JUVENILE *LITOPENAEUS VANNAMEI*

An assessment of the efficacy of different commercial sources of supplemental methionine over the growth performance of young whiteleg shrimp

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While some studies have demonstrated that the bio-efficacy of the crystalline amino acids (CAAs) is similar to that found in protein-bound amino acids, other researchers have found lower bio-efficacy values for the former.

DL-Met is a racemic mixture of D- and L-isomers of Met and despite its wide application in aquaculture feeds, a debate still remains among nutritionists on its bio-efficacy (biological efficiency).

The lowest CAA bio-efficacies were found in studies with marine shrimp due to water leaching issues. Among different supplemental sources of methionine, studies have indicated that a methionine-hydroxy analogue (OH-Met) can be as efficient as DL-Met in meeting the dietary Met requirement of juvenile whiteleg shrimp, Litopenaeus vannamei, when fed low fishmeal diets (Foster and Dominy, 2006).

The present study aimed at validating the similar bio-efficacy of DL-Met and OH-Met in fishmeal-restrained diets fed to juvenile L. vannamei reared under high density in a green-water rearing system.

The study was conducted in the experimental aquaculture facilities of the Laboratory of Aquatic Animal Nutrition of Labomar (Figure 1), the Marine Sciences Institute of the Federal University of Ceará in Portugal.

Composed of independent outdoor tanks

The rearing system adopted in this study was composed of independent outdoor tanks (1.5 m^3) subjected to natural fluctuations in temperature and light (Figure 1). In order to mimic pond water conditions, water preparation consisted of applying liquid sugar-cane molasses along with ground commercial shrimp feed at a 1:1 ratio (20 g/m^3, as is basis) over a five-day period.

Shrimp of 1.32 ± 0.07g body weight (BW) were transferred to outdoor tanks and stocked under 100 animals/m^2 (165 shrimp/tank). After stocking, shrimp were allowed to grow for 11 days on a single commercial feed after being fed the experimental diets.

A total of 3 experimental feeds were prepared. Feed composition and analysed nutrients are shown in Table 1. One diet acted as control without any supplemental Met. In this case, analysed total dietary Met reached 0.46% and the total Met + Cys reached 0.94%.

From the CONT diet, two other diets were prepared to contain different supplemental sources of Met. For both feeds, analysis showed a total Met of 0.67%, and total Met + Cys of 1.23-1.29%. Total dietary lysine (Lys) and threonine (Thr) were found at 1.92-1.97% and 1.29-1.36%, respectively, in all experimental feeds. Pellet water stability was measured using a horizontal orbital shaker.

Initially, shrimps were fed using a feeding dispenser device. The feeding dispenser operated by dropping feed over the water surface during a 10-h period from 07:30 AM to 05:30 PM. Feed rations were adjusted daily assuming an estimated 0.38 percent weekly drop in shrimp survival across all the diets.

Feed rations were adjusted daily assuming an estimated 0.38% weekly drop in survival across groups and by biweekly weighing of 10 shrimp per tank. No feed leftovers were collected during the rearing period. Water quality parameters (i.e., pH, temperature, and salinity) were measured once daily starting at 0900 in all tanks.

One-way analysis of variance (ANOVA) was used for mean comparison. In case of significance, means were compared two-by-two with Tukey's HSD test.

A significant level of five percent was set in all statistical analyses, with the Statistical Package for Social Sciences (SPSS) package 23 used.

The variation in water salinity was consistent

Mean water salinity, pH and temperature reached 32 ± 2 g/L (23-39, n = 2,200), 8.2 ± 0.3 (7.3-8.6; n = 2,200), and 25.6 ± 0.6°C (24.0-28.8°C, 2,200). Values did not differ statistically between dietary treatments ($P > 0.05$).

However, these parameters varied significantly during culture (Figure 2). Variation in water salinity was consistent with the dry period that occurred during the trial. Although tanks were sheltered and protected with a lid on top to avoid shrimp escaping, high water temperature during the day and the limited water exchange favoured an increase in salinity.

As a result, salinity increased from 31 g/L on day one of culture to 34 g/L a day prior to shrimp harvest. The is-osmotic point for the whiteleg shrimp ranges from 21 to 26 g/L salinity. However, the species is widely farmed in salinities that can range from less than three to more than 50 g/L.

Although observed salinities during the trial period did not fall within the optimal range for L. vannamei, it did not appear to be detrimental to shrimp performance.

Weekly variations in salinity ranged from less than one consistent drop as the culture period progressed. In the first

Figure 1. Outdoor tanks (1.5 m3) used in the present study

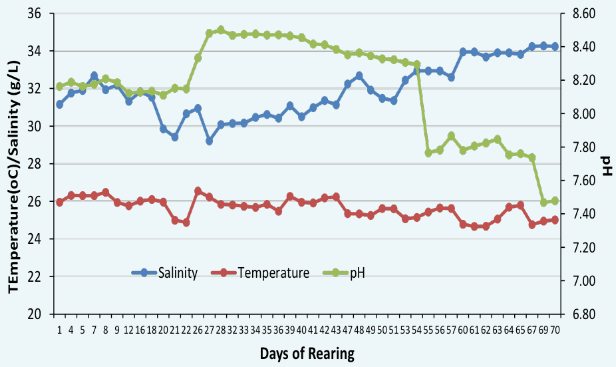

Figure 2. Variation in water salinity (g/L), temperature (°C) and pH over the culture period. Each data point represents the mean of 50 readings

month of culture, the temperature ranged from 24.9 to 26.3 °C but dropped to 25.2°C in the last week of rearing.

Variations in water pH likely reflected the accumulation of feed remains and shrimp excreta in the tank bottom during culture. No pH values were detected below seven or above nine during the experimental period.

Water stability for all feeds remained above the recommended level of 80 percent (Figure 3). The CONT feed had the lowest stability compared to the OH-Met and DL-Met supplemented feeds ($P < 0.05$). Importantly, the water stability of both Met supplemented feeds was statistically similar. Since physical stability remained high for all feeds and more than adequate levels (80%), it is unlikely that differences played any effect on shrimp performance.

Shrimp survival at harvest was high with a mean of 89.7 ± 7.3 percent (Figure 4) and was not significantly affected by dietary treatment. Likewise, no statistical differences were found in growth performance between the two methionine sources.

In relation to the non-supplemented group, weight gain was around eight and four percent higher in the groups supplemented with OH-Met and DL-Met, respectively. A similar numerical trend was detected in SGR, with improvements of four percent and two percent by OH-Met and DL-Met, respectively. Only OH-Met showed a numerical and positive improvement of six percent in relation to non-supplementation.

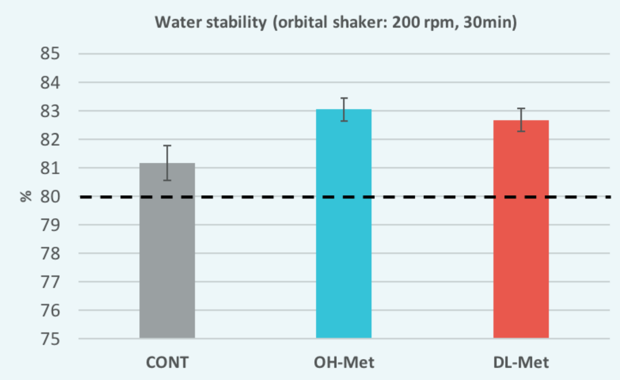

Figure 3. Mean (± standard error) water stability of tested feeds as measured by the orbital shaker method. Dotted line at recommended level of 80%. Columns with the same letter denote non-statistically significant differences at p< 0.05.

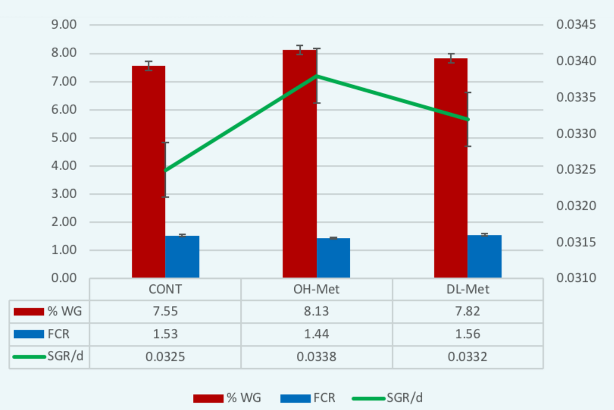

Figure 4. Effect of dietary treatments on weight gain (WG%) feed conversion ratio and specific growth rate/day (SGR/d) of white leg shrimp (Litopenaeus vannamei). No significant differences between means (p>0.05).

No difference between OH-Met and DL-Met was observed

In conclusion, the present study has shown that juvenile white leg shrimp fed diets formulated to contain a total of 0.67% Met (1.23 -1.29% Met + Cys) with supplemented sources of Met achieved a higher BW than those fed a non-supplemented diet with 0.50 percent Met (0.94% Met + Cys).

Among the different supplemental Met sources, no difference between OH-Met and DL-Met was observed, demonstrating the equal bio-efficacy of these Met sources in maintaining the growth performance of white leg shrimps.

Table 1. Ingredient composition and analyzed nutrients of experimental feeds

INGREDIENTS (%)	CONT	OH-Met	DL-Met
Soybean meal	38.00	38.00	38.00
Salmon meal	10.00	10.00	10.00
Wheat flour	25.00	25.00	25.00
Soy protein concentrate	3.73	3.73	3.73
Vital wheat gluten meal	2.50	2.50	2.50
Salmon oil	3.00	3.00	3.00
Cassava starch	2.99	2.67	2.70
Yellow kaolin	3.00	3.00	3.00
Calcium carbonate	2.20	2.20	2.20
Soy lecithin oil	2.48	2.48	2.48
Vitamin-mineral premix	1.00	1.00	1.00
Sodium monophosphate	1.62	1.62	1.62
L-Lysine, 54.6%	0.71	0.71	0.71
Potassium chloride	0.82	0.82	0.82
Magnesium sulphate	1.25	1.25	1.25
Synthetic binder	0.50	0.50	0.50
Salt coarse	0.85	0.85	0.85
L-Threonine, 98.5%	0.30	0.30	0.30
Stay C, 35%	0.06	0.06	0.06
OH-Met Liquid, 88%	-	0.32	-
DL-Methionine, 99%	-	-	0.28
ANALYSED NUTRIENTS			
Moisture (%)	9.39	9.45	10.42
Ash (%)	12.01	12.15	12.02
Crude Protein (%)	33.63	33.68	33.47
Crude Fat (%)	7.17	6.75	7.05
Methionine	0.46	0.67	0.67
Methionine + cystine	0.94	1.23	1.29
Lysine	1.97	1.92	1.92
Threonine	1.29	1.36	1.36