulations of short chain fatty acids (SCFA), particularly butyrate (Jung et al., 2022), which is used as an energy source for mucin production. Fermentation of protein and nitrogenous compounds produces indoles and polyamines amongst other compounds, which are generally considered detrimental to gut health but in both cases these compounds have been associated with an increase in mucin production (Sittipo et al., 2019). Increased levels of insoluble fibre can markedly increase erosion of mucus from the loose layer and as a result demand greater rates of synthesis in the GIT which provides more mucin in the large intestine for fermentation (Duangnumsawang et al., 2021).

Mucins entering the large intestine are accompanied by other indigestible proteins but mostly of dietary origin. Such dietary sources predominantly arise from structural animal proteins as well as plant sources which are often encapsulated within intact cells by complex carbohydrates (CHO). Indigestible dietary protein largely succumbs to fermentation by the large intestinal microflora; however, the resulting amino acids and metabolites released into the lumen are expected to be overwhelmingly recovered in support of the existing population. Gastro-intestinal mucosa secretions, which include mucins, are thought to contribute up to 64% to 83% of daily endogenous losses, and although 70% to 90% of endogenous losses are estimated to be reabsorbed by the terminal ileum, little of this is mucin due to their proteolytic resistance which is attributed to the presence of large quantities of threonine and serine which project *O*-linked oligosaccharides from the protein core effectively blanketing the three-dimensional structure of mucin (Lien et al., 2001). Microbes lack competence at cleaving many of these *O*-linked saccharides thereby denying proteolytic access to the core (Lien et al., 2001). Thus, the majority of endogenous losses entering the large intestine are derived from mucins, and from this point on they disappear in the large intestine either due to microbial fermentation or absorption by the host (Lien et al., 2001; Montagne et al., 2004). The extent of mucin fermentation can be significant, especially in low fibre diets which seem to favour mucin-degrading bacterial species as other sources of fermentable carbohydrate are limited (Montagne et al., 2004).

Mucins in the large intestine other than those entering as endogenous losses have been shown to appear as two layers on the large intestine's mucosa. The first layer is loosely associated with the lumen surface that appears to continually exchange representatives among the microbial population. The second layer or unstirred water layer (USWL) is formed by the collaboration of enterocytes with goblet cells that create a completely separate mucin barrier. By having a distinct internal structure, the entry of microbes from within the loose layer is restricted while simultaneously limiting the size of nutrient forms that can pass through for absorption (Corfield, 2015; Johansson et al., 2008; Moran, 2016). Preferential use of amino acids by microbes within the lumen not only minimizes their potential absorption and villus use, but a separate modified and limited large intestinal vascular system within the mucosa further discourages access from body sources (Ahmadinejad et al., 1991; Wille and Schenk, 1997). As a consequence, large intestinal enterocytes and goblet cells have meager accessibility to amino acids for their own use.

Little has been established about the metabolism of any nitrogen (N) source in the large intestine from the point where it enters from the ileum until exiting via the rectum or cloaca. Shimotoyodome et al. (2005) have shown that mammalian fecal pellets are covered with a uniform layer of mucin that is similar among animals, but its total amount and source lacks definition. Hendricks et al. (2012) reviewed the literature regarding the quantity of N realized by the large intestine of different animals. The disappearance of crude protein (CP, N $\times$ 6.25) between the terminal ileum and the rectum was used to represent the amount of N recovered by the large intestine's mucosa. Values approximating 14% to 39% N of total dietary intake were reported to be absorbed for several non-ruminant herbivores which was considerably greater than the 8% average for pigs and -1% with chickens. The particularly low values for fowl can be attributed to additional N being retro-peristaltically conveyed from the urodeum into the ceca, thereby increasing the apparent N content.

Most sources of absorbed N have been assumed to appear as ammonia (NH_3) which can be eliminated in the urine, whereas fecal N is largely considered to be microbial protein. Based on the actual disappearance of total amino acids between the ileum and those reaching the rectum in swine, Sauer et al. (1980) noted that the resulting their pattern of apparent absorption paralleled that of mucin, regardless of dietary feedstuffs employed. Although absorptive sites for amino acids and peptides have been identified within the large intestine's mucosa, classical measurements have failed to establish that they are absorbed from this section of the intestine in sufficient quantities to enter the host's portal system (Darragh et al., 1994; Jarvis et al., 1977; Obst and Diamond, 1989). However, such measurements neglect the indirect benefits for the host if they had been quantitatively used for villus maintenance.

The pig's GIT is representative of simple stomached mammals, particularly the human, whereas fowl convey that of domestic avian systems. Swine focus on the use of their colon for nutrient recovery from the large intestine, whereas fowl defer recovery to both ceca (Moran, 2022). Double layering of mucin associated with the large intestine's mucosa has been substantiated with mammals (Szabóová et al., 2018); however, Duangnumsawang et al. (2021) review of mucins throughout the fowl's intestine lacked available research meaningfully convey double layering in the ceca. The following rationalizes that the release of endogenous mucins from the small intestine and their transit into the large intestine may well be purposeful with both animal types. Presumably, ileal mucins being viscous likely capture microbial representatives during motility to form a composite that becomes loosely adherent to the lumen's USWL. The slow microbial release of amino acids from within the loose barrier mucin is presumed to improve the mucosal absorption and maintenance of the USWL. A cursory description of the small intestine's mucosa and the creation of endogenous loss is first given to provide a perspective of the large intestine and speculative use of endogenous mucin. The breadth of this topic requires dependence on many reviews for brevity of referencing while considerable poetic license is employed to assemble a hypothesis covering a complex topic.

2. Small intestinal mucosa

Every animal's small intestine has the task of retrieving nutrients from digesta previously prepared by the oral and gastric

systems. The primary structure of the small intestine parallels that of the large intestine by having four layers with the mucosa being inner most to the lumen followed by the submucosa, primary circular-longitudinal muscles then the serosa. The mucosal layer is particularly dynamic as a result of its continuous replacement of epithelial cells that are responsible for nutrient absorption while providing the greatest part of endogenous mucins that continue to the large intestine.

Two types of cells dominate the small intestine's villus as it projects into the lumen. Enterocytes and goblet cells evolve from stem cells in the crypt that mature during movement to the apex in a manner that is similar for both swine (Slupecka et al., 2010) and fowl (Uni et al., 1998). When mature these cells approximate one-third of the upper villus where they collaborate with each other to retrieve nutrients from the lumen. An extensive vascular network exists within the mucosa that begins as an arteriole proceeding from the submucosa through to the villus apex. Oxygen is carried by the arteriole to act as support for energy generation throughout the system. Thereafter, a perfuse network of venules that are porous to nutrients cascade down from the villus tip to "feed" surface epithelia en route to the portal system. Absorbed nutrients not only support the immediate needs of cells at the apex but subsequently provide for those in the process of developing. Considerable "information" is concurrently relayed by the pattern of nutrients absorbed such that cellular adjustments during maturation accommodate conditions that are continually changing in the lumen. The assembly of digestive enzymes, the necessary transport systems on microvilli, and the presentation of the glycocalyx are but a few items in continual need of adjustment. Such items within the chick's jejunum appear to be reacting to this messaging independently of one another while adjusting to lumen conditions (Shehata et al., 1984). Once formed, fowl enterocytes have prominent microvilli but present a sparse glycocalyx compared to mammals; however, overall cell appearances are similar in all other respects (Michael and Hodges, 1973).

Motility is central to the convective exchange of digestion products in the lumen with the USWL and then nutrient absorption. The glycocalyx is a membrane associated mucin that has an intricate relationship within microvilli (Carraway et al., 2003; Mooseker and Tilney, 1975). The glycocalyx influences internal actin filaments that move the microvilli in response to lumenal nutrients favorable to their convection. Such motility is complemented by minor longitudinal muscle fibers projecting from the *muscularis mucosae* that rotate the whole villus in the lumen. This movement further creates a "pumping" that moves lymph in a central lacteal to the vena cava.

Significant convection within the intestine of mammals arises from periodic segmentation created by the major overlying circular muscles. The minor circular fibers within the adjacent *muscularis mucosae* superimpose additional contractions on the surface during segmentation that create Kerkring Valves that accentuate lumen exposure. Fowl, on the other hand, do not have perceptible *muscularis mucosae*. Mammals export dietary fat from the enterocytes as chylomicrons and their large size necessitates transfer into the lymph. Fowl absorb fat as very low-density lipoproteins that enter venules, and in turn, lacteals are absent. Villi shape further influences the effectiveness of motility. Swine generally present cylindrical villi into the lumen that readily realize convective exposure from segmentation; however, fowl employ reflexive peristalsis along a mucosa that is predominantly presented as "wavy-shaped" villi and receive minimal advantage from Kerkring Valves.

Goblet cells are placed in a mosaic pattern with enterocytes on the villi where they release a soluble mucin that spreads over adjacent microvilli. Nutrient retrieval from the underlying venules by goblet cells high on the villus is expected to be significant as they have an extensive nutrient need in order to support continual release of large amounts of soluble mucin. Conversely, the nutrient requirements for the comparatively small amounts of membrane-associated mucin secreted as the glycocalyx would be far less for those enterocytes undergoing maturation low on the villus. Dietary threonine, methionine-cystine, serine–glycine, glutamate-glutamine, and proline would be particularly useful for mucin synthesis. Such "first-pass" removal of venule contents before entry into the portal system reduces subsequent amino acid availability for the body at-large with both swine (Fang et al., 2009; Lambert et al., 2006; Schaart et al., 2005) and fowl (Bartell and Batal, 2007; Wils-Plots and Diger, 2013; Zhang et al., 2016).

Mucin synthesis not only requires amino acids for its core protein but glucose in conjunction with glutamine are necessities to form the associated oligosaccharides. Glucose proceeds through glycolysis to fructose-6-phosphate where the γ-NH_3 of glutamine is transferred to form glucosamine-6-phosphate thereafter initiating the synthesis of an array of oligosaccharides (Durand et al., 2008). Swine small intestinal mucus has been shown to generally contain galactose, mannose, *N*-acetylgalactosamine, *N*-acetylglucosamine, *C*-6-sulfated-*N*-glucosamine, fucose, and sialic acid that may be sulfated as well (Choi et al. 1991; Karlsson et al., 1996). Sialic acid and fucose are frequently located at positions where their bio-enzymatic removal is particularly difficult thereby interfering with mucin degradation. Keto acids arising from a multitude of transaminations in the process of mucin synthesis represent the primary source of energy for the intestinal epithelia upon entering the Kreb's Cycle (Wu, 1998).

The structure of mucin produced by enterocytes is very different from goblet cells (Forstner, 1995). The core protein from the enterocyte lends to linear polymers involving threonine–serine projecting *O*-oligosaccharides. Collections of these linear polymers are used to assemble membrane associated mucins such as those destined to be the glycocalyx. Goblet cells separately interconnect core sections of mucin proteins by employing cystine to form exceptionally large "fish-net" like molecules (Ambort et al., 2011; Park et al., 2009). These cells then employ Ca^{++} to moderate surface charges arising from sialic acid and sulfated saccharides enabling molecular condensations that create secretory granules having reduced size (Ambort et al., 2012). These granules once released into the lumen become solubilized to greatly expand more than 1,000-fold due to water uptake by their dominant oligosaccharides.

Solubilized mucin from goblet cells is spread across adjacent enterocyte microvilli in a manner facilitated by overall motility thereby initiating nutrient absorption. Maintaining solubilized mucin at the surface seems particularly dependent on its "entangling" with the microvillus glycocalyx. Staining of goblet cells relates to the prominence of neutral and acidic mucins within the small intestine's secretory granules that eventually entangle with the glycocalyx. Pastor et al. (1988) observed both sialo and sulfomucins to be well associated with goblet cells of the chicken's jejunum while Morè et al. (1987) noted that both mucins also occur in the pig's small intestine and that these change in proportions with the nature of feed.

Mucin also functions as a localized buffer that optimizes the terms for final enzyme digestion and active transport while small molecular neutralizations enable direct membrane transfer (Moran, 2016). Protein digestion by pepsin and the array of subsequent pancreatic enzymes generally leads to the release of free essential amino acids while the non-essentials largely appear as peptides. Similarly, di- and tri-saccharides arise from amylase activity on starch. All products from digestion in the lumen must pass through the soluble mucin's micro-sieve in order to be accommodated at the microvillus surface.

The contribution of mucins from the various sections to the GIT that become part of the endogenous loss can vary with the feedstuff being digested. Lien et al. (2001) noted that approximately 75% of the mucins from endogenous loss with swine were derived from the small intestine when using a protein-free diet, whereas the inclusion of wheat reduced intestinal contribution to 45% while gastric mucin increased to 45%–50%. The absence of fluid saliva and oral mastication together with the use of the proventriculus-gizzard that affects gastric digestion can be expected to alter mucin contributions from these sections differently than those of swine.

Soluble type mucins predominate from all GIT locations to collectively overwhelm those types present at ileal release. Release from the small intestine is expected to be substantial and result from two separate situations. Solubilized mucin leaving the goblet cell may not be "captured" by the glycocalyx during convective exchanges at the lumen interface. Separately, a more extensive mucin release probably results from surface cell senescence. Epithelia turnover approximates 3 1/2 days from crypt to apex which involves both enterocytes and goblet cells (Duangnumsawang et al., 2021; Montagne et al., 2004). Holman (1975) described the structural changes during extrusion of enterocytes in the small intestine of chickens. The final stage entailed lumen release of residual parts of the cell after hydrolytic activity by resident fibroblasts and macrophages. Such residual appears to be "pushed" into the lumen as remnants of microvilli which is expected to be much less as glycocalyx than the extended volumes of soluble mucin.

3. Large intestinal mucosa

Endogenous mucins entering the large intestine are accompanied by several other forms of indigesta that require fermentation to realize their nutritional value. The development of a functional population of strict anaerobes is most facilitated when they are placed at favorable locations within the large intestine. Swine have all indigesta enter directly from the ileum into the cecum (Fig. 1), whereupon the composite continuously encounters microbial action throughout an extensive helicoidal colon (Argenzio and Southworth, 1974). Conversely, fowl eject endogenous and other indigesta directly into a short colon where fluids and finer materials are retro-peristaltically returned into two ceca for fermentation (Fig. 2). Concurrently, coarse materials move caudally to collect in the cloaca for fecal excretion (Józefiak et al., 2004).

Placement of the most labile indigesta occurs adjacent to mucosa where microflora are most competent at fermentation thereby optimizing nutrient recovery. Swine gather their longitudinal muscle fibers into two groups opposite each other through the length of the helicoidal colon. Motility initiates a "bulging" of the circular muscle in the absence of overhead stabilization by longitudinal fibers (Barbiers et al., 1994; Huizinga et al., 1983; Thornton et al., 1983). The net result of this bulging is to peripherally assemble readily fermentable materials into haustrae pockets while collecting difficult coarse fiber at the core. Each pocket develops in the cecum during entry of ileal indigesta whereupon circular muscle moves the contents within each bulge caudally. Simplistically, the mucosa at any one point is exposed to the contents of the next haustra as aboral movement (Lentle and Janssen, 2008; Moran, 2022). From another perspective, mucosa corresponding to each location would be fixed in place and expected to retain the microbial population associated within the loose layer of mucin (Moran and Bedford, 2022). Hypothetically, microbes in the loose layer at each haustra's location would be those most relevant to increased CHO complexity created by the previous haustra's fermentation from which volatile fatty acids (VFA) are produced and absorbed (Fig. 3) through the mucin layers (Moran and Bedford, 2022). Separation of coarse material into the core infers that less nutritional value can be expected and hence the core experiences a more rapid movement to the rectum (Brunsgaard, 1998).

Colloids and fine materials circulate within each haustra's lumen where they encounter microflora and are convectively conveyed to the mucosal surface. The size of the haustrae is expected to diminish during its caudal progression as nutrients from fermentation are absorbed. Resistant starch and other microbiologically fragile CHO are the first to disappear (Tiawari et al., 2019). Again, having the microbes retained within the loose mucin permits a progressive accommodation to the increasingly complex composition of contents during colon transit. Eventually, difficult cellulosics and lignin collect in the pocket as a residual "pellet." Once the distal colon approaches and longitudinal muscle fibers return around the intestinal circumference, haustrae bulging recedes. The mucin which has been shown to cover fecal pellets at evacuation which would seem to arise from encapsulation by the mucosa's loose mucin during final haustra contact (Shimotoyodome et al., 2005). These pellets once formed may now "press" onto the coarse core leading to nodulation of resulting feces (Moran, 2022).

The strategy in the positioning of ileal indigesta within the fowl's large intestine is vastly different from swine. Circular and longitudinal muscles remain in their respective layers throughout the fowl's large intestine. Circular muscle fibers are extensively employed during retroperistalsis of fluids and finer materials from colon into proximal ceca (Duke, 1989; Lai and Duke, 1978). The entrance to the fowl caeca is constricted in size and effectively prevents entry of large particulates and even large, soluble viscous fibers (Choct et al., 1996; Svihus et al., 2012). This might therefore restrict mucin entry into the caeca and as a result mucin fermentation in the hind gut in fowl may be restricted to the colon. However, there are some indications that fermentation of mucin in the caeca takes place, suggesting such sieving does not quantitatively exclude all mucins (Apajalahti and Vienola, 2016; Parsons et al., 1983) but this clearly needs further investigation. Prominent villi in the proximal ceca extract available nutrients and water (Svihus et al., 2012) using segmentation that decreases fluidity of contents before conveyance from proximal to distal ceca. The lumen volume of the distal ceca largely remains constant despite repetitive entries ensuing from the proximal ceca (Danziger, 1989; Strong et al., 1989). The volume of indigesta continually entering the distal ceca is largely balanced by removal of VFA (volatile fatty acids) and water. A perpetual microbial action within this fixed volume progressively expends the labile substrates during fermentation while concentrating the crude forms (Moran and Bedford, 2022). Spent contents eventually accrue to provoke a peristaltic rush that evacuates the entire large intestine as a separate cecal excreta (Takahashi et al., 2004). "Fresh" material then refills the ceca with frequency of each cycle and duration for its completion being commensurate with feed intake and associated fiber.

Thickening of distal ceca contents as each cycle progresses suggests modification from representation as simple to complex hemicellulosics. Such disappearance of simple with accrual of resistant materials would seem to necessitate a continual microbial adaptation. An extensive mucin layer at the mucosal surface is known to contain an extensive microbial population with fowl (Fuller and Turvey, 1971; Salanitro et al., 1978). The exchange of microbes from the mucosal surface to the lumen is expected to occur as does their adaptation to substrate complexity given the many hours involved with each cycle. Tsirtsikos et al. (2012) compared the mucin composition within duodenum, ileum, and cecum in broilers (Table 1). Differences in the proportions of

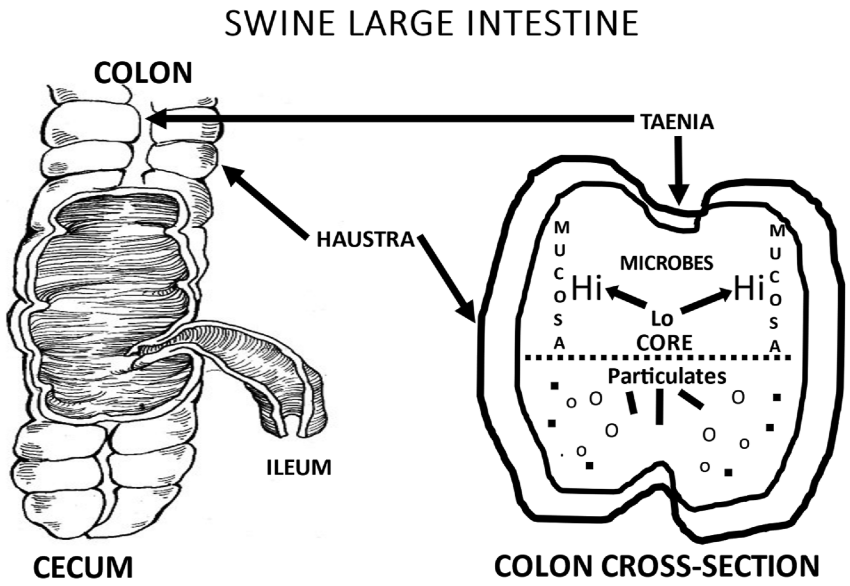

Fig. 1. Schematic representation of the pig's anterior large intestine and cross-section of the anterior colon. Bulging of circular muscle during motility creates haustra where readily fermentable material collects with microflora as coarse particulates concentrate in the core. Selected material redrawn from Moran (1982, 2022). Hi = high density microbial populations; Lo = low density microbial populations.

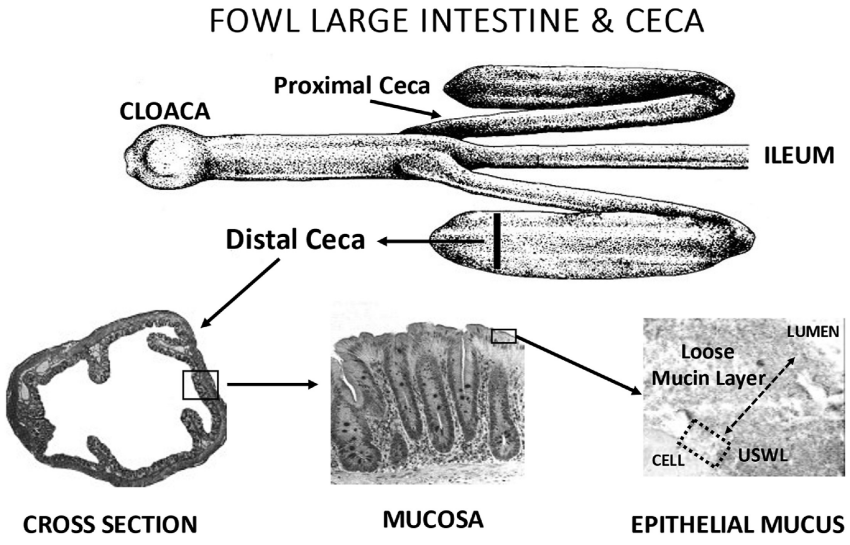

Fig. 2. Schematic representation of the fowl's ceca with appearances of a distal cross-section, its mucosa, and speculated location of two mucus layers (loose and unstirred water layer [USWL]). Selected diagrams redrawn from Salanitro et al. (1978), Moran (1982), Moran and Bedford (2022).

monosaccharides between sections were minimal; however, the amount of fucose present in the cecum was greater than that in the small intestine. Given mucin fucose concentrations increased with transit through the intestinal tract it suggests this sugar is associated with specific mucins that seem to defy fermentation more so than others (Tsirtsikos et al., 2012), indirectly suggesting an alteration of the microbial population.

Close adjacency of villi within the pig's colon and the fowl's distant ceca creates a comparatively “flat” surface compared to the small intestine's “shag rug” profile. Cells immediate to the lumen surface continue to present enterocytes with goblet cells in a mosaic pattern. These goblet cells predominantly have an acidic staining in the pig (Morè et al., 1987) as well as fowl (Pastor et al., 1988). Again, the two layers of mucin existing between the open lumen contents and epithelia have been well documented in mammals but alluded to more than substantiated with fowl. The top layer of mucin typically has a much greater depth while presenting a loose character that may either envelope and/or release members associated with the microbial community (Johansson et al., 2011). Whilst the pore size of the top layer of mucin is considered to be between 0.5 and 2 μm, that of the membrane-associated mucin is less than 0.5 μm which precludes penetration by bacteria (Birchenough et al., 2015; Duangnumsawang et al., 2021). The soluble portion acts in parallel to the USWL of the small intestine as a nutritional microfilter. Measurements relating to mucin thickness of the mucosa with fowl are much less than that noted for the pig (Tables 1 and 2) suggesting that measurements employed with fowl differed from those with swine.

The nutritional wherewithal to generate mucin by the large intestine's mucosa lacks clarity compared to the small intestine. Energy does not appear to be an obstacle as indigestible CHO avail considerable amounts of by-product VFA. While these VFA have

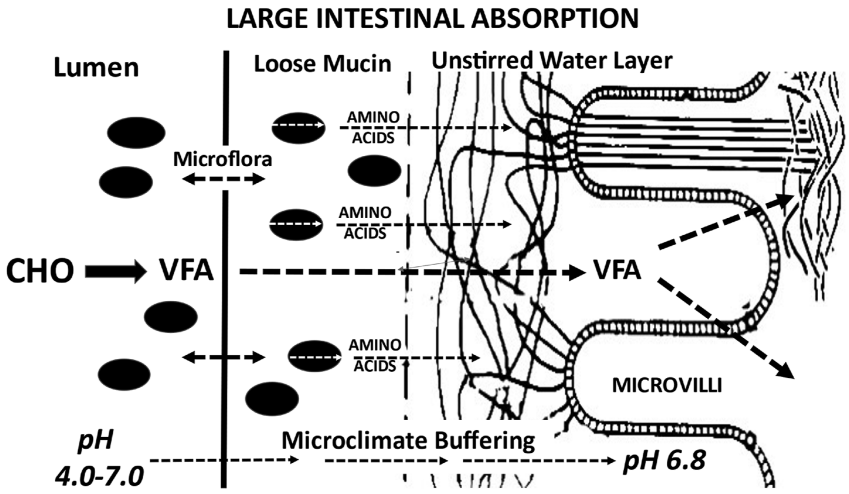

Fig. 3. Schematic representation of volatile fatty acid (VFA) and amino acid absorption through two mucus layers of the pig's colon. Redrawn from Moran and Bedford (2022). CHO = carbohydrates.

Table 1
Mucin monosaccharide molar ratios in the 42-day-old broiler intestine.[1]

Mucin monosaccharide	Total mucin monosaccharide, %		
	Duodenum	Ileum	Ceca
N-acetyl-glucosamine	33.1	34.8	30.8
N-acetyl-galactosamine	10.9	8.9	8.7
Galactose	24.1	27.2	28.2
Mannose	4.2	2.5	3.6
Fucose	12.2	12.0	22.2
N-acetyl-neuraminic acid	15.6	14.7	7.0
Mucus thickness, μm	14.6	16.8	16.1

[1] Selected data from broilers receiving common feed (Tsirtsikos et al., 2012).

been shown to generously pass-through epithelia to the host, mucosal cells preferably retain butyric acid as a source of energy that distinctively supports formation of oligosaccharides for mucin formation (Darcy-Vrillon et al., 1993; Mentschel and Claus, 2003; Morel et al., 2005). Tsukahara et al. (2003) demonstrated that dietary fructooligosaccharides were particularly stimulating at butyrate production in the cecum and proximal colon of piglets while simultaneously decreasing pH as VFA is collectively escalating (Table 3).

The presentation of amino acids to the epithelial cells for the assembly of either mucin layer is not apparent. The capacity of amino acid and monosaccharide absorption is apparent early in life, but little recovery based on classical measurements can be established with either animal once microflora were apparent in the lumen (Darragh et al., 1994; Jarvis et al., 1977). Nyström et al. (2021) offered that loose mucin could form as a plume in mucosal crevices by lateral goblet cells and then rise to cover the surface. A speculative hypothesis presented here is that the loose mucin does not arise from epithelial cell secretions but is directly secured from ileal mucin. As implied with the introduction, endogenous mucins when released from the ileum are viscous and it is proposed here that such viscosity in conjunction with motility likely envelope microbes in transit and perhaps multiple placements of this composite progressively generates the loose layer in the lower large intestine.

Multiple layering of this free “floating” composite above the USWL can vary with location along the large intestine. Poor gel structure is suggested from loose mucin's known weak adherence and fracturing encountered during microscopic efforts (Johansson et al., 2008; Röhe et al., 2018; Sellers et al., 1991). Layering of loose mucin would initially be thin at the cecum to subsequently thicken with a diminishing accrual in the colon until haustra content is depleted. On the other hand, Varum et al. (2010) citing Sousa et al. (2008) attributed mucus thinning at the cecum to result from extensive microbial activity, but no potential for loose mucin's initial presence was forthcoming.

The USWL is unlikely to form from loose mucin but must be created by the underlying epithelia. Epithelial synthesis is defensible in terms of available energy as VFA, but not without accessible amino acids. Potentially, the loose mucin may not only act as a lumen barrier but provide amino acids released during microbial fermentation. The ability of large intestinal microbes to degrade mucin is known to be highly restricted (Arike et al., 2017; Etzold and Juge, 2014; Tailford et al., 2015); however, Parsons et al. (1983) observed twice the amount of glucosamine and galactosamine, indicators of the presence of mucin, were present in cecectomized rooster excreta than intact, suggesting that a substantial mucin degrading capacity exists in the cecum. Location of these amino acids being adjacent to enterocytes and goblet cells would improve the likelihood of their absorption and immediate use. A complete consumption of these amino acids for mucin resynthesis as the USWL likely circumvents detection by measurements relying on their portal appearance.

Table 2
Mucus thickness (μm) of each section of the pig's gastrointestinal tract.[1]

Item	Thickness
Stomach	
Antrum	56.1
Body	67.9
Fundus	51.5
Small intestine	
Duodenum	25.9
Jejunum	28.6
Ileum	31.0
Large intestine	
Cecum	19.4
Anterior colon	31.9
Rectum	40.8

[1] Selected data from Varum et al. (2010). Values are the average of 9 adult swine.

Table 3
Effect of dietary fructooligosaccharides on the piglet colon, pH and volatile fatty acids.[1]

Dietary treatment	Large Intestine	Moisture, %	Lumen pH	Volatile fatty acid, mmol/kg			
				Total	Acetate	Butyrate	Propionate
Control diet	Cecum	79.0	6.5	84.6	44.3	9.1	27.0
	Ant. colon	73.1	6.9	96.0	50.0	10.2	27.7
	Post. colon	69.1	6.8	68.3	33.4	6.8	21.0
	Rectum	65.2	6.6	50.4	30.7	5.0	15.5
Control diet with 10% fructo-oligo-saccharides	Cecum	86.9	6.1	144.2	68.0	28.8	12.7
	Ant. colon	82.9	6.1	147.5	71.3	35.0	32.2
	Post colon	74.8	6.5	99.7	46.3	19.2	21.3
	Rectum	74.7	6.5	67.7	34.4	10.5	14.2

Ant. colon = anterior colon; Post. colon = posterior colon.
[1] Selected data from Tsukahara et al. (2003). A total of 40-day-old castrated piglets (20 per treatment) were sampled after 7 days dietary adaptation. Control feed was based on standard ingredients.

4. Conclusions

Endogenous loss occurs as a cost of food digestion throughout the upper GIT. Swine are good representatives of mammals while fowl favor the avian species with both species consuming similar foods. The primary objective of measurements on endogenous loss to date has been their use to correct for dietary amino acid availabilities. The proposal here attempts to rationalize that endogenous loss is not a waste but would provide useful products that enhance overall large intestinal functioning.

Effective operation of the large intestine depends on a thriving population of strict anaerobes to ferment the contents from the ileal effluent. Sustaining satisfactory terms of operation for anaerobes would seem to be an opportune use of endogenous mucins. Mucins throughout the upper GIT provided selective barrier functions between lumen and mucosa. An intensive anaerobic population in the large intestine would require an extensive barrier that must not only protect the host but provide a microbial environment which limits the encroachment of oxygen. Having two layers of mucin exist on the mucosa serves both purposes.

The placement of ileal mucin as a loose wall facing the large intestinal lumen could provide a first layer with its by-product amino acids relieving deficits needed to form the second layer. Epithelia must synthesize the USWL. Microflora within the loose layer likely ferments its mucin but only to a limited extent. Although marginal amounts of amino acids are likely to result, the location of these amino acids immediate to the epithelia optimizes absorption and immediate use at synthesizing the second layer. Endogenous loss is continual and probably commensurate with mucin amounts needed to sustain the large intestine's mucosa.

Author contributions

Edwin T. Moran and **Michael R. Bedford**: Conceptualization, reviewing and editing. **Edwin T. Moran**: Tables and Figures visualization.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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