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Effect of shrimp stocking density and graded levels of dietary methionine over the growth performance of *Litopenaeus vannamei* reared in a green-water system

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ABSTRACT

This study evaluated the effect of shrimp stocking density, dietary methionine (Met) content and their interaction over the growth performance of juveniles of *Litopenaeus vannamei* reared under a green-water system. Five diets were formulated to contain increasing levels of Met (0.48, 0.62, 0.72, 0.81 or 0.94% on a dry matter basis, DM) and Met + Cysteine (Cys) (0.96, 1.09, 1.19, 1.28 or 1.40% DM, respectively) by supplementing a basal diet low (5%) in fishmeal with graded levels (0, 0.11, 0.21, 0.31, and 0.41% as fed basis) of DL-methionyl-DL-methionine, a dipeptide of Met. Each of the five diets were fed four times daily for 70 days to five replicate groups of 1.97 ± 0.14 g initial body weight shrimp, stocked at 50, 75 or 100 animals m^{-2}. At harvest, both dietary Met and shrimp stocking density statistically affected shrimp performance. The increase in stocking density significantly increased FCR and yield, while reducing shrimp weekly growth and final body weight. Final shrimp survival was higher than 90% and unaffected by stocking density. Dietary Met and Met + Cys content by supplementation with the dipeptide of Met, positively affected final shrimp survival, apparent feed intake, FCR and yield. Growth rate of shrimp was significantly affected by an interaction between dietary Met content and stocking density, being highest (1.56 ± 0.04 g $week^{-1}$) in shrimp fed diets with 0.62% Met (1.09% Met + Cys) reared at a stocking density of 50 animals m^{-2}. Within groups of 50 and 100 shrimp m^{-2}, final shrimp body weight was progressively increased up to 0.72% Met (maximum of 16.80 ± 1.95 g). Within 75 shrimp m^{-2}, final shrimp weight was highest at 0.81% Met (16.59 ± 1.88 g). Beyond this level, no benefit could be detected in final shrimp weight. Natural food collected from tank walls contained between 21.5 and 26.2% CP, 0.25–0.28% Met and 0.58–0.69% Met + Cys and may have thus contributed to meeting shrimp Met requirements, at least at lower stocking densities. This study has shown that under green water, an enhanced shrimp performance was obtained in diets containing between 0.72 (1.19% Met + Cys) and 0.81% Met (1.28% Met + Cys), being 29 and 38% of this total Met provided as supplemental dipeptide of Met.

Statement of relevance: Shrimp stocking density affects dietary methionine level.

1. Introduction

In industrially manufactured shrimp feeds, methionine is considered the most limiting essential amino acid (EAA). In animals, methionine plays a major role in the structure and synthesis of various metabolites, neurotransmitters, hormones and required substances. It acts as a precursor for molecules such as glutathione peroxidase, the most important anti-oxidative system in the body (NRC, 2011; Stipanuk, 2004). Shrimp obtains methionine and other EAA through the hydrolysis of dietary protein in the food or through the breakdown of body protein as it enters the metabolic pool (Kaushik and Seiliez, 2010).

Traditionally, formulators have relied on intact sources of methionine to meet shrimp requirements. However, with the strong movement towards fishmeal replacement for alternate sources of proteins, shrimp feeds are expected to make a greater use of crystalline amino acids in the coming years, especially methionine. Dietary methionine requirement has been estimated for two commercially relevant farm-reared penaeids, the Kuruma shrimp, *Marsupenaeus japonicus* (0.7% of the diet or 1.4% of the crude protein, Teshima et al., 2002) and the black tiger shrimp, *Penaeus monodon* (0.89% of the diet or 2.4% of the crude protein, Millamena et al., 1996). Some studies have shown that dietary methionine requirement of the Pacific white shrimp,

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Litopenaeus vannamei, may range from 1.9 to 2.9% of the crude protein (Fox et al., 2010; Lin et al., 2015).

Essential amino acid requirements in penaeid shrimp have been most commonly investigated under clear-water rearing systems with no access to naturally available food sources using purified or semi-purified diets (NRC, 2011). However, many exogenous factors such as feeding regime, stocking density, water quality rearing conditions have the potential to affect the dietary amino acid requirements of aquatic organisms. Very few studies have attempted to associate these factors with dietary amino acid requirement in penaeids (Liu et al., 2014; Xie et al., 2014). The objective of the present study was to evaluate the effect of shrimp stocking density and graded levels of dietary methionine over the growth performance of juvenile *Litopenaeus vannamei* reared under a green-water rearing system.

2. Material and methods

2.1. Feed design

Feeds were formulated to vary the dietary methionine content (0.48, 0.62, 0.72, 0.81, and 0.94% of the diet, on a dry matter basis, DM) and subsequently Met + Cys (methionine + cysteine, 0.96, 1.09, 1.19, 1.28 or 1.40% DM). Five replicate tanks were designated for each treatment, totaling 75 experimental units, randomly assigned under a green-water outdoor rearing system.

Experimental diets were designed with a minimum inclusion of fishmeal and other marine ingredients (Table 1). The dietary inclusion of salmon by-product meal, sardine hydrolysate, krill meal and salmon oil were locked at 5.00%, 2.00%, 0.50%, and 3.18% of the diet (as is basis), respectively. Soybean meal was the major protein component in the formulas included at levels varying from 34.52 to 35.13%. Wheat flour varied slightly from 36.64 to 36.75%. Soybean oil was used from 0.01 to 0.02% to keep dietary lipid levels consistent (Table 2).

Methionine supplementation followed graded levels. First, a control diet was designed to contain 0.48% of total methionine (as is basis) originating only from intact sources. From this diet, four nearly similar diets were formulated to contain DL-methionyl-DL-methionine (AQUAVI® Met-Met, Evonik Industries AG, Hanau, Germany) at 0.11, 0.21, 0.31 and 0.41% of the diet (as is basis, Table 1). DL-methionyl-DL-methionine was the major methyl-group donor in the supplemented experimental diets. Any other potential methyl-group donors derived from intact sources. These have not varied between experimental diets. Diets were manufactured with laboratory equipment as described in Nunes et al. (2011).

Table 1
Ingredient composition of the experimental diets (% of the diet, as is basis).

Ingredients/dietary Met content	Diets/composition (%, as is)				
	0.48%	0.62%	0.72%	0.81%	0.94%
Soybean meal[a]	35.13	34.97	34.82	34.67	34.52
Wheat flour[b]	36.64	36.68	36.70	36.73	36.75
Salmon by-product meal[c]	5.00	5.00	5.00	5.00	5.00
Soy protein concentrate[d]	4.12	4.12	4.12	4.12	4.12
Wheat bran	4.50	4.50	4.50	4.50	4.50
Krill meal[e]	0.50	0.50	0.50	0.50	0.50
Sardine hydrolasate[f]	2.00	2.00	2.00	2.00	2.00
Salmon oil[c]	3.18	3.18	3.18	3.18	3.18
Soybean oil[a]	0.00	0.00	0.01	0.01	0.01
Soybean lecithin	3.13	3.13	3.13	3.13	3.13
Bicalcium phosphate	2.00	2.00	2.00	2.00	2.00
Mineral-vitamin premix[g]	1.00	1.00	1.00	1.00	1.00
L-Lysine, 50.7%[h]	1.39	1.39	1.40	1.41	1.42
DL-Met-Met, 99.0%[i]	0.00	0.11	0.21	0.31	0.41
L-Threonine, 98.5%[j]	0.47	0.48	0.48	0.48	0.49
L-Arginine, 90.5%[k]	0.32	0.32	0.33	0.34	0.34
Synthetic binder[l]	0.50	0.50	0.50	0.50	0.50
Cholesterol, 91%[m]	0.08	0.08	0.08	0.08	0.08
Ascorbic acid, 35%[n]	0.04	0.04	0.04	0.04	0.04

[a] Bunge Alimentos S.A. (Luiz Eduardo Magalhães, Brazil).
[b] J.Macedo (Fortaleza, Brazil).
[c] Pesquera Pacific Star S.A. (Puerto Montt, Chile).
[d] Sementes Selecta S.A. (Goiânia, Brazil).
[e] Qrill™ meal (AkerBiomarine ASA, Oslo, Norway);
[f] AP50 295 (Aquativ, Descalvado, Brazil).
[g] Rovimix® Camarões Intensivo (DSM Produtos Nutricionais Brasil Ltda., São Paulo, Brazil).
[h] AQUAVI® Lys(Evonik Industries AG, Hanau, Germany).
[i] AQUAVI® Met-Met, DL-methionyl-DL-methionine (Evonik Industries AG, Hanau, Germany).
[j] ThreAMINO® (Evonik Industries AG, Hanau, Germany).
[k] Sigma-Aldrich Co. (St. Louis, USA).
[l] Nutri-Bind Aqua Veg Dry, Nutri-Ad International NV (Dendermonde, Belgium).
[m] Cholesterol SF, Dishman Netherlands B.V. (The Netherlands).
[n] Rovimix® Stay C® 35 (DSM Produtos Nutricionais Brasil Ltda., São Paulo, Brazil).

2.2. Shrimp and rearing conditions

Post-larvae (PL) of *L. vannamei* were obtained from a commercial hatchery (Aquatec Industrial Pecuária Ltda., Canguaretama, Brazil). Initially, thirty-thousand animals were reared from PL10 to juvenile stage in three outdoor nursery tanks of 23 m^3. For the study, shrimp of 1.97 ± 0.14 g (mean ± standard deviation; $n = 4932$, CV = 7.1%) were stocked under 50, 75 and 100 shrimp m^{-2} in 75 circular polypropylene tanks of 1 m^3 with a bottom surface area of 1.02 m^2.

The outdoor rearing system used in this study was described by Nunes et al. (2011). Initially, rearing tanks were filled with sand-filtered sea water at 30 ± 0.5 g L^{-1} salinity. No water fertilization was required to achieve green-water conditions. Feed remains and shrimp feces acted as natural fertilizers, reducing water transparency from 50 ± 10 cm in the first week of culture to 30 ± 3 cm in the last week before shrimp harvest. Thus, all potential natural food sources available in water was either naturally grown in the rearing system or obtained through water exchange, kept at 14.2% $tank^{-1}$ per day. Rearing tanks were provided with continuous aeration to reach near saturation of dissolved oxygen. Water temperature, salinity, pH, and transparency remained relatively stable during culture, at 29.6 ± 0.76 °C ($n = 4425$), 35 ± 1.4 g L^{-1} ($n = 4425$), 8.05 ± 1.36 ($n = 4425$), and 32 ± 8.7 cm ($n = 825$), respectively.

Shrimp were fed experimental diets daily at 7:00 am, 10:00 am, 01:00 pm and 04:00 pm, exclusively in feeding trays (area of

Table 2
Crude protein and amino acid composition of experimental diets (% of the diet, on a dry matter basis).

Amino acid/dietary Met content	Diets/composition (% of the diet, DM)				
	0.48%	0.62%	0.72%	0.81%	0.94%
Crude protein	36.02	36.75	36.27	35.29	35.91
Essential amino acids (EAA)					
Arginine	2.38	2.45	2.44	2.36	2.39
Histidine	0.74	0.76	0.76	0.73	0.73
Isoleucine	1.37	1.43	1.40	1.34	1.36
Leucine	2.36	2.45	2.41	2.31	2.34
Lysine	2.37	2.46	2.44	2.37	2.41
Methionine	0.48	0.62	0.72	0.81	0.94
Met + Cys[a]	0.96	1.09	1.19	1.28	1.40
Phenylalanine	1.59	1.63	1.61	1.55	1.57
Threonine	1.64	1.71	1.68	1.63	1.66
Tryptophan	0.37	0.39	0.38	0.37	0.37
Valine	1.50	1.56	1.53	1.47	1.50
Non-essential amino acids (NEAA)					
Alanine	1.46	1.51	1.49	1.44	1.45
Cystine	0.48	0.47	0.46	0.45	0.46
Glycine	1.62	1.68	1.66	1.62	1.64
Serine	1.54	1.60	1.57	1.51	1.53
Proline	1.92	1.95	1.93	1.89	1.91
Aspartate	3.16	3.27	3.22	3.07	3.13
Glutamine	6.22	6.41	6.31	6.09	6.18

[a] TSAA, total sulfur amino acids.