convert HMB to L-met. Two tissues were of particular interest: intestine, because it is the first tissue to encounter nutrients, and skeletal muscle, because it is the target tissue for nutritional supplements in meat producing animals.

When chick small intestine was incubated for 1 hr with ^{14}C-HMB, tissue extraction confirmed conversion of HMB to L-met by the isolated enterocytes (Knight and Dibner, 1984). Similar studies using isolated chick femoral muscle after short-term tissue culture confirmed the conversion of ^{14}C-HMB to ^{14}C-L-met by this tissue (Dibner, 1985). These results confirmed that the intact mitochondria in isolated skeletal muscle produce L-met from D-HMB. Similar experiments were conducted using pectoral and cardiac muscles of broiler chick, and femoral and pectoral muscles of ducks, geese and turkeys. All isolated muscles showed conversion of D-HMB to L-met in short-term culture (data not shown).

The extensive use of HMB by peripheral tissues has also been described in ruminants (Lobley *et al.*, 2001). In these studies, conversion by liver, kidney, intestine and mammary gland were reported. The data indicate that about 30% of the absorbed (*i.e.* portal blood) HMB was retained by the liver, with the rest going directly into the systemic circulation. The liver of ruminants was shown to convert HMB and incorporate it into protein such as albumin and metabolic enzymes. Some of this protein was secreted as plasma protein but the majority was retained by the liver for its own metabolic purposes. Other tissues were also found to convert HMB to L-met and incorporate it into tissue protein. Interestingly, in ruminants, it appears that the major source of plasma free methionine derived from HMB is the kidney, which secretes free methionine into the blood stream (Lobley *et al.*, 2001). Other tissues use the L-met from HMB for protein synthesis and only release free HMB-derived L-met at times of relative L-met excess.

Summary and conclusion

This research was undertaken to describe the metabolism of HMB by broiler chicks in order to define the optimum conditions for its use as a source of supplemental L-met. It is important to emphasize that the work described above was only a part of this effort. Performance studies, although few are reported here, were an integral part of this metabolism research, and were used to test the validity of hypotheses suggested by biochemistry or cellular biology.

Polymer availability was studied using two *in vitro* models of intestinal digestion. These model systems confirmed that enzyme-mediated dimer hydrolysis rates are sufficient to account for complete hydrolysis of the polymers present during its residence time in the chick intestine. Studies also showed that most of the dimer is already hydrolysed before it is ingested, following its application on feed. Finally, performance studies confirmed that polymer fractions extracted are fully efficacious when compared to the un-extracted supplement and DL-met.

Two enzymes for HMB conversion were described which can simultaneously catalyse D and L isomer reactions, thus providing a kinetic mechanism for biochemical equivalence. The oxidase enzymes for L-HMB and D-met were found to be very similar in that they are both peroxide producing flavo-enzymes found primarily in peroxisomes of the liver and kidney. The enzymes also produce the same keto intermediate from their different substrates. In contrast to these, D-HMB was converted by an enzyme found throughout the body in mitochondria. Conversion of radiolabeled HMB to L-met was demonstrated in short-term cultures of isolated intestine and skeletal muscle. Thus D-HMB could be converted and used by any tissue and in this respect resembles L-met itself.

Another major area of research was HMB absorption. Conflicting data in the literature were clarified by the finding that HMB and L-met differ in mechanism of absorption and

site of maximum absorption. HMB absorption was determined to be primarily concentration-dependent, carrier-independent and energy-independent with a minor component of proton-dependent, energy-independent but carrier-mediated uptake using a lactic acid transport system. In contrast, L-met absorption was primarily energy-dependent, concentration-independent and carrier-mediated. L-met and D-met compete for the same transport system(s). Maximum absorption of HMB occurred in the duodenum, while the site of maximum absorption of L-met was in the ileum. When plasma radioactivity was tested, no difference in overall rate of absorption between the HMB product and L-met could be detected, despite these differences in mechanism and site. Intact animal studies indicated no inefficiencies in HMB absorption and insignificant loss associated with renal excretion.

Primary cultures of chick and porcine liver cells were used to compare HMB and DL-met as sources of L-met for protein synthesis. These studies demonstrated that the racemic mixtures DL-HMB and DL-met were equivalent in terms of incorporation into polypeptides by the hepatocytes, but the four individual stereoisomers were significantly different from one another. When the isomers were compared in terms of their ability to support protein synthesis, L-met was superior to the other three isomers, D and L-HMB were virtually identical, and D-met was significantly poorer than any of these. It can be concluded from this work that, although the DL-HMB and DL-met can be considered to be equivalent sources of L-met for hepatocyte protein synthesis, the individual isomers, particularly L-met and D-met, are actually quite different from one another in this and probably other respects.

Controversy about the efficacy of the supplement as a source of methionine arises from the fact that when it is ingested, it is fundamentally different from methionine. When ingested, HMB is chemically an organic acid and its properties in the gut are much more like lactic acid or formic acid than they are like an amino acid such as methionine. Once the HMB molecule enters the animal's cells, however, it is rapidly converted to L-met only, and its properties, functions and availability are identical to L-met from any other source, including synthetic DL-met or intact protein.

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