Hydroxymethionine:

An efficient source of methionine for aquafeeds

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Plant-based aquaculture feeds and amino acid supplementation

Fishmeal has been the preferred protein source in aquatic feed formulations, but it is becoming less available and more expensive. Cheaper plant-based protein sources have been popularised within the aquaculture feed industry, particularly soybean- and corn-based meals. Although the crude protein content of plant-based ingredients might be comparable to that of fishmeal, such ingredients are generally less digestible and deficient in one or more essential amino acids (EAA). In particular, soybean meal-based aquafeeds are deficient in total sulfur amino acids (TSAA) and cornmeal-based aquafeeds are deficient in lysine (Lys).

Moreover, deficiency in TSAA increases with increasing levels of plant ingredients in aquafeed formulations (Goff and Gatlin III, 2004). Thus, crystalline amino acids, mainly Lys and methionine (Met), are increasingly being used to fill EAA deficiencies in plant-based aquafeeds in order to sustain protein synthesis and optimal performance.

Because Met is a precursor of cysteine (Cys), the levels of dietary Cys will affect the requirement of dietary Met. Thus, these two EAA show overlapping functions, and many studies determine the TSAA requirement (i.e., Met + Cys) instead of the Met requirement alone [National Research Council (NRC), 2011].

Although both the Met requirement alone and the TSAA requirement should be considered in feed formulation, as they vary widely among species (see Table 1; NRC, 2011), EAA requirements have generally been determined under optimal experimental conditions (i.e. temperature, oxygen supply, available food, and population density) but no information is available for EAA requirements under natural production conditions.

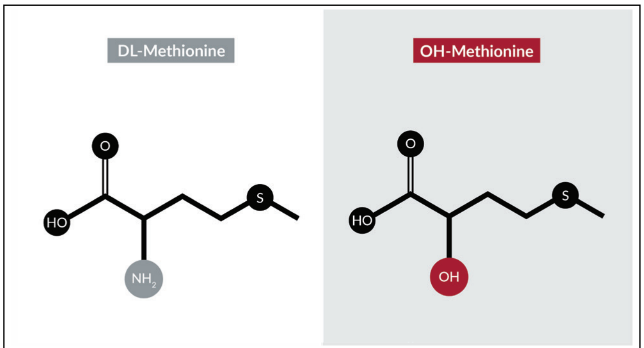

Figure 1: Chemical structures of DL-methionine and hydroxymethionine (OH-Methionine)

Table 1: Total sulfur amino acids and lysine requirements of farmed fish and crustaceans (Adapted from NRC, 2011).
NT= not tested; Lys, Lysine; Met, Methionine; Cys, Cysteine

Dry Matter (%)	Lys	Met	Met+Cys
Atlantic salmon	2.40	0.70	1.10
Common carp	2.20	0.70	1.00
Rohu	2.30	0.70	1.00
Tilapia	1.60	0.70	1.00
Channel catfish	1.60	0.60	0.90
Hybrid striped bass	1.60	0.70	1.10
Rainbow trout	2.40	0.70	1.10
Pacific salmon	2.20	0.70	1.10
Asian seabass	2.10	0.80	1.20
Cobia	2.30	0.80	1.10
European seabass	2.20	NT	1.10
Flounder	2.60	0.90	NT
Japanese grouper	2.80	NT	NT
Red drum	1.70	0.80	1.20
Yellowtail	1.90	0.80	1.20
Kuruma prawn	1.90	0.70	1.00

Methionine supplementation

Methionine can be supplemented in three different synthetic forms available on the market: L-Met (99 percent active substance), the biologically-active form of Met in vegetable or animal proteins; DL-Met (99 percent active substance), a racemic mixture of 50 percent D-Met and 50 percent L-Met, produced by chemical synthesis and used as a feed additive for decades, and hydroxymethionine (OH-Met), a hydroxyl acid with a hydroxyl group (OH) instead of an amine group (NH2) at the asymmetric carbon (See Figure 1). OH-Met is available either as a liquid product containing 88 percent of active substance and 12 percent of water or as a calcium salt.

Differences in the chemical structure lead to differences in the absorption, conversion, and utilisation of these molecules. Similar to other EAA, DL-Met is absorbed via active transport. In contrast, because OH-Met is an organic acid, it is absorbed by both passive diffusion and active transport (Martín-Venegas et al., 2007), which is Na+/H+ dependent and operated by monocarboxylate transporter 1.

Moreover, while both D- and L-Met are absorbed along the gastrointestinal tract, OH-Met absorption is completed at the end of the duodenum (Richards et al., 2005; Jendza et al., 2011). The conversion of L- and D-OH-Met to L-Met is a two-step process, each compound being converted first to ketomethylthio-butanoic acid (KMB) and then transaminated to L-Met (Vázquez-Añon et al. 2017) (See Figure 2). This conversion is driven by different

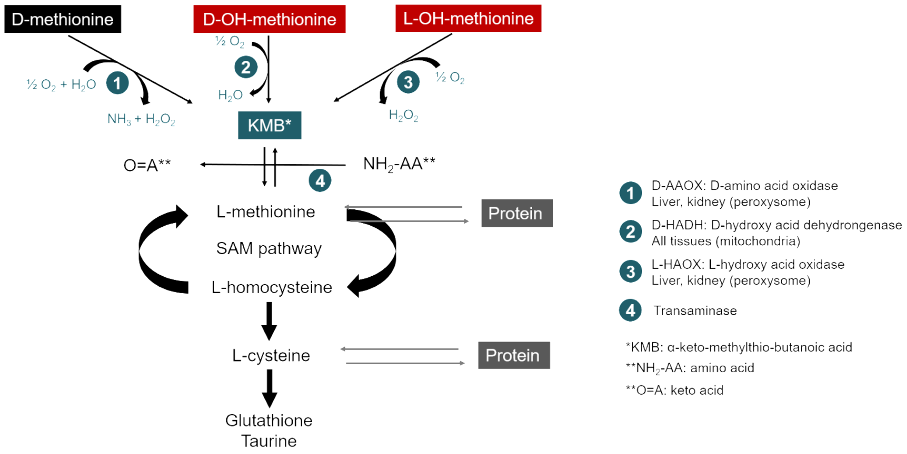

Figure 2: Conversion of DL-hydroxymethionine (DL-OH-Met) and DL-methionine (DL-Met) into L-methionine (L-Met), which is then used to synthesise proteins as well as a precursor for the synthesis of Cys, taurine and glutathione

enzymes present in the gastrointestinal tract, liver and kidney (Fang et al. 2010).

The effects of Met supplementation go beyond protein synthesis. The different metabolism of OH-Met contributes to improve the anti-oxidative capacity and immune system of animals, as well as to alleviate stress responses (Métayer et al., 2008). The concept of a better antioxidant effect of OH-Met compared to DL-Met is associated with its greater tendency to be involved in the transsulfuration cycle, and to form larger quantities of Cys, taurine and glutathione (Martín-Venegas et al., 2006) as well as to increase the reduced glutathione/oxidised glutathione ratio (Willemsen et al., 2011).

In teleost fish, the protective role of OH-Met has been associated with taurine production and with the improvement of the physical barrier in the intestine and gills and of the antioxidant status and immunity (Wu et al., 2018). Cys and glutathione act as chain-breaking elements of the oxidative processes (Métayer et al., 2008). Their enhanced production by OH-Met improves hydroxyl radical–scavenging ability and enzymatic antioxidant capacity, thereby improving meat and fillet quality via mitigation of protein and lipid oxidation (Xiao et al., 2012; Lebret et al., 2018).

Similar bio-efficacy of methionine sources in aquatic animals

Despite the low number of studies comparing methionine sources in aquatic animals, multiple studies show that fish are able to use DL-Met or OH-Met equally as effectively as L-Met to meet their TSAA requirement. Because the estimation of the bio-efficacy (i.e. biological efficiency in terms of growth performance such as body weight gain and feed conversion ratio) of DL-Met and OH-Met does not always consider the differences in feed intake, contradictory results are found in the literature.

Dietary Met affects feed intake and thereby performance (Vázquez-Añón et al., 2017). Given the close association between circulating Met levels and voluntary feed intake and the fact that OH-Met is delivered to tissues as OH-Met rather than Met, this metabolic difference could result in different ad libitum feeding patterns between OH-Met and DL-Met-supplemented animals (Vázquez-Añón et al., 2017).

Therefore, the amount of Met ingested by an animal (Met intake = dietary Met x feed intake) should be considered when comparing Met sources, as this criteria has been followed with other amino acids such as lysine (Smiricky-Tjardes et al. 2004). Under such consideration, both OH-Met and DL-Met were 100 percent equivalent to L-Met (Agostini et al., 2015; Vázquez-Añón et al., 2017).

Among the 54 peer-reviewed studies reporting the bioefficacy of Met supplementation published from 1978 to 2019, 24 compared the supplementation of different Met sources at different doses to a Met-deficient diet, but only 17 of these studies were performed in conditions allowing an accurate comparison among Met sources.

In these studies, the different Met sources were supplied on equal sulfur or equimolar basis for obtaining similar amounts of Met + Cys in all experimental treatments, as well as similar levels of the other nutrients. The different sources of Met led to similar growth performances. For example, Goff and Gatlin III (2004) reported L-Met, DL-Met, and OH-Met as equally effective sources of Met for red drum (Sciaenops ocellatus) (See Figure 3). Forster and Dominy (2006) determined that these sources could also be used to meet the Met requirement of Pacific white shrimp (Litopenaeus vannamei). Ma et al. (2013) found that turbot (Psetta maxima) can use OH-Met as effectively as, or better than, L-Met to achieve a maximum performance.

At sub-optimal dietary levels, fish fed L-Met performed better than fish fed OH-Met; in contrast, the latter performed better at near the Met requirement level. For channel catfish (Ictalurus punctatus), Zhao et al. (2017) found that supplementation of Met-deficient diets with OH-Met and microencapsulated DL-Met, rather than crystalline DL-Met, could equally improve the growth performance of this species (1.54 <

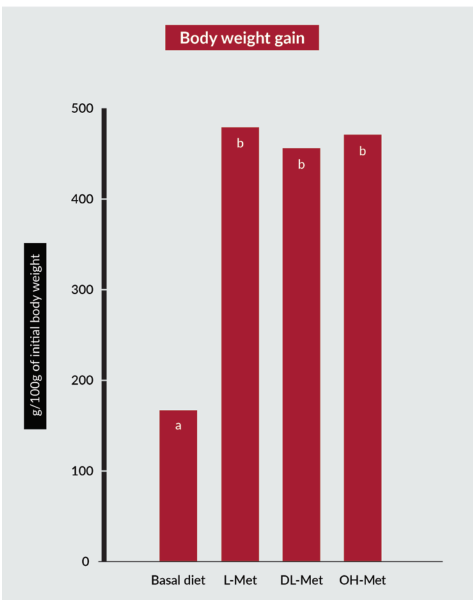

Figure 3: Body weight gain (g/100 g of initial body weight) response to L-methionine (Met), DL-Met, or hydroxymethionine (OH-Met) supplementation compared to a basal diet in red drum. Different letters on the top of bars indicate significant differences (Adapted from Goff & Gatlin III, 2004)

FCR < 1.69).

The supplementation of 0.1 percent OH-Met rather than crystalline Met to a Met-deficient diet also promoted the growth and feed utilisation of Pacific white shrimp, indicating that this species can effectively utilise OH-Met (Chen et al., 2018).

Similar bioefficacies are observed when different aquafeeds are supplemented with either DL-Met or OH-Met. Figure 4 shows that similar FCR results were obtained in studies addressing the supplementation of fish or shrimp feeds with different Met forms. Despite the large variability among studies, which can be attributed to the different experimental settings, feed formulation, and shrimp or fish species, similar FCR values were reported for both Met forms.

In conclusion, there is scientific evidence supporting that OH-Met can be efficiently used by aquatic species as an L-Met precursor. It is important to acknowledge the structural differences between DL-Met and OH-Met that result in different mechanisms for the absorption and utilisation of these two Met sources, and that their bioefficacy should be compared based on Met intake. Thus, DL-Met and OH-Met are similarly effective as Met supplements for plant-based aquafeeds.

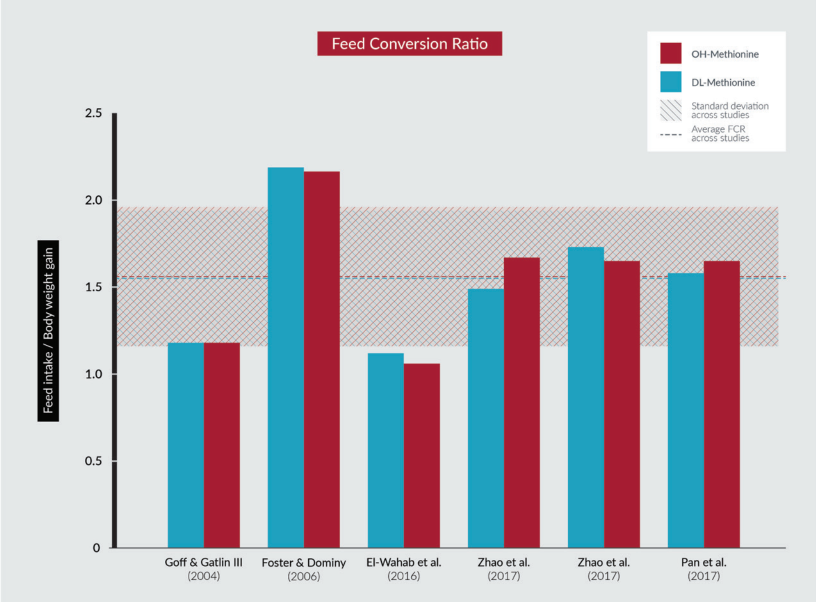

Figure 4: Feed conversion ratio (FCR) response to the supplementation of either DL-methionine (Met) or hydroxymethionine (OH-Met) in studies comparing different Met sources supplemented to fish or shrimp species. The average and standard deviation of FCR results observed for each Met form across studies are also shown. Different Met sources within each study were supplied on equimolar basis in order to achieve similar amounts of Met + Cys across treatments, as well as similar levels of the other nutrients