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# Bioavailability of different dietary supplemental methionine sources in animals

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## 1. ABSTRACT

Dietary methionine is indispensable for animal maintenance, growth and development. L-methionine (L-Met), and its synthetic forms DL-methionine (DL-Met) and 2-hydroxy-4 (methylthio) butanoic acid (HMTBA) are common supplemental methionine sources in animal diets. There are different characteristics for cellular absorption, transport, metabolism and bio-efficiency between these three dietary methionine sources. Moreover, there are differences in their utilization among various species such as chickens, pigs and ruminants. As a methionine precursor, HMTBA is efficacious in the promotion of growth in animals. It is absorbed mainly by monocarboxylate transporter 1 (MCT1), coupled with the activity of the $Na^+/H^+$ exchanger (NHE3), while DL-Met uptake occurs via multiple carrier-mediated systems. Liver, kidney and small intestine can metabolize D-Met and HMTBA to L-Met through oxidation and transamination. In ruminants, the non-hepatic tissues act as major sites of HMTBA conversion, which are different from that in chickens and pigs. HMTBA also has additional benefits in anti-oxidation. Understanding the characteristics of uptake and metabolism of different methionine sources will greatly benefit the industry and bioscience research.

## 2. INTRODUCTION

Methionine (Met) is an essential amino acid in mammals and avians and thus its dietary uptake is indispensable for animal maintenance, growth and development. Methionine is a precursor for the synthesis of proteins and serves as the predominant amino acid for translation initiation. In addition, methionine is an intermediate in the biosynthesis of other important molecules such as cysteine, carnitine and taurine and can be converted to S-adenosylmethionine (SAM), the major methyl donor in cells (1), indirectly participating in the epigenetic regulation of gene expression. Thus the study of methionine can be related to various bioscience research areas.

In animal diets especially poultry diets, methionine is typically considered to be the first limiting amino acid. The dietary supplemental methionine sources include L-methionine (L-Met), and its synthetic forms DL-methionine (DL-Met) and 2-hydroxy-4 (methylthio) butanoic acid (HMTBA) (Figure 1). Conversion of HMTBA to 2-keto-4(methylthio) butanoic acid (KMB) by oxidases and subsequent conversion of KMB to Met by transaminases are described in detail in a later section. An overall view of transport, conversion and utilization of HMTBA and DL-Met is shown in Figure 2.

As the metabolically active isomer of methionine, the industrial-scale fermentation method for the manufacture of L-Met is still under exploration. Today L-Met is manufactured on an industrial scale by enzymatic reactions using the DL-Met products via an N-acetyl derivative (2). DL-Met is one of the amino acids that is most widely manufactured by chemical synthesis, using acrolein, methyl mercaptan and hydrogen cyanide, and sold as a powder (2). In the manufacturing process, a racemic mixture of D- and L-Met is synthesized. The Met analogue, HMTBA, is available as an 88%

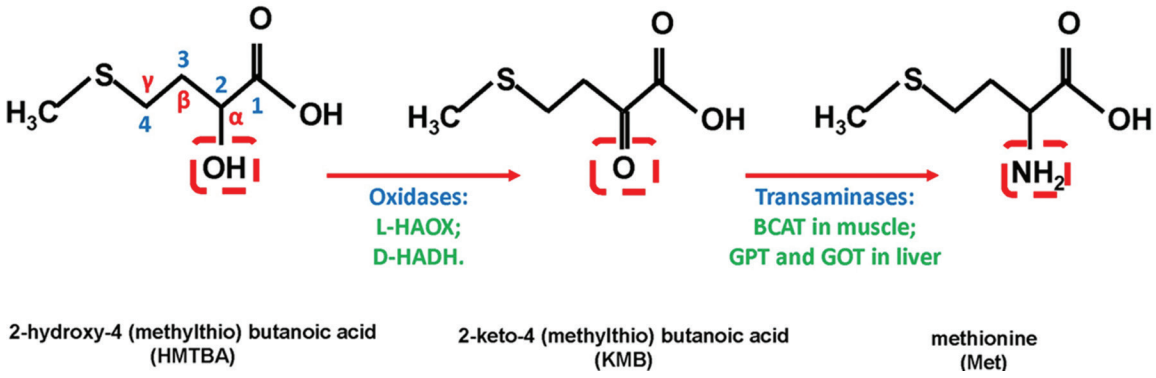


**Figure 1.** Chemical structures of 2-hydroxy-4 (methylthio) butanoic acid, 2-keto-4 (methylthio) butanoic acid and methionine. The α-hydroxyl group in HMTBA can be oxidized to the α-keto group in KMB by L-HAOX or D-HADH, and can then be converted to α-amino group by transaminases. The major transaminases reported in animals include BCAT in skeletal muscle, with amino group donors of branched-chain amino acids, and GPT and GOT in liver, with amino group donors of glutamate. BCAT: branched-chain amino acid transaminase; D-HADH: D-2-hydroxy acid dehydrogenase; GPT: glutamate-pyruvate transaminase; GOT: glutamic oxaloacetic transaminase; L-HAOX: L-2-hydroxy acid oxidase.

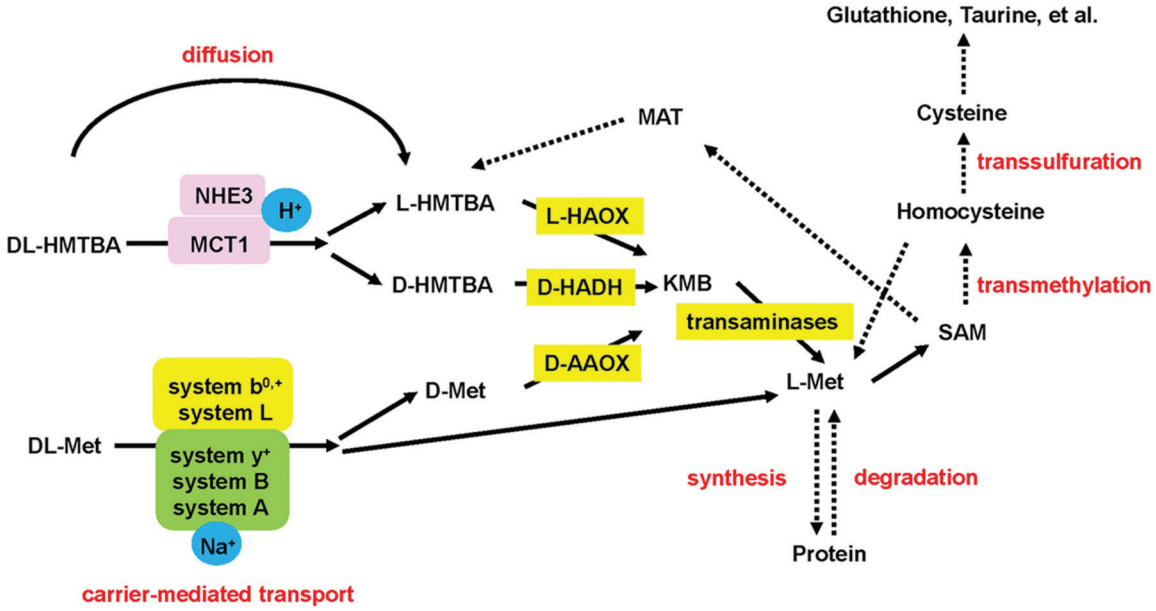


**Figure 2.** Absorption, transport and metabolism of L-Met, D-Met and HMTBA in non-ruminants. L-Met, D-Met and HMTBA can absorbed through diffusion or multiple carrier-mediated transport systems in the small intestine. D-Met and HMTBA must be metabolized to L-Met for utilization. The product L-Met can be used for protein synthesis, or undergo transmethylation or transsulfuration. D-AAOX: D-amino acid oxidase; D-HADH: D-2-hydroxy acid dehydrogenase; D-Met: D-methionine; HMTBA: 2-hydroxy-4 (methylthio) butanoic acid; KMB: 2-keto-4 (methylthio) butanoic acid; L-HAOX: L-2-hydroxy acid oxidase; L-Met: L-methionine; MCT1: monocarboxylate transporter 1; MTA: 5'-deoxy-5'-methylthioadenosine; NHE3: Na+/H+ exchanger; SAM: S-adenosylmethionine.

liquid concentrate in water as a free acid or as an 84% preparation of the calcium salt (3). HMTBA lacks an amino group and thus is not a true amino acid but rather an amino acid precursor.

After absorption in the GI tract, biological utilization of both D-Met and HMTBA relies on their conversion to L-Met. Thus there are different absorption, transport and metabolism mechanisms for utilization of different methionine sources. In the past few decades, there has been a continuing controversy centered on the absorption and bio-efficacy of DL-Met and HMTBA. This review summarizes studies about different absorption, transport and metabolism characteristics of L-Met, DL-Met and HMTBA in several animal models (poultry, swine, and ruminants), and their bio-efficacy in growth and development with a focus on poultry and pigs. Understanding the characteristics of different methionine sources will greatly benefit the animal nutrition industry as well as bioscience research.

**Table 1.** Small intestinal epithelial cell transporters for methionine in mammals and avians

| System | Protein | Gene | Location[1] | Notes |
|---|---|---|---|---|
| A | SAT1 | SLC38A1 | BL | $Na^+$ - dependent short-chained neutral amino acid transporter |
| | SAT2 | SLC38A2 | BL | $Na^+$ - dependent neutral amino acid transporter. Sensitive to low pH. Ubiquitous expression |
| $B^{0,+}$ | $ATB^{0,+}$ | SLC6A14 | BBM | $Na^+$ - dependent cationic amino acid transporter |
| $b^{0,+}$ | $b^{0,+}AT$ | SLC7A9 | BBM | $Na^+$ - independent cationic and neutral amino acid exchanger |
| L | LAT1 | SLC7A5 | BL | $Na^+$ - independent neutral amino acid transporter, exchanger for large hydrophobic amino acids. Ubiquitous expression |
| | LAT2 | SLC7A8 | BL | Same as LAT1. Not present in the chicken genome |
| $y^+L$ | $y^+LAT1$ | SLC7A7 | BL | $Na^+$ - dependent cationic/neutral amino acid exchanger |
| | $y^+LAT2$ | SLC7A6 | BL | $Na^+$ - dependent cationic/neutral amino acid exchanger |
| / | 4F2hc | SLC3A2 | BL | Dimerizes with LAT1, LAT2, $y^+LAT1$ and y+LAT2 |
| [1]BBM: brushborder membrane, BL: basolateral membrane | | | | |

## 3. UTILIZATION OF DIFFERENT SUPPLEMENTAL METHIONINE SOURCES IN POULTRY

### 3.1. Absorption and transport of dietary L-Met, DL-Met and HMTBA in poultry

Because HMTBA does not enter the lumen of the gastrointestinal tract (GI tract) in the form of an amino acid, it is likely absorbed differently from D-Met and L-Met. Knight and Dibner used an *in vitro* intestine model and *in vivo* ligated intestinal segment plus crop intubation method to explore the absorption of (1-$^{14}$C)-HMTBA and L-Met in 8 to 16 day-old chickens (4). Intestinal uptake of L-Met was inhibited by the electron transport uncoupler 2, 4-dinitrophenol and its uptake conformed to Michaelis-Menten kinetics; whereas, the *in vitro* uptake of HMTBA was linear relative to concentration. These results led to the conclusion that L-Met absorption was a concentration and energy dependent process while HMTBA absorption was only concentration dependent (4). The *in situ* experiment also illustrated that the absorption rate of the two methionine sources varied in different small intestinal locations. L-Met was absorbed more quickly in the ileum, while HMTBA was absorbed more rapidly in the proximal loop of the duodenum and mid-jejunum (4).

Richards *et al*. demonstrated that HMTBA was absorbed along the entire GI tract (from crop to cloaca) in chickens, primarily in the proximal GI tract prior to the small intestine (5). They also showed that the uptake rate of HMTBA was equal to or greater than DL-Met using an *in vitro* uptake experiment (5). In this same study HMTBA was shown to be completely absorbed, whereas, Maenz and Engele-Schaan showed that HMTBA was partially broken down to non-absorbed, non-methionine products during passage down the small intestine in chickens (6). This discrepancy can be explained by the *in vitro* brush border membrane vesicle (BBMV) model used by Maenz and Engele-Schaan. The BBMV method tends to minimize HMTBA absorption by diffusion, which accounts for a large proportion of HMTBA uptake *in vivo* (5). In contrast, the intestinal slices model is more efficient at measuring the uptake of HMTBA through both diffusion and carrier-mediated absorption (4,5).

Nevertheless, the BBMV model could be used to study the specific transporters on the small intestinal brush border membrane. Brachet and Puigserver reported that the transport of both L-HMTBA and D-HMTBA were $Na^+$ independent and electroneutral (7). L-lactate, a structural analogue, could act as a competitor to L-HMTBA transport. The HMTBA uptake by chicken intestine had similar characteristics to uptake in rats (7). Maenz and Engele-Schaan confirmed this conclusion by showing that HMTBA was absorbed by a $H^+$-dependent, non-stereospecific, monocarboxylic acid transport system (8). Taking advantage of a high affinity transport and low affinity competitive inhibition model, L- and D-isomers of methionine were shown to be transported by a broadly specific, $Na^+$-dependent system B type transporter (8). L-Met was transported mainly by $Na^+$-independent carrier-mediated mechanisms, using BBMV isolated from the jejunum of 6-week-old chickens (9). Only a small portion of the transmembrane movement of L-Met was through passive diffusion (10).

There are several known transport systems for DL-Met in the chicken jejunum: the $Na^+$-independent $b^{o,+}$ and system L, and the $Na^+$-dependent system $y^+$, system B and system A (9). However, it remains unknown whether L-Met and D-Met share the same transporter systems. Some of the transporters reported to transport methionine include $b^{0,+}AT$, LAT1/LAT2, $y^+LAT1/y^+LAT2$, $ATB^{0,+}$, SAT1/SAT2, 4F2hc. (11, 12). (Table 1) The transport system for DL-HMTBA is the monocarboxylate transporter 1 (MCT1) (13, 14). Because transport of HMTBA by MCT1 is $H^+$-dependent, it is coupled to the

activity of the $Na^+/H^+$ exchanger (NHE3) to maintain the proton gradient at the brush border membrane of the enterocyte. This research was conducted using Caco-2 cell lines, which demonstrated the uptake of HMTBA through the apical membrane of human small intestinal epithelia. No related results have been reported using a chicken cell model.

Following GI tract uptake, avian liver also has the capacity to absorb HMTBA. Liver can remove circulating HMTBA in excess of that needed for metabolism. Wang *et al*. observed no sign of toxicity from infusion of pharmacological levels of DL-HMTBA directly into the broiler hepatic portal vein, while feeding a diet containing normal supplemental levels of HMTBA (15). They concluded that liver is a major site for HMTBA removal from circulation and metabolism in chickens.

### 3.2. Conversion and metabolism of dietary L-Met, DL-Met and HMTBA in poultry

For DL-Met, D-Met must be converted to L-Met following transport into the enterocyte. D-Met is first oxidatively deaminated to the α-keto analogue of L-Met, KMB (Figure 2), by the enzyme D-amino acid oxidase (D-AAOX; EC 1.4.3.3.), which is a peroxisomal enzyme containing flavin adenine dinucleotide (FAD) as a cofactor (16). The KMB is converted to L-Met by the transfer of nitrogen from donor amino acids catalyzed by an unknown number of transaminases. Transaminase is ubiquitous and is not thought to be a limiting step in the transformation process, although this has not been definitively demonstrated.

HMTBA utilization includes the following steps: absorption, transport, conversion to L-methionine and incorporation into polypeptides. Both isomers of HMTBA can be used as methionine sources at similar rates for hepatocyte protein synthesis and are biochemically equivalent to DL-Met in chickens (17). The stereospecific pathway of HMTBA conversion to L-Met was demonstrated by Dibner and Knight using chicken liver homogenates (18).

The first step is oxidation of the α-hydroxyl group yielding the keto intermediate KMB, catalyzed by two enzyme systems according to the substrate isomers (Figure 1). L-HMTBA is the substrate of L-2-hydroxy acid oxidase (L-HAOX; EC 1.1.3.1.5.), a hydrogen peroxide ($H_2O_2$)-producing flavoenzyme found in peroxisomes of liver and kidney in chickens. There are two isoforms of L-HAOX, HAO1 and HAO2. HAO1 is rich in liver and preferentially oxidizes short-chain aliphatic 2-hydroxyacids (19). HAO2 is abundant in kidney and catalyzes the oxidation of long-chain aliphatic or aromatic 2-hydroxyacids (19). Thus HAO1 is assumed to be involved mainly in L-HMTBA oxidation. D-HMTBA is oxidized through an entirely different mechanism, catalyzed by the mitochondrial D-2-hydroxy acid dehydrogenase (D-HADH; EC 1.1.9.9.6), producing $H_2O_2$ as a byproduct. Because D-HADH is located in mitochondria, D-HMTBA could be used by any organ for protein synthesis, including small intestine and skeletal muscle (18, 20). FAD and flavin mononucleotide (FMN) are common cofactors for both L-HAOX and D-HADH. L-HAOX also has oxidative decarboxylation activity *in vitro*, which uses nicotinamide adenine dinucleotide (NADH) (21).

In both cases the resulting keto intermediate KMB is transaminated to L-Met by transaminase, the second step in HMTBA metabolism (Figure 1). In rat liver, KMB is transaminated by the enzyme transaminase using glutamine or asparagine as substrates to produce L-Met (22). In rat skeletal muscle, the branched-chain amino acids play an important role in transamination (23,24). However, in chicken the physiology is more complicated (25). Kidney is the most active tissue for conversion of KMB to L-Met, the liver and small intestinal mucosa are intermediate, and skeletal muscle has the lowest activity. All amino acids can serve as substrates for transamination of KMB in chicken. Branched-chain amino acids, glutamic acid and asparagine are more effective substrates in tissues other than the small intestinal mucosa. In mitochondria, the preferred substrates are glutamate in liver mitochondria, isoleucine and alanine in kidney mitochondria and branched-chain amino acids and glutamic acid in skeletal muscle mitochondria. In Caco-2 cells, the branched-chain amino acid leucine is the preferred amino acid group donor (26).

Conversion of DL-HMTBA to methionine occurs principally in the liver, because both L-HAOX and D-HADH are highly expressed in this organ. At super physiological concentrations, DL-HMTBA is oxidized principally in skeletal muscle (27). However, growing broilers have more than enough biochemical capacity for DL-Met and HMTBA conversion, thus no accumulation of dietary methionine sources might occur (28). HMTBA conversion also takes place in small intestine, following the process of oxidation and transamination (26,29). This process is up-regulated by HMTBA, but the transamination is not affected by HMTBA availability (26). Under fasting or methionine deficient conditions, brain and liver showed enhanced rates of DL-HMTBA and DL-Met conversion (30). In non-ruminants, oxidation is the major pathway for HMTBA catabolism. In ruminants, HMTBA can also be degraded directly by microorganisms.

Besides dietary HMTBA, HMTBA is also a naturally occurring compound in chickens through a salvage pathway to regenerate L-Met from 5'-deoxy-5'-methylthioadenosine (MTA), although KMB does not participate in this process (31, 32). Even though the naturally occurring HMTBA may not be an obligatory intermediate in the cytosolic MTA salvage pathway, it can still be used as an L-Met precursor in chicken liver (31).

L-Met can be directly used to synthesize SAM or can be degraded through pathways such as transamination (33). L-Met can also be degraded or utilized by the bacteria in the lumen of the small intestine (34). SAM can undergo the transmethylation pathway to synthesize homocysteine or the transsulfuration pathway, with the products cysteine, glutathione, taurine, etc. In skeletal muscle, methionine can regulate the ribosomal protein S6 kinase (S6K1) pathway and its translational targets, i.e. ribosomal protein S6 (S6) and eukaryotic elongation factor 2 (eEF2), and regulate protein accretion and synthesis (35,36). In quail muscle QMY myoblasts incubated in methionine-free medium, S6K1 signaling was inactivated without increasing eukaryotic translation initiation factor 2α (eIF2α) phosphorylation. Activation of S6K1 is also induced by KMB but not DL-HMTBA or D-Met (36).

### 3.3. The bio-efficacy of L-Met, DL-Met and HMTBA in poultry

By the early 1980s, numerous studies had already shown that HMTBA was nutritionally effective in broiler chickens (37,38), both in liquid form (Alimet) and dry/calcium salt form (MHA). Many other studies showed no significant differences among the three commercial methionine sources in affecting weight gain and feed conversion ratio (3,39,40), while a few indicated HMTBA to be less effective than L-Met or DL-Met in promoting growth (3). This was thought to be due to the transformation process of HMTBA to L-Met (40).

More recently, the methodology was altered for comparing different methionine sources, by defining their own response curve model within each source and determining their relative performance by comparing the predictions of each model (41). Vazquez-Anon *et al*. imposed linear, quadratic and exponential equations to body weight gain and selected the best model (with best goodness of fit) to estimate the gain responses to feeding different doses of HMTBA vs. DL-Met to male chicks for 6-7 weeks (41). They concluded that these two methionine sources had a different dose-response, with HMTBA outperforming DL-Met at commercial levels and DL-Met outperforming HMTBA at total dietary sulfur-containing amino acid (TSAA) deficient levels (41). Similar results were observed in turkeys, with feeding supplemental HMTBA to young male turkeys for 21 days leading to a lower growth but greater maximum response compared with DL-Met at TSAA deficient levels (42).

In 1997, Littell *et al*. proposed the concept of relative bioavailability (RBV), in which each supplementation level at a given dose is related in a nonlinear common plateau asymptotic regression (43). RBV was shown to be more conclusive than the previous concept of 'relative responses' as a measure of efficiency, since an 'effectiveness' of one Met source in relation to another is impossible to derive from individual supplementation levels (44, 45). Elwert *et al*. assessed the RBV of HMTBA in comparison to DL-Met in both male Ross 308 and Cobb 500 chickens fed a methionine + cysteine deficient basal diet for 38 days (44). The results suggested a superiority of DL-Met RBV over HMTBA RBV in performance parameters (44), in accordance with the conclusions discussed above. Sauer *et al*. used meta-analysis with the same nonlinear mixed model and also illustrated 79% and 87% relative biological effectiveness of HMTBA over DL-Met for the response variables average daily gain and gain to feed ratio, respectively (46). Vedenov and Pesti applied an economic analysis to these meta-analysis results and reported relative economic values (cost ratio, HMTBA: DL-Met) between 81%-86%, depending on the value of a broiler and the cost of feed and DL-Met (47). However, the authors concluded that these results were questionable because the profit-maximizing levels of DL-Met and HMTBA in this trial were so far above the levels studied in most of the trials (47).

Besides the effects on body weight gain and feed conversion ratio, HMTBA and DL-Met may also differ in effects on protein and fat deposition when methionine is clearly limiting. Esteve-Garcia *et al*. showed that the amount of breast muscle yield was greater for DL-Met supplemented chickens than an equivalent amount of HMTBA supplement, while HMTBA supplementation resulted in greater abdominal fat deposition at 41 days for male broilers deficient in TSAA, even if live body weight was the same (48). Lemme *et al*. also reported less efficacious carcass yield and breast muscle yield from 42-day-old broilers that were fed additional liquid HMTBA compared to DL-Met (49).

In trials where methionine was supplemented to protein-deficient diets, some researchers also observed the same molar bio-efficacy for growth performance and carcass quality between HMTBA and DL-Met (50). This may be due to the different TSAA levels in the trial diets. Even though HMTBA showed similar effects to DL-Met on body weight gain, feed efficiency and muscle deposition, Liu *et al*. reported superior breast and thigh muscle color in HMTBA-fed birds compared to DL-Met-fed birds (50).

The bio-efficacy of DL-Met and L-Met was not different in both purified and practical-type low-protein diets of varying TSAA contents fed to chickens from 8 to 20 days old (51). Furthermore, the addition of 0.2.% of L-cysteine in a methionine + cysteine deficient diet improved body weight gain efficiency but was associated with anorexic behavior in the chickens (51) This depression in feed intake was thought due to a unique nutritional imbalance, and the improvement in gain:feed ratio mediated through reduced feed intake is a rare event in nutritional studies. More research is needed to provide a physiological basis for this phenomenon (51). A summary of the biological equivalencies of the Met sources in multiple species is shown in Table 2.

**Table 2.** Summary of the biological equivalencies of the Met sources in livestock and poultry species

| Species | Bio-efficacy of Met Sources | Traits measured | Criteria | References Include ref by numbers below |
|---|---|---|---|---|
| Broiler chicken & Turkey | HMTBA was more effective than DL-Met at commercial levels and DL-Met was more effective than HMTBA at TSAA deficient levels | Body weight gain and feed efficiency | Comparison of estimated gain responses using the best model (linear, quadratic or exponential) within each Met source | |
| Broiler chicken | DL-Met was more effective than HMTBA at TSAA deficient levels | Body weight gain and feed efficiency | Comparison of relative bio-availability by nonlinear common plateau asymptotic regression; meta-analysis | |
| | DL-Met was more effective than HMTBA at TSAA deficient levels | Carcass and breast muscle yield | Comparison of estimated parameters using nonlinear exponential model | |
| | HMTBA was as effective as DL-Met at TSAA deficient levels | Body weight gain, feed efficiency, and muscle accretion | Comparison of estimated parameters using the best model (linear, quadratic or exponential) within each Met source | |
| | DL-Met was as effective as L-Met at TSAA deficient levels | Body weight gain and feed efficiency | Comparison of estimated parameters using nonlinear exponential model | |
| Swine | HMTBA was as effective as DL-Met at TSAA deficient levels | Growth performance | Comparison of estimated parameters using multiple linear regression model | |

## 4. UTILIZATION OF DIFFERENT DIETARY METHIONINE SOURCES IN OTHER LIVESTOCK SPECIES

### 4.1. Utilization of dietary L-Met, DL-Met and HMTBA in piglets

On the basis of the net portal appearance, methionine rather than lysine is considered to be one of the most limiting amino acids for young piglets. L-Met supplementation improved the maintenance of the integrity and barrier function of the small-intestinal mucosa in post-weaning piglets (52). Studies on dietary methionine metabolism and systemic homocysteine regulation using piglets as models may benefit the clinical treatment of cardiovascular disease and stroke (53).

Early research showed that HMTBA and DL-Met provided equimolar levels of methionine activity in early-weaned pigs (54). Recently, Jendza *et al* indicated that when cost per mole of methionine activity is not different, HMTBA is a better choice in low-fiber pig diets due to increased apparent ileal digestibility of acid and neutral detergent fiber and several other amino acids, while DL-Met is preferred in high-fiber pig diets because of the negative interaction between HMTBA and wheat middlings on digestibility of other amino acids (55).

Gut and liver are the main organs involved in the utilization of dietary methionine for cysteine synthesis in piglets, which can be regulated by dietary methionine status and dietary methionine sources. There is little Met catabolism to form cysteine in enterocytes of the piglet small intestine (56). With sufficient methionine supply, the GI tract metabolizes 20% of dietary methionine intake in neonatal pigs, which is mainly transmethylated to homocysteine and transsulfurated to cysteine. In contrast, TSAA deficiency coordinates methionine metabolism, such that protein synthesis is preserved over methionine transmethylation and the methionine pool is preserved by up-regulation of homocysteine re-methylation and suppression of transsulfuration (57). Supplementation of dietary HMTBA, which is all absorbed by the end of the duodenum in pigs (55), increased circulating plasma taurine concentrations compared to DL-Met supplementation, indicating the greater potential of HMTBA over DL-Met to promote the transsulfuration of dietary methionine (58). Dietary HMTBA may also up-regulate portal blood flow and net portal absorption of amino acids in piglets, with signs of increase in both concentrations of intestinal short-chain fatty acids and expression of proglucagon, glucagon-like-peptide 2 receptor and endothelial nitric-oxide synthase genes (59). All of these might imply an additional nutritional effect of HMTBA on swine growth. A summary of the plasma L-Met concentrations of livestock and poultry fed dietary supplemental Met sources is shown in Table 3 (59-61).

The conversion of DL-HMTBA and D-Met to L-Met in pigs follows the same pathway as that in chickens. Moreover, the extent of conversion of dietary methionine precursors is different among tissues. On the basis of activity distribution and mRNA abundance of relevant enzymes, the liver and kidney are the major sites for HMTBA conversion, with the highest L-HAOX and D-HADH activities and mRNA abundance. The stomach can also convert DL-HMTBA to L-Met, while the small intestine, with higher D-AAOX expression, contains a relatively higher capacity to convert D-Met than to convert HMTBA (62). Furthermore, this provides a

**Table 3.** Concentrations of L-Met in the plasma of livestock and poultry species receiving dietary supplemental Met sources

| Species | Treatment | Plasma L-Met concentration |
|---|---|---|
| Broiler chicken | Basal diet | 22.4. μM[a] |
| | Basal diet+DL-Met | 27.2. μM[b] |
| | Basal diet+HMTBA | 26.5. μM[b] |
| | SEM | 5.5. |
| Pig | Basal diet+DL-Met | 5.4.6 mole/100 mole amino acid[c] |
| | Basal diet+HMTBA | 5.7.7 mole/100 mole amino acid[c] |
| | SEM | 0.3.7 |
| Dairy cow | Basal diet | 19.1. μM[d] |
| | Basal diet+DL-Met | 21.8. μM[d] |
| | Basal diet+HMTBA | 17.0. μM[d] |
| | SEM | 2.4. |

[a,b,c,d]Mean values with different superscript letters within each species are significantly different ($P$<0.0.5). Data obtained from references 59-61

biological basis for the similar portal appearance of L-Met in piglets fed DL-Met- and HMTBA-supplemented diets.

### 4.2. Utilization of dietary HMTBA in ruminants

Compared to non-ruminants, the absorption and metabolism of HMTBA by ruminants are different. There are several destinations in the GI tract for dietary HMTBA after oral administration. First, HMTBA can be directly absorbed, mainly through the ruminal, omasal or abomasal epithelia. McCollum *et al*. clearly indicated that the former two tissues account for at least a portion of the absorption of HMTBA in sheep (63). A number of factors can determine the absorption amount available, such as the retention time (the inverse of liquid outflow rate) within the rumen (64). Second, the forestomach tissues may convert HMTBA to methionine during the absorption process (65). This would increase the net availability of methionine for absorption or support tissue protein synthesis to the animals (64). But it is still unknown whether this conversion within the digestive tract is dose dependent or represents a fixed value linked to the enzyme capacity within the tissues. Third, microbes in the rumen may degrade HMTBA and the products would continue to form during passage of fluid between the rumen and abomasum.

Lobley *et al*. pointed out that less than 30% of the oral dose was absorbed as HMTBA, the majority of HMTBA having been either oxidized or converted to other products (64). The HMTBA converting enzymes were found in ruminal and omasal epithelia, liver and kidney in sheep, demonstrating the possible metabolism sites of HMTBA in ruminants (63). Actually, the non-hepatic tissues rather than the liver act as major sites of synthesis of methionine from HMTBA (64, 66). Approximately 65%-75% of absorbed HMTBA passed beyond the liver for subsequent metabolism by peripheral tissues in lambs (67). Most of the synthesized methionine is preferably retained to support tissue protein synthesis with little returned to the plasma. As a consequence, only a small increase in plasma methionine was observed even when enough dietary HMTBA was supplemented. This would yield an energy savings through using passive diffusion of HMTBA rather than active transport of methionine (63). This also indicates that the plasma methionine concentrations cannot accurately predict methionine availability in ruminants (65).

In ruminants, the hepatic HMTBA can undergo similar fates as in non-ruminants: catabolism (oxidation), conversion to methionine and export or use by cells, or conversion to other metabolic products such as cysteine and glutathione through the transsulfuration pathway (67). To ensure maintenance of aminoacidemia and prevent methionine toxicity, the liver plays a key role in removal of extra methionine (66). There also appears to be preferential use of D-HMTBA by ruminant tissue, with D-HADH more active (by 45% to 75%) than L-HAOX in ovine omasum, rumen and kidney but not in liver (65).

In lactating dairy cows, dietary HMTBA supplementation has been proposed as a means to increase milk protein yield, considering that it is more resistant to rumen microbial degradation than DL-Met (61). Approximately 15% of methionine incorporated into milk protein originated from direct conversion of HMTBA to methionine, and the remaining 85% was provided indirectly, where methionine synthesized from HMTBA within peripheral tissues was used to support intracellular protein synthesis, allowing methionine released from protein breakdown to be exported for use by the mammary gland (66).

## 5. ANTIOXIDANT EFFECTS OF HMTBA

It is now well established that HMTBA is a safe and efficacious precursor of methionine widely used in animal diets (68). A summary of the practical doses of supplemental HMTBA in animal diets is shown in Table 4 (54, 61, 69). Furthermore, HMTBA has antioxidant effects and thus can improve the anti-oxidative capacity, enhance the immune function and alleviate the stress response (e.g. caused by heat) in animals.

Tang *et al*. showed that supplementation of 0.2.% HMTBA brought about a significant improvement in antioxidant defenses in high fat diet-fed male C57BL/6 mice (70). Excessive fat intake can induce the hyper-secretion of insulin, which then increases feed intake and subsequently increases electron flow along the mitochondrial respiratory chain, resulting in oxidative stress. The supplementation of 0.2.% HMTBA can

**Table 4.** Practical doses of supplemental HMTBA and DL-Met in the diets of livestock and poultry species

| Species | DL-Met supplementation (%) | HMTBA supplementation (%) |
|---|---|---|
| Broiler chicken | 0.0.2-0.4 | 0.0.2-0.4 |
| | 0.2.2-0.3. (optimal) | 0.2.2-0.3. (optimal) |
| Pig | 0.0.5-0.1. | 0.0.5-0.1. |
| Dairy cow | 0.0.88 (dry matter basis) | 0.1.0-0.1.3 (dry matter basis) |
| Data obtained from references 54, 61, 69 | | |

restore these changes, indicating the potential of HMTBA as a dietary supplement to considerably improve certain metabolic disorders and correct the redox imbalance (70).

In Caco-2 cells, pre-incubation with HMTBA partially prevented the inflammation induced by $H_2O_2$ or inflammatory cytokine tumor necrosis factor α, while pre-incubation with DL-Met did not significantly improve the antioxidant capacity of the Caco-2 cells (71). The protective role of HMTBA on intestinal epithelial barrier function is correlated with the higher level of taurine and reduced glutathione (GSH), which are products of L-Met conversion after transsulfuration, suggesting that HMTBA might be preferentially diverted to the transsulfuration pathway (29). Also, taurine was reported to have the capacity to protect epithelial barrier function from disruption by oxidative stress generated by docosahexaenoic acid enrichment (71). This may partially explain the antioxidant effect of HMTBA. Moreover, the protection of barrier function by dietary HMTBA reduced the passage of macromolecules such as antigens and pathogens through the paracellular pathway, thus contributing to the quality of animal products for human consumption (71).

In chickens, DL-HMTBA supplementation partially prevented the growth-depressing effect of heat exposure (32 °C - 33 °C) and alleviated oxidative damage caused by heat stress on broiler chickens (72, 73). This effect was accompanied by a reduced hepatic total GSH to total glutathione ratio, which may be responsible for the positive effect of HMTBA (72). Also, HMTBA mitigated the decreased feed utilization and decreased humoral and nonspecific immunocompetence of broiler chickens due to methionine deficiency (74).

Furthermore, recent studies in ducks showed that plasma homocysteine of birds fed DL-HMTBA supplemented diets was significantly lower than in ducks fed equimolar DL-Met supplemented diets, suggesting the lower toxicity of HMTBA relative to DL-Met (75). Plasma homocysteine, a key product of methionine metabolism via the transmethylation pathway, is strongly associated with oxidative stress and cardiovascular disease in humans (76). A recent study on turbot fish also demonstrated higher serum ascorbic acid concentration associated with dietary HMTBA supplementation, demonstrating an antioxidant function of HMTBA (77).

Although it still remains unknown whether the health of the human GI tract benefits from HMTBA, DL-HMTBA is currently being marketed as an enteral product, in conjunction with a low-protein diet, for patients suffering with chronic renal insufficiency (53,68, 78,79). HMTBA shows promise as a possible additive to alleviate some metabolic diseases to improve both animal performance and human health.

## 6. SUMMARY AND PERSPECTIVE

In this review, we summarized the absorption, transport, metabolism and bio-efficiency of three dietary methionine sources, L-Met, DL-Met and HMTBA in different species including poultry, pigs and ruminants. HMTBA is an efficacious methionine precursor in the promotion of growth in chickens and pigs. It is absorbed mainly by MCT1, coupled with the activity of the $Na^+/H^+$ exchanger (NHE3), while uptake of D-Met and L-Met takes place via multiple carrier-mediated systems. HMTBA is absorbed along the entire GI tract, especially the upper GI tract. Intestine, liver and kidney all can remove D-Met and HMTBA from circulation and metabolize them to L-Met through oxidation and transamination.

Dietary methionine is indispensable in the animal nutrition industry, and can be utilized to promote animal growth and development and improve the quality of animal products for human consumption. With the antioxidant capacity, it is promising that the dietary methionine sources, especially HMTBA, can be more widely used to benefit human health in the future.

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**Key Words:** L-Met, DL-Met, HMTBA, Bio-efficiency, Absorption, Transport, Metabolism, Review

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