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ARTICLE

Methionine Metabolism in Piglets Fed DL-Methionine or Its Hydroxy Analogue Was Affected by Distribution of Enzymes Oxidizing These Sources to Keto-Methionine

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Previous evidence shows that the extensive catabolism of dietary essential amino acids (AA) by the intestine results in decreased availability of these AA for protein synthesis in extraintestinal tissues. This raises the possibility that extraintestinal availability of AA may be improved by supplying the animal with an AA source more of which can bypass the intestine. To test this hypothesis, six barrows (35-day-old, 8.6 ± 1.4 kg), implanted with arterial, portal, and mesenteric catheters, were fed a DL-methionine (DL-MET) or DL-2-hydroxy-4-methylthiobutyrate (DL-HMTB) diet once hourly and infused intramesenterically with 1% *p*-amino hippurate. Although the directly available L-MET in DL-MET diet was about 1.2-fold that in DL-HMTB diet, the net portal appearance of L-MET was not different between the two diets. Compared with the low mRNA abundance and low activity of D-2-hydroxy acid dehydrogenase (D-HADH) and L-2-hydroxy acid oxidase (L-HAOX) in the intestine, the high mRNA abundance and high activity of D-AA oxidase (D-AAOX) indicated that the intestine had a relatively higher capacity of D-MET utilization than of DL-HMTB utilization to L-MET synthesis and its subsequent metabolism. However, in contrast to the much lower D-AAOX activity (nmol/g tissue) in the stomach than in the liver and kidney, both D-HADH and L-HAOX activity in the stomach was comparable with those in the liver and/or kidney, indicating the substantial capacity of the stomach to convert DL-HMTB to L-MET. Collectively, the difference in distribution of activity and mRNA abundance of D-AAOX, D-HADH, and L-HAOX in the piglets may offer a biological basis for the similar portal appearance of L-MET between DL-MET and DL-HMTB diets, and thus may provide new important insights into nutritional efficiency of different L-MET sources.

KEYWORDS: DL-2-hydroxy-4-methylthiobutyrate; D-amino acid oxidase; D-2-hydroxy acid dehydrogenase; L-2-hydroxy acid oxidase; net portal appearance of amino acids

INTRODUCTION

The primary fate of essential amino acids (AA) is presumably for protein synthesis; however, intriguing data have shown that catabolism dominates the first-pass utilization of these AA by the gut (*1*). This novel concept has important implications for protein and AA nutrition in animals because the extensive catabolism of dietary essential AA by the intestine results in decreased nutritional efficiency (*1*, *2*). The regulation of this metabolism generates particular interest among researchers in animal production and clinical treatment of gut disease. It has been proposed that lowering intestinal AA metabolism without compromising gut absorptive capacity or protective functions may be promising for improving AA utilization efficiency (*3*).

Given evidence that the extensive catabolism of dietary essential AA is driven directly by their local availability in enterocytes (*4*, *5*), we hypothesized that AA utilization efficiency might be improved by supplying the animal with an AA source more of which can bypass the intestine. To test this hypothesis, DL-2-hydroxy-4-methylthiobutyrate (DL-HMTB) and DL-methionine (DL-MET) were taken as paradigms for "more" and "less" bypass-the-intestine AA, respectively. Indeed, both DL-HMTB and D-MET must be converted to L-MET before they can be used by the intestine (*6*). The rate-limiting enzymes for conversion of D-MET, D-HMTB, and L-HMTB to L-MET are D-AA oxidase (D-AAOX), D-2-hydroxy acid dehydrogenase (D-HADH), and L-2-hydroxy acid oxidase (L-HAOX), respectively (*7*). Previous studies in broilers indicate that the intestine possesses considerable capacity to convert D-MET and D-HMTB to L-MET (*7*, *8*). In contrast, the oxidation capacity of the intestine toward L-HMTB may be quite low because the activity of L-HAOX is

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mainly observed in the liver and kidney (*7*). It would appear that compared with equal moles of DL-MET, DL-HMTB might produce less L-MET following transport across the enterocytes.

Interestingly, previous studies reported that plasma concentration of DL-HMTB was higher than that of L-MET in broilers fed DL-HMTB or L-MET at an equal molar basis (*9*, *10*), but little information exists about the biological basis for this observation. A recent study revealed that 52% of dietary methionine was extracted by the intestine in the first pass (*1*). Furthermore, the methionine requirement was 30% lower in parenterally fed than enterally fed piglets fed methionine alone or in combination with excess cysteine, which provides further evidence for the extensive first-pass utilization of dietary methionine by the intestine (*11*, *12*). The above observation combined with the potentially low capacity of DL-HMTB utilization by enterocytes to L-MET synthesis raises the possibility that DL-HMTB may contribute to improved extraintestinal availability of L-MET via decreasing the first-pass metabolism of dietary methionine by the intestine. Therefore, DL-HMTB and DL-MET were used in the present study to determine whether the extraintestinal availability of dietary methionine could be affected by methionine sources. The distribution of mRNA abundance and the specific activities of D-AAOX, D-HADH, and L-HAOX in the piglets were also examined, which may provide a biological basis for the observed similarity or difference in L-MET availability between sources.

MATERIALS AND METHODS

Animals and Diets. The protocol of this study was approved by the Animal Care and Use Committee of College of Animal Sciences and Technology, Huazhong Agricultural University, and was carried out in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals. Ten 21-day-old piglets from the same litter [Large White × Landrace, 6.3 ± 1.1 kg bodyweight (BW)], were individually housed in metabolic cages (1.5 m × 0.75 m) located in the same air-conditioned room. Pigs were fed one of two diets (DL-HMTB vs DL-MET diet) with 5 pigs per diet during the experimental period from 21 to 35 days of age. The two diets (**Table 1**), formulated to meet National Research Council (*13*) nutrient requirements, had the same amounts of corn, dried whey, porcine plasma, and dehulled soybean meal. The only difference between the two diets was that 30% of the total dietary methionine was provided by DL-HMTB and DL-MET, respectively. Pigs were offered the meal at a rate of 50 g feed·kg BW^{-1}·day^{-1}, which supplied 8.81 g crude protein·kg^{-1}·day^{-1} and 725 kJ gross energy·kg^{-1}·day^{-1}. The crude protein in the diet was analyzed by using Kjeldahl nitrogen determination method as described (*14*). Pigs had free access to water at all times. After 7 days of adaption, all pigs received a surgery as described (*15*). Briefly, after overnight food deprivation, pigs were surgically implanted with catheters in one carotid artery (Tygon tubing, 2.41 mm OD, a kind gift from Institute of Subtropical Agriculture, The Chinese Academy of Sciences, Changsha, China), the portal vein (polyethylene tubing, 1.78 mm OD, Wuhan Qianmao Medical Treatment Appliance Co., Ltd., Wuhan, China), and the mesenteric vein (Silicone tubing, 1.02 mm OD, a kind gift from Institute of Subtropical Agriculture) under isoflurane anesthesia and strict aseptic conditions. The catheters were filled with sterile saline containing heparin (2.0×10^5 U/L). All of the catheters were protected with gauze pads and secured with an elastic bandage. The animals received an intramuscular injection of analgestic (0.1 mg/kg of butorphenol tartrate) and antibiotic (20 mg/kg of ampicillin sodium) twice daily during the first 3 days postsurgery. After the surgery, the piglets were offered parenteral nutrition for 12−24 h and were then progressively returned to their preceding level of dietary intake for ≥5 days before the experimental protocol. At a postnatal age of 35 days, 6 barrows in good health and with well-kept catheters were studied for a 7-h experimental period. Their body weight was 8.6 ± 1.4 kg.

Infusion Protocol and Blood Collection. The piglets were deprived of feed from 1800 to 0800 h. At 0655 h, a 1% solution of *p*-amino hippurate (pAH, sodium salt, diluted in sterile saline at pH 7.5) was infused continuously with a screw-driven syringe constant-infusion pump (WZS-50F6, Medical Instrument Corporation of Zhejiang University, China) into the mesenteric catheters at a rate of 3.820 mL/min for 5 min and then 0.788 mL/min for 7 h (*15*). At ∼0745 h, baseline arterial and portal blood samples (5 mL each) were withdrawn into heparinized tubes. From 0800 to 1400 h, pigs were offered meals at hourly intervals, and the meal was the equivalent of one twenty-fourth of the daily intake (45 g/kg BW). Water was freely accessible throughout the sampling period. From 0900 to 1400 h, arterial and portal blood samples (5 mL each) were taken at hourly intervals, and all the blood samples were immediately placed on ice. Within 10 min of collection, the blood was centrifuged at 4 °C and 2550*g* for 15 min to separate plasma from cells. An aliquot of plasma was refrigerated and assayed within 24 h for pAH concentration. Another aliquot was stored at −80 °C until analyzed for AA concentration.

Table 1. Composition of Experimental Diets (As-Fed Basis)

ingredients		amino acids	amino acid concentration (g/kg of protein)[d]
corn	68.13	threonine	56
dried whey	11.50	tryptophan	16
soybean oil	2.00	leucine	84
dehulled soybean meal	4.50	lysine	84
porcine plasma	10.00	phenylalanine	52
methionine premix[a]	0.14	valine	58
lysine HCl	0.45	methionine	22
threonine	0.10	isoleucine	46
tryptophan	0.01	histidine	31
isoleucine	0.17	proline	52
valine	0.05	arginine	55
limestone	1.02	tyrosine	28
dicalcium phosphate	1.14	cysteine	28
vitamin premix[b]	0.30	alanine	60
mineral premix[c]	0.49	serine	53
total	100	glutamate plus glutamine	149
		aspartate plus asparagine	85
		glycine	41

[a] Supplied 0.12% methionine activity at the expense of corn as DL-methionine (99%) or Alimet (88%, DL-2-hydroxy-4-methylthiobutyrate, Novus International, St. Louis, MO). [b] Provided per kg of diet: vitamin A, 13500 IU; vitamin D3, 3000 IU; vitamin E, 24 mg; menadione, 3 mg; thiamine, 3 mg; riboflavin, 7.5 mg; niacin, 30 mg; D-panthothenic acid, 15 mg; vitamin B6, 3 mg; vitamin B12, 22.5 μg; D-biotin, 120 μg; folic acid, 1.5 mg. [c] Provided per kg of diet: copper, 140 mg; iron, 150 mg; manganese, 50 mg; zinc, 140 mg; iodine, 0.8 mg; selenium, 0.4 mg. flavours, 200 mg; antioxidant, 100 mg. [d] The crude-protein content was analyzed to be 176 g per kg diet.

Collection of Tissue Samples. Immediately after completion of blood samples, pigs were killed with an arterial injection of sodium pentobarbital (50 mg/kg BW) and sodium phenytoin (5 mg/kg BW). Immediately after death, the animals were rinsed with 70% ethanol, and the *longissimus dorsi* muscle was collected rapidly. Then the abdomen was opened to collect tissue samples representing the jejunum and ileum as described (*16*). Duodenal samples were obtained by cutting a 2-cm section of tissue, the upper terminal of which is 5-cm away from the pylorus. The samples of stomach, liver, and kidney were also collected as described (*17*). Samples were obtained as quickly as possible, snap frozen in liquid nitrogen, and stored at −80 °C for subsequent RNA isolation.

pAH Analysis. pAH in plasma samples was analyzed as described (*15*). The portal plasma flow rate was calculated as the following equation: $PPF = C_i \times IR \times [(pAH_p - pAH_a) \cdot BW]^{-1}$ where PPF is portal plasma flow rate (L·kg^{-1}·h^{-1}), C_i is the concentration of infused pAH solution (g/L), IR is the infusion rate (L/h) of pAH, pAH_p, and pAH_a are the concentration (g/L) of pAH detected in the portal vein and artery, respectively, and BW is the bodyweight (kg).

AA Analysis. For AA analysis, the diet was hydrolyzed in 6 N HCl for 24 h at 110 °C in evacuated, sealed tubes. The frozen plasma samples were thawed at 4 °C and deproteinized using 2.5 mL of 7.5% (w/v)

trichloroacetic acid per milliliter of plasma. The resulting mixture was centrifuged at 4 °C and 27000*g* for 15 min to obtain the supernate for subsequent AA analysis. For methionine and cysteine analysis, the dietary protein was oxidized with performic acid for 16 h at 4 °C before hydrolysis in 6 N HCl for 24 h at 110 °C. The AA concentration of deproteinized plasma and hydrolyzed protein was determined by ion-exchange chromatography with an L8800 high-speed amino acid analyzer (Hitachi, Tokyo, Japan). Net portal mass balances ($\mu mol \cdot kg^{-1} \cdot h^{-1}$) of AA were calculated by the following formula: portal mass balance = PPF × ($C_p - C_a$) where PPF is the portal plasma flow rate ($L \cdot kg^{-1} \cdot h^{-1}$) and C_p and C_a are the portal and arterial plasma concentrations of AA (μmol/L). Fractional mass balance (percentage of intake) = portal balance × 100/input. The methionine input is the total methionine (dietary L-MET plus D-MET or DL-HMTB) intake.

RNA Isolation. Total RNA was extracted using the TRIzol reagent (Invitrogen Corp., Carlsbad, CA, USA) according to the manufacturer's specifications. The RNA samples were quantified with an ultraviolet spectrophotometer (Eppendorf, Hamburg, Germany) at 260 and 280 nm. The ratio of absorbance at 260 nm to absorbance at 280 nm between 1.8 and 2.0 was acceptable. The quality of RNA was also checked by 1.0% agarose gel electrophoresis and staining with 1 μg/mL ethidium bromide.

The Relative mRNA Abundance Analysis. A two-step semiquantitative reverse transcription polymerase chain reaction (PCR) method (*18*) was used to measure the relative mRNA abundance of *DAO1*, *HADH*, and *HAO1* at the time of slaughter. There is growing evidence that 18S rRNA is superior to β-actin, GAPDH, and ubiquitin as a standard in the comparative study of mRNA expression among different tissues or organs (*19*). The observation that 18S rRNA expression was not significantly different between treatments further confirmed the feasibility of using this gene as endogenous standards. Thus, in the reverse transcription reaction, random primers were added in order to using 18S rRNA as an internal control. Briefly, individual samples of total RNA (2 μg), 1 μL of random decamer primers (25 mM; Toyobo, Osaka, Japan), 4 μL of oligo-$(dT)_{20}$ (10 mM; Toyobo), and 0.1% diethylpyrocarbonate (DEPC)-treated water were made up to a total volume of 15 μL in thin-walled PCR microtubes (ABgene, Surrey, UK). This RNA mix was denatured at 70 °C (10 min) and then immediately stored on ice. In a separate tube, a reverse transcription reaction master mix consisting of 5× first-strand buffer [250 mM TriszHCl (pH 8.3), 375 mM KCl, 15 mM $MgCl_2$; Toyobo], 40 U of RNasin ribonuclease inhibitor (Promega, Madison, Wisconsin, USA), 150 U of Maloney's murine leukemia virus reverse transcriptase (Toyobo), and dNTP mix (10 mM each dGTP, dATP, dTTP, and dCTP; Toyobo) was prepared, and 35 μL was added to each RNA mix. The RNA was reverse transcribed into cDNA by incubation at 42 °C (60 min) and 95 °C (5 min) and cooled on ice. The cDNA stock was stored at −20 °C.

The PCR reaction was performed on a 2720 PCR machine (Applied Biosystems, CA, USA). One microliter of the cDNA from the reverse transcription reaction was added to 24 μL of a PCR master mix consisting of 10 × PCR buffer minus Mg [200 mM TriszHCl (pH 8.4), 500 mM KCl; Toyobo], 10 mM dNTP mix, 50 mM $MgCl_2$ (Life Technologies, Carlsbad, CA, USA), autoclaved distilled water and 1 U of Taq DNA polymerase (Toyobo), and specific sense and antisense oligonucleotides at 0.5 mM each. The samples for *DAO1*, *HADH*, and *HAO1* genes were denatured at 94 °C for 4 min and underwent amplification cycles with denaturation at 94 °C for 40 s, annealing for 30 s at 54−57 °C, and extension for 40 s at 72 °C. An additional extension at 72 °C for 10 min was performed and the samples were then cooled to 4 °C. The linear amplification range for each gene was tested on the adjusted cDNA. The optimal cycle number was considered to be two cycles lower than the highest cycle of linearity. The PCR samples were electrophoresed on a 2% agarose gel and stained with ethidium bromide (10 μg/mL). The gel images were digitally captured with G:BOX-HR Gel Documentation System (Syngene, Cambridge, UK) and densitometry values were measured using the Gene Tool software (Syngene). mRNA abundance was expressed as band intensity relative to 18S rRNA band intensity. Data for each replicate represented the mean of three individual reverse transcription PCR.

Primers. Primer sequences and optimal PCR annealing temperatures are listed in **Table 2**. Oligonucleotides to recognize the pig *DAO1* were designed from cDNA sequences in pigs (accession number NM_214066). The sense and antisense oligonucleotides corresponded to nucleotides 177−196 and 531−550, respectively. Oligonucleotides to identify the pig *HAO1* were designed from regions of high homology between the *HAO1* cDNA sequences in rat (accession number NM_001107780) and human (NM_017545). The sense and antisense oligonucleotides corresponded to nucleotides 92−110 and 374−394, respectively, in human. Oligonucleotides to recognize the pig *HADH* were designed as described (*20*). Briefly, a human *HADH* cDNA sequence (AF113251) was used to search available ESTs in the dbEST nonhuman and nonmouse subdivision (EST_others, http://www.ncbi.nlm.nih.gov/BLAST/) by BLAST. A primer pair was designed from the porcine EST consensus sequence (BP141590, BP441486, BX926265, CJ000139). The sense and antisense oligonucleotides corresponded to nucleotides 23−42 and 384−403, respectively, in pigs (BP141590).

Table 2. Oligonucleotide Sense and Antisense Sequences, Annealing Temperatures, and Cycle Number

oligonucleotides	annealing temperatures and cycles	sequences
DAO1	55 °C, 30 cycles	
sense		5′-CCCTCAGCCAACTACTCAAT-3′
antisense		5′-GGAAGACTGGACCAAATGAC-3′
HAO1	54 °C, 33 cycles	
sense		5′-CTGAGTGGGTGCCAGAATG-3′
antisense		5′-TCAGGATGCAAGTCCATTTCT-3′
HADH	57 °C, 30 cycles	
sense		5′-GTGCATGAGGCGAGTGAGAC-3′
antisense		5′-AATCGGCGACAGGTGGTGAG-3′

Enzyme Assays. Tissues samples including the stomach, duodenum, jejunum, ileum, liver, kidney, and muscle were dissected free from connective tissue and/or intraperitoneal fat, rinsed, and homogenized into four volumes of homogenizing buffer and diluted 1:1 with potassium phosphate buffer as described (*6*). The homogenizing buffer consisted of 0.25 M sucrose, 0.01 M phenylmethyl sulfonyl fluoride and 0.02 M potassium phosphate. The potassium phosphate buffer consisted of 0.02 M potassium phosphate, pH 7.5. Protein levels in the homogenates were quantified using a modified biuret protein assay as described (*6*). The specific activity of the enzymes involved in the conversion of DL-HMTB and D-MET to keto-methionine in each of the tissues was determined as described (*7*). The D- and L-isomer of HMTB used as substrates in enzyme reaction were a kind gift from Novus International (St. Louis, Missouri, USA). One unit of enzyme activity was defined as that amount yielding 1 nanomole of keto-methionine per gram of tissue or per milligram of protein under standard reaction conditions.

Statistics. Data were statistically analyzed as described.(*21*) The main effects on the enzyme activities and relative mRNA abundance of tested genes consisted of tissue and treatment and were analyzed using the GLM procedures of SAS statistical package (V8.1, SAS Institute Inc., Cary, NC). Least-squares means comparison was used to evaluate differences among tissues and between treatments for each enzyme and mRNA abundance. For comparisons of portal mass balance and fractional portal balance of methionine between the DL-MET and DL-HMTB group, a repeated two-way ANOVA (V8.1, SAS Institute Inc.) analysis was performed with time of pAH infusion as the repeated effect.

RESULTS

Net Portal Appearance of Methionine. There was no significant ($P > 0.05$) difference in the net portal mass balance of methionine between groups over the 0−3 h, 3−6 h, or 0−6 h (**Table 3**). The fractional net portal mass balance of methionine was also not significantly ($P > 0.05$) different between groups over the time courses evaluated.

***DAO1* mRNA Abundance.** There were significant ($P < 0.05$) differences in *DAO1* mRNA abundance among tissues evaluated (**Figure 1**). *DAO1* mRNA abundance was significantly ($P < 0.05$) higher in the kidney than in the other tissues, significantly ($P < 0.05$) higher in the duodenum, jejunum, and ileum than in the liver, but significantly ($P < 0.05$) lower in the muscle than in the other tissues. The dietary treatments had also a significant ($P < 0.05$) effect on the expression of *DAO1* in the piglets (**Figure 1**).

Table 3. Intake (μmol·kg^{-1}·h^{-1}), Net Portal Mass Balance (μmol·kg^{-1}·h^{-1}) and Fractional Net Portal Mass Balance (% of Intake) of Methionine in Piglets Fed Once Hourly with DL-Methionine (DL-MET) or DL-2-Hydroxy-4-methylthiobutyrate (DL-HMTB)

		portal balance			fractional portal balance		
diets	intake[a]	0−3 h	3−6 h	0−6 h	0−3 h	3−6 h	0−6 h[b]
DL-MET	43 ± 1	22 ± 8	23 ± 8	22 ± 8	51 ± 19	52 ± 17	51 ± 18
DL-HMTB	45 ± 5	25 ± 9	27 ± 5	26 ± 7	57 ± 20	62 ± 15	59 ± 18
P-value	0.60	0.27	0.17	0.10	0.40	0.25	0.18

[a] Ingested total methionine (dietary L-MET plus D-MET or DL-HMTB) was taken as the methionine intake. [b] The data were overlapped with that presented in our earlier paper (*15*).

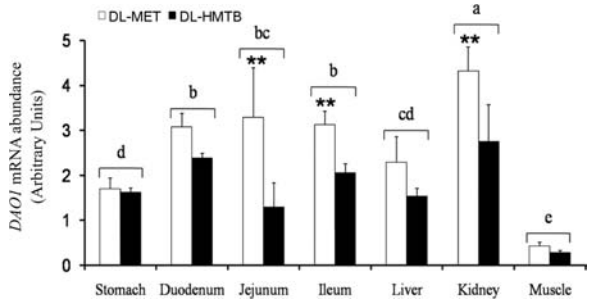

Figure 1. Relative mRNA abundance of *DAO1* in the stomach, duodenum, jejunum, ileum, liver, kidney, and muscle of DL-HMTB fed pigs (solid bars) compared with that of DL-MET fed pigs (open bars). mRNA abundance, measured by semiquantitative RT-PCR, is expressed relative to 18S rRNA. *Significant difference between treatments ($P < 0.05$). Letters and horizontal lines (top) indicate body tissues for which values lacking common letters differ significantly ($P < 0.05$).

DAO1 mRNA abundance in the jejunum, ileum, and kidney was significantly ($P < 0.05$) higher in DL-MET group than in DL-HMTB group.

***HADH* mRNA Abundance.** There were significant ($P < 0.05$) differences in *HADH* mRNA abundance among tissues evaluated (**Figure 2**). *HADH* mRNA abundance was significantly ($P < 0.05$) lower in the stomach, jejunum, and ileum than in the liver and kidney. However, *HADH* mRNA abundance in the muscle was similar to that in the liver and kidney. The dietary treatments had also a significant ($P < 0.05$) effect on the expression of *HADH* in the piglets (**Figure 2**). *HADH* mRNA abundance in the duodenum, ileum, and muscle was significantly ($P < 0.05$) higher in DL-HMTB group than in DL-MET group.

***HAO1* mRNA Abundance.** There were significant ($P < 0.05$) differences in *HAO1* mRNA abundance among tissues evaluated (**Figure 3**). *HAO1* mRNA abundance in the liver and kidney was about three times that in the other tissues. *HAO1* mRNA abundance in the stomach, duodenum, jejunum, and ileum was similar to that in the muscle. The dietary treatments had also a significant ($P < 0.05$) effect on the expression of *HAO1* in the piglets (**Figure 3**). *HAO1* mRNA abundance in the duodenum, jejunum, and muscle was significantly ($P < 0.05$) higher in DL-HMTB group than in DL-MET group.

D-AAOX Activity. Among the tissues evaluated (**Table 4**), the kidney had the highest D-AAOX activity, about twice that in the liver, while the D-AAOX activity in the liver was significantly ($P < 0.05$) higher than that in gastrointestinal tissues and muscle. D-AAOX activity in the duodenum was about twice that in the ileum or muscle. In contrast, D-AAOX activity in the stomach and jejunum was similar to that in the duodenum. Overall, a similar distribution of D-AAOX activity in the piglets was observed when enzyme activity was expressed as nmol per milligram of protein. The dietary treatments had a significant ($P < 0.05$) effect on D-AAOX activity. D-AAOX activity in both the duodenum and kidney was significantly ($P < 0.05$) higher in DL-MET group than in DL-HMTB group, whereas dietary treatments had no effect ($P > 0.05$) on D-AAOX activity in other tissues.

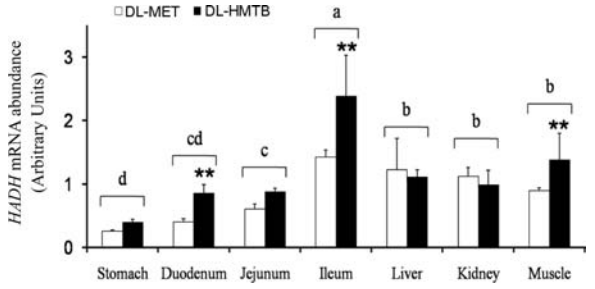

Figure 2. Relative mRNA abundance of *HADH* in the stomach, duodenum, jejunum, ileum, liver, kidney, and muscle of DL-HMTB fed pigs (solid bars) compared with that of DL-MET fed pigs (open bars). mRNA abundance, measured by semiquantitative RT-PCR, is expressed relative to 18S rRNA. *Significant difference between treatments ($P < 0.05$). Letters and horizontal lines (top) indicate body tissues for which values lacking common letters differ significantly ($P < 0.05$).

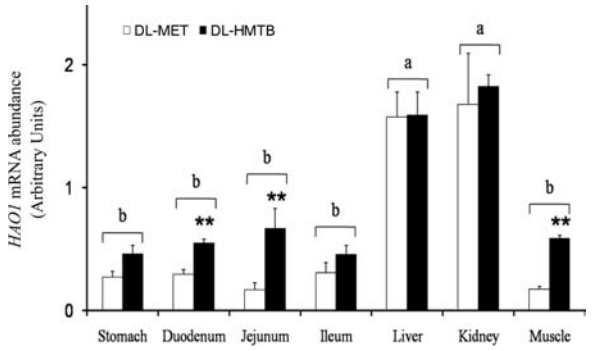

Figure 3. Relative mRNA abundance of *HAO1* in the stomach, duodenum, jejunum, ileum, liver, kidney, and muscle of DL-HMTB fed pigs (solid bars) compared with that of DL-MET fed pigs (open bars). mRNA abundance, measured by semiquantitative RT-PCR, is expressed relative to 18S rRNA. *Significant difference between treatments ($P < 0.05$). Letters and horizontal lines (top) indicate body tissues for which values lacking common letters differ significantly ($P < 0.05$).

D-HADH Activity. D-HADH activity in the kidney and muscle was significantly ($P < 0.05$) higher than that in other tissues (**Table 5**). D-HADH activity in the stomach and ileum was comparable with that in the liver, but significantly ($P < 0.05$) higher than that in the duodenum and jejunum. Interestingly, when enzyme activity was expressed as nmol per milligram of protein, D-HADH activity in the liver and kidney was significantly ($P < 0.05$) lower than that in gastrointestinal tissues and muscle. The dietary treatments had a significant ($P < 0.05$) effect on D-HADH activity. D-HADH activity in both the stomach and jejunum was significantly ($P < 0.05$) higher in DL-HMTB group than in DL-MET group, whereas dietary treatments had no effect ($P > 0.05$) on D-HADH activity in other tissues.

L-HAOX Activity. L-HAOX activity in the kidney was significantly ($P < 0.05$) higher than that in other tissues (**Table 6**). L-HAOX activity in the liver and muscle was similar to that in the stomach, but significantly higher than that in the duodenum, jejunum, and ileum. When enzyme activity was expressed as nmol per milligram of protein, L-HAOX activity in the liver and kidney

Table 4. Distribution of D-Amino Acid Oxidase Activity in Gastrointestinal Tissues, Liver, Kidney and Muscle of Piglets Fed DL-Methionine (DL-MET) or DL-2-Hydroxy-4-Methylthiobutyrate (DL-HMTB)

	treatments[a]		average activity[b]	
tissue	DL-MET[c]	DL-HMTB[c]	nmol/g of tissue	nmol/mg of protein
stomach	54.00 ± 19.49 (4.35 ± 2.33)	56.34 ± 15.78 (2.01 ± 0.52)	55.17 ± 15.91^{cd}	3.18 ± 1.98^{b}
duodenum	136.53 ± 47.68^{y} (4.46 ± 0.92)^{y}	69.18 ± 26.14^{x} (2.04 ± 0.45)^{x}	102.86 ± 47.67^{c}	3.25 ± 1.48^{b}
jejunum	99.81 ± 10.25 (2.68 ± 0.84)	67.54 ± 26.89 (1.51 ± 0.64)	83.67 ± 25.37^{cd}	2.09 ± 0.93^{bc}
ileum	46.82 ± 19.85 (1.20 ± 0.48)	32.30 ± 15.65 (0.78 ± 0.38)	39.56 ± 17.86^{d}	0.99 ± 0.45^{c}
liver	244.02 ± 36.61 (2.39 ± 0.19)	249.78 ± 64.62 (3.03 ± 1.24)	246.90 ± 47.68^{b}	2.71 ± 0.87^{b}
kidney	642.63 ± 55.12^{y} (8.51 ± 2.15)^{y}	491.16 ± 103.38^{x} (6.39 ± 1.56)^{x}	566.90 ± 111.23^{a}	7.45 ± 2.04^{a}
muscle	39.10 ± 9.27 (0.98 ± 0.32)	33.81 ± 5.50 (0.93 ± 0.22)	36.45 ± 7.41^{d}	0.95 ± 0.24^{c}

[a] Values within same rows lacking common superscripts differ significantly ($P < 0.05$). [b] Values within same columns lacking common superscripts differ significantly ($P < 0.05$). [c] Values with brackets and with no brackets represent activities contained per milligram of protein (nmol/mg of protein) and per milligram of tissue (nmol/g of tissue), respectively.

Table 5. Distribution of D-Hydroxy Acid Dehydrogenase in Gastrointestinal Tissues, Liver, Kidney and Muscle of Piglets Fed DL-Methionine (DL-MET) or DL-2-Hydroxy-4-methylthiobutyrate (DL-HMTB)

	treatments[a]		average activity[b]	
tissue	DL-MET[c]	DL-HMTB[c]	nmol/g of tissue	nmol/mg of protein
stomach	56.20 ± 7.05^{x} (4.30 ± 0.67)^{y}	71.52 ± 6.66^{y} (2.56 ± 0.08)^{x}	63.86 ± 10.39^{ab}	3.43 ± 1.05^{a}
duodenum	42.41 ± 5.22 (1.51 ± 0.65)	45.70 ± 7.88 (1.37 ± 0.49)	44.06 ± 6.24^{c}	1.44 ± 0.52^{bc}
jejunum	39.12 ± 2.92^{x} (1.06 ± 0.38)	51.16 ± 4.18^{y} (1.18 ± 0.34)	45.14 ± 7.34^{c}	1.12 ± 0.33^{cd}
ileum	54.42 ± 7.73 (1.45 ± 0.59)	59.46 ± 5.86 (1.44 ± 0.16)	56.94 ± 6.72^{b}	1.44 ± 0.39^{bc}
liver	60.61 ± 7.57 (0.59 ± 0.05)	55.36 ± 4.36 (0.65 ± 0.06)	57.99 ± 6.23^{b}	0.62 ± 0.06^{e}
kidney	70.44 ± 9.06 (0.91 ± 0.09)	69.85 ± 6.30 (0.91 ± 0.11)	70.15 ± 6.99^{a}	0.91 ± 0.09^{de}
muscle	66.60 ± 4.34 (1.67 ± 0.41)	73.08 ± 5.19 (2.00 ± 0.42)	69.84 ± 5.56^{a}	1.84 ± 0.41^{b}

[a] Values within same rows lacking common superscripts differ significantly ($P < 0.05$). [b] Values within same columns lacking common superscripts differ significantly ($P < 0.05$). [c] Values with brackets and with no brackets represent activities contained per milligram of protein (nmol/mg of protein) and per milligram of tissue (nmol/g of tissue), respectively.

Table 6. Distribution of L-Hydroxy Acid Oxidase Activity in Gastrointestinal Tissues, Liver, Kidney and Muscle of piglets fed DL-Methionine (DL-MET) or DL-2-Hydroxy-4-methylthiobutyrate (DL-HMTB)

	treatments[a]		average activity[b]	
tissue	DL-MET[c]	DL-HMTB[c]	nmol/g of tissue	nmol/mg of protein
stomach	33.28 ± 5.46 (2.54 ± 0.45)^{y}	32.72 ± 1.52 (1.17 ± 0.03)^{x}	33.00 ± 3.60^{bcd}	1.86 ± 0.80^{a}
duodenum	28.13 ± 10.41 (0.91 ± 0.11)	25.44 ± 4.73 (0.74 ± 0.18)	26.79 ± 7.38^{d}	0.83 ± 0.16^{bcd}
jejunum	21.41 ± 1.47 (0.58 ± 0.19)	25.68 ± 2.18 (0.59 ± 0.17)	23.55 ± 2.87^{d}	0.58 ± 0.16^{de}
ileum	25.44 ± 4.67 (0.68 ± 0.31)	30.51 ± 3.26 (0.74 ± 0.09)	27.97 ± 4.55^{cd}	0.71 ± 0.21^{cde}
liver	44.09 ± 0.86 (0.44 ± 0.04)	38.32 ± 8.52 (0.45 ± 0.07)	41.20 ± 6.27^{b}	0.44 ± 0.05^{e}
kidney	81.05 ± 29.67 (1.02 ± 0.20)	68.31 ± 10.31 (0.89 ± 0.16)	74.68 ± 21.06^{a}	0.95 ± 0.18^{bc}
muscle	39.48 ± 10.82 (1.03 ± 0.52)	39.86 ± 7.35 (1.11 ± 0.39)	39.67 ± 8.28^{bc}	1.07 ± 0.42^{b}

[a] Values within same rows lacking common superscripts differ significantly ($P < 0.05$). [b] Values within same columns lacking common superscripts differ significantly ($P < 0.05$). [c] Values with brackets and with no brackets represent activities contained per milligram of protein (nmol/mg of protein) and per milligram of tissue (nmol/g of tissue), respectively.

was significantly ($P < 0.05$) lower than that in the stomach and muscle. The dietary treatments had no effect ($P > 0.05$) on L-HAOX activity in tissues evaluated.

DISCUSSION

The Specific Activity of D-AAOX, D-HADH, and L-HAOX. In the present study, DL-HMTB and DL-MET were taken as paradigms for "more" and "less" bypass-the-intestine AA, respectively. But this notion is challenged by a question, namely, whether the intestine has a relatively lower capacity of DL-HMTB utilization than D-MET utilization to L-MET synthesis. To answer this question, we first compared the capacity of the intestine to convert DL-HMTB and D-MET to L-MET in terms of enzyme activity of D-HADH, L-HAOX, and D-AAOX. Given that liver and kidney are the major sites for conversion of D-MET and DL-HMTB (*6*, *7*, *9*), enzyme activity in the target tissues in comparison to that in the liver and kidney has been commonly used as an indicator to reflect the capacity of the target tissues to convert methionine sources (*8*, *17*). Regardless of treatments, highest or comparable enzyme activities of D-AAOX, D-HADH, and L-HAOX were observed in the liver and kidney among tissues evaluated, which agreed well with the results of previous studies in broilers (*7*,*8*) and ruminants (*17*). These results further confirmed the predominant role of liver and kidney in the conversion of D-MET and DL-HMTB. Because D-AAOX and L-HAOX are mainly produced in peroxisomes and D-HADH are mainly produced in mitochondria, the fact that both peroxisomes and mitochondria are abundant in the liver and kidney (*22*) provides a biological basis for the high D-AAOX, D-HADH, and L-HAOX activity in these two organs. D-AAOX activity in the duodenum was about half of that in the liver, indicating that the duodenum mucosa possesses substantial oxidative activity toward D-MET. The result was similar to that reported in broilers (*8*). Among the tissues evaluated, the lowest D-AAOX activity in the muscle

suggested the minor capacity of the muscle to convert D-MET. This may be associated with the relatively high oxidative activity the intestine and liver possess toward D-MET and thus low amounts of D-MET could reach the muscle tissue. In contrast, for D-HADH and L-HAOX the lowest activity was observed in the duodenum and jejunum, suggesting the lower capacity of the intestine toward DL-HMTB compared with that of other tissues. These results reveal that D-MET is a methionine precursor more ready to be converted by the intestine than DL-HMTB. Furthermore, it was noteworthy that DL-MET and DL-HMTB were supplemented at an equal molar basis. Because D-MET (comprising 50% of DL-MET), D- and L-HMTB must be converted to L-MET before they can be used by the intestine (*6*), methionine precursors that need converting in a DL-HMTB diet was twice that in a DL-MET diet. The more methionine precursor that needs converting in DL-HMTB than in DL-MET, combined with the relatively lower capacity of DL-HMTB utilization than of D-MET utilization by the enterocytes to L-MET synthesis, provided a biological evidence for the notion that compared with DL-MET, DL-HMTB might produce less L-MET when they were transported across the enterocytes.

Another important finding in the present study was that both D-HADH and L-HAOX activity (nmol/g tissue) in the stomach was comparable with those in the liver and/or kidney, indicating the substantial capacity of DL-HMTB utilization by gastric tissues to L-MET synthesis. Moreover, compared with DL-MET group, the remarkable increase in D-HADH activity in DL-HMTB group mainly occurred in the stomach. However, compared with DL-HMTB group, the remarkable increase in D-AAOX activity in DL-MET group mainly occurred in the duodenum. This may be explained by the difference in the absorption mechanism between methionine sources and thus the difference in the sites where methionine precursors are absorbed. Like other AA, DL-MET is absorbed primarily by active transport and to a lesser degree by carrier-mediated transport; both mechanisms occur principally in the small intestine (*10*). However, until it is converted to L-MET, DL-HMTB is an organic acid, not an AA (*23*). It is established that DL-HMTB is taken up by a combination of diffusion and lactic acid carrier-mediated uptake (*24*, *25*). Further study in broilers reveals that DL-HMTB absorption occurs along the entire length of the gastrointestinal tract but primarily in the proximal gastrointestinal tract prior to the small intestine (*26*). Because 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 (*27*), there is reason to believe that DL-HMTB may be also very efficiently absorbed in the upper gastrointestinal tract of piglets. It would appear that compared with piglets fed DL-MET, the piglets fed DL-HMTB having relatively higher D-HADH activity in the stomach and relatively lower D-AAOX activity in the duodenum was a result of manipulation by the substrate availability of these enzymes.

mRNA Abundance of *DAO1*, *HADH* and *HAO1*. Consistent with the results of McCollum et al. (*17*), the specific activity of L-HAOX and D-HADH, when expressed on a per milligram of protein basis, was considerably higher in the stomach than in the liver and kidney. Although this may be explained by a lower content of protein in the stomach tissue (*17*), it is not clear whether the specific enzyme activity is associated with the protein abundance of these oxidation enzymes. Furthermore, the result of enzyme activities supported that the specific activities of D-AAOX and D-HADH were regulated by their substrates. Considering that there is generally a correlation between mRNA and protein abundance (*28*), we further examined mRNA levels for *DAO1*, *HADH* and *HAO1*, which may provide further explanation for the mechanism underlining the difference in the distribution of activities of these genes' products. Regardless of treatments, mRNA abundance for *DAO1*, *HADH*, and *HAO1* both in the liver and kidney was considerably higher than or at least comparable with those in the other organs evaluated. Consistent with this result, the specific activities of D-AAOX, D-HADH, and L-HAOX in the liver and kidney are higher than or comparable with those in the other organs evaluated. The considerably lower mRNA abundance for *DAO1* in the duodenum than that in the kidney may provide in part an explanation for the lower enzyme activity in the duodenum than that in the kidney. Similarly, the relatively low mRNA abundance for *DAO1* and high mRNA abundance for *HAO1* and *HADH* in the muscle may provide a biological basis for the viewpoint that the muscle may possess a lower oxidation activity toward D-MET than toward DL-HMTB. Collectively, these results suggested a positive correlation between mRNA abundance and enzyme activities.

Net Portal Methionine Appearance. The net appearance of dietary AA in the portal blood is dependent on the degree to which they are utilized by the enterocytes in the first pass and thus has been commonly used as an indicator for the evaluation of intestinal metabolism and extraintestinal availability of dietary AA (*1*, *15*). Given the difference in distribution of oxidation enzymes for conversion of DL-HMTB and D-MET in the piglets as discussed above, another end point in the present study was to determine whether the net portal appearance of methionine would differ between piglets after ingestion of equal amounts of total methionine (dietary L-MET plus D-MET or DL-HMTB). The comparison between treatments was therefore focused on the net portal mass balance and fractional portal balance within a given period. All of the data were novel except for the fractional portal balance within 0−6 h (**Table 3**), which was overlapped with the data presented in our earlier paper (*15*). The results indicated that although the directly available L-MET intake in the DL-MET group was about 1.2-fold that in the DL-HMTB group (85% vs 70% of total methionine), the net portal appearance of L-MET did not differ between the two groups over the time courses evaluated. Furthermore, the fraction of L-MET extracted by the intestine in its first-pass was similar between DL-HMTB and DL-MET diets as we previously determined using stable isotope 1-^{13}C-methionine (*29*). These results suggested that the more the input of L-MET in enterocytes, the more the absolute amount of L-MET that was extracted by the intestine. This notion was further supported by the fact that transsulfuration reaction of methionine in splanchnic tissues was affected by dietary methionine levels (*30*). As discussed previously, the more methionine precursors that need converting in DL-HMTB than in DL-MET, combined with the relatively lower capacity of DL-HMTB utilization than D-MET utilization by the enterocytes to L-MET synthesis, suggested less L-MET input in enterocytes and thus less extraction of methionine by the intestine, from the DL-HMTB diet than from the DL-MET diet following transport of these sources across the intestinal membrane cell. In addition, the great potential of the stomach to absorb DL-HMTB as discussed previously may further decrease the amount of methionine sources that can be accessed by the intestine in their first-pass. Collectively, DL-HMTB as compared with DL-MET is more favorable for absorption by the stomach, and methionine and its precursors from DL-MET diet as compared with those from the DL-HMTB diet are more likely to be metabolized by the intestine, thereby resulting in a similar net portal appearance of L-MET between the two diets.

In summary, piglets had a substantial capacity to efficiently utilize methionine precursors, but the extent to which methionine precursors can be converted was different among tissues. On the basis of activity distribution and mRNA abundance of enzymes

oxidizing methionine sources to keto-methionine, the liver and kidney are the major sites for conversion of supplemented sources, and the stomach also plays an important role in conversion of DL-HMTB. The intestine contains a relatively higher capacity to convert D-MET than that to convert DL-HMTB, whereas the stomach contains a relatively higher capacity to convert DL-HMTB than that to convert D-MET. These results may offer a biological basis for the similar portal L-MET appearance between DL-MET and DL-HMTB diets, and thus may provide new important insights into nutritional efficiency of different methionine sources.

LITERATURE CITED

(1) Stoll, B.; Henry, J.; Reeds, P. J.; Yu, H.; Jahoor, F.; Burrin, D. G. Catabolism dominates the first-pass intestinal metabolism of dietary essential amino acids in milk protein-fed piglets. *J. Nutr*. **1998**, *128*, 606–614.

(2) Wu, G. Intestinal mucosa amino acid metabolism. *J. Nutr*. **1998**, *128*, 1249–1252.

(3) Bertolo, R. F. P.; Pencharz, P. B.; Ball, R. O. Role of intestinal first-pass metabolism on whole-body amino acid requirements. In Burrin, D. G.; Mersmann, H. J., Eds.; *Biology of Metabolism in Growing Animals*; Elsevier Science: London, 2005; pp 127−156.

(4) Bos, C.; Stoll, B.; Fouillet, H.; Gaudichon, C.; Guan, X.; Grusak, M. A.; Reeds, P. J.; Tome, D.; Burrin, D. G. Intestinal lysine metabolism is driven by the enteral availability of dietary lysine in piglets fed a bolus meal. *Am. J. Physiol. Endocrinol. Metab*. **2003**, *285*, E1246–E1257.

(5) van der Schoor, S. R.; van Goudoever, J. B.; Stoll, B.; Henry, J. F.; Rosenberger, J. R.; Burrin, D. G.; Reeds, P. J. The pattern of intestinal substrate oxidation is altered by protein restriction in pigs. *Gastroenterology* **2001**, *121*, 1167–1175.

(6) Dibner, J. J.; Ivey, F. J. Capacity in the liver of the broiler chick for conversion of supplemental methionine activity to L-methionine. *Poult. Sci*. **1992**, *71*, 1695–1699.

(7) Dibner, J. J.; Knight, C. D. Conversion of 2-hydroxy-4-(methylthio)-butanoic acid to L-methionine in the chick: A stereospecific pathway. *J. Nutr*. **1984**, *114*, 1716–1723.

(8) Brachet, P.; Puigserver, A. Regional differences for the D-amino acid oxidase-catalysed oxidation of D-methionine in chicken small intestine. *Comp. Biochem. Physiol. B*. **1992**, *101*, 509–511.

(9) Saroka, J. M.; Combs, G. F. Comparison of the utilization of methionine and its hydroxyl analogs by the chick. *Poult. Sci*. **1983**, *62*, 1496.

(10) Knight, C. D.; Dibner, J. J. Comparative absorption of 2-hydroxy-4-(methylthio) butanoic acid and L-methionine in the broiler chick. *J. Nutr*. **1984**, *114*, 2179–2186.

(11) Shoveller, A. K.; Brunton, J. A.; House, J. D.; Pencharz, P. B.; Ball, R. O. Dietary cysteine reduces the methionine requirement by an equal proportion in both parenterally and enterally fed piglets. *J. Nutr*. **2003**, *133*, 4215–4224.

(12) Shoveller, A. K.; Brunton, J. A.; Pencharz, P. B.; Ball, R. O. The methionine requirement is lower in neonatal piglets fed parenterally than in those fed enterally. *J. Nutr*. **2003**, *133*, 1390–1397.

(13) NRC, Nutrient requirements of swine, 10th ed.; Washington, DC: National Academic Press. 1998.

(14) AOAC, Official methods of analysis, 15th ed.; Association of Official Analytical Chemists; Arlington, VA, 1990.

(15) Fang, Z. F.; Luo, J.; Qi, Z. L.; Huang, F. R.; Zhao, S. J.; Liu, M. Y.; Jiang, S. W.; Peng, J. Effects of 2-hydroxy-4-methylthiobutyrate on portal plasma flow and net portal appearance of amino acids in piglets. *Amino Acids* **2009**, *36*, 501–509.

(16) Tappenden, K. A.; Drozdowski, L. A.; Thomson, A. B. R.; McBurney, M. I. Short-chain fatty acid−supplemented total parenteral nutrition alters intestinal structure, glucose transporter 2 (GLUT2) mRNA and protein, and proglucagon mRNA abundance in normal rats. *Am. J. Clin. Nutr*. **1998**, *68*, 118–125.

(17) McCollum, M. Q.; Vázquez-Añón, M.; Dibner, J. J.; Webb, K. E. Jr. Absorption of 2-hydroxy-4-(methylthio)butanoic acid by isolated sheep ruminal and omasal epithelia. *J. Anim. Sci*. **2000**, *78*, 1078–1083.

(18) Spencer, W. E.; Christensen, M. J. Multiplex relative RT-PCR method for verification of differential gene expression. *Biotechniques* **1999**, *27*, 1044–1052.

(19) Zhu, L.; Altmann, S. W. mRNA and 18S−RNA coapplication−reverse transcription for quantitative gene expression analysis. *Anal. Biochem*. **2005**, *345*, 102–109.

(20) Yu, J.; Liu, B.; Fan, B.; Zhu, M. J.; Xiong, T. A.; Yu, M.; Li, K.; Zhao, S. H. The porcine FBXO32 gene: map assignment, SNP detection and tissue expression. *Anim. Genet*. **2005**, *36*, 435–462.

(21) Steel, R. G. D.; Torrie, J. H. *Principles and Procedures of Statistics*; McGraw-Hill: New York, NY, 1980.

(22) de Duve, C.; Baudhuin, P. Peroxisomes (microbodies and related particles). *Physiol. Rev*. **1966**, *45*, 323–357.

(23) Dibner, J. J.; Buttin, P. Use of organic acids as a model to study the impact of gut microflora on nutrition and metabolism. *J. Appl. Poult. Res*. **2002**, *11*, 453–463.

(24) Brachet, P.; Puigserver, A. Na^+-independent and nonstereospecific transport of 2-hydroxy 4-methylthiobutanoic acid by brush border membrane vesicles from chick small intestine. *Comp. Biochem. Physiol*. **1989**, *946*, 157–163.

(25) Brachet, P.; Puigserver, A. Transport of methionine hydroxy analog across the brush border membrane of rat jejunum. *J. Nutr*. **1987**, *117*, 1241–1246.

(26) Richards, J. D.; Atwell, C. A.; Vázquez-Añón, M.; Dibner, J. J. Comparative in vitro and in vivo absorption of 2-hydroxy-4-(methylthio) butanoic acid and methionine in the broiler chicken. *Poult. Sci*. **2005**, *84*, 1397–1405.

(27) Walter, A.; Gutknecht, J. Monocarboxylic acid permeation through lipid bilayer membranes. *J. Membr. Biol*. **1984**, *77*, 255–264.

(28) Greenbaum, D.; Colangelo, C.; Williams, K.; Gerstein, M. Comparing protein abundance and mRNA expression levels on a genomic scale. *Genome Biol*. **2003**, *49*, 117–124.

(29) Fang, Z. F.; Huang, F. R.; Luo, J.; Wei, H. K.; Ma, L. B.; Jiang, S. W.; Peng, J. Effects of DL-2-hydroxy-4-methylthiobutyrate on the first-pass intestinal metabolism of dietary methionine and its extraintestinal availability. *Br. J. Nutr*. **2009**, DOI:10.1017/S0007114509992169.

(30) Storch, K. J.; Wagner, D. A.; Burke, J. F.; Youngm, V. R. [1−13C; methyl-2H3]methionine kinetics in humans: methionine conservation and cystine sparing. *Am. J. Physiol*. **1990**, *258*, E790–E798.

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