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Research Article

Beta-Agonist, Ractopamine Hydrochloride, Improves Growth, Alters Body Composition, and Suppresses Gonadal Maturation in All-Female Giant Freshwater Prawn, *Macrobrachium rosenbergii*

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All-female giant freshwater prawn *Macrobrachium rosenbergii* has a significant growth limitation due to its early sexual maturity. A 60-day trial was carried out to determine the effect of beta-agonist, ractopamine hydrochloride (RAC), on the growth performance, body composition, and gonadal maturation of all-female prawns. Prawn juveniles (4.39 ± 0.40 g initial weight) were stocked at 10 prawns/m^2 in 15 plastic tanks (1000 liters each) in a completely randomized design with four treatments and a control (0, 5, 10, 15, and 20 mg/kg) in triplicate. The significantly ($p < 0.05$) highest values of final weight, weight gain, daily weight gain, and specific growth rate (SGR) were observed in the female prawns fed with the diet containing 10 mg/kg RAC, while the lowest values were found in the control tank. Similarly, survival rate, feed conversion rate (FCR), and condition factors were observed among all treatments, which were significantly better than the control (0 mg/kg). Significantly greater yield and lower gonadosomatic index (GSI) values were obtained in prawns fed with a 10 mg/kg RAC-containing diet than in all other treatments, except for the diet containing 15 mg/kg RAC, while the lowest yield was produced by the control tank. A diet containing 10 mg/kg RAC had significantly improved the whole-body protein content in prawns, while diets containing 10 and 15 mg/kg RAC exhibited lower lipid content in prawns than in the other treatments. A significant increase in the number of Stage 1 (virgin) females (up to 54% at 10 mg/kg RAC diet) was found in all RAC treatments, while the control had a significantly higher number of females at various maturity stages, indicating that RAC administration effectively suppresses gonadal maturity. Residue analysis of whole prawn samples at the end of the trial indicated no trace of ractopamine. The quadratic regression analysis of weight gain, SGR, FCR, and yield revealed that the optimum dietary ractopamine level for all-female prawn culture should be between 10 and 15 mg/kg.

1. Introduction

Giant freshwater prawn (GFP) *Macrobrachium rosenbergii* is one of the largest farmed Palaemonid prawns with considerable aquaculture importance. However, despite its high nutritional and economic value, size heterogeneity and early sexual maturity, particularly in a mixed-sex farmed cohort, are two major obstacles in farming this prawn in ponds or tanks. All-male and all-female prawn farming has become a practical alternative to mixed-sex farming to overcome some of these challenges. Previous studies on all-female prawn farming have highlighted its benefits in growing

uniform-sized prawns with a high survival rate [1]. However, early maturation continues to be a significant barrier in all-female GFP cultures. When female prawns attain a weight of 18-26 g, they are considered as mature as they begin preparing for reproduction [2]. The gonadal maturation process of prawns significantly reduces the growth rate due to the reallocation of energy resources towards gonad development [3]. If allowed to proceed, maturation soon reduces the flesh quality due to the loss of a significant portion of lipids, carotenoids, and protein.

Measures to retard gonadal maturation and direct energy towards growth have received considerable attention to overcome the irregular growth patterns in prawns, particularly in the monosex populations [4]. Ractopamine is a phenylethylamine-derived adrenergic agonist with composition and pharmacological properties comparable to endogenous catecholamines: adrenaline and noradrenaline [5]. Ractopamine improves growth in farmed animals by decreasing lipid content [6, 7]. It is an artificial substance that can shift nutrients away from adipose tissue and stimulate the deposition of skeletal muscle ([8]; Almeida, [9]). Growth and feed efficiency improve as energy efficiency increases by nutrient redirection [10]. Ractopamine acts in adipose tissue to reduce fat accumulation by enhancing lipolysis and decreasing lipogenesis [8, 11]. It may have an anabolic impact on protein metabolism, causing muscle fiber growth and frequent changes in muscle fiber type [12]. Ractopamine has been tested in a few species of fish, such as channel catfish [13, 14], rainbow trout [15–19], pacu [20, 21], Hungarian carp [22], tilapia [5], and calbasu [23], with both positive and contradictory results. The objective of this study was thus to evaluate the effect of ractopamine as a dietary additive on the growth performance and gonadal maturity of all-female prawns.

2. Materials and Methods

The experiment was set up in an indoor prawn rearing unit at the Aquaculture Field Laboratory, Asian Institute of Technology (AIT), consisting of 15 polyethylene tanks, each with 1 m^2 bottom area holding 1000 liters of freshwater with continuous aeration for 60 days from November to December 2020. Plastic nets were provided in each tank as shelters for the prawns to reduce cannibalism.

2.1. Experimental Animal. The juveniles of all-female prawn were collected from a commercial prawn nursery in Samut Prakan Province, Thailand. The juveniles were transported to the experimental area in oxygenated polythene bags. Before stocking, the prawns were properly acclimated in freshwater with aeration at the AIT's hatchery unit in a concrete tank ($8 \times 3 \times 1$ m) for seven days and fed with a commercial prawn feed (brand-CP, 35% protein and 4% lipid), which was later used as the control feed (containing no ractopamine). Feeding was provided three times (6:00, 12:00, and 18:00 h) daily, and 50% of tank water was exchanged daily. Before starting the experiment, the prawns were kept without feeding for 24 h.

2.2. Experimental Feed Preparation. Experimental feed was prepared by mixing ractopamine hydrochloride (RAC) (Sigma Aldrich, code-90274-24) with the commercial prawn feed (brand-CP, 35% protein and 4% lipid). Five levels of RAC were used, namely, 0, 5, 10, 15, and 20 mg/kg. The RAC in powder form was mixed with ethanol (95%), sprayed over the feed, and dried at room temperature [24, 25]. After drying, the pellets were packed in black plastic bags, sealed, and stored at 4°C until use.

2.3. Experimental Procedure. Five treatments with three replications were used to stock the prawns (initial weight 4.39 ± 0.40 g and length 5.80 ± 0.45 cm) distributed into different treatment groups at a stocking density of 10 prawns/m^2, in a completely randomized design (CRD). Prawns were fed three times daily at 4-6% of their total body weight. The daily allocated feed was divided into three rations: about 30% was offered in the morning (7:00 h), 30% in the afternoon (13:00 h), and 40% provided in the evening (7:00 h). Tanks were siphoned daily to remove uneaten feed and fecal matter without disturbing the animal. Twenty percent of water in each tank was changed every two days to maintain optimum water quality parameters such as temperature, pH, and dissolved oxygen (DO) measured daily, while ammonia, nitrite, alkalinity, and hardness were measured weekly. At the end of the experiment, prawns were harvested, and length-weight data were recorded individually and used for subsequent analysis.

2.4. Growth Index Analysis. The initial weight was calculated for the whole prawn stock (150 prawns) by randomly weighing 60 prawns. Before readjusting the feeding rate, at least 50% of prawns were randomly sampled biweekly using a scoop net to assess their general health condition and growth (length and weight). Prawns were handled carefully during the sampling to avoid stress. After the termination of the experiment, all the surviving prawns from each tank were harvested for measurements of different growth indices and gonadal maturation, as well as biochemical analysis. Growth parameters were calculated as follows:

$$\begin{aligned}
\text{Survival rate (\%)} &= \frac{\text{number of live prawns}}{\text{number of prawns stocked}} \times 100,\\
\text{Weight gain (g)} &= \text{Avg.final body weight} - \text{Avg.initial weight},\\
\text{Daily weight gain (DWG)}\left(\frac{\text{g}}{\text{day}}\right) &= \frac{\text{final weight} - \text{initial weight}}{\text{culture duration}},\\
\text{Specific growth rate (SGR) in } \frac{\%}{\text{day}} &= \frac{\ln(\text{final weight}) - \ln(\text{initial weght})}{\text{culture duration}} \times 100,\\
\text{Feed conversion ratio (FCR)} &= \frac{\text{total amount of feed given (g)}}{\text{weight gain (g)}}.
\end{aligned} \tag{1}$$

The condition factor was calculated using the following equation:

$$\text{Condition factor} = \frac{(\text{weight})}{(\text{length})^3} \times 100. \tag{2}$$

2.5. Evaluation of Prawn Gonadal Maturity. The prawns'

gonads were examined every two weeks to determine their maturation stages. Identification of different maturation stages of females was made according to Chang and Shih [26]. The gonadosomatic index of prawns was also calculated using the following equation:

$$\text{Gonadosomatic index (GSI)} = \frac{(\text{gonad weight})}{(\text{body weight})} \times 100. \quad (3)$$

2.6. Analysis of Proximate Composition. The whole-body proximate composition of all-female prawns was analyzed, following the AOAC protocols [27]. At the end of the experiment, four prawn samples were collected randomly from each tank to analyze the percentage of moisture, ash, protein, and lipid. The micro-Kjeldahl method was used to estimate crude protein content ($N = 6.25$) using the FOSS Kjeltec 8100 instrument (FOSS Analytical AB, Hoganas, Sweden). The Soxhlet method was used to determine crude lipid utilizing petroleum ether (boiling point 40–60°C) as a solvent with a FOSS Soxtec 2043 apparatus (FOSS Scino Co. Ltd, Suzhou, China). The total moisture percentage in the prawn body was determined using the air oven method in a Memmert UF 110 oven (Memmert GmbH+ Co., Schwabach FRG, Germany) at 105°C and ash content using a Lab Tech LEF-115S-1 muffle furnace (Daihan Labtech Co. Ltd, Namyangju, Korea) at 550°C for 24 h.

2.7. Residue Analysis. The presence of ractopamine residues in the prawn body was analyzed using LC/MS, following Williams et al. [28]. After complete harvesting, the prawn samples from different replications of each treatment were collected to prepare a composite sample. A composite sample of each treatment was divided into three for residue analysis. The values obtained from each composite sample analysis were combined to make a single representative and expressed as mean ± SD (standard deviation).

2.8. Statistical Analysis. All collected and processed data were analyzed using SPSS version 23.0. One-way analysis of variance (ANOVA) was used to compare the means of all growth parameters. The differences among the means were assessed by Tukey's HSD test at a $p < 0.05$ level of significance. The optimum RAC level was determined via quadratic regression equation as follows:

$$Y_i = aX_i^2 + bX_i + \varepsilon_i, \quad (4)$$

where Y_i represents the growth parameter for tank i, X_i is the dosage of RAC (mg/kg), and ε_i is the random error term. Using the obtained coefficients, the optimum dosage was estimated on a continuous scale as follows [29]:

$$X^* = -\frac{b}{2a}. \quad (5)$$

The Kruskal-Wallis H test was performed to examine the different gonadal stages of prawns in different treatments, for which Dunn's post hoc test was performed to calculate a statistic based on the differences in rank pairs to determine which treatments gave the difference.

3. Results

Prawns were maintained within optimum water quality parameters across all the treatments, which included the dissolved oxygen level of 6.34-8.55 mg/l, temperature ranges of 27.34–31.49°C, pH values of 6.97–8.41, total ammonia levels of 0.00–1.24 mg/l, nitrite levels of 0.00–1.35, alkalinity levels of 117.34–152.61 mg/l, and hardness levels of 157.54-193.81 mg/l.

3.1. Growth Indices. The data on growth performance and gonadal development of GFP in each dietary treatment over 60 days are presented in Table 1. Dietary incorporation of 10 mg/kg RAC resulted in a significantly higher ($p < 0.05$) final body weight (35.41 g) than all other treatments, while the control had the lowest final body weight. The final length of the prawn showed a similar trend. Weight gain, SGR, and daily weight gain were also significantly higher ($p < 0.05$) when prawns were fed with 10 mg/kg RAC. The survival rate of prawns in different treatments showed no significant difference, while a diet with 10 (93.3%) and 15 mg/kg (90.0%) RAC gave significantly greater survival rates ($p < 0.05$) than the control. FCRs in all RAC treatments were significantly lower ($p < 0.05$) than in the control. The FCR was the lowest in the prawns fed with 15 mg/kg RAC (1.43), which was not significantly different from other treatments, while the highest FCR was found in the control (2.13).

The diet with 10 mg/kg RAC showed the highest yield (289.56 g/m^2). This was not significantly different from the yield with 15 mg/kg RAC, but different from the two other treatments and the control diet, which had the lowest yield (97.65 g/m^2) (Figure 1).

The quadratic regression results indicate that, as RAC dosage increased, the values of final weight, weight gain, SGR, survival rate, and yield ($r^2 = 0.82$) had also increased up to a certain level and then decreased. The trend was the opposite for FCR, as minimization is the purpose of FCR. With the obtained coefficients, the optimum dietary RAC dosage for maximum weight gain, SGR, yield, and minimum FCR was estimated to be 12.47, 12.72, 12.00, and 11.78, respectively. The predicted reduction in growth rate and total yield in prawns fed with diets containing 15 and 20 mg/kg of RAC indicates that the overdose of RAC would not be advantageous (Figure 2), which was confirmed for all growth indices measured.

Fulton's condition factor (K) for all-female prawns in the current study ranged from 0.94 to 1.06. K values for the 10 mg/kg RAC diet were the maximum (1.06) and showed no significant difference from the other treatments, except for the control (0.94).

Proximate analysis of all-female prawns indicated a significantly higher percentage of protein content when fed with a diet containing 10 and 15 mg/kg RAC for 60 days. The crude protein of the prawn body was the highest when fed with 10 mg/kg RAC and the lowest with no RAC in the diet. In addition, feeding at 10 mg/kg RAC revealed

Table 1: Growth performance parameters and gonadosomatic index (GSI) of all-female prawn fed with dietary RAC for 60 days ($n = 3$).

Parameter	Ractopamine levels (mg/kg)					Regression analysis		
	0	5	10	15	20	Coeff. a (p value)	Coeff. b (p value)	R^2
Initial length (cm)	5.80 ± 0.45	5.80 ± 0.45	5.80 ± 0.45	5.80 ± 0.45	5.80 ± 0.45			
Final length (cm)	$12.50 \pm 0.26_{d}$	13.60 ± 0.27^{c}	14.96 ± 0.42^{a}	14.70 ± 0.30^{ab}	14.16 ± 0.15^{bc}			
Initial weight (gm)	4.39 ± 0.40	4.39 ± 0.40	4.39 ± 0.40	4.39 ± 0.40	4.39 ± 0.40			
Final weight (g)	$18.34 \pm 1.20_{d}$	24.97 ± 0.63^{c}	35.41 ± 1.58^{a}	30.60 ± 1.55^{b}	27.65 ± 0.68^{bc}	-0.0983 (0.0xx)	2.451 (0.0xx)	0.65
Weight gain (g)	$13.95 \pm 1.20_{d}$	20.58 ± 63^{c}	31.02 ± 1.58^{a}	26.21 ± 1.55^{b}	23.26 ± 0.68^{c}	-0.0983 (0.0xx)	2.451 (0.0xx)	0.65
DWG (g/day)	0.23 ± 0.02^{d}	0.34 ± 0.01^{b}	0.52 ± 0.03^{a}	0.44 ± 0.03^{b}	0.39 ± 0.01^{bc}	-0.0016 (0.0xx)	0.041 (0.0xx)	0.65
SGR (% bw/day)	2.38 ± 0.11^{d}	2.89 ± 0.04^{b}	3.48 ± 0.07^{a}	3.23 ± 0.09^{b}	3.07 ± 0.04^{bc}	-0.0063 (0.0xx)	0.161 (0.0xx)	0.69
Survival rate (%)	70.0 ± 0.00^{b}	$83.3.8 \pm 5.77_{ab}$	93.3 ± 5.77^{a}	90.0 ± 10.00^{a}	80.0 ± 10.00^{ab}	-0.1714 (0.0xx)	3.962 (0.0xx)	0.64
FCR	2.13 ± 0.20^{b}	1.46 ± 0.03^{a}	1.43 ± 0.09^{a}	1.66 ± 0.19^{a}	1.60 ± 0.15^{a}	0.0043 (0.0xx)	0.102 (0.0xx)	0.58
Yield (g/m^2)	$97.65 \pm 8.38_{d}$	$171.69 \pm 16.45_{c}$	$289.41 \pm 20.62_{a}$	$236.56 \pm 36.01_{ab}$	$185.64 \pm 17.92_{bc}$	-1.2014 (0.0xx)	28.845 (0.0xx)	0.82
Condition factor	0.94 ± 0.08^{b}	0.99 ± 0.06^{ab}	1.06 ± 0.07^{a}	0.97 ± 0.09^{ab}	0.97 ± 0.09^{ab}			
GSI	3.47 ± 1.42^{c}	2.95 ± 1.30^{bc}	1.22 ± 0.64^{a}	1.58 ± 0.53^{ab}	3.05 ± 0.72^{bc}			

Each numerical value is mean ± SD; $n = 3$. The mean values of the different alphabetical superscripts within the same row indicate statistically significant differences at $p < 0.05$ (one-way ANOVA followed by post hoc Tukey test).

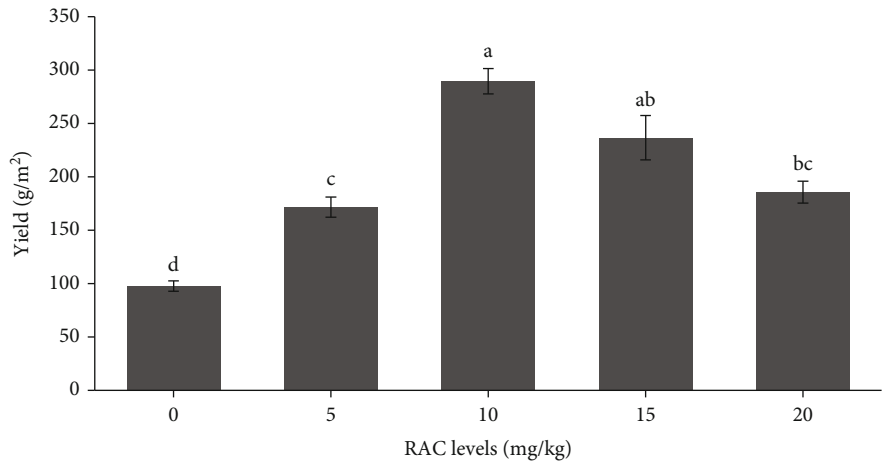
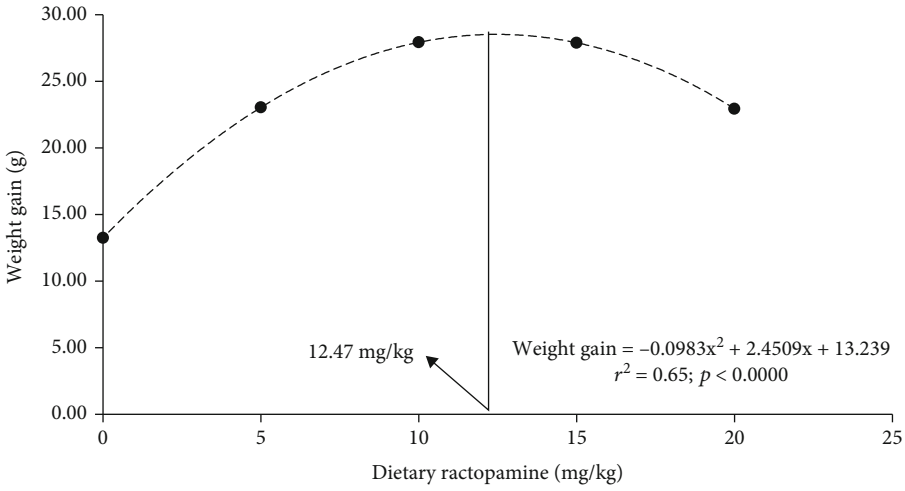


Figure 1: Yield of all-female prawns at different RAC levels ($n = 15$).

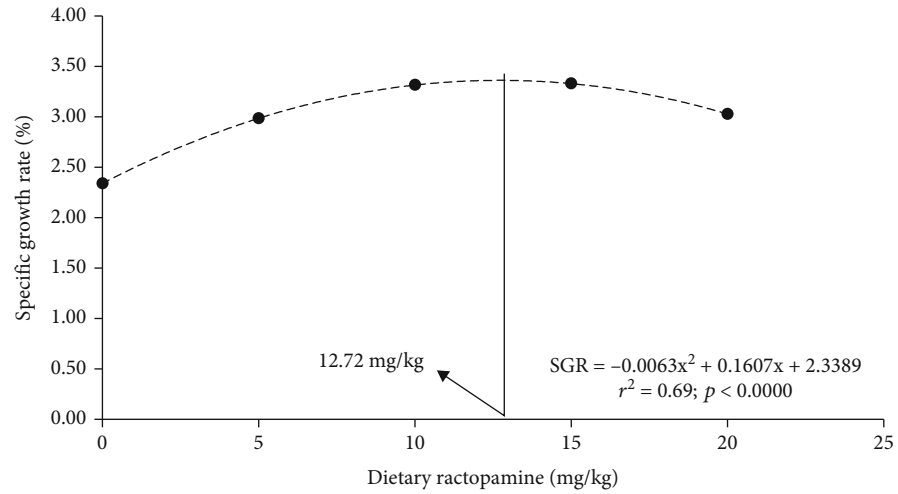
significantly lower lipid content in the prawn body over all other treatments, except the diet containing 15 mg/kg RAC. Lipid contents of 5 and 20 mg/kg RAC diet-fed prawns were similar (Table 2).

3.2. Evaluation of Gonadal Maturity. The one-way ANOVA revealed that the diet with 10 mg/kg RAC showed a minimum GSI value (1.22), which was significantly lower ($p < 0.05$) than all other treatments except the diet containing 15 mg/kg RAC. On the other hand, the 15 mg/kg RAC diet had no significant difference from the diet containing 5 and 20 mg/kg RAC but was significantly higher ($p < 0.05$) than the control.

Gonadal maturation stages of all-female prawns at different levels of RAC are shown in Figure 3. The gonadal maturation stages indicated that 19% of the prawns in the control (0 mg/kg RAC) diet had an absence of apparent ovarian tissue (Stage 1). In contrast, 81% of them had presumably passed through successive gonadal developmental stages. In the case of the diet containing 5 mg/kg RAC, a higher proportion of prawns (76%) had undergone various gonadal maturation phases, while the rest (24%) were in Stage 1. Most of the prawns remained in Stage 1 (53.6%), while 46.7% had passed through different ovarian developmental stages when fed a 10 mg/kg RAC diet. In another treatment, when prawns were fed with a 15 mg/kg RAC diet,



(a)



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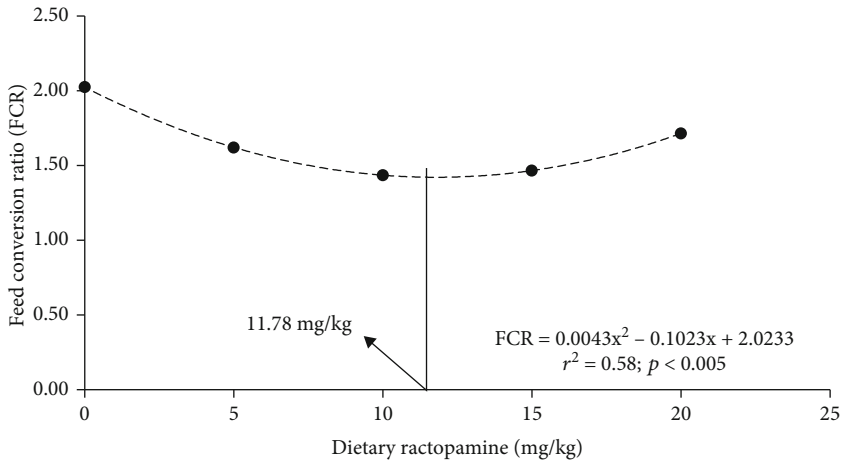

(c)

Figure 2: Continued.

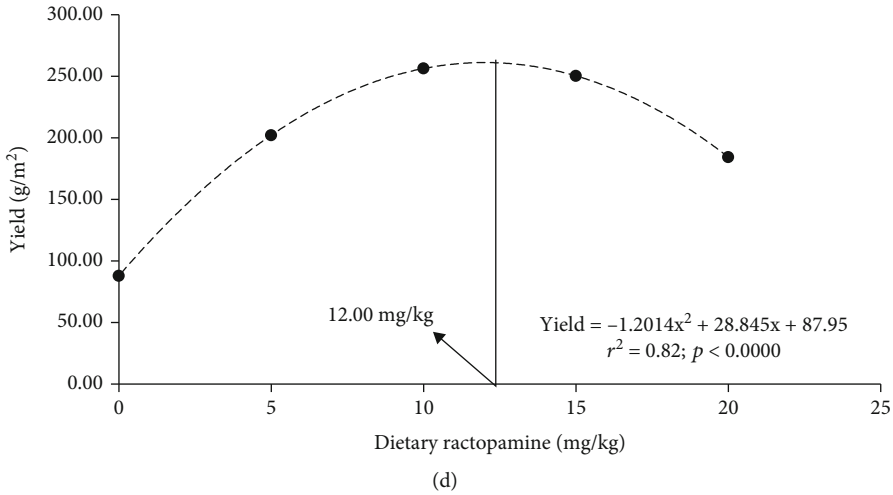

Figure 2: Second-degree polynomial model fitting of (a) weight gain, (b) SGR, (c) FCR, and (d) yield to dietary RAC levels in female prawns in three replicates ($n = 75$).

Table 2: Whole body composition of all-female prawns fed different levels of RAC ($n = 3$).

Treatments	Proximate composition			
	Moisture	Protein	Lipid	Ash
0 (control)	78.73 ± 1.57^{a}	55.83 ± 1.77^{c}	6.59 ± 0.51^{c}	16.98 ± 1.51^{a}
5	76.56 ± 2.42^{a}	62.97 ± 0.89^{b}	5.56 ± 0.45^{bc}	16.41 ± 0.94^{a}
10	75.34 ± 3.00^{a}	66.57 ± 0.76^{a}	3.98 ± 0.13^{a}	17.65 ± 1.02^{a}
15	77.54 ± 2.51^{a}	62.43 ± 1.28^{b}	4.67 ± 0.50^{ab}	15.87 ± 0.82^{a}
20	76.42 ± 2.83^{a}	61.27 ± 1.04^{b}	6.70 ± 0.46^{c}	17.43 ± 1.29^{a}

All values mean ± SD. The means with different alphabetical superscripts within the same row are significantly different at $p < 0.05$ (one-way ANOVA followed by the post hoc Tukey test).

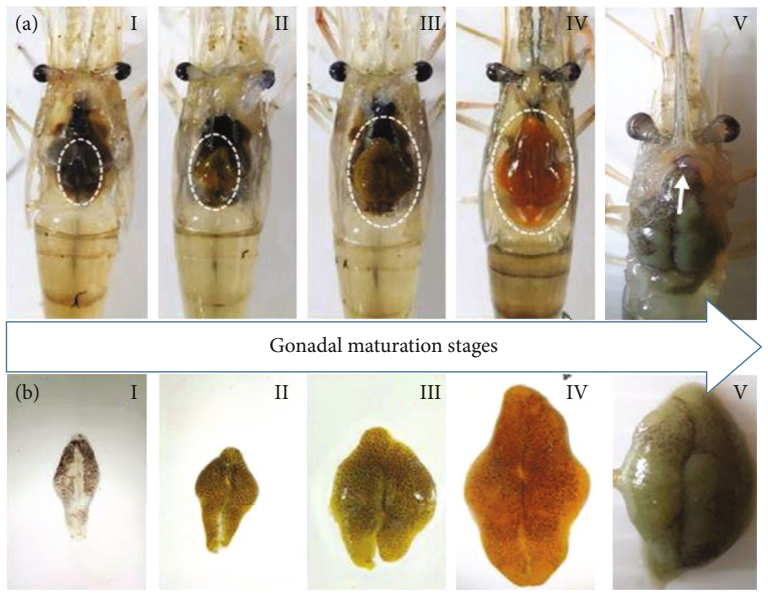

Figure 3: Gonadal maturation stages of all-female prawns at different dietary RAC levels.

48.1% of them were in Stage 1, while 52.9% of the female prawns were gravid or in different ovarian developmental stages. In the treatment with 20 mg/kg RAC diet, the majority of the prawns (70.8%) had undergone various gonadal maturation stages, whereas 29.2% had no ovarian tissue in their gonads (Stage 1) (Figure 4).

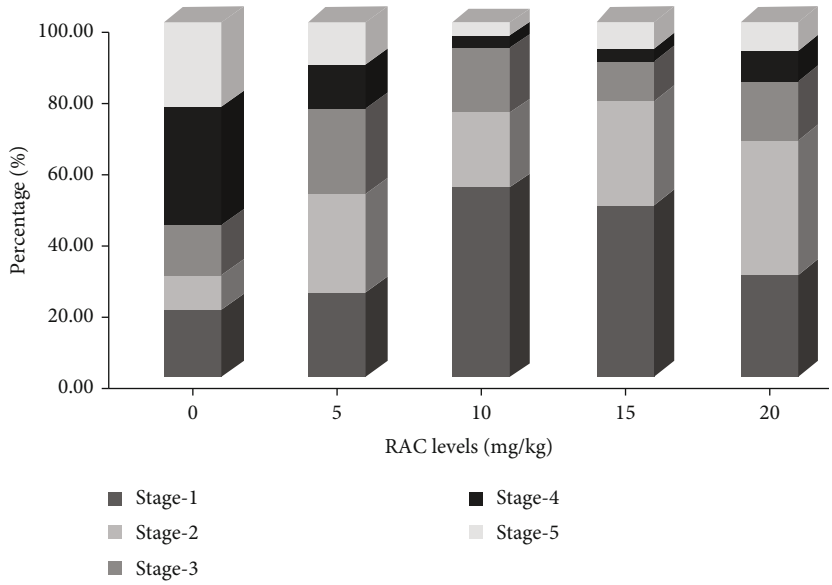

Figure 4: Gonadal maturation as a proportion of various ovarian stages in all-female prawn fed different levels of dietary RAC ($n = 125$).

Gonadal maturation stages of all-female prawns in five treatments were tested by the Kruskal-Wallis H test, which revealed significant differences ($p = 0.001$) among the different doses of RAC when the different stages of gonadal maturation were compared. Dunn's post hoc test revealed that the 10 mg/kg RAC diet showed a significant difference from the control (0 mg/kg RAC) but not from the other treatments. Similarly, the 15 mg/kg RAC-containing diet did not significantly differ from the other treatments apart from the control (0 mg/kg RAC).

3.3. Residue Level. The residue of RAC was not detectable in the prawn body at any of the treatments when they were fed with different doses of RAC-containing feed.

4. Discussion

In the current study, all-female prawn juveniles responded positively to the ractopamine-containing diet up to a certain threshold. Mustin and Lovell [14] observed positive effects of ractopamine on younger channel catfish, while Bicudo et al. [20] found similar results in pacu (*Piaractus mesopotamicus*) fingerlings. Vandenberg et al. [10] and Singh et al. [23] observed that rainbow trout juveniles and calbasu also exhibited similar trends. The present study revealed that feeding all-female prawns with a diet containing the beta-agonist RAC resulted in a substantial growth increase at a dosage of 10 mg/kg of feed. An increase in RAC level from 0 to 10 mg/kg increased prawn growth considerably, while the growth decreased at higher levels of RAC, indicating that excess dosage of RAC in diets does not generate beneficial effects. Webster et al. [30] reported that the beta-adrenergic agonist family of chemicals acts as repartitioning agents in intermediate metabolism, controlling muscle protein breakdown and promoting muscle accretion through hypertrophy. In the current study, improvement was observed in some of the growth performance indices, viz., weight gain, SGR, survival rate, and FCR, when all-female prawns were fed with a RAC-containing diet, whereas the control (0 mg/kg RAC diet) showed inferior growth performance. The condition factor K of all-female prawns fed with RAC-treated diet also improved compared to the untreated control groups.

Ractopamine has been shown to increase feed efficiency by increasing growth rate and lowering feed intake [6, 31, 32] or by enhancing utilization efficiency by increasing muscle mass and directing feed energy into lean growth rather than fat deposition [7]. The present study concurred with the findings of Mustin and Lovell [13] that channel catfish fed with feed containing 20 mg/kg ractopamine gained greater weight than fish fed with a diet without ractopamine. In another study, when channel catfish were fed with ractopamine, they exhibited a significant response to weight gain [14], while Vandenberg et al. [10] observed an increase in rainbow trout feed efficiency.

Furthermore, 1 g/kg L-carnitine and 10 mg/kg ractopamine helped rainbow trout and calbasu increase their growth performance [16, 23]. Satpathy et al. [31] found that salbutamol, a beta-adrenergic agonist, enhanced growth effectively and contributed to efficient nutrient utilization at a 3 mg/kg dietary incorporation level, indicating its potential for use in formulated diets for the Indian major carp, rohu, under culture conditions. Williams et al. [7] reported that dietary inclusion of ractopamine resulted in a weight gain of up to 15% in other animals.

At higher doses, ractopamine was not found to improve fish growth. The addition of ractopamine up to 40 mg/kg in the diet did not influence growth performance and body composition, though it positively affected the hematological and biochemical parameters of juvenile pacu [20]. Similarly, ractopamine did not influence any growth parameters measured in Hungarian carp (*Cyprinus carpio*) [22] and tilapia (*Oreochromis niloticus*) towards the end of their grow-out phase (750-920 g market size) [5].

Dietary ractopamine supplementation was found to be beneficial in lowering body lipid and raising body protein in all-female prawns in our study, as it has been previously reported for blue catfish *I. furcatus* [30] and channel catfish *I. punctatus* [13] fed with the beta-adrenergic agonist compounds L644, 969, and ractopamine, respectively. Drumond et al. [33] reported the reduction of lipid deposition between muscle fibers in pacu. Similarly, the addition of 8 mg/kg ractopamine to the feed altered the abdominal muscle chemical composition of Nile tilapia, where the treated fish had a 23.3% lower lipid content than the control fish [5]. They also reported that a ractopamine-rich diet had no impact on the lipid content of other fish tissues such fillets, liver, and viscera, as well as crude protein and ash. In the fillets, protein content increased with a higher dose of 45 mg/kg ractopamine, while a lower dose of 11.25 mg/kg ractopamine lowered ether extract levels. Supplementing with ractopamine and L-carnitine enhances muscle protein accretion and nitrogen retention through increased synthesis, while preventing protein degradation and accelerating fatty acid oxidation [34, 35]. This suggests that beta-adrenergic agonists influence the prawn's body composition by shifting nutrients from lipid reserves to muscle production.

The present study demonstrated that RAC-fed diets significantly retarded the maturation of all-female prawns, as evidenced by the significantly higher proportion of virgin (Stage 1) females (53.6%) compared to those that had passed through various ovarian stages (46.7%) when prawns were fed 10 mg/kg RAC. In comparison, the control group had the lowest proportion of virgin females (19%), while up to 81 percent had progressed through various maturity stages, showing unabated gonadal development when the prawns were fed the control diet even in the absence of males in the tanks. Although the proportion of prawns in their non-reproducing stage was greatest on the 10 mg/kg RAC diet, when higher dosages of RAC (15 and 20 mg/kg) were administered, the percentage of Stage 1 females decreased, with a simultaneous increase in the proportion of prawns at advanced stages of gonadal maturity. This was consistent with the trend of increasing growth in response to RAC feeding initially, which tended to decline after a certain threshold limit was reached following a 10 mg/kg RAC diet. Additionally, the whole-body lipid content of the tested prawns followed a similar pattern, with the lowest level being found in prawns fed 10 mg/kg RAC and increasing in 15 and 20 mg/kg RAC treatments. This study demonstrates conclusively that RAC inhibits gonadal maturation in all-female prawns. However, additional research is needed to characterize the specific interaction of RAC when fed to a mixed-sex prawn population in which males and females are grown together as in a traditional prawn culture system.

Data on the effect of beta-agonists on the reproductive performance of animals, particularly crustaceans and fishes, are scarce. Gonadal development is critical in all-female prawn farming because gonadal maturation consumes the majority of energy. According to Cavalli et al. [36], somatic growth at maturation is hindered because the major energy allocation focuses on the reproductive phase. During the different stages of gonadal maturation of female prawns, the role of lipid deposition is critical and essential for reproduction and larval development because, in crustacean metabolism, lipids are known to perform several vital roles [36–38]. Adeola et al. [39] and Williams et al. [7] found beta-adrenergic agonists to cause a spike in protein deposition due to reduced lipogenesis and increased lipolysis in adipose tissue, which led to protein synthesis in muscles. According to Reeds and Mersmann [40], the lower rate of fat deposition might be due to a shift in amino acid catabolism to ATP synthesis and an increase in total energy expenditure. The increased protein retention efficiency and lower lipid retention efficiency evidenced in this study might indicate similar effects of feeding ractopamine that impacted the different gonadal maturation stages of all-female prawns due to reduced levels of lipid deposition. Gonadal development of female prawns was delayed when fed with 5 and 10 mg/kg RAC containing diet, while maturation accelerated with a further increase in ractopamine dose in the feed.

Although ractopamine as a feed additive has been authorized by the US Food and Drug Administration and the Codex Alimentarius, its residues have been a topic of public concern, given the possibility of harmful effects on human health if drug-treated animals are fed to humans. The drug's maximum residue limits (MRLs) have previously been established. More than 20 nations, including the United States, Canada, and Brazil, have approved ractopamine as a feed additive for animal production [41]. Although there is little research on ractopamine residues in aquatic species, there are a few publications on ractopamine residues in livestock. In the present study, ractopamine residue was not detected in the prawn body after 60 days of exposure to feeding with 0, 5, 10, 15, and 20 mg ractopamine-containing diet. According to Almeida et al. (2012), ractopamine could be found in the urine of sheep and cattle after short-term exposure. Qiang et al. [41] examined ractopamine residues in pig tissues following a 28-day dose of 20 ppm dietary ractopamine, finding that residues in muscles and fat were not detectable 24 hours after the final agonist exposure.

The results from the present study indicate that ractopamine incorporation up to certain thresholds is beneficial in improving growth rate while suppressing gonadal maturation in all-female prawns, which is a significant outcome in farming these prawns.

5. Conclusion

In the present research, all-female giant prawns fed with diets containing 10–15 mg/kg ractopamine (RAC) resulted in positive growth indices such as final weight (30.60–35.41 g), SGR (3.23–3.48 percent/day), and enhanced protein content, while decreasing fat deposition in the body. When prawns were fed a RAC-containing diet, over half of the females remained in a non-reproducing stage (virgins), but most of those on a RAC-free diet matured normally, suggesting that RAC in the feed can impede reproduction and gonadal maturity in all-female prawns. The optimal dietary RAC dose for maximum weight increase, SGR, yield, and minimum FCR of all-female prawns was predicted to be 12.47, 12.72, 12.00, and 11.78 mg/kg, respectively, based

on the quadratic regression analysis. This implies that the optimal dietary RAC level in all-female prawns grown under culture conditions appears to be higher than 10 but lower than 15 mg/kg. Increased protein sparing properties of lipids may be associated with improved growth performance and protein retention. Research with a more extensive scope and dimension is recommended in light of the paucity of information on the use of hormonal and non-hormonal growth enhancers as feed additives in crustaceans.

Data Availability

The data used for the analyses in this study will be made available upon reasonable request to the corresponding author.

Ethical Approval

The experimental prawns were handled with care at every stage of this experiment. All aspects of the care and use of animals were performed in conformity with all applicable international, national, and institutional rules and regulations. All animal research methodologies employed in this study have adhered to the Asian Institute of Technology's ethical standards. Additional approval was obtained from the AIT's institutional ethics committee and research advisory board members.

Conflicts of Interest

The authors declare that there are no financial or personal relationships with other people or organizations that could improperly influence their work and that they have no professional or other personal interest in any product, service, or company that could be perceived as influencing the position presented in, or the review of, the manuscript entitled.

Authors' Contributions

Md. Moshiur Rahman planned and designed this study, conducted the experiment, analyzed data, and wrote the first draft. Krishna R. Salin was involved in planning and designing this study, helped with data analysis, edited the manuscript, and was responsible for the overall supervision of this research. Takuji W. Tsusaka helped in research design, manuscript editing, review, and data analysis. Amararatne Yakupitiyage contributed to experimental design, review, editing, and final correction.

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