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Minimum water exchange spares the requirement for dietary methionine for juvenile *Litopenaeus vannamei* reared under intensive outdoor conditions

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Abstract

We examined if minimum water exchange could spare dietary methionine (Met) required for maximum growth performance of juvenile *Litopenaeus vannamei* reared in an intensive outdoor system. Shrimp of 1.98 ± 0.13 g were stocked at 70 animals/m^2 and reared for 72 days in 50 tanks of 1 m^3 under flow-through (14.4% a day) and static (1.4%–2.9% a day) green-water conditions at 32.0 ± 3.7 g/L salinity. Five diets with a minimum inclusion of fishmeal supplemented with a dipeptide, DL-methionyl-DL-methionine, were formulated to contain increasing levels of Met, 4.8, 6.2, 7.2, 8.1 or 9.4 g/kg (on a dry matter basis). Each of the five diets were fed four times daily to five replicate groups. Dietary Met and water exchange significantly influenced shrimp survival, gained yield, apparent feed intake, food conversion ratio and final body weight ($p < .05$). Raising shrimp under limited water exchange, i.e., static versus flow-through spared the dependence on higher levels of dietary Met to maximize shrimp body weight, from 9.4 g/kg to 8.0 g/kg (14.0 and 12.6 g/kg Met+Cys respectively). In an intensive rearing system, a reduction in water exchange is desirable as it leads to a lower need for supplemental dietary Met.

KEYWORDS
growth performance, *Litopenaeus vannamei*, methionine, water exchange

1 | INTRODUCTION

Amino acid requirements of marine shrimp have been determined under flow-through clear-water rearing systems (Lin et al., 2015; Millamena & Bautista-Teruel, 1996; Millamena, Bautista-Teruel, & Kanazawa, 1996; Teshima, Alam, Koshio, Ishikawa, & Kanazawa, 2002) using purified or semi-purified diets (Millamena et al., 1996; Teshima et al., 2002). These experimental procedures are adopted to avoid negative effects of metabolites, to provide maximum control over the nutrient composition of experimental diets and to allow comparisons with results already published (NRC, 2011). However, these conditions do not account for specific practical farming situations where natural food or other pond production variables, e.g., water quality, feed management, stocking density, have the potential to interfere with shrimp nutrition and their growth performance.

Very few studies have attempted to quantify the effects of these variables on shrimp amino acid nutrition (Façanha, Oliveira-Neto, Figueiredo-Silva, & Nunes, 2016; Liu et al., 2014). We have previously shown the response of *Litopenaeus vannamei* to dietary Met+Cys (methionine + cysteine) content closely relates to shrimp stocking density and natural food availability. In systems where natural food is scarcer or stocking densities are higher, an increase in dietary Met+Cys in a total basis, seems to be required to sustain maximum growth of juvenile shrimp. Shrimp growth rate was shown to be significantly affected by the interaction between dietary Met (Met+Cys) content and stocking density, being highest (1.49 g/week) in shrimp fed diets containing 7.2 g of Met per kg of feed (11.9 g/kg Met+Cys, on a dry matter basis, DM) reared at 50 animals/m^2. Yet, response of shrimp to dietary Met+Cys was less clear above 50 animals/m^2, namely 75 and 100 animals/m^2, and related with reduced weight gain and possibly feed restriction and crowding (Façanha et al., 2016).

In intensive pond culture, water is exchanged to reduce nitrogen load and toxic metabolites, derived from uneaten feed, dead algae and shrimp feces (Verdegem, 2013). In recent years, there has been a growing trend to reduce pond water discharge to a minimum, for increased biosecurity (Tal et al., 2009), more stable water quality conditions (Schneider, Sereti, Eding, & Verreth, 2005) and decreased risk of escapees (Zohar et al., 2005). As water exchange is reduced, primary pond productivity increases (Lorenzen, Struve, & Cowan, 1997; Martinez-Cordova, Villarreal-Colmenares, & Porchas-Cornejo, 1995), which in turn, can serve as a food source and positively contribute to the growth performance of marine shrimp (Kaweekietyota, Taparhudee, Limsuwan, & Chuchird, 2007; Kent, Browdy, & Leffler, 2011; Verdegem, 2013). Our study was conducted to evaluate if reducing water exchange could spare dietary Met (Met+Cys) required for maximum growth performance of juvenile *L. vannamei* reared in an intensive outdoor culture system under 32.0 ± 3.7 g/L salinity.

2 | MATERIALS AND METHODS

2.1 | Experimental design

The study was carried out with juveniles of the white shrimp, *L. vannamei*, reared in an outdoor green-water system (described by Façanha et al., 2016). The work consisted in the evaluation of five diets formulated to contain graded levels of dietary methionine (Met), from 5 to 9 g/kg (10–14 g/kg Met+Cys, respectively; in a dry matter basis, DM). Diets were evaluated under two water exchange regimes, flow-through (FL) and static (ST) water. Five round tanks of 1 m^3 were assigned for each treatment, totalling 50 tanks. Shrimp were stocked at 70 animals/m^2 (72 shrimp per tank) and reared for 72 days.

2.2 | Diets

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 50, 20, 5 and 32 g/kg of the diet (as is basis) respectively. Soybean meal was the major protein component in the formulas included at levels varying from 345 to 351 g/kg. Use of wheat flour reached a mean of 367 g/kg. Soybean oil was kept to a minimum included only to homogenize dietary lipid levels.

TABLE 1 Ingredient composition of diets

	Diets[a]/Composition (g/kg of the diet, as is)				
Ingredients	4.8	6.2	7.2	8.1	9.4
Soybean meal[b]	351	350	348	347	345
Wheat flour[c]	366	367	367	367	368
Salmon by-product meal[d]	50	50	50	50	50
Soy protein concentrate[e]	41	41	41	41	41
Wheat bran	45	45	45	45	45
Krill meal[f]	5	5	5	5	5
Sardine hydrolasate[g]	20	20	20	20	20
Salmon oil	32	32	32	32	32
Soybean oil	—	—	0.1	0.1	0.1
Soybean lecithin	31	31	31	31	31
Dicalcium phosphate[h]	20	20	20	20	20
Mineral-vitamin premix[i]	10	10	10	10	10
L-Lysine[j]	14	14	14	14	14
DL-Met-Met[k]	—	1.1	2.1	3.1	4.1
L-Threonine[l]	4.7	4.8	4.8	4.8	4.9
L-Arginine[m]	3.2	3.2	3.3	3.4	3.4
Synthetic binder[n]	5.0	5.0	5.0	5.0	5.0
Cholesterol[o]	0.8	0.8	0.8	0.8	0.8
Ascorbic acid[p]	0.4	0.4	0.4	0.4	0.4

[a]Dietary methionine (g/kg, dry matter basis).
[b]Bunge Alimentos S.A. (Luiz Eduardo Magalhães, Brazil). 525.8 g/kg crude protein (CP, on a total dry matter basis), 31.0 g/kg lysine (Lys), 6.9 g/kg methionine (Met), 14.6 g/kg methionine + cysteine (Met+Cys), 20.5 g/kg threonine (Thr), 37.6 g/kg arginine (Arg).
[c]133.7 g/kg CP, 2.8 g/kg Lys, 2.1 g/kg Met, 5.2 g/kg Met+Cys, 3.5 g/kg Thr, 5.3 g/kg Arg.
[d]Pesquera Pacific Star S.A. (Puerto Montt, Chile). 701.4 g/kg CP, 45.8 g/kg Lys, 17.3 g/kg Met, 22.9 g/kg Met+Cys, 27.1 g/kg Thr, 41.8 g/kg Arg.
[e]Sementes Selecta S.A. (Goiânia, Brazil). 672.9 g/kg CP, 41.7 g/kg Lys, 9.3 g/kg Met, 18.8 g/kg Met+Cys, 27.1 g/kg Thr, 50.2 g/kg Arg.
[f]Qrill™ meal, AkerBiomarine ASA (Oslo, Norway). 618.2 g/kg CP, 42.7 g/kg Lys, 18.2 g/kg Met, 23.3 g/kg Met+Cys, 27.5 g/kg Thr, 36.8 g/kg Arg.
[g]P50 295, SPF do Brasil Ltda. (Descalvado, Brazil).
[h]Serrana Foscálcio20, Bunge Fertilizantes S.A. (Cubatão, Brazil). 205.0 g/kg calcium, 202.0 g/kg total phosphorous.
[i]Rovimix® Camarões Intensivo, DSM Produtos Nutricionais Brasil Ltda. (São Paulo, Brazil). See Sá, Sabry-Neto, Cordeiro-Júnior & Nunes (2013) for composition.
[j]Aquavi® Lys, Evonik Nutrition & Care GmbH (Hanau, Germany). 507.0 g/kg lysine.
[k]Aquavi® Met-Met, DL-methionyl-DL-methionine, Evonik Nutrition & Care GmbH (Hanau, Germany). 950.0 g/kg methionine,
[l]ThreAMINO®, Evonik Nutrition & Care GmbH (Hanau, Germany). 985.0 g/kg threonine,
[m]Sigma-Aldrich Co. (St. Louis, USA), 985.0 g/kg arginine.
[n]Nutri-Bind Aqua Veg Dry, Nutri-Ad International NV (Dendermonde, Belgium).
[o]Cholesterol SF, Dishman Netherlands B.V. (Veenendaal, Netherlands). 910.0 g/kg cholesterol.
[p]Rovimix® Stay C® 35, DSM Produtos Nutricionais Brasil Ltda. (São Paulo, Brazil). 350.0 g/kg phosphorylated vitamin C.

In order to achieve graded levels of dietary Met, a basal diet was first designed to contain a minimum level of Met originating only from intact sources. From this diet, four nearly similar diets were supplemented with a dipeptide, DL-methionyl-DL-methionine (AQUAVI® Met-Met, Evonik Nutrition & Care GmbH, Hanau, Germany). All diets were formulated on an ideal protein concept basis using lysine (Lys) as the first limiting and reference amino acid to maximize protein utilization (NRC, 2011). Therefore, diets were supplemented with L-Lysine (Biolys®, Evonik Nutrition & Care GmbH), L-Threonine (ThreAMINO®, Evonik Nutrition & Care GmbH) and L-Arginine (Sigma-Aldrich do Brasil Ltda., São Paulo, Brazil). The following Lys: EAA (essential amino acid) ratios were adopted (NRC, 2011): 100 Lys:67 Thr (threonine), and 100 Lys:95 Arg (arginine). Diets were manufactured with laboratory equipment as described by Nunes, Sá, and Sabry-Neto (2011).

Diets were analysed for dry matter (drying in a convection oven for 24 hr at 105°C), and crude protein (Kjeldahl method of nitrogen estimation) following standard methods (AOAC, 2002). Determination of dietary amino acid concentration followed procedures described by Figueiredo-Silva, Lemme, Sangsue, and Kiriratnikom (2015). Diets contained a mean crude protein content of 361 ± 5.3 g/kg. Total dietary Met reached 4.8, 6.2, 7.2, 8.1, and 9.4 g/kg (DM basis) and subsequently Met+Cys at 9.6, 10.9, 11.9, 12.8 and 14.0 g/kg respectively (Table 2). Dietary cystine originated only from intact sources, but levels were kept relatively constant throughout all diets. Dietary variation (CV, coefficient of variation) of all other amino acids were kept at less than 3%.

TABLE 2 Dry matter, crude protein and amino acid composition of diets

Nutrient composition	Diets[a]/Composition (g/kg of the diet, dry matter basis) 4.8	6.2	7.2	8.1	9.4
Dry matter	894.9	907.4	906.0	909.7	915.8
Crude protein	360.2	367.5	362.7	352.9	359.1
Essential amino acids (EAA)					
Arginine	23.8	24.5	24.4	23.6	23.9
Histidine	7.4	7.6	7.6	7.3	7.3
Isoleucine	13.7	14.3	14	13.4	13.6
Leucine	23.6	24.5	24.1	23.1	23.4
Lysine	23.7	24.6	24.4	23.7	24.1
Methionine	4.8	6.2	7.2	8.1	9.4
Met+Cys[b]	9.6	10.9	11.9	12.8	14.0
Phenylalanine	15.9	16.3	16.1	15.5	15.7
Threonine	16.4	17.1	16.8	16.3	16.6
Tryptophan	3.7	3.9	3.8	3.7	3.7
Valine	15.0	15.6	15.3	14.7	15.0
Non-essential amino acids (NEAA)					
Alanine	14.6	15.1	14.9	14.4	14.5
Cystine	4.8	4.7	4.6	4.5	4.6
Glycine	16.2	16.8	16.6	16.2	16.4
Serine	15.4	16	15.7	15.1	15.3
Proline	19.2	19.5	19.3	18.9	19.1
Aspartate	31.6	32.7	32.2	30.7	31.3
Glutamine	62.2	64.1	63.1	60.9	61.8

[a]Dietary methionine (g/kg, dry matter basis).
[b]TSAA, total sulphur amino acids.

2.3 | Shrimp and rearing conditions

Post-larvae (PL) of *L. vannamei* were obtained from a commercial hatchery (Aquatec Aquacultura Ltda., Canguaretama, Brazil) and nursery-reared in the lab from PL10 (post-larvae) to juvenile size. Shrimp of 1.98 ± 0.13 g (mean ± *SD*; *n* = 3,600, CV = 6.8%) were individually weighed for culling. Shrimp were stocked in outdoor tanks of 1 m^3 with a bottom surface area of 1.02 m^2 (0.74 m height).

The rearing system, water preparation and feed management were the same as adopted by Nunes et al. (2011) and Façanha et al. (2016). Initially, rearing tanks were filled with sand-filtered seawater at 35.0 ± 0.1 g/L salinity and 7.95 ± 0.01 pH. Green-water conditions as described by Façanha et al. (2016) was achieved regardless of the water exchange regime. No artificial fertilizers were applied. The static (ST) system operated by exchanging water at a daily rate of 1.4 to a maximum of 2.9% of total tank volume. Water exchange in the flow-through (FL) system was carried out at a rate of 100 mL/s (14.4% a day). Rearing tanks were provided with continuous aeration to reach near saturation of dissolved oxygen. Temperature and pH values were monitored using a tester (pHmeter Hanna Instruments, model HI 98128). Water salinity was measured using an optical refractometer. Equipment was calibrated once a week. The pH meter was calibrated by first placing the electrode in a pH 7 solution, followed by a pH 2 buffer solution. The salinity refractometer was calibrated using a 35 g/L refractive index standard solution. Water quality parameters were monitored daily in each tank at 01:00 pm by sampling 10 cm below water surface. Water temperature, salinity and pH remained relatively stable during culture, at 30.1 ± 1.7°C (*n* = 2,600), 32.0 ± 3.7 g/L (*n* = 2,600), and 8.15 ± 0.22 (*n* = 2,600) respectively.

Shrimp were fed daily under a regular feed allowance using feeding trays. Feeding rates were based on the formula $MM = 0.0931BW^{0.6200}$, where MM is the maximum amount of feed that can be eaten daily by one individual per body weight (BW; Nunes & Parsons, 2000). This equation has been successfully applied to estimate daily feeding rates of *L. vannamei* (Lemos & Nunes, 2008; Nunes, Sá, Carvalho, & Sabry-Neto, 2006; Silva-Neto, Nunes, Sabry-Neto, & Sá, 2012). In the first 14 days of rearing, meals were adjusted on a daily basis following an estimated weight gain of 100 mg day^{-1} $shrimp^{-1}$ and a 0.5% weekly drop in shrimp survival across all diets. Biweekly (days 15, 30, 45 and 60 of rearing), rations were corrected by weighing individually 10 animals/tank. Until the next weight check, feed ration was adjusted according to calculated daily weight shrimp gains for each tank maintaining a 0.5% weekly drop in survival. Feeding times were the same as adopted by Façanha et al. (2016).

2.4 | Growth performance and statistical analyses

Determination of shrimp growth performance and feed efficiency followed the methodology described by Nunes et al. (2011) and Façanha et al. (2016). Two-way analysis of variance (ANOVA) was applied to determine the interaction between graded levels of dietary Met and water exchange regimes (FL versus ST). Two-by-two comparisons with the Tukey's HSD test was used when statistical differences were observed. Linear and quadratic regression was used to analyse the correlation between final shrimp body weight and dietary Met plus cysteine levels (Met+Cys) under the FL and ST systems respectively. All statistical analyses were performed using SPSS 15.0 (Illinois, USA). The significant level of 5% was set in all statistical analyses.

3 | RESULTS

Water exchange regime significantly influenced shrimp final survival, final body weight, gained yield, apparent feed intake (AFI), and food conversion ratio (FCR) (Table 3, $p < .05$, ANOVA). These variables were also statistically affected by dietary Met content, except final survival and weekly growth rate. Overall, shrimp survival was high with a mean (±standard deviation) of 95.4 ± 5.5 and 86.4 ± 8.4% for the flow-through (FL) and static (ST) water systems respectively ($p < .05$). Rearing shrimp under the FL system resulted in a higher gain in yield compared with the ST system, but only when shrimp were fed with 7.2 g/kg dietary Met ($p < .05$). In the FL system, shrimp yield increased significantly with higher levels of dietary Met, up to 6.2 g/kg ($p < .05$). Under the ST system, no significant effect could be observed in gained yield when dietary Met was increased from 4.8 to 9.4 g/kg ($p < .05$).

In the FL system, AFI significantly increased as dietary Met was raised from 4.8 to 8.1 g/kg ($p < .05$). Comparatively, shrimp reared under the FL system consumed less feed (16.8 ± 0.12 g of feed shrimp^{-1}) than those under the ST system (17.1 ± 0.36 g of feed shrimp^{-1}), particularly when fed 6.2 g/kg Met ($p < .05$). With 7.2 g/kg dietary Met, there was a statistically lower FCR in the FL (1.73 ± 0.01) compared with the ST system (1.94 ± 0.04). There was a significant reduction in FCR when dietary Met was raised from 4.8 to 9.4 g/kg in the FL system ($p < .05$). In the ST system, no effect on FCR could be observed as a result of graded levels of dietary Met ($p > 0.05$).

Final shrimp body weight (BW, g) was in excess of 11.5 g at harvest. Dietary Met content and water exchange regime had a combined effect over final shrimp BW ($p < .05$, Two-Way ANOVA). In the FL system, there was a Met dose–response effect on final shrimp BW. It increased gradually when shrimp were fed from 4.8 to 6.2 g/kg Met, and then raised significantly with 8.1 g/kg dietary Met ($p < .05$). There was a significant interaction between dietary Met level and water exchange regime for shrimp BW ($p < .05$, Figure 1). BW under the FL system responded linearly to increasing doses of dietary Met ($y = 10.6916 + 1.8297x$, $R^2 = .876$; Figure 1) and

TABLE 3 Growth performance and feed utilization of juvenile *Litopenaeus vannamei* fed graded levels of dietary methionine (Met) under a flow-through and static water system for 72 days. Data represent mean (±*SD*) of five tanks. Lowercase letters indicate differences between dietary Met within the same water exchange regime ($p < .05$). Capital letters refer to statistical differences between water exchange regimes within the same level of dietary Met ($p < .05$)

Performance	Dietary met (g/kg)	Water exchange regime: Flow-through (FL)	Static water (ST)
Survival (%)	4.8	94.4 ± 2.6A	85.6 ± 4.0A
	6.2	93.1 ± 4.4A	85.3 ± 3.9A
	7.2	97.8 ± 1.1A	84.7 ± 4.0B
	8.1	96.7 ± 0.8A	89.6 ± 4.6A
	9.4	95.0 ± 2.0A	87.5 ± 4.2A
Final body weight (g)	4.8	11.48 ± 0.93aA	11.98 ± 0.76aB
	6.2	12.03 ± 1.42bA	12.58 ± 0.60bB
	7.2	11.92 ± 0.36bA	12.81 ± 1.29bA
	8.1	12.14 ± 0.30bcA	12.82 ± 1.41bB
	9.4	12.42 ± 0.73cA	12.65 ± 1.21bA
Weekly growth (g)	4.8	1.06 ± 0.05	1.12 ± 0.04
	6.2	1.13 ± 0.07	1.18 ± 0.03
	7.2	1.11 ± 0.02	1.21 ± 0.06
	8.1	1.13 ± 0.01	1.20 ± 0.08
	9.4	1.16 ± 0.04	1.20 ± 0.06
Gained yield (g/m^2)	4.8	626 ± 10aA	583 ± 23A
	6.2	651 ± 15bA	618 ± 24A
	7.2	684 ± 7bA	626 ± 12B
	8.1	688 ± 12bA	664 ± 11A
	9.4	693 ± 6bA	642 ± 25A
Apparent feed intake (AFI) (g of feed per shrimp)	4.8	16.4 ± 0.24aA	16.8 ± 0.20A
	6.2	16.7 ± 0.06abA	17.1 ± 0.11B
	7.2	16.8 ± 0.19abA	17.1 ± 0.15A
	8.1	17.2 ± 0.11bA	17.3 ± 0.15A
	9.4	16.8 ± 0.11abA	16.9 ± 0.18A
Food Conversion Ratio (FCR)	4.8	1.85 ± 0.03aA	2.05 ± 0.09A
	6.2	1.82 ± 0.05abA	1.96 ± 0.07A
	7.2	1.73 ± 0.01abA	1.94 ± 0.04B
	8.1	1.76 ± 0.03abA	1.84 ± 0.03A
	9.4	1.71 ± 0.01bA	1.87 ± 0.08A
Two-Way ANOVA	Met	Water Regime	Met x Water Regime
Survival	ns[a]	< 0.0001	ns
Final body weight	< 0.0001	< 0.0001	0.001
Weekly growth	ns	ns	ns
Gained yield	0.001	< 0.0001	ns
AFI	0.011	0.005	ns
FCR	0.022	< 0.0001	ns

[a]ns, not statistically significant ($p > 0.05$).

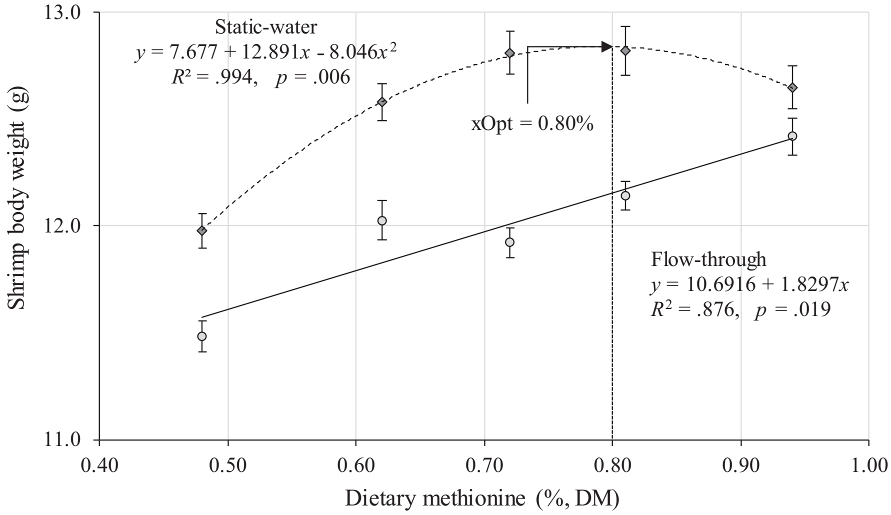

FIGURE 1 Regression analysis between dietary methionine content (g/kg, dry matter) and shrimp final weight (g) in a flow-through (FL) and static (ST) water system

Met+Cys ($y = 9.759 + 1.891x$, $R^2 = .868$). The maximum shrimp BW was reached with a dietary Met and Met+Cys level of 9.4 and 14.0 g/kg (on a dry matter basis) respectively. Comparatively, under the ST system, final BW increased from 4.8 to 6.2 g/kg dietary Met and then remained consistent along higher doses. Under ST system, maximum BW in response to dietary Met and Met+Cys were reached at 8.0 g/kg ($y = 7.677 + 12.891x - 8.046x^2$, $R^2 = .994$; Figure 1) and 12.6 g/kg ($y = -2.595 + 24.573x - 9.778x^2$, $R^2 = .999$) of the diet (as a dry matter basis) respectively.

4 | DISCUSSION

Growth performance of *L. vannamei* was significantly affected by water exchange regime and dietary Met content, possibly as a result of natural food availability and water quality. The best growth performance and feed utilization in the flow-through (FL) and static (ST) water exchange regimes were observed when shrimp were fed diets containing a total Met content of 9.4 g/kg (14.0 g/kg Met+Cys) and 8.1 g/kg Met (12.8 g/kg Met+Cys) respectively. Regression analysis estimated that maximum shrimp growth in these water regimes was achieved with 9.4 g/kg (14.0 g/kg Met+Cys) and 8.0 g/kg Met (12.6 g/kg Met+Cys). On a dietary crude protein (CP) basis, this is equivalent to 26.1 g/kg and 22.2 g/kg of the diet for the FL and ST regimes respectively. The dietary Met and Met+Cys levels expressed on CP basis allow comparisons between different studies. It was calculated as (amino acid optimal level/total dietary CP level) × 1,000. Met values for maximum shrimp growth observed for the FL regime are within the range of other work carried out under high water exchange conditions, such as 26.0 g/kg of dietary CP for Kuruma shrimp (*Marsupenaeus japonicus*; Teshima et al., 2002), and 24.0 g/kg of dietary CP for black tiger shrimp (*Penaeus monodon*; Millamena et al., 1996). On the other hand, results obtained under the ST regime correspond to reports carried out under limited water exchange conditions, such as 19.8–22.9 g/kg of the dietary CP for *L. vannamei* (Façanha et al., 2016). Therefore, observed variability in Met levels between different studies were not only due to differences among species, animal size, diets, but also on water exchange regime. As reported by the NRC (2011), there are clear differences in amino acid requirements of the major commercially-farmed penaeid shrimp, i.e., *L. vannamei*, *P. monodon*, and *M. japonicus*. For example, the latter two species have higher requirements for lysine compared with the white shrimp (19 and 21 versus 16 g/kg of the diet, DM basis). Similarly, within the same shrimp species, amino acid requirements are higher in earlier stages of development, as shown by Lin et al. (2015) in *L. vannamei* of 0.55 ± 0.01, 4.18 ± 0.05 and 9.77 ± 0.08 g. According to authors, maximum growth performance was achieved with 9.1, 6.7 and 6.6 g/kg dietary Met (on a DM basis) respectively.

Based on previous studies, Met requirement of *L. vannamei* tends to be lower in green-water than in controlled clear-water conditions. This can be explained by the availability of natural food that may act as a source of essential nutrients, including methionine. According to Façanha et al. (2016), natural food in a green-water system contained 247 g/kg CP, 77.8–96.0 g/kg of total essential amino acid content (ΣEAA), 2.7 ± 0.2 g/kg of Met and 6.2 ± 0.3 g/kg of Met+Cys content (on a DM basis). Therefore, the presence of natural food in green-water may contribute to meeting Met requirements of farmed penaeid shrimp.

The static (ST) system may have led to a higher phytoplankton biomass in rearing tanks and a greater amount of accumulated organic material on the tank bottom due to a limited water exchange. In fact, this seems to be corroborated by the higher final shrimp body weight in the ST system (12.57 ± 0.34 g) compared with the FL system (12.00 ± 0.34 g). Kaweekietyota et al. (2007) analysed the number of plankton (cells/cc) under two water exchange regimes: low (10% once a week) and high (10% twice a week). Authors reported that under low water exchange, the number of plankton cells are 44% higher than under high water exchange. Thus, water exchange regime appears to contribute to different levels of natural food availability in water. If that is the case, it may have influenced the lower amount of dietary Met required for maximum growth in ST system (8.0 g/kg Met and 12.6 g/kg Met+Cys). Tacon et al. (2002) found that shrimp reared in the presence of natural food grew 3.4 times faster than those deprived of natural food items. Porchas-Cornejo et al. (2011) concluded that enhancing the growth of naturally grown biota in semi-intensive shrimp ponds significantly improves shrimp yields. These results and those obtained from various studies suggest that shrimp has the ability to filter, ingest and digest natural food present under green-water culture systems (Anand et al., 2014; Gamboa-Delgado, 2014; Kent et al., 2011; Roy, Davis, & Whitis, 2012) reducing their dependency on Met provided by artificial food.

It is not clear, however, if the higher final shrimp body weight in the ST system compared with the FL was driven by a greater availability of natural food or by a lower final stocked shrimp biomass. Under a lower stocked biomass, shrimp tend to grow faster and achieve larger body weights (Araneda, Pérez, & Gasca-Leyva, 2008; Sookying, Da Silva, Davis, & Hanson, 2011; Wyban, Lee, Sato, Sweeney, & Richards, 1987). The possibility that a higher shrimp biomass, as observed in the FL system, has resulted in feed restriction and ultimately limited growth can also not be ruled out, deserving future consideration.

The FL system promoted a higher shrimp final survival (95.4%) compared with the ST system (86.4%). This may have been the driving factor behind the differences seen in gained yield, AFI and FCR detected between shrimp raised under the two water exchange regimes. Although the FL system did not represent a clear-water condition, it operated under continuous water exchange. Water in this system had a higher transparency compared with the ST system. This suggests that the FL system provided a less stressful culture environment to stocked shrimp. Taking into account that accumulation of organic matter in the tank bottom tends to increase under limited water exchange, the ST regime may be prone to greater water quality deterioration. Although our work was not designed to investigate the effects of water quality on shrimp performance, it is well-known that the accumulation of nitrogen compounds has a pronounced effect on the success of crops (Hargreaves, 1998). Moderate or high levels of ammonium and nitrate have the potential to directly affect shrimp metabolism, deteriorating its growth performance and damaging important tissues (Fregoso-López et al., 2017; Romano & Zeng, 2013).

Our findings are corroborated by Roy et al. (2012) who reported that shrimp reared in a flow-through system had better survival and FCR than those in a static system. Nunes et al. (2011) compared the growth performance of *L. vannamei* fed krill-meal supplemented diets containing 9.6 g/kg of dietary Met over 10 weeks in a clear- versus green-water culture system. Authors observed that clear-water tanks (continual water recirculation overnight) under indoor conditions promoted a more challenging culture environment to *L. vannamei*. Shrimp displayed a significantly slower growth (1.00 ± 0.06 versus 1.04 ± 0.09 g/week) and lower final survival (81.4 ± 7.3 versus 91.4 ± 5.4%) in clear compared with green water.

In the present study, supplementation of DL-methionyl-DL-methionine (DL-Met-Met) at any level in diets with 50 g/kg salmon by-product meal and about 348.0 g/kg of soybean meal, containing a total Met content of 4.8 g/kg led to no improvement in performance when shrimp was raised under a ST water exchange regime. However, in this system, there was a positive boosting effect in shrimp final weight when DL-Met-Met was supplemented at 1.1 g/kg (as is basis), which resulted in a diet with 6.2 g/kg dietary Met (DM basis). Above this level, DL-Met-Met supplementation did not prove necessary in the ST water regime. On the other hand, in the FL system where natural food was possibly less available, supplementation of DL-Met-Met at 1.1 g/kg was effective in promoting a greater gain in shrimp yield, feed intake, a reduced FCR and a higher final body weight. A further improvement was detected in shrimp final body weight when DL-Met-Met was supplemented at 3.1 g/kg, resulting in a diet with 8.1 g/kg of total Met. These results demonstrate the ability of juvenile whiteleg shrimp to utilize crystalline dipeptide forms of methionine. Similar ability was observed in *L. vannamei* fed with diets supplemented with other forms of crystalline amino acids (Lin et al., 2015; Xie et al., 2012; Zhou, Wang, Wang, & Tan, 2013; Zhou et al., 2012).

5 | CONCLUSIONS

Results from this work carried out under intensive outdoor conditions indicate an interaction between dietary Met content and water exchange regime. Raising shrimp under limited, i.e., static, versus flow-through water exchange regime spared the dependence on higher levels of dietary Met to maximize whiteleg shrimp growth, from 9.4 g/kg to 8.0 g/kg (14.0 and 12.6 g/kg Met+Cys respectively). In an intensive rearing system stocked under 70 shrimp/m^2, a reduction in water exchange is desirable as it leads to a lower need for supplemental dietary Met.

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