

Uptake of DL-2-Hydroxy-4-methylthio-butanoic Acid (DL-HMB) in the Broiler Liver In Vivo

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ABSTRACT The methionine source DL-2-hydroxy-4-methylthio-butanoic acid (DL-HMB; Alimet™ feed supplement) is widely used in the poultry industry. The purpose of this study was to determine the capacity of the broiler liver to remove DL-HMB from the circulation. Cannulae were implanted in the carotid artery and hepatic and hepatic portal veins in anesthetized male broilers (3.33 ± 0.13 kg BW). In Experiment 1, birds (n = 5) were infused with DL-HMB solutions (diluted in saline, pH 7.2 to 7.4) into the hepatic portal vein at rates ranging from 4.4 to 22 mg/min per kg BW, whereas in Experiment 2, birds (n = 6) were infused with DL-HMB at rates ranging from 2.2 to 4.4 mg/min per kg BW. Plasma samples from each vessel were obtained before and after each 10-min DL-HMB infusion period with a 10-min clearance period

allowed between each DL-HMB infusion. Regression analysis revealed a highly significant correlation in the amount of DL-HMB entering the liver via afferent vessels (afferent DL-HMB) and DL-HMB removed by the liver ($y = 0.86(x) - 173$, $r^2 = 0.98$). The slope of this regression indicates that 86% of DL-HMB entering in afferent blood (i.e. from both the hepatic artery and hepatic portal vein) was removed or that the liver apparently metabolized 86% of the DL-HMB that entered the liver. The results indicate that the broiler liver has the capacity to remove DL-HMB from the circulation far in excess of that needed to metabolize DL-HMB that would enter the liver following gastrointestinal absorption in birds fed a conventional poultry diet. In addition, present results implicate the liver as a major site of removal from circulation and further metabolism of DL-HMB in chickens.

(Key words: Alimet™, methionine, liver, broilers)

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INTRODUCTION

As poultry diets are typically deficient in sufficient methionine to support maximum rates of protein synthesis for growth and production, commercial diets are typically supplemented with a source of methionine either as DL-methionine or a methionine precursor DL-2-hydroxy-4-[methylthio]-butanoic acid (DL-HMB, Alimet™ feed supplement). The liver is the major site of methionine metabolism, an amino acid vital for protein synthesis. Although protein synthesis is the most important role of L-methionine, through contribution of functional moieties such as methyl, sulfur, and amino groups, L-methionine is also critically important in many anabolic reactions (Finkelstein, 1990). In addition, the liver is the major site of

conversion of DL-HMB and D-methionine to the biochemically active form, L-methionine (Dibner and Knight, 1984; Dibner and Ivey, 1991).

Incorporation of DL-HMB and DL-methionine into protein has been observed in the liver (Dibner and Ivey, 1991) and peripheral tissues (Saunderson, 1985, 1987). An equal rate of incorporation of DL-HMB and DL-methionine into protein is reflected in equivalent animal performance (Waldroup et al., 1981; Elkin and Hester, 1983; Garlich, 1985). In spite of these similarities, DL-HMB and DL-methionine are transported in the chick by different mechanisms in gut mucosal cells (Knight and Dibner, 1984). Uptake of DL-methionine is mainly carrier-specific and Na⁺-dependent (Dibner et al., 1992; Maenz and Engele-Schaan, 1996) and DL-HMB uptake is mainly by diffusion (Dibner et al., 1992) or by Na⁺-independent transport (Brachet and Puigserver, 1989).

Although the biochemistry of DL-HMB has been well characterized (Langer, 1965; Knight and Dibner, 1984; Dibner et al., 1990), the capacity of the liver to remove DL-HMB from circulation in vivo is unknown. We devel-

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Abbreviation Key: DL-HMB = DL-2-hydroxy-4-methylthio-butanoic acid; MABP = mean arterial blood pressure.

oped a technique that enables simultaneous blood sampling from the hepatic portal vein (prehepatic) and hepatic vein (posthepatic) in anesthetized birds (Wang et al., 1998; Song et al., 2000). This technique is a powerful research tool for assessing hepatic metabolism *in vivo* and represents a bridge between *in vitro* hepatocyte culture and whole animal feeding studies. Therefore, the major objective of this study was to utilize the *in vivo* liver model (Wang et al., 1998) to determine the capacity of the liver to remove DL-HMB from the circulation by monitoring DL-HMB levels in pre- and posthepatic blood vessels following infusions of DL-HMB directly into the hepatic portal vein of broilers.

MATERIALS AND METHODS

Animals

In Experiments 1 and 2, male broiler chicks (Cobb 500, Experiments 1 and 2), obtained from a local hatchery,⁴ were placed in cages (>30 cm² per bird) and provided a corn-soybean meal based starter diet (24% protein, 3,400 kcal ME/kg; containing 0.25% DL-HMB) and water. The diet was formulated to meet or exceed National Research Council (1994) recommendations. Birds had *ad libitum* access to feed and water until they were selected for the study. Birds (3.33 ± 0.13 kg BW) raised in floor pens were randomly selected between 5 and 8 wk of age and were assigned to one of the two experimental groups described below. Only one bird was used per day due the extensive time required for surgical preparation. Birds used in the studies did not have access to feed or water 4 to 6 h prior to blood sample collections described below. All animal handling and surgical procedures were approved by the University of Arkansas IACUC committee (#96-302).

Surgical Protocol and Treatments

All birds were anesthetized with an injection of allobarbitol (5,5-diallyl-barbituric acid,⁵ 50 mg/kg BW) into the pectoralis muscle. Each bird was fastened in dorsal recumbency on a heated surgical board that was thermostatically regulated to maintain body temperature. The top of the board was elevated to a 30-degree head-up angle. The right wing vein was cannulated with PE 50 tubing,⁶ and mannitol (2.5%, 0.1 mL/min) was infused through this cannula to hydrate the bird during the entire experiment (Wideman and Gregg, 1988).

Cannulae were implanted in blood vessels according to Wang et al. (1998). A diagram of cannulae placement is provided in Figure 1. All cannulae were filled with heparin-saline (0.85% NaCl containing 200 IU heparin/mL). Briefly, after the right carotid artery was cannulated

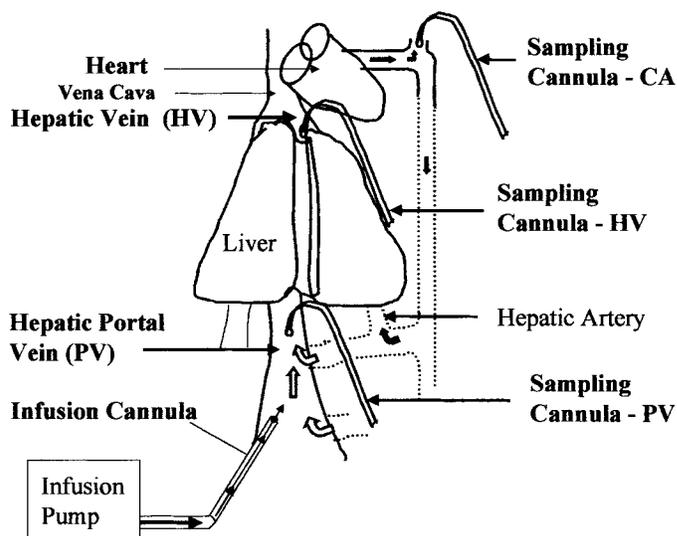


FIGURE 1. Diagram depicting surgical placement of cannulae to facilitate blood sampling from the hepatic vein (HV), hepatic portal vein (PV), and carotid artery (CA), as well as for infusion into the hepatic portal vein.

(PE 60 tubing), an abdominal incision was made to expose the abdominal cavity. The pericardial membrane dorsal to the right lobe of the liver was carefully transected to expose the hepatic vein. A single cannula attached to a curved needle (18-ga) was inserted into the hepatic vein, and two cannulae attached to bent needles (20-ga) were inserted into the hepatic portal vein, one for infusion and one for obtaining blood samples. The cannulae (PE 90) were filled with heparin-saline (0.85% NaCl containing 200 IU heparin/mL). The abdominal incision was closed to prevent tissue dehydration and temperature loss. Verification of correct needle placement in the hepatic vein and the hepatic portal vein was accomplished by visual inspection following careful dissection of birds upon completion of each experiment. Mean arterial blood pressure (MABP, mm Hg) was determined by electronic dampening of the pressure oscillations in the carotid arterial cannula using a Gilson blood pressure transducer⁷ and monitored on a strip chart recorder. Hepatic portal and hepatic venous pressures (cm H₂O) were recorded by measurement of fluid height in open-ended cannulae inserted in each of these vessels.

In the context of this paper, clearance or uptake are used as general terms to denote metabolism or usage by the liver. The objective of the two experiments was to assess the capacity of the broiler liver to clear or remove DL-HMB from hepatic portal venous blood. In Experiment 1, pharmacological levels of DL-HMB were infused directly into the hepatic portal vein. The reasons for using very high levels of DL-HMB in these experiments were twofold: 1) to ensure that changes in plasma DL-HMB levels could be monitored, as basal levels of DL-HMB in the plasma are usually very close to limits of detection, and 2) to truly challenge the liver to establish the capacity for uptake.

⁴Randall Road Hatchery, Tyson Foods, Inc., Springdale, AR 72762.

⁵Sigma Chemical Co., St. Louis, MO 63178-9916.

⁶All cannulae consisted of PE tubing obtained from Beckton Dickinson, Parsippany, NJ 07054.

⁷Gilson Medical Electronics, Middleton, WI 53131.

The DL-HMB solutions used in these studies were made by mixing Alimet™ (88% DL-HMB and 12% water) in physiological saline (0.85% NaCl) and adjusting the pH to 7.2 to 7.4 with 1 N NaOH. In Experiment 1, an initial blood sample (0.5 mL) was obtained from each vessel followed by 10-min infusions of DL-HMB solutions that were calculated to deliver 4.4, 5.5, 7.2, 11.0, and 22.0 mg DL-HMB/min per kg BW. A 10-min recovery was allowed between each DL-HMB infusion. Blood samples (0.5 mL) were taken from each blood vessel at 10-min intervals throughout each study at the end of each 10-min infusion and 10-min recovery. The samples were immediately centrifuged (9,000 × g for 1 min). Plasma was immediately separated from cells and stored at -80 C for biochemical analyses. The protocol for Experiment 2 was similar to Experiment 1 with the exception that the DL-HMB solutions infused into the hepatic portal vein were calculated to deliver 2.2, 2.8, 3.6, and 4.4 mg DL-HMB/min per kg BW during each 10-min infusion. Levels of DL-HMB used in this study were calculated to deliver DL-HMB in a 10-min period that would be 20- to 100-fold higher (Experiment 1) and 10- to 20-fold higher (Experiment 2) than what a broiler would be expected to consume over a 24-h period.⁸

Biochemical Analysis of plasma DL-HMB

After deproteinization with an equal amount of 6% trichloroacetic acid (vol/vol), DL-HMB levels in plasma were determined by HPLC. Briefly, plasma DL-HMB was separated on a Waters Symmetry C-18 column (150 × 2.1 mm) attached to a Waters 2690 Alliance chromatographic system.⁹ Samples were eluted using a gradient mobile phase consisting of water and acetonitrile, each containing 0.05% (vol/vol) trifluoroacetate. Initial column conditions were 2.5% (vol/vol) acetonitrile for 10 min (flow rate of 0.3 mL/min). Acetonitrile was raised to 100% for 5 min and held for 3 min before returning to starting conditions. DL-HMB was detected using a Waters programmable UV detector set a 210 nm.

Calculation of Apparent Utilization of DL-HMB by the Avian Liver

A simple way to determine uptake or metabolism of compound A by an organ is to subtract the venous concentration of the compound multiplied by venous blood flow (Q, in mL/min) from the arterial concentration, multiplied by arterial blood flow (Equation 1):

$$([A]_{\text{artery}} \times Q_{\text{artery}}) - ([A]_{\text{vein}} \times Q_{\text{vein}}) \quad [1]$$

= DL-HMB uptake/mL per min.

In an extensive review of hepatic blood flow measurements made with several different methodologies in sev-

eral animal species, Richardson and Withrington (1981) indicated that liver blood flow (which would be equal to hepatic venous blood flow) is approximately 2 mL/min per g liver with the hepatic arterial (HA) and hepatic portal venous (HPV) flows contributing 30 and 70%, respectively, to total hepatic blood flow. Multiplying these percentages by the total hepatic flow yields 0.6 mL/min per g liver from the hepatic artery, and 1.4 mL/min per g liver from the hepatic portal vein. Finally, as there are no capillary beds between the heart and any artery, and transit time is very rapid, little change in concentration of a compound occurs between the carotid artery (CA) and any major systemic artery. Thus, in this study, plasma concentrations of DL-HMB in the carotid and hepatic arteries were assumed to be equal.

Based on assumptions made above, estimations of hepatic uptake or use of DL-HMB in these studies were made in the following manner. The amount of DL-HMB entering the liver per min (DL-HMB In) was determined as

$$\begin{aligned} \text{DL-HMB In} &= \text{amount of DL-HMB} \\ &\text{entering the liver/min per g liver} \quad [2] \\ &= ([\text{DL-HMB}]_{\text{CA}} \times 0.6 \text{ mL/min per g liver}) \\ &\quad + ([\text{DL-HMB}]_{\text{HPV}} \times 1.4 \text{ mL/min per g liver}). \end{aligned}$$

The amount of DL-HMB exiting the liver (DL-HMB Out) was calculated as

$$\begin{aligned} \text{DL-HMB Out} &= \text{amount of DL-HMB exiting} \\ &\text{the liver/min per g liver} \quad [3] \\ &= ([\text{DL-HMB}]_{\text{HV}} \times 2.0 \text{ mL/min per g liver}). \end{aligned}$$

Therefore, an estimate of net hepatic uptake of DL-HMB would be the difference (as in Equation 1) in the amounts of DL-HMB entering (Equation 2) and exiting (Equation 3) the liver (Equation 4):

$$\text{DL-HMB Removed} = \text{DL-HMB In} - \text{Out.} \quad [4]$$

Statistical Analysis

Mean comparisons between time points were assessed by multiple *t*-tests. The data were analyzed by general linear model procedure of SAS® software (SAS Institute, 1996). Regression analysis of calculated amounts of DL-HMB entering (Equation 2) and exiting (Equation 3) the liver was used to estimate the amount of uptake or utilization of these compounds in the avian liver. A probability level of $P \leq 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The results of Experiments 1 and 2 are presented in Tables 1 and 2, respectively. Several similarities between the two experiments are evident from these data. With regard to vascular pressures (presented on the right hand side of each table), MABP was maintained above 80 mm Hg throughout both experiments. While HVP pressure

⁸Based on the amount 3 kg broiler would consume (of a diet containing 0.25% HMB) per day.

⁹Waters and Associates, Milford, MA 01757.

TABLE 1. Concentrations of DL-2-hydroxy-4-methylthio-butanoic acid (DL-HMB) ($\mu\text{g/mL}$) in the hepatic portal vein (PV), carotid artery (CA), and hepatic vein (HV) and mean arterial blood pressure (MABP), hepatic venous pressure (HVP), and portal venous pressure (PVP) in birds following 10-min infusions of DL-HMB (into the portal vein) and at the end of 10-min recovery following each infusion in Experiment 1¹

Time ²	Period ³	PV	CA	HV	MABP	HVP	PVP
		$(\mu\text{g DL-HMB/mL})$			(mm Hg)	(cm H ₂ O)	(cm H ₂ O)
0	Initial	13 \pm 4	12 \pm 2	10 \pm 2	92 \pm 5	6.1 \pm 0.4	11.1 \pm 0.2
10	4.4 mg DL-HMB	182 \pm 68 ^{a,x}	61 \pm 7 ^a	72 \pm 12 ^a	92 \pm 4	5.8 \pm 0.2	10.6 \pm 0.3
20	Recovery	41 \pm 6*	31 \pm 4*	39 \pm 9*	91 \pm 5	5.4 \pm 0.3	10.3 \pm 0.1
30	5.5 mg DL-HMB	307 \pm 129 ^{a,x}	69 \pm 7 ^a	97 \pm 25 ^a	90 \pm 5	5.6 \pm 0.3	10.0 \pm 0.6
40	Recovery	52 \pm 9*	39 \pm 8*	64 \pm 13*	89 \pm 5	5.5 \pm 0.2	9.4 \pm 0.3*
50	7.2 mg DL-HMB	503 \pm 275 ^{a,x}	176 \pm 63 ^a	113 \pm 33 ^a	89 \pm 4	5.5 \pm 0.3	9.1 \pm 0.2*
60	Recovery	60 \pm 10*	63 \pm 6*	61 \pm 8*	88 \pm 4	5.6 \pm 0.3	8.9 \pm 0.3*
70	11.0 mg DL-HMB	665 \pm 282 ^{a,x}	156 \pm 13 ^a	154 \pm 33 ^a	87 \pm 5	5.7 \pm 0.4	8.8 \pm 0.3*
80	Recovery	97 \pm 13*	83 \pm 9*	75 \pm 10*	85 \pm 5	5.5 \pm 0.3	8.7 \pm 0.3*
90	22.0 mg DL-HMB	1,365 \pm 679 ^{a,x}	295 \pm 39 ^a	245 \pm 80 ^a	86 \pm 5	5.4 \pm 0.3	8.7 \pm 0.3*

^aMeans within a column are different from preceding value ($P < 0.05$).

^xMean plasma DL-HMB concentrations in the hepatic portal vein (PV) are higher than values in the carotid artery (CA) or hepatic vein (HV) ($P < 0.05$).

¹Values represent the mean \pm SEM ($n = 5$).

²Elapsed time of the experiment, in minutes.

³Plasma samples were obtained at the start of the experiment; at the end of 10-min infusions of DL-HMB dilutions to deliver 4.4, 5.5, 7.2, 11.0, and 22.0 mg/min per kg BW; and at the end of 10-min recovery periods between each DL-HMB infusion.

*Means within a column are different from values at 0 min ($P < 0.05$).

was maintained, a decrease in PVP was observed after 30 min in both experiments. However, a sufficient pressure gradient across the hepatic vasculature (i.e. transhepatic pressure gradient) was maintained, indicating a viable liver preparation throughout the studies. Hematocrit was not monitored in Experiment 1. In Experiment 2, hematocrit declined during the study presumably due to a combination of blood sampling and constant infusion of mannitol during each study (Table 2).

Many similarities were also observed in plasma DL-HMB data between the two experiments (first three data

columns of Tables 1 and 2). Initial values of DL-HMB for each vessel were similar between experiments and across blood vessels. Each infusion of DL-HMB into the hepatic portal vein produced elevations of plasma DL-HMB in all vessels. There was considerable individual bird variability in response to DL-HMB infusions, as indicated by the large standard errors for plasma DL-HMB at the end of each 10-min infusion, especially with regard to DL-HMB concentrations in the hepatic portal vein. However, it could also be clearly observed that plasma DL-HMB values in the hepatic portal vein were much higher at the end

TABLE 2. Concentrations of DL-2-hydroxy-4-methylthio-butanoic acid (DL-HMB) ($\mu\text{g/mL}$) in the hepatic portal vein (PV), carotid artery (CA), and hepatic vein (HV) and mean arterial blood pressure (MABP), hepatic venous pressure (HVP), portal venous pressure (PVP), and hematocrit (HCT) in birds following 10-min infusions of DL-HMB (into the portal vein) and at the end of 10-min recovery following each infusion in Experiment 2¹

Time ²	Period ³	PV	CA	HV	MABP	HVP	PVP	HCT
		$(\mu\text{g DL-HMB/mL})$			(mm Hg)	(cm H ₂ O)	(cm H ₂ O)	(%)
0	Initial	14 \pm 2	11 \pm 3	6 \pm 3	90 \pm 3	7.3 \pm 0.4	12.6 \pm 0.2	30 \pm 1
10	2.2 mg DL-HMB	235 \pm 87 ^{a,x}	51 \pm 14 ^a	62 \pm 14 ^a	90 \pm 3	6.7 \pm 0.2	11.8 \pm 0.3	29 \pm 1
20	Recovery	34 \pm 4*	24 \pm 4*	23 \pm 4*	88 \pm 3	6.8 \pm 0.3	11.3 \pm 0.1	28 \pm 1
30	2.8 mg DL-HMB	309 \pm 111 ^{a,x}	75 \pm 14 ^a	87 \pm 15 ^a	86 \pm 3	6.8 \pm 0.3	11.0 \pm 0.6	28 \pm 1
40	Recovery	102 \pm 58 ^{a,x}	40 \pm 7*	37 \pm 7*	85 \pm 3	7.0 \pm 0.2	10.8 \pm 0.3*	28 \pm 1
50	3.6 mg DL-HMB	330 \pm 132 ^{a,x}	102 \pm 19 ^a	93 \pm 19 ^a	84 \pm 3	6.8 \pm 0.3	10.5 \pm 0.2*	27 \pm 1
60	Recovery	75 \pm 13*	89 \pm 25*	79 \pm 25*	83 \pm 3	6.7 \pm 0.3	10.4 \pm 0.3*	27 \pm 1
70	4.4 mg DL-HMB	563 \pm 270 ^{a,x}	139 \pm 29 ^a	145 \pm 29 ^a	81 \pm 3*	6.6 \pm 0.4	10.4 \pm 0.3*	25 \pm 1*
80	Recovery	101 \pm 45*	73 \pm 28*	68 \pm 27*	82 \pm 3*	6.7 \pm 0.3	9.6 \pm 0.7*	25 \pm 2*

^aMeans within a column are different from preceding value ($P < 0.05$).

^xMean plasma DL-HMB concentrations in the hepatic portal vein (HV) are higher than values in the carotid artery (CA) or hepatic vein (HV) ($P < 0.05$).

¹Values represent the mean \pm SEM ($n = 6$).

²Elapsed time of the experiment.

³Plasma samples were obtained at the start of the experiment, at the end of 10-min infusions of DL-HMB dilutions (2.2, 2.8, 3.6, and 4.4 mg DL-HMB/min per kg BW), and at the end of 10-min recovery periods between each DL-HMB infusion.

*Means within a column are different from values at 0 min ($P < 0.05$).

of each 10-min infusion, when compared to values in the carotid artery and hepatic vein. This finding indicated that a large amount of DL-HMB had been cleared from the circulation by the liver. Evidence that DL-HMB is metabolized to methionine and secreted as free methionine into the plasma was observed recently (Song et al., 2001). Clearance of DL-HMB infused into the hepatic portal vein was not 100%, as plasma DL-HMB values in each vessel did not return to values obtained at the onset of the experiment (at 0 min). For example in Experiment 2, DL-HMB values in each vessel during the last recovery period were 7- to 11-fold higher than initial baseline values. As DL-HMB levels in the carotid and hepatic arteries were assumed to be equal, the increase in arterial DL-HMB levels during the experiment indicates that an additional DL-HMB load would be placed on the liver during the course of the experiment due to entry of DL-HMB via the hepatic artery. Thus, the apparent utilization of DL-HMB by the liver would be greater than that demonstrated by the simple difference of DL-HMB between the hepatic portal vein and hepatic vein alone.

Further demonstration of the apparent ability of the broiler liver to take up large amounts of DL-HMB is presented in Figure 2. In this bird, DL-HMB concentrations in the hepatic portal vein increase dramatically following a constant infusion of DL-HMB (4.4 mg/min per kg BW) for 50 min. Despite the increase in hepatic portal vein DL-HMB, hepatic vein and carotid artery DL-HMB levels remain fairly constant and are lower than values of DL-HMB in the hepatic portal vein. Values in the hepatic portal vein returned to values found in the other 2 vessels within 10 min after DL-HMB infusion had ceased.

The relationship between the amount of DL-HMB entering the liver (DL-HMB In, Equation 2) and the amount of DL-HMB removed by the liver (Equation 4) is shown in Figure 3. The slope of the regression equation in Figure 3 ($y = 0.86(x) - 173$, $r^2 = 0.98$, $P < 0.001$) indicates that the liver was able to remove 86% of the DL-HMB that arrived via afferent blood. From Experiments 1 and 2, it is appar-

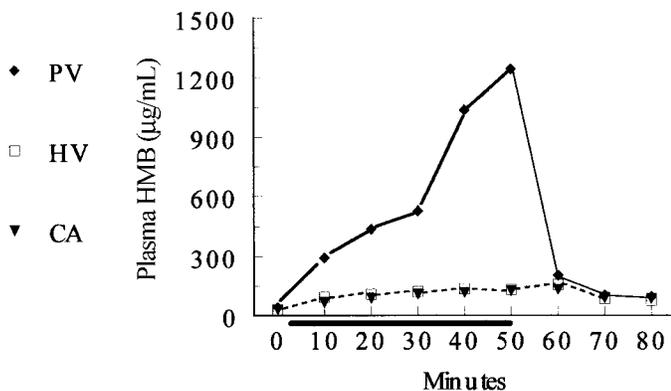


FIGURE 2. Effect of 50-min constant infusion of DL-2-hydroxy-4-methylthio-butanoic acid (DL-HMB) (4.4 mg DL-HMB/min per kg BW) into the hepatic portal vein of a 3.6-kg broiler on plasma concentrations of DL-HMB in the hepatic portal vein (PV, solid diamond), hepatic vein (HV, open square), and carotid artery (CA, solid triangle). The DL-HMB infusion period is denoted by the heavy line from 0 to 50 min.

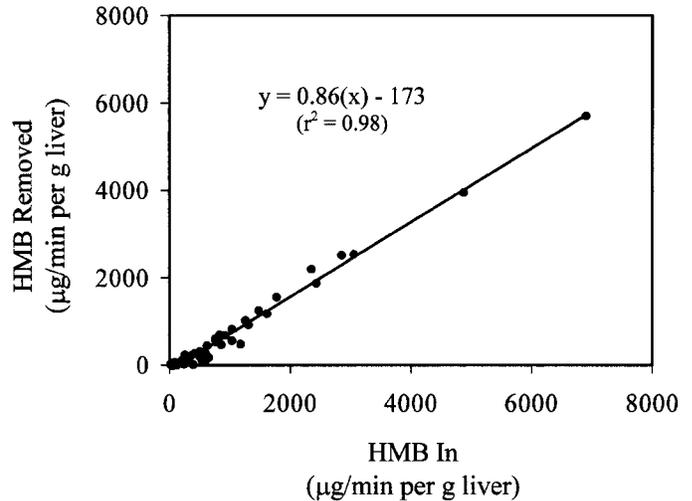


FIGURE 3. Regression analysis of calculated amounts of DL-2-hydroxy-4-methylthio-butanoic acid (DL-HMB) entering the liver (DL-HMB In, $\mu\text{g}/\text{min per g liver}$, Equation 2) and removed by the liver (DL-HMB removed, $\mu\text{g}/\text{min}/\text{g liver}$, Equation 4). The regression equation shown was significant ($P \leq 0.001$).

ent that the broiler liver has the ability to use DL-HMB far in excess of what would be expected to be presented to the liver following gastrointestinal absorption of DL-HMB in birds fed a diet containing a typical dietary level of DL-HMB.

The liver is the major site of synthesis of constitutive and export proteins, such as albumin, that would provide amino acids to the peripheral tissues. In addition, the liver is the site of major anabolic reactions of methionine (Finkelstein, 1990). In the present study, no attempt was made to determine the fate of DL-HMB in the liver. However, the presence of the enzymes involved in the conversion of DL-HMB to methionine (Dibner and Knight, 1984) and rapid incorporation of HMB into liver (Saunderson, 1987) and plasma proteins (Larbier and Perrot, 1984) proteins in birds administered with $1\text{-}^{14}\text{C}$ -DL-HMB suggests that the liver plays a major role in DL-HMB removal from circulation, conversion, and further metabolism of the methionine derived from DL-HMB. Evidence for conversion of DL-HMB into methionine by liver and intestinal tissue has also been demonstrated in vivo in a recent study (Song et al., 2001).

In summary, results provide evidence that the avian liver has the capacity to utilize DL-HMB far in excess of that expected to be encountered in birds consuming a diet containing a typical level of DL-HMB supplementation. Despite infusions of pharmacological levels of DL-HMB directly into the hepatic portal vein, no signs of toxicity were observed. In addition, the results from this study implicate the liver as a major site of uptake and further metabolism of DL-HMB in chickens.

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REFERENCES

- Brachet, P., and A. Puigserver, 1989. Na⁺-independent and non-stereospecific transport of 2-hydroxy 4-methylthiobutanoic acid by brush border membrane vesicles from chick small intestine. *Comp. Biochem. Physiol.* 94A:157–163.
- Dibner J. J., C. A. Atwell, and F. J. Ivey, 1992. Effect of heat stress on 2-hydroxy-4-(methylthio) butanoic acid and DL-methionine absorption measured in vitro. *Poultry Sci.* 71:1900–1910.
- Dibner, J. J., R. C. Durley, J. G. Kostelc, and F. J. Ivey, 1990. 2-Hydroxy-4-(methylthio)butanoic acid is a naturally occurring methionine precursor in the chick. *J. Nutr.* 120:553–560.
- Dibner, J. J., and F. J. Ivey, 1992. Capacity of the liver of the broiler chick for conversion of supplemental methionine activity to L-methionine. *Poultry Sci.* 71:700–708.
- Dibner, J. J., and C. D. Knight, 1984. Conversion of 2-hydroxy-4-(methylthio)butanoic acid to L-methionine in the chick: a stereo-specific pathway. *J. Nutr.* 114:1716–1723.
- Elkin, G. R., and P. Y. Hester, 1983. A comparison of methionine sources for broiler chickens fed corn-soybean meal diets under simulated commercial grow-out conditions. *Poultry Sci.* 62:2030–2034.
- Finkelstein, J. D., 1990. Methionine metabolism in mammals. *J. Nutr. Biochem.* 1:228–237.
- Garlich, J. D., 1985. Response of broilers to DL-methionine hydroxy analog free acid, DL-methionine and L-methionine. *Poultry Sci.* 64:1541–1545.
- Knight, C. D., and J. J. Dibner, 1984. Comparative absorption of 2-hydroxy-4-(methylthio) butanoic acid and L-methionine in the broiler chick. *J. Nutr.* 114:2179–2186.
- Larbier, M., and I. Perrot, 1984. A comparison of the metabolism of L-methionine, DL-methionine, and methionine hydroxy analog (MHA) by domestic fowl chicks. *Arch. Geflugelk.* 48:113–116.
- Langer, B. W., 1965. The biochemical conversion of 2-hydroxy-4-methylthiobutyric acid into methionine by the rat in vitro. *Biochem. J.* 95:683–687.
- Maenz, D. D., and C. M. Engele-Schaan, 1996. Methionine and 2-hydroxy-4-methylthiobutanoic acid are transported by distinct Na⁺-dependent and H⁺-dependent systems in the brushborder membrane of the chick intestinal epithelium. *J. Nutr.* 126:529–536.
- National Research Council, 1994. *Nutrient Requirements of Poultry*. 9th Ed. National Academy Press, Washington DC.
- Richardson, P. D. I., and P. G. Withrington, 1981. Liver blood flow. I. Intrinsic and nervous control of liver blood flow. *Gastroenterology* 81:159–173.
- Saunderson, C. L., 1985. Comparative metabolism of L-methionine, DL-methionine and DL-2-hydroxy 4-methylthiobutanoic acid by broiler chick. *J. Nutr.* 54:621–633.
- Saunderson, C. L., 1987. Effect of fasting and of methionine deficiency of L-methionine, DL-methionine and DL-2-hydroxy-4-methylthiobutanoic acid metabolism in broiler chicks. *Br. J. Nutr.* 57:429–437.
- SAS Institute, 1996. *SAS User's Guide*. Version 6.12 Edition. SAS Institute Inc., Cary, NC.
- Song, Z., K. Beers, J. J. Dibner, M. Vázquez-Añón, R. McNew, and W. Bottje, 2001. The hepatic extraction of plasma free amino acids and response to hepatic portal venous infusion of methionine sources in anesthetized SCWL males (*Gallus domesticus*). *Comp. Biochem. Physiol. (B)* 130:237–250.
- Song, Z., D. Cawthon, K. Beers, and W. G. Bottje, 2000b. Hepatic and extra-hepatic stimulation of glutathione release by norepinephrine in vivo. *Poultry Sci.* 79:1632–1639.
- Waldroup, P. W., C. J. Mabray, J. R. Blackman, P. J. Slagter, R. J. Short, and Z. B. Johnson, 1981. Effectiveness of the free acid of methionine hydroxy analogue as a methionine supplement in broilers diets. *Poultry Sci.* 60:438–441.
- Wang, S., W. G. Bottje, D. Cawthon, C. Evenson, K. Beers, and R. McNew, 1998. Hepatic export of glutathione and uptake of constituent amino acids, glutamate and cysteine, in broilers in vivo. *Poultry Sci.* 77:1556–1564.
- Wideman, R. F., Jr., and C. M. Gregg, 1988. Model for evaluating avian hemodynamics and glomerular filtration rate autoregulation. *Am. J. Physiol.* 254:R925–R932.