

Intestinal Uptake and Metabolism of Threonine: Nutritional Impact

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■ Introduction

Intestinal metabolism of dietary essential amino acids and its potential impact on their availability for whole-body growth has been the focus of many investigators' research over several decades. One of the major questions was concerned with the discrepancy between the disappearance of dietary essential amino acids from the small intestinal lumen and appearance in the portal circulation despite the fact that most commonly fed proteins are almost completely digestible. The earlier pioneering studies with adult pigs made clear that the disappearance of essential amino acids from the lumen of the small intestine overestimates their availability to peripheral tissues of the host (Rerat et al., 1988; 1992).

Much of what we know now about intestinal amino acid utilization is derived from studies that have measured the metabolic exchange of amino acids across the portal-drained viscera (PDV) of young pigs (review: Burrin and Stoll, 2005). The PDV, a collective term for the entity of the small and large intestine, stomach, pancreas, and spleen, contribute approximately 5% to the whole-body weight in pigs, yet they account for 20-35% of whole body protein turnover and energy expenditure.

The disproportionate impact of gastrointestinal tissues on whole-body metabolism is a function of their relatively high fractional rates of protein synthesis and oxygen consumption. The high rates of metabolism and nutrient utilization in the gut are directly linked to the high rates of proliferation, protein secretion, and cell death. In young pigs, epithelial cells have a life span of about three to ten days. These epithelial cells are in fact a mixture of four lineages of differentiated cells derived from stem cells located in the proliferative crypt compartment within the intestinal epithelium: absorptive enterocytes, mucin-producing goblet cells, antibacterial peptide-producing

Paneth cells, and enteroendocrine cells. Studies in vitro have shown that these cell types exhibit high rates of protein synthesis and glutamine metabolism. In particular, goblet cells utilize substantial amounts of nutrients for the synthesis of mucins, which are a major component of endogenous secretions that are fermented in the colon.

Considering the multitude of functions of the gut tissues, including nutrient absorption, proliferation, and protection of the host, their intense metabolic activity is not surprising. Hence, it seems logical that the metabolic fate of

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dietary essential amino acids utilized by the intestinal tissues will have a critical influence on their availability for growth and thus, on their requirement. This might become of even greater importance for the essential amino acids threonine, lysine, and methionine, the first limiting amino acids in milk-based formulas and/or cereal diets for growing pigs.

■ Intestinal Metabolic Fate of Dietary Threonine

Once taken up by the intestinal tissues, amino acids can be utilized for three major metabolic purposes: (1) incorporation into protein; (2) conversion via transamination into other amino acids, metabolic substrates and biosynthetic intermediates; and (3) complete oxidation to CO₂. In the first two pathways, amino acids can be deposited and recycled by the body for purposes of growth or other biological functions. In the case of some amino acids, namely threonine and cysteine, incorporation into endogenous secretions that are fermented in the large intestine represents a nutritional loss. Likewise, if the amino acids are completely oxidized to CO₂ by the mucosal cells, this is also a nutritional loss, especially in the case of essential amino acids.

Net Portal Balance

The net portal balance technique, which involves measurements of amino acid concentrations in arterial and portal-venous blood as well as portal blood flow rates, has been extensively used to measure the appearance of dietary amino acids in the portal circulation (Rerat et al., 1988; Simoes-Nunes et al., 1991; Stoll et al., 1998). Consequently, the difference between dietary intake and portal appearance reflects the portion of dietary amino acids that must have been utilized by the PDV. Studies in pigs (Stoll et al., 1998; van Goudoever et al., 2000; van der Schoor et al., 2002) and dogs (Yu et al., 1990) have shown

that on average 50% of the dietary essential amino acid intake is extracted by the PDV in first-pass, and less than 20% of this fraction is incorporated into constitutive protein. In the case of threonine, intestinal first-pass extraction ranges from 60-80%, and thus, makes threonine the single most utilized essential amino acid by the PDV. In agreement with this enormous intestinal requirement for threonine, a recent study (Bertolo et al., 1998) demonstrated that the threonine requirement of piglets maintained by intravenous nutrition, during which many intestinal activities and functions are silenced or suppressed, was 60% lower than that of piglets receiving enteral feedings. Moreover, a subsequent report found that feeding threonine-deficient diets to piglets significantly reduces intestinal mass, goblet cell numbers, and mucin content, and this suppression of intestinal growth cannot be fully restored by providing threonine intravenously (Ball et al., 1999).

*Enormous intestinal
requirement for
threonine*

However, in order to quantify the partitioning of different pathways of threonine metabolism, catabolism and utilization for other metabolic purposes, subsequent studies administered isotopically labeled threonine.

Utilization

Although some essential amino acids are known to be oxidized by the PDV (van Goudoever et al., 2000; van der Schoor et al., 2001; Riedijk et al 2005), catabolism of essential amino acids does not seem to be their major fate within the intestinal tissues. Threonine catabolism through L-threonine 3-dehydrogenase pathway is a minor component within the splanchnic tissues with no contribution from the intestine (Le Floc'h and Sève, 2005, Schaart et al., 2005). However, intestinal oxidation measured by $^{13}\text{CO}_2$ production from ^{13}C -labeled threonine accounts for 2% of intestinal threonine utilization and 13% of whole-body threonine oxidation (Schaart et al., 2005).

These findings suggest that dietary threonine utilization by the intestinal mucosa of pigs is dominated by protein synthesis (Le Floc'h and Sève, 2005, Schaart et al., 2005), most likely through incorporation into mucins. Mucins (review: Montagne et al., 2004) are polymeric glycoproteins representing an important component of the mucus layer that covers the epithelium of the gastrointestinal tract, as well as all epithelia of mammals. Mucins can be membrane bound or secreted. The secretory mucins play a key role in the innate immune defense of the mucosa, and the core protein of the major intestinal mucins contains a large amount of threonine. In rats, the fractional synthesis rate of mucin glycoproteins was demonstrated to be relatively constant along the length of the intestine (range 112-138%/day), but substantially higher than the total mucosal protein synthesis rate, especially in the ileum (77%/day) and colon (44%/day) (Faure et al., 2002).

Mucins account for approximately 11% of endogenous protein in ileal digesta of pigs with threonine contributing 30% to mucin protein (Lien et al., 1997). Amino acids from endogenously secreted proteins, reaching the large intestine, are lost by the animal. Yet, secretion of mucins, erosion of the mucus, and subsequent recovery of mucins in endogenous ileal losses is dependent on many dietary factors including fiber, protein, and anti-nutritional factors (Montagne et al., 2004). Thus, the secretion, recycling, and loss of intestinal mucins most likely have a substantial impact on the requirement for threonine, and this perhaps contributes to the energy needs of the organism. In a recent study (van der Schoor et al., 2002), involving a 12-h-feeding and a 12-h-fasting period, intestinal recycling of amino acids from secretory proteins was proposed to be an important regulatory mechanism for the systemic availability of dietary amino acids. However, no significant threonine recycling from mucosal protein in the portal circulation could be detected, suggesting that these proteins could be very resistant to digestion, or that recycled threonine might be immediately reincorporated into mucosal protein.

*No significant
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Impact of Threonine Restriction and Disease

The importance of threonine specifically for the intestine and its implications for intestinal health and nutritional requirements has been demonstrated in a number of recent studies (Bertolo et al., 1998; Ball et al., 1999; Schaart et al., 2005; Faure et al., 2005). In piglets, isocaloric protein restriction (40% of normal intake) decreased whole-body growth while the small intestinal mass index (g per kg body weight) remained unchanged (Schaart et al., 2005). Although the absolute amount of threonine utilized by the PDV was reduced during protein restriction, the fraction of dietary intake utilized by the PDV was not different from that in control piglets. Moreover, the fraction of dietary threonine incorporated into intestinal protein represented 86% and 57% of the amount utilized by the PDV in protein-restricted and control animals, respectively. Low-protein feeding did not affect intestinal threonine oxidation, whereas whole-body threonine oxidation rates were reduced by almost 50%. Hence, intestinal oxidation rates accounted for 50% and 13% of whole-body oxidation of threonine in protein-restricted and control piglets, respectively.

A study in rats (Faure et al., 2005) demonstrated that isonitrogenous threonine restriction alone (30% of requirement), specifically decreased the fractional protein synthesis rates of mucins in all segments of the small intestine, reaching a maximal reduction of 40% in the duodenum. However, the overall amino composition of multiple intestinal mucins did not change, with threonine being the single largest contributor (up to 29% of total amino acid composition).

Small and large intestinal mucin production was also evaluated in a rat model of chronic colitis (Faure et al., 2003). A marked decrease in the threonine content of mucins was observed all along the gut of diseased compared to pair-fed rats, suggesting the predominant production of mucins less enriched in this amino acid. This implies, that a mucus gel layer with altered physiochemical properties may be produced impairing its integrity and protective function.

■ Summary

Amino acid metabolism by the splanchnic tissues, which include the liver and the portal-drained viscera (PDV), determines amino acid availability for protein deposition in peripheral tissues and thus whole-body growth. Threonine, lysine and methionine are the first limiting amino acids in milk-based and cereal diets of growing pigs. Studies have shown that both dietary essential and non-essential amino acids are extensively utilized by intestinal tissues for the generation of energy, protein synthesis, and other biosynthetic purposes. Threonine, with 60-80% extraction in first-pass of the dietary intake, is the single most utilized essential amino acid by the PDV. This can most likely be attributed to the incorporation of threonine into membrane-associated and secretory mucosal glycoproteins, the so-called mucins, which are an important component of the protective mucus covering the gut epithelium. In pigs, mucin represents 11% of the basal endogenous ileal losses of protein with threonine contributing approximately 30% to the total amino acid content. Thus, the secretion, recycling, and loss of intestinal mucins have a substantial impact on the maintenance requirement for threonine. Factors that increase the production of mucin will increase threonine requirements and consequently, decrease the availability of threonine and energy for growth and production. Furthermore, the availability of threonine may limit intestinal mucin synthesis and therefore reduce gut barrier function. This highlights the importance of threonine for maintaining intestinal integrity, which is essential for regulating dietary amino acid supply for the whole body.

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