

Nutrition and health relevant regulation of intestinal sulfur amino acid metabolism

Zhengfeng Fang · Kang Yao · Xiaoling Zhang · Shengjun Zhao · Zhihong Sun · Gang Tian · Bing Yu · Yan Lin · Biquan Zhu · Gang Jia · Keying Zhang · Daiwen Chen · De Wu

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Abstract Sulfur amino acids (SAA), particularly methionine and cysteine, are critical for the gut to maintain its functions including the digestion, absorption and metabolism of nutrients, the immune surveillance of the intestinal epithelial layer and regulation of the mucosal response to foreign antigens. However, the metabolism of SAA in the gut, specifically the transmethylation of methionine, will result in a net release of homocysteine, which is shown to be associated with cardiovascular disease and stroke. Furthermore, the extensive catabolism of dietary methionine by the intestine or by luminal microbes may result in a decrease in nutritional efficiency. Therefore, the regulation of SAA metabolism in the gut is not only nutritionally relevant, but also relevant to the overall health and well-

being. The superiority of DL-2-hydroxy-4-methylthiobutyrate to DL-methionine in decreasing homocysteine production, alleviating stress responses, and reducing the first-pass intestinal metabolism of dietary methionine may provide a promising implication for nutritional strategies to manipulate SAA metabolism and thus to improve the nutrition and health status of animals and perhaps humans.

Keywords Gut · Health · Metabolism · Nutrition · Sulfur amino acids

Abbreviations

AA	Amino acids
BCAA	Branched-chain amino acids
D-AAOX	D-Amino acid oxidase
D-HADH	D-2-Hydroxy acid dehydrogenase
DL-HMTB	DL-2-Hydroxy-4-methylthiobutyrate
DL-MET	DL-Methionine
L-HAOX	L-2-Hydroxy acid oxidase
PDV	Portal drained viscera
SAA	Sulfur amino acids

Z. Fang · G. Tian · B. Yu · Y. Lin · B. Zhu · G. Jia · K. Zhang · D. Chen · D. Wu (✉)
Key Laboratory for Animal Disease Resistance Nutrition of the Ministry of Education of China, Animal Nutrition Institute, Sichuan Agricultural University, Ya'an 625014, People's Republic of China
e-mail: pig2pig@sina.com

Z. Fang
e-mail: fangzhengfeng@hotmail.com

K. Yao · Z. Sun
Institute of Subtropical Agriculture,
The Chinese Academy of Sciences,
Changsha 410128, People's Republic of China

X. Zhang
College of Life Science and Technology,
Sichuan Agricultural University,
Ya'an 625014, People's Republic of China

S. Zhao
Department of Feed Science, Wuhan Polytechnic University,
Wuhan 430023, People's Republic of China

Introduction

There is growing evidence that sulfur amino acids (SAA), methionine and cysteine, play a metabolically and functionally important role in human health and disease (Shoveller et al. 2005). For example, methionine is a nutritionally indispensable AA for vertebrates (Wang et al. 2009), but on the other hand is also toxic to animals and humans (Garlick 2006). Cardiovascular disease in adults (Stipanuk 2004; Selhub 1999) and increased risk of stroke

in infants and children (Hogeveen et al. 2002; Van Beynum et al. 1999) have been shown to be strongly associated with enhanced plasma levels of homocysteine, a key product of methionine metabolism via transmethylation pathway (Brosnan and Brosnan 2006). Recent evidence indicates that the gut is a significant site of net homocysteine release (Riedijk et al. 2007; Bauchart-Thevret et al. 2009a, b). Dietary methionine deficiency (Bauchart-Thevret et al. 2009a) or excess (Velez-Carrasco et al. 2008) directly effects on plasma homocysteine concentrations. The balance of dietary SAA (Shoveller et al. 2004), the route of feeding (enteral vs. parenteral) and the source of methionine (methionine vs. methionine hydroxy analogue) (Xie et al. 2007) were also demonstrated to affect plasma homocysteine concentrations. Thus, how to regulate the methionine metabolism with the objective of controlling the systemic homocysteine generates particular interest among researchers in clinical treatment of cardiovascular disease and stroke.

Notably, recent evidence supports the view that tissues of the intestine capture, transform, and degrade absorbed AA before their entry into portal circulation (Fang et al. 2009, 2010a; Riedijk and van Goudoever 2007; van Goudoever et al. 2006) and thus form a critical first line of defense against the vagaries of the dietary AA supply (van Goudoever et al. 2008), acting as a “gatekeeper” to defend the organism against AA toxicity (Baracos 2004). However, whether and how SAA are metabolized by the gut in the first pass remains an open question, the elucidation of which may have implications for nutritional strategies to manipulate homocysteine metabolism as a means to reduce the risk of cardiovascular disease and stroke. Furthermore, given that the intestinal epithelium obtains a substantial fraction of its metabolic energy from the catabolism of dietary AA and thereby results in decreased availability of these AA to extraintestinal tissues (Wu et al. 2005; Wu 1998; Stoll et al. 1998), the regulation of this metabolism may also have implications for nutritional efficiency of dietary AA. Therefore, the purpose of this article is to discuss the recent evidence regarding the intestinal metabolism of dietary SAA and the potential strategies to manipulate this metabolism.

New insights into intestinal metabolism of dietary SAA

Historically, it is assumed that dietary AA be absorbed from the lumen into the portal blood without degradation. However, this notion was questioned by the intriguing finding (Stegink and Besten 1972) that there was dropped plasma cysteine concentration when diets with methionine as the sole SAA were administered parenterally, whereas the concentration rose immediately when feeding switched

to the oral route. Given that the parenteral feeding make the infused solution enter the circulation directly without first passing through the gut and liver, these results revealed that splanchnic tissues (mainly the gut and liver) may play an important role in the utilization of dietary methionine to cysteine synthesis. Shoveller et al. (2003a, b) also found that regardless of dietary cystine deficiency or excess, methionine requirements are ~30% lower in parenterally fed than in enterally fed piglets, further confirming the important first-pass metabolic demand of dietary methionine by splanchnic tissues. But from these studies, the relative contribution of the gastrointestinal tissues to the metabolism of dietary methionine remains unclear. With the increasing evidence that the gut may play an essential role in altering the concentrations and profiles of AA such as glutamine absorbed from the lumen before their entry into the portal blood (Windmueller 1982), the first-pass metabolism of dietary methionine in the gut generates growing interest among nutritional researchers. Stoll et al. (1998) implanted young piglets with portal and arterial catheters for determination of the portal balance of AA, and demonstrated that only 48% of dietary methionine intake was absorbed into the portal blood. On the basis of the net portal appearance, methionine rather than lysine was considered to be one of the limiting AA for young piglets. Fang et al. (2010a) used piglets implanted with arterial and portal catheters combined with the duodenal infusions of stable isotope tracer methionine ($1\text{-}^{13}\text{C}$ -methionine) to measure the appearance across and the use of the tracer by the portal drained viscera (PDV) (stomach, intestine, pancreas and spleen). The results confirmed that 30% of dietary methionine was metabolized by the intestine in the first pass. The earlier studies in rat (Finkelstein 2000) and recent studies in piglets (Bauchart-Thevret et al. 2009a) both demonstrate that the gastrointestinal tissues possess the significant activities of enzymes necessary to metabolize methionine to cysteine, which provided a further biochemical basis for the extensive utilization of methionine by the intestine (Bauchart-Thevret et al. 2009b; Shoveller et al. 2005).

However, recent studies (Chen et al. 2007, 2009) in vitro with isolated enterocytes seem contradictory to the view that the gut may be quantitatively important site for conversion of dietary methionine to both homocysteine and cysteine. In the studies conducted by Chen et al. (2009), enterocytes isolated from the jejunum of 0-, 7-, 14-, and 21-day-old piglets were incubated in Krebs buffer containing plasma concentrations of AA and one of the following tracer AA plus tracers: lysine, methionine, threonine, tryptophan, histidine, phenylalanine, and branched-chain AA (BCAA) including leucine, isoleucine and valine, to test whether the mucosal cells of the neonatal small intestine could degrade nutritionally indispensable

AA. The results indicated that BCAA were extensively transaminated and 15–50% of decarboxylated branched-chain α -ketoacids were oxidized to CO_2 depending on the age of piglets. In contrast to BCAA, catabolism of methionine and phenylalanine was negligible and that of other indispensable AA was absent in enterocytes from all ages of piglets due to the lack of key enzymes. The authors (Chen et al. 2009) concluded that previously observed extensive *in vivo* catabolism of methionine, histidine, lysine, phenylalanine, threonine or tryptophan by the small intestine (Stoll et al. 1998) may result from the action of luminal microbes in the intestinal mucosa (Blachier et al. 2007). In agreement with this notion, recent evidence demonstrated that dietary and endogenous AA were the main contributors to microbial protein in the upper gut of normally nourished pigs (Libao-Mercado et al. 2009), which means that microbial protein, if subsequently not digested and absorbed, may make a net loss to the host's AA supply. However, it is also possible that ^{13}C -methionine oxidation reported *in vivo* in pigs occurs by other cell types and tissues with the PDV, such as lymphocytes, fibroblast, pancreatic tissue, spleen tissue, or stomach as they all drain into the portal vein. In support of this view, Riedijk et al. (2007) demonstrated that 20% of dietary methionine intake was metabolized by the intestine and 40% of this was oxidized to CO_2 . One demand for intestinal methionine oxidation may be for synthesis of cysteine-rich mucins secreted by goblet cells involved in innate immune function (Van Klinken et al. 1998). Similarly, the observation that ^{14}C radioactivity in the pancreas was about twice that in the intestine after administration of L-1- ^{14}C -methionine (Saunderson 1985) may imply the significant role of pancreas in the metabolism of dietary methionine, which remains to be defined.

In addition, it should also be cautioned that studies *in vitro* with isolated enterocytes have its own limitation and thus may not always reflect the case *in vivo*. Firstly, because the apical and basolateral membranes of each enterocyte are chemically, biochemically, and physically distinct (Madara 1991), the enterocytes can selectively receive nutrients from two sources: the arterial blood across its basolateral membrane and the intestinal lumen across its brush border membrane (Wu et al. 2005; Stoll and Burrin 2006). There is growing evidence that the intestine may preferentially oxidize the methionine derived from the artery rather than that absorbed from the lumen (Riedijk et al. 2007), further confirming the importance of that the bi-polar nature of the gut is stimulated *in vitro*, in particular using chamber systems as well as some cell systems such as Caco-2. In this regard, *in vitro* culture of isolated primary intestinal epithelial cells (Chen et al. 2009) may limit the utilization of methionine by these cells. Secondly, recent evidence (Riedijk et al. 2007)

suggests that the high rate of methionine transsulfuration in the gut is driven by cysteine needs for glutathione synthesis because of the oxidant stress associated with the high metabolic activity of proliferating epithelial cells. Thus, the potential difference in the oxidation status associated with proliferation rate of epithelial cells between *in vitro* and *in vivo* studies may also result in difference in methionine metabolism. Thirdly, previous studies (Dangin et al. 2001) in young men demonstrate that a single meal of free AA mimicking casein composition compared with a single meal of slowly digested casein induced a stronger, more rapid and transient increase of AA oxidation. Therefore, the existing status (AA vs. protein) of nutrients used in the *in vitro* and *in vivo* studies combined with the dynamic access rate (fast vs. slow) of enterocytes to these nutrients may also result in difference in methionine metabolism between *in vitro* and *in vivo* studies. Thus, the relative contribution of PDV tissues and luminal microbes to the metabolism of dietary methionine remains to be elucidated.

The regulation of intestinal SAA metabolism

It is established that the gut is a significant site of net homocysteine release into the circulation (Riedijk et al. 2007; Bauchart-Thevret et al. 2009a, b) and moreover, the transmethylation and transsulfuration in splanchnic tissues are manipulated by dietary SAA (Shoveller et al. 2003a; Di Buono et al. 2003). Thus, the regulation of intestinal SAA metabolism may have implications for nutrition and health.

The regulation of intestinal SAA metabolism by dietary SAA status

The pioneering studies in humans (Stegink and Besten 1972) indicated that circulating plasma total cysteine concentrations were significantly greater in enterally than in parenterally fed men administered methionine as the sole dietary SAA. Further studies in piglets (Shoveller et al. 2003a) revealed that plasma cysteine concentrations were positively associated with SAA intake in enterally but not parenterally fed piglets. These results have two implications: firstly, splanchnic tissues may play an important role in the conversion of dietary methionine to cysteine in the first pass and secondly, dietary SAA status including the component (methionine or cysteine alone, or their combinations) and intake may effect on the transmethylation and transsulfuration of methionine and thus the net cysteine release from the splanchnic tissues. In support of this view, adult humans fed methionine alone [24 mg/(kg day)] had higher rates of transmethylation and transsulfuration and lower rates of remethylation than adults fed 13 mg methionine/(kg day) and 11 mg methionine/(kg day) (Di Buono

et al. 2003). The studies in humans with isotopic tracers also showed indirectly that a substantial fraction of the dietary methionine transsulfuration occurs via first-pass splanchnic metabolism (Hiramatsu et al. 1994; Raguso et al. 1997). However, on the basis of these results, the role of the gut in SAA metabolism cannot be discriminated from that of the liver which is usually considered to be the major site of methionine transmethylation and transsulfuration. Most recently, Burrin and co-workers (Riedijk et al. 2007) using duodenal and intravenous infusion of stable isotope tracer methionine in infant piglets demonstrate that the gastrointestinal tract metabolizes 20% of dietary methionine intake, which is mainly transmethylated to homocysteine and transsulfurated to cysteine during conditions of diet supply with sufficient SAA. In contrast, under SAA-deficient conditions, methionine metabolism is prioritized in a coordinate manner, such that protein synthesis is preserved over methionine transmethylation and the methionine pool is preserved by upregulation of homocysteine remethylation and suppression of transsulfuration (Bauchart-Thevret et al. 2009a). These results make it clear that the gut is also a significant site of methionine metabolism and moreover, the metabolic fate (remethylation or transsulfuration) of methionine is regulated by dietary SAA status.

The potential regulation of SAA metabolism by methionine sources

Except for glycine, all AA can have L- and D-isomers (Wu 2009). Most D-AA, except for D-arginine, D-cystine, D-histidine, D-lysine, and D-threonine, can be converted into L-AA in animals via widespread D-AA oxidases (D-AAOX) and transaminases (Baker 2009; Fang et al. 2009). However, because the efficiency of D-AA utilization, on a molar basis of the L-isomer, may be 20–100%, depending on substrates and species (Baker 2009), not all D-isomers can be widely used for nutritional purpose. In contrast, some D-isomers such as D-methionine (D-MET) have been widely accepted as a methionine source due to its nutritional efficiency and some specific functional role in health. Evidence in rats (Campbell et al. 1996) indicates that D-MET provides excellent protection from cisplatin ototoxicity. This is considered to be associated with that the D-MET is less well-metabolized than L-methionine (L-MET) in humans, so it may remain more available for cisplatin binding, thereby protecting the L-MET for needed protein synthesis, cell activation and metabolism. In support of this view, parenteral administration of DL-methionine (DL-MET) in humans results in higher plasma levels of the D-MET than that of the L-MET (Printen et al. 1979).

Another exciting new development in SAA nutrition is the discovery of the superiority of DL-2-hydroxy-4-methylthiobutyrate (DL-HMTB), the hydroxy analogue of

DL-MET, to DL-MET in decreasing homocysteine production and alleviating stress responses. There is evidence that plasma ceruloplasmin, α -1 acid glycoprotein concentration, and heterophil to lymphocyte ratio in blood after lipopolysaccharide injection were lower in chicks fed a DL-HMTB diet than in chicks fed a DL-MET diet, which suggested that dietary DL-HMTB had a potential to alleviate certain stress responses (Matsushita et al. 2007). Furthermore, recent studies in ducks (Xie et al. 2007) indicated that plasma homocysteine of birds fed DL-HMTB-supplemented diets was significantly lower than birds fed equimolar DL-MET-supplemented diets, suggesting the less toxicity of DL-HMTB relative to DL-MET. However, there is limited biochemical evidence for these differences. The original studies (Martín-Venegas et al. 2006) in vitro with isolated chicken enterocytes showed that cysteine and taurine synthesis after incubation with DL-HMTB was higher when compared with L-MET incubation, indicating that cysteine and taurine formation by chicken enterocytes could be favored when DL-HMTB was used as a methionine source. Given that the high rate of methionine transsulfuration in the gut may be driven by cysteine needs for synthesis of mucins secreted by goblet cells involved in innate immune function (Van Klinken et al. 1998), the favored cysteine production following DL-HMTB administration may account for the potential of DL-HMTB to alleviate stress responses induced by lipopolysaccharide as evidenced by Matsushita et al. (2007). Recent studies (Fang et al. 2010a) in vivo with piglets implanted with arterial, portal and mesenteric catheters found that there was increased circulating plasma taurine concentration in DL-HMTB fed than in DL-MET fed piglets, further confirming the greater potential of DL-HMTB than of DL-MET to promote the transsulfuration of dietary methionine. Given that the increased taurine concentration was mainly observed in the arterial plasma rather than in the portal plasma, the liver may play a major role in methionine transsulfuration. Thus, that DL-HMTB might be preferentially diverted to the transsulfuration pathway by splanchnic tissues may in part account for the lower circulation homocysteine in DL-HMTB fed than DL-MET fed animals such as ducks (Xie et al. 2007).

However, based on previous reports (Martín-Venegas et al. 2006) that cysteine and taurine formation by isolated enterocytes could be favored when DL-HMTB rather than L-MET was used as a methionine source, DL-HMTB fed piglets should have higher portal plasma taurine concentration than DL-MET fed piglets, which was not observed in the studies of Fang et al. (2010a). The inconsistency between the in vitro and in vivo studies may be explained by the novel evidence that DL-HMTB absorption occurred primarily in the proximal gastrointestinal tract prior to the small intestine (Richards et al. 2005). The fact that until it

is converted to L-MET, DL-HMTB is an organic acid, not an amino acid (Dibner and Buttin 2002), may provide a biochemical basis for the absorption of DL-HMTB in the upper gastrointestinal tract including the stomach. Furthermore, it is established that diffusion into cells represents a major route of organic acid uptake and occurs most rapidly at low pH when more of the acid will be protonated and lipophilic (Walter and Gutknecht 1984). In this regard, the low pH (<4.3) and considerable retention time (~2 h) of digesta in the stomach of piglets (Wilson and Leibholz 1981) may facilitate the absorption of DL-HMTB by the gastric tissues. Therefore, it is likely that DL-HMTB is high efficiently absorbed in the upper gastrointestinal tract of piglets, thereby reducing the amount of methionine sources that can be accessed by the intestine or luminal microbes in the first pass, which may imply the greater potential of DL-HMTB than AA such as DL-MET to bypass the first-pass intestinal metabolism as discussed below.

DL-HMTB, a methionine source with greater potential to bypass the first-pass intestinal metabolism

In addition to health relevant SAA metabolism, the recent intriguing data show that intestinal metabolism of methionine and its precursors may also have important implications for nutritional efficiency (Fang et al. 2009, 2010a, b). Given evidence that the extensive catabolism of dietary indispensable AA by the small intestine results in a decrease in their nutritional efficiency (Wu et al. 2005; Wu 1998; Stoll et al. 1998), there is growing interest in the question whether the catabolism of AA represents a functional requirement by the intestine for purposes of growth and function or if it is driven simply by the local availability of these substrates to the mucosal enterocytes (Fang et al. 2010a). Previous evidence (van Goudoever et al. 2000) indicated that intestinal oxidation of enteral lysine contributed one-third of total body lysine oxidation in growing pigs fed a high-protein diet, but was virtually absent in pigs fed a low-protein diet. In agreement with this notion, there was a substantial suppression of AA oxidation by the intestine during a low protein intake (van der Schoor et al. 2001). Further study revealed that the first-pass utilization rate of dietary lysine by the intestine was directly increased by the enteral lysine availability (Bos et al. 2003). These results indicate adaptive regulation of intestinal protein and AA metabolism. Therefore, it is likely that the high rate of mucosal AA catabolism in the intestine is under nutritional regulation, particularly by the protein intake of the animals (Fang et al. 2010a).

On the basis of that the small intestinal capacity to digest and absorb protein and AA is substantially greater than possible dietary inputs (Burrin et al. 1999), much of

this organ's demand for AA for maintenance may be an unnecessary burden (Bertolo et al. 2005). This raises a possibility that lowering intestinal AA metabolism without compromising gut absorptive capacity or protective functions may be promising for improving AA nutritional efficiency (Fang et al. 2010a). To test this hypothesis, Peng and co-workers (Fang et al. 2009, 2010a, b) conducted a series of studies with piglets implanted with arterial, portal and mesenteric catheters as the animal model and with DL-HMTB and DL-MET taken as the potential paradigms for "more" and "less" bypass-the-intestine AA, respectively. The results indicated that despite the difference in methionine sources, the first-pass utilization of dietary methionine by the intestine remained at ~30% of intake (Fang et al. 2010a). Similar results in piglets fed milk protein have been obtained in a previous study (Bos et al. 2003), in which dietary methionine intake is about 1.5-fold that administered by Fang et al. (2010a). These results suggested that the fraction of methionine might be more constant than the absolute amount of methionine that extracted by the intestine in its first-pass. It would appear that the more the local availability of dietary methionine absorbed into enterocytes, the more the absolute amount of methionine that metabolized by the intestine.

The distribution of activity of oxidation enzymes for conversion of DL-HMTB and D-MET may provide a biological basis for the difference in metabolism between sources (Fang et al. 2010b). Firstly, it was noteworthy that both D- and L-HMTB compared with only D-MET (comprising 50% of DL-MET) must be converted to L-MET before they can be used by the intestine (Dibner and Ivey 1992), such that the methionine precursor that needs converting in DL-HMTB diet was twice that in DL-MET diet (Fig. 1). Secondly, the rate-limiting enzymes for conversion of D-MET, D-HMTB and L-HMTB to L-MET are D-AAOX, D-2-hydroxy acid dehydrogenase (D-HADH) and L-2-hydroxy acid oxidase (L-HAOX), respectively (Dibner and Knight 1984) (Fig. 1). Studies in broilers (Dibner and Ivey 1992; Brachet and Puigserver 1992), piglets (Fang et al. 2010b) and ruminants (Lobley et al. 2006; McCollum et al. 2000) indicate that the intestine possesses considerable capacity to convert D-MET and D-HMTB to L-MET. In contrast, the enzyme activity values for D-AAOX were about fourfold that for L-HAOX and twofold that for D-HADH in both the duodenum and jejunum where DL-MET was mainly absorbed (Fig. 2). These results suggested that the intestine might possess a relatively higher capacity of D-MET utilization than of DL-HMTB utilization to L-MET synthesis. Thus, the more methionine precursor that needs converting in DL-HMTB than in DL-MET combined with the relatively lower oxidative activity the intestine possesses toward DL-HMTB may provide a biochemical evidence for that DL-HMTB might produce

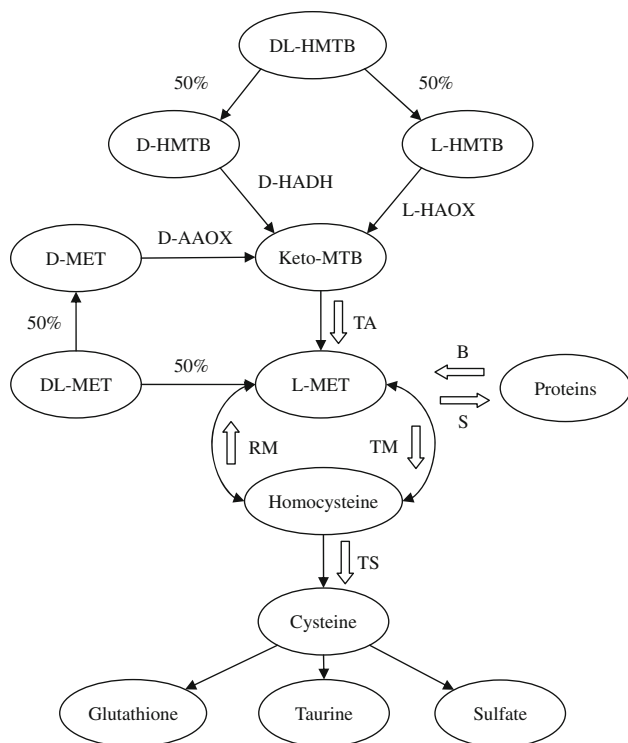


Fig. 1 Schematic illustration of metabolic pathway of methionine and its precursors via transamination (TA), transmethylation (TM), transsulfuration (TS), remethylation (RM), and protein synthesis (S), and breakdown (B)

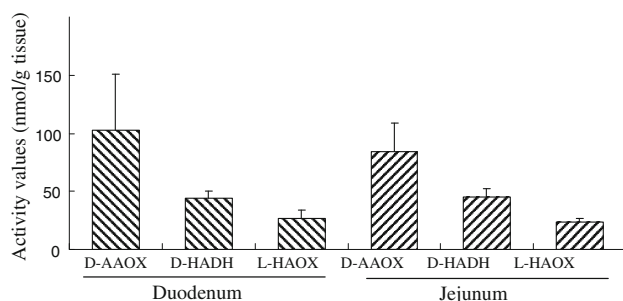


Fig. 2 Activity values for D-AAOX, D-HADH and L-HAOX in the duodenum and jejunum of piglets fed diets with 30% of the total methionine supplied by DL-HMTB or DL-MET. Data were adapted from Fang et al. (2010b)

less L-MET than DL-MET when they were transported across the enterocytes, thereby reducing the first-pass metabolism of dietary methionine by the intestine (Fang et al. 2010b). In this regard, it is not surprised that there was no difference in the net portal balance of L-MET between piglets fed diets with 30% of the total methionine supplied by DL-MET or DL-HMTB (Fang et al. 2009), although the directly available L-MET in DL-MET diet was about 1.2-fold (85 vs. 70% of the total dietary methionine) that in DL-HMTB diet. Among the tissues evaluated, the

liver and kidney possessing the highest L-HAOX and D-HADH activities indicates the sufficient capacity of methionine source utilization by the piglets (Fang et al. 2010b), although a major amount of these sources may be absorbed into the portal blood with few conversion occurring during the transport process (Lobley et al. 2006).

Conclusion and perspectives

The gut plays a key role not only in the digestion, absorption and metabolism of nutrients, but also in the immune surveillance of the intestinal epithelial layer and regulation of the mucosal response to foreign antigens. SAA, particularly methionine and cysteine, are critical for the gut to maintain these functions. However, the metabolism of SAA, specifically the transmethylation of methionine, will result in a net release of homocysteine, which is shown to be associated with cardiovascular disease and stroke. Furthermore, the extensive catabolism of dietary methionine by the intestine or by luminal microbes may result in a decrease in nutritional efficiency. Therefore, the regulation of SAA metabolism in the gut is not only nutritionally relevant, but also relevant to the overall health and well-being. The superiority of DL-HMTB to DL-MET in decreasing homocysteine production, alleviating stress responses, and reducing the first-pass intestinal metabolism of dietary methionine may provide a promising implication for nutritional strategies to manipulate SAA metabolism and thus to improve the nutrition and health status of animals and perhaps humans.

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