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L-Tryptophan Mitigates Cannibalism and Improves Growth of Asian seabass, *Lates calcarifer* Reared in a RAS System

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Abstract: Severe cannibalism can result in a significant loss of productivity during the nursery phase of Asian seabass, *Lates calcarifer*. The present study aimed to determine the effect of dietary tryptophan on growth, feed utilization, cannibalism, survival, and muscle proximate composition of Asian seabass juveniles (initial size, 2.77 ± 0.04 cm in length and 0.29 ± 0.01 g in weight) in a recirculating aquaculture system (RAS) at different stocking densities. The tryptophan levels were set at 0.41% (control diet, standard dosage for normal growth and survival of Asian seabass), 1.00% (Diet 1), and 1.50% (Diet 2), while the stocking densities were set at 0.5 ind./L and 1.5 ind./L. The results indicated that dietary supplementation with L-tryptophan (TRP) and fish stocking density had a significant effect on fish growth parameters, feed utilization, cannibalism, survival, and muscle lipid content ($p < 0.05$) over the 45-day trial. The maximum length, weight, WG, and SGR were 11.64 ± 0.35 cm, 22.93 ± 2.67 g, 22.64 ± 2.67 g, and 9.63 ± 0.27%, respectively, in the fish fed Diet 2, and 11.35 ± 0.22 cm, 24.38 ± 1.28 g, 24.09 ± 1.28 g, and 9.82 ± 0.11% at a 1.5 ind./L stocking density. The lower FCR (0.81 ± 0.04) and higher PER (2.98 ± 0.16) ensured better utilization of Diet 1 than the other diets. Moreover, significant interaction effects between diet and stocking density were observed in total yield, cannibalism, and survival of the Asian seabass. Significantly higher survival rates of 76.11 ± 3.90% in the Diet 1 group and 76.28 ± 2.88% in 0.5 ind./L stocking density were obtained, which is promising. The study concludes that dietary supplementation with 1.00% TRP was effective in reducing cannibalism and increasing the survival of the Asian seabass nursery reared in RAS at a lower stocking density (i.e., 0.5 ind./L), whereas 1.50% supplemental TRP at a higher stocking density (i.e., 1.5 ind./L) significantly increased the cannibalism and growth, which in turn reduced the survival rate.

Keywords: L-tryptophan; cannibalism; mitigation; growth performance; survival

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1. Introduction

The Asian seabass, *Lates calcarifer* (Bloch, 1790), more widely known as barramundi, is euryhaline [1], protandry hermaphrodite [2], and a highly cannibalistic fish [1]. The species is considered an economically important food fish of tropical and subtropical origin [3] and is distributed in the Indo–Western Pacific region [4]. Asian seabass is well-accepted in aquaculture for its rapid growth rate, ability to tolerate varying environmental conditions, and high market price [2]. Hatchery production of seabass seed is well-established, and the farming of this species is gradually increasing in many Asian countries [5]. While the Asian seabass is considered the most promising species for possible intensive fish farming in Southeast Asia, it is also a highly carnivorous and opportunistic predator [6], posing significant obstacles to commercial hatchery and nursery management.

Cannibalism is an intra-specific predatory feeding strategy primarily affected by several interactive and non-interactive elements, such as environmental factors (environmental structure, temperature, and light intensity) and population factors (density and size dimorphism), respectively [7]. Food availability and quality are also significant in influencing the degree to which cannibalism occurs [8–11]. While it is impossible to eliminate intra-cohort cannibalism, routine size grading, and frequent ad libitum feeding are common strategies for mitigating cannibalism during the nursery culture of Asian seabass. However, grading is time-consuming and stressful to fish, leading to additional stock loss. Similarly, ad libitum feeding increases production costs and nitrogenous waste, deteriorating water quality in intensive fish culture.

In intensive fish culture, a high stocking density is fundamental for optimizing productivity. However, excessive stocking density in aquaculture can influence the physiology and behavior of fish [12]. Increased stocking density may cause irreversible stress associated with adverse social interactions [13,14]. This chronic stress will deplete brain serotonin levels in teleost fish and may exacerbate behavioral aggressiveness [15,16], the incidence of cannibalism [17,18], poor survival [19], and defective growth [20]. Fish aggression results in physical injuries and increased mortality.

Dietary intervention can be an alternative strategy for limiting cannibalistic behavior in animals [21,22]. Dietary L-tryptophan (TRP), an essential amino acid, is a precursor to synthesizing serotonin (5-hydroxytryptamine, 5-HT) in the brain and gut and melatonin in the pineal gland [23]. Brain serotonin is crucial for abating aggressiveness in fish [24,25] and aquatic invertebrates [26,27]. According to Coloso et al. [28], the dietary tryptophan requirement of juvenile Asian sea bass is 0.41% for normal growth and survival. Previous studies have suggested that acute stress from higher stocking density can increase fish aggressiveness by reducing brain serotonin from the required levels in teleost fish [15,16]. However, supplementation of tryptophan content in the feed can enhance brain serotonergic activity in fish, which boosts stress resistance against higher stocking densities [16,29,30] or alleviates aggression [16,24,25]. On the downside, enhanced serotonin levels through additional tryptophan intake tend to suppress food intake, depressing fish growth [24,31,32].

Growth is the most common criterion for determining ideal nutrient levels and is a reliable predictor of dietary adequacy [33]. However, there is a dearth of research on the effects of dietary TRP on the growth performance and cannibalistic behavior of Asian seabass at various stocking densities. The objective of this study was to examine the effects of dietary TRP on growth performance, feed utilization, cannibalism, survival, and muscle composition of juvenile Asian seabass reared at different stocking densities in a recirculating aquaculture system.

2. Materials and Methods

2.1. Experimental Fish

Asian seabass fry with an initial total length of 2.77 ± 0.04 cm and body weight of 0.29 ± 0.01 g were collected from a seabass farm in Chonburi, Thailand. The experiment was conducted for 45 days in a RAS at the Aquaculture and Aquatic Resource Management Hatchery complex, Asian Institute of Technology, Pathumthani, Thailand. The experimental fish were acclimatized in brackish water (10‰) for 48 h before stocking, following previously established procedures [34].

2.2. Experimental Design

Healthy Asian seabass fry were randomly distributed into six treatments with three replicates using a (2 × 3) factorial design with two stocking densities of fish and three levels of dietary TRP (Table 1) as follows: T1 at a stocking density of 0.5 ind./L + 0.41% tryptophan, T2 at a stocking density of 0.5 ind./L + 1.00% tryptophan, T3 at a stocking density of 0.5 ind./L + 1.50% tryptophan, T4 at a stocking density of 1.5 ind./L + 0.41% tryptophan, T5 at a stocking density of 1.5 ind./L + 1.00% tryptophan, and T6 at a stocking density of 1.5 ind./L + 1.50% tryptophan. Two hundred fish were stocked in each tank for the 0.5 ind./L stocking density and six hundred fish for the 1.5 ind./L stocking density.

Table 1. Experimental design for the different stocking densities and the doses of L-tryptophan (TRP).

Stocking Density	Doses of Dietary TRP (%)		
	Control (Diet)	Diet-1 (1.00%)	Diet-2 (1.50%)
0.5 ind./L	0.41 * + 0	0.41 + 0.59	0.41 + 1.09
1.5 ind./L	0.41 * + 0	0.41 + 0.59	0.41 + 1.09

Note: * 0.41 is the tryptophan content of the commercial feed used in the experiment, and + indicates the amount of crystalline TRP topped up. The percentage of dietary inclusion is shown in parenthesis.

2.3. Experimental Feeds

A commercial nursery feed (D-LIGHT, 1042, floating feed for seabass nursery, Thai Union Feed Mill, Thailand) containing 10% moisture, 42% crude protein, 3% crude fiber, and 10% crude lipid was used as the control diet, and as the basal feed for preparing the experimental diets labeled Diet 1 and Diet 2. The tryptophan level in the basal feed was assayed using sodium hydroxide hydrolyzation and an amino acid analyzer (HPLC, Model: Agilent 1100 Series, WI-19-028, Agilent Technologies, Hewlett-Packard-Strasse 8, 76337, Waldbronn, Germany) at the Central Laboratory, Bangkok, Thailand. The basal feed contained 0.41% tryptophan on a DM basis (0.58% of dietary protein). Two experimental diets supplemented with tryptophan (TRP) were prepared using the sprinkle method [21]. For this, TRP (TRP 99%, Thermo Fisher Scientific, Heysham, UK) was weighed, dissolved in hot water and ethanol (80%), then sprinkled onto the commercial diet at amounts of 100 and 150 mg TRP/100g (1.00% TRP; 0.84% dietary protein and 1.50% TRP; 1.55% dietary protein) for Diet 1 and Diet 2, respectively. The control diet was sprinkled with ethanol solution without tryptophan. The diets were dried at room temperature and stored at 4 °C in separate plastic bags until use.

2.4. Feeding Trial

Eighteen rectangular, white-colored plastic tanks (length: 1.08 m, width: 0.78 m, and height: 0.66 m), each tank holding 400 L of the working volume, were used for the RAS, with a flow rate maintained at 3 L/min [34]. The fish were fed with the experimental diets three times (07:00 h, 12:00 h, and 17:00 h) at 10% of their body weight for the first 15 days and then 8% for the remaining days of the experiment. The regular feeding was adjusted at 15-day intervals based on the feeding rate and total survival of the fish in each tank. Dead fish were recorded and removed daily. The rearing tanks were cleared of unconsumed food and fish excreta daily by siphoning, and the tank water was topped up from a prepared saline water storage tank. The water salinity was maintained at 10 ± 1‰ throughout the experiment and measured using a refractometer (ATAGO, Japan). The water temperature was recorded twice daily (8:00 h and 16:00 h) using a Celsius glass thermometer. Water pH and dissolved oxygen (DO) were measured once a day using a digital DO meter with a combined pH reading (DO II, Trading Tech Asia Co., Ltd., Tokyo, Japan). The ammonia–nitrogen (phenate method) and nitrite–nitrogen (colorimetric method) of the tank water were measured following the standard procedure for the examination of water and wastewater [35].

2.5. Estimation of Growth Parameters and Cannibalism

The growth parameters of the Asian seabass juveniles were calculated after the experimental period. The individual total body length and wet body weight were weighed from a random sample of approximately 25% of the stocked fish from each tank using a measuring scale and digital weighing balance (nearest 0.1 g, Ohaus Corporation, 7 Campus Dr # 310, Parsippany-Troy Hills, USA). Weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), and fish yield per tank were calculated following Khan et al. [36] and Khan et al. [34] as follows:

$$\text{WG (g)} = W_f - W_i$$

where W_f is the final body weight, and W_i is the initial body weight of the seabass.

$$\text{SGR (\%, per day)} = [\ln(W_f) - \ln(W_i)] \times 100/t$$

where t is the duration of the experiment in days.

$$\text{FCR} = [\text{feed applied (dry weight)}]/[\text{live fish weight gain}]$$

$$\text{PER} = \text{wet weight gain of fish}/\text{protein intake}$$

The fish yield was calculated as follows:

$$\text{Yield (g/tank/45 days)} = \text{final fish biomass at harvest (g)} - \text{initial fish biomass stocked (g)}$$

Cannibalism, mortality, and survival rate (SR) were measured following Baras et al. [37]:

$$\text{Cannibalism (\%)} = [(N_i - (N_f + N_d))/N_i] \times 100$$

where N_i and N_f are the initial and final numbers of fish, respectively, and N_d is the number of dead fish.

$$\text{Mortality (\%)} = (\text{number of truncated fish}/N_i) \times 100$$

where N_i is the initial number of fish.

$$\text{Survival rate (SR) (\%)} = 100 \times N_f/N_i$$

where N_i and N_f are the initial and final numbers of fish, respectively.

The coefficient of variation (CV) of total length and body weight used to represent the size heterogeneity was calculated as follows:

$$CV_{TL}\ (\%) = [SD(TL)/A(TL)] \times 100,$$

and

$$CV_{BW}\ (\%) = [SD(BW)/A(BW)] \times 100$$

where SD is the standard deviation, A is the average, TL is the total length of fish (cm), and BW is the wet body weight of fish (g).

2.6. Biochemical Analysis of Fish Samples

At the end of the experiment, 60 individual fish from each treatment (20 from each replicate) were randomly sampled and anesthetized with clove oil following Mylonas et al. [38]. Fresh flesh was collected from the fish using a disposable medical surgical stainless steel scalpel blade (Shanghai Even Medical Instruments Co., Ltd., Shanghai, China). The fish muscle was stored at −20 °C in separate plastic bags for biochemical analysis. The biochemical composition of the fish muscle was determined according to the standard method [39]. The moisture content of fish muscle was evaluated by drying it in an oven at 105 °C until it reached a constant weight. The ash content was determined by burning the samples in a muffle furnace at 550 °C for 4 h. Crude lipid was extracted using a Soxhlet extractor with petroleum ether [40,41]. After confirming the extraction was complete, petroleum ether evaporated, and the residue was dried to a constant weight at 105 °C. The crude protein was analyzed using the Kjeldahl method with a conversion factor 6.25 to convert total nitrogen into crude protein. The crude fiber was quantified using 2 g samples previously boiled in dilute H_2SO_4 (0.3 N). The mixture was filtered and washed with 200 mL of boiling water and NaOH (0.5 N). After washing with boiling distilled water and acetone, the residue was re-extracted and then dried at 105 °C to a constant weight. The material was heated at 550 °C for 3 h and then weighed.

2.7. Statistical Analysis

A two-way full factorial ANOVA was performed to analyze the collected data. Tukey's HSD test was used as a post hoc test when the overall differences were statistically significant, according to the ANOVA. All statistical analyses were performed using IBM SPSS Statistics 23.0. All descriptive statistics in the text and tables used the untransformed mean ± standard error (SE).

3. Results

No significant differences were observed in mean water temperature among the experimental diets and stocking densities (Table 2). On the other hand, DO, pH, ammonia–nitrogen, and nitrite–nitrogen differed significantly ($p < 0.001$) between the two stocking densities. The DO level was significantly lower in Diet-1 than in the other diets ($p < 0.01$).

Table 2. Water quality parameters at different dosages of L-tryptophan diets and two stocking densities during the nursery period of *Lates calcarifer* juveniles: full factorial ANOVA ($n = 18$).

Parameter	Mean ± SE					Interaction Effects (Two-Way Factorial *p*-Value)
	Experimental Diet			Stocking Density		
	Control	Diet-1	Diet-2	0.5 ind./L	1.5 ind./L	
Temperature (°C)	29.10 ± 0.00	29.10 ± 0.00	29.10 ± 0.00	29.10 ± 0.00	29.10 ± 0.00	0.300
DO (mg/L)	6.78 ± 0.06^{b}	6.69 ± 0.06^{a}	6.73 ± 0.05^{ab}	6.86 ± 0.02^{B}	6.61 ± 0.02^{A}	0.120
pH	7.55 ± 0.00	7.55 ± 0.00	7.55 ± 0.00	7.55 ± 0.00^{B}	7.54 ± 0.00^{A}	0.397
NH_3 (mg/L)	0.06 ± 0.00	0.06 ± 0.00	0.07 ± 0.00	0.06 ± 0.00^{A}	0.07 ± 0.00^{B}	0.178
NO_2 (mg/L)	0.55 ± 0.03	0.56 ± 0.03	0.53 ± 0.03	0.49 ± 0.01^{A}	0.60 ± 0.01^{B}	0.451

Note: Different superscripts (lower case for experimental diet and upper case for different stocking densities) within a row indicate significant differences in means based on Tukey's HSD test at a 5% significance level.

3.1. Growth Parameters

The growth parameters of the juvenile Asian seabass reared in the RAS significantly differed among the three dosages of TRP used in their diets and the two stocking densities ($p < 0.05$) (Table 3). Diet 2 resulted in a considerably greater final body length (TL_f), body weight (BW_f), WG, and SGR than the other experimental diets ($p < 0.01$). The same parameters had significantly higher values in 1.5 ind./L than in 0.5 ind./L stocking density ($p < 0.01$). Diet 1 had a significantly lower FCR and a significantly higher PER ($p < 0.05$), indicating a higher feed utilization efficiency than the other experimental diets. Reduced FCR and increased PER were also found at 1.5 ind./L stocking density compared to 0.5 ind/L ($p < 0.001$). Diet 1 produced significantly greater fish yields than the other diets, while the yields were significantly greater at 1.5 ind./L than at 0.5 ind./L stocking densities. Finally, there were significant interaction effects between the TRP dosage and stocking density on the fish yield ($p < 0.01$). The results indicated that the effect of Diet 1 and Diet 2 increased the yield by 1617 and 1236 g, respectively, while maintaining a constant stocking density. When the stocking density was raised from 0.5 to 1.5 ind./L, the yield increased by 6253 g, while the dosages of TRP remained unchanged. A significantly higher CV for weight was found in Diet 1 and the 1.5 ind./L stocking density ($p < 0.001$), and the CV for length was higher in the 1.5 ind./L than 0.5 ind./L stocking density.

Table 3. Growth performance, feed utilization, cannibalism, and survival of *Lates calcarifer* juveniles at different dosages of L-tryptophan diets and two stocking densities: full factorial ANOVA (n = 18).

Dependent Variable	Experimental Diet			Stocking Density		Interaction Effects (Two-Way Factorial p-Value)
	Control	Diet-1	Diet-2	0.5 ind./L	1.5 ind./L	
Final body length (TL_f, cm)	10.43 ± 0.16 ^{a}	10.68 ± 0.26 ^{a}	11.64 ± 0.35 ^{b}	10.48 ± 0.25 ^{A}	11.35 ± 0.22 ^{B}	0.708
Final body weight (BW_f, g)	16.61 ± 1.96 ^{a}	18.35 ± 2.67 ^{a}	22.93 ± 2.67 ^{b}	14.21 ± 1.10 ^{A}	24.38 ± 1.28 ^{B}	0.432
Weight gain (WG, g)	16.32 ± 1.96 ^{a}	18.06 ± 2.67 ^{a}	22.64 ± 2.67 ^{b}	13.92 ± 1.10 ^{A}	24.09 ± 1.28 ^{B}	0.432
Specific growth rate (SGR, % day^{-1})	8.91 ± 0.27 ^{a}	9.09 ± 0.34 ^{a}	9.63 ± 0.27 ^{b}	8.60 ± 0.26 ^{A}	9.82 ± 0.11 ^{B}	0.371
Feed conversion ratio (FCR)	0.94 ± 0.04 ^{b}	0.81 ± 0.04 ^{a}	0.87 ± 0.07 ^{ab}	0.96 ± 0.03 ^{B}	0.79 ± 0.03 ^{A}	0.163
Protein efficiency ratio (PER)	2.55 ± 0.10 ^{a}	2.98 ± 0.16 ^{b}	2.82 ± 0.20 ^{ab}	2.50 ± 0.07 ^{A}	3.07 ± 0.12 ^{B}	0.086
Yield (g)	4245 ± 1069 ^{a}	5862 ± 1715 ^{b}	5481 ± 1462 ^{b}	2069 ± 99 ^{A}	8322 ± 508 ^{B}	0.003
Cannibalism (%)	26.36 ± 5.18 ^{b}	17.03 ± 3.35 ^{a}	35.00 ± 2.34 ^{c}	18.28 ± 3.11 ^{A}	33.98 ± 2.56 ^{B}	0.001
Mortality (%)	7.17 ± 0.53	6.86 ± 0.76	5.83 ± 0.78	5.44 ± 0.50 ^{A}	7.80 ± 0.32 ^{B}	0.363
Survival rate (SR, %)	66.47 ± 5.52 ^{b}	76.11 ± 3.90 ^{c}	59.17 ± 3.01 ^{a}	76.28 ± 2.88 ^{B}	58.22 ± 2.49 ^{A}	0.006
CV for length (TL_f, %)	16.14 ± 3.84	21.51 ± 3.51	21.61 ± 1.93	14.14 ± 2.06 ^{A}	25.37 ± 1.53 ^{B}	0.397
CV for weight (BW_f, %)	46.88 ± 13.23 ^{a}	74.88 ± 7.44 ^{b}	67.04 ± 7.67 ^{b}	42.78 ± 6.74 ^{A}	82.69 ± 3.02 ^{B}	0.050

Note: Different superscripts (lower case for experimental diet and upper case for different stocking densities) within a row indicate significant differences in means based on Tukey's HSD test at a 5% significance level.

3.2. Cannibalism, Mortality, and Survival

The data on cannibalism, mortality, and survival rates of the seabass fed with different experimental diets and stocking densities are presented in Table 3. Significantly lower cannibalism was observed in fish fed with Diet 1 and at a stocking density of 0.5 ind./L ($p < 0.001$). Dietary composition and stocking density significantly interacted with fish cannibalism ($p < 0.01$). Specifically, the effect of Diet 1 resulted in reduced cannibalism by 9.33% points, and the effect of Diet 2 resulted in increased cannibalism by 8.64% points when stocking density was held constant. For Diet 2, increasing the stocking density from 0.5 to 1.5 ind./L increased cannibalism by 15.7% points, keeping the same dosages of TRP. In particular, increasing the level of TRP by 0.59% (Diet 1) would reduce cannibalism by 5 and 13.67% points, whereas increasing the TRP by 1.09% (Diet 2) would increase cannibalism by 15.34% and 1.94% points in the 0.5 ind./L and 1.5 ind./L stocking density, respectively (Figure 1).

There were no significant differences in fish mortality among the different diets ($p > 0.05$), and lower mortality was found at the 0.5 ind./L than at the 1.5 ind./L stocking density ($p < 0.01$) (Table 3). Additionally, diet and stocking density had no interaction effect on mortality. A significantly higher survival rate (76.11 ± 3.90%) was found in the fish fed with Diet 1 than in the fish fed the other diets, and in 0.5 ind./L (76.28 ± 2.88%) than 1.5 ind./L stocking density ($p < 0.001$). Significant interaction effects of the diets and stocking density were observed on survival rate ($p < 0.01$). The results showed that Diet 1 increased the survival rate by 9.64% points, and Diet 2 decreased survival by 7.3% when maintaining a constant stocking density. Increasing the stocking density from 0.5 to 1.5 ind./L resulted in an 18.06%-point decrease in the survival rate when maintaining the same dosages of TRP in the diets. For 0.5 and 1.5 ind./L stocking densities, Diet 1 enhanced survival by 5.83 and 13.44% points, respectively, while Diet-2 decreased survival by 13 and 1.61% points, respectively (Figure 2).

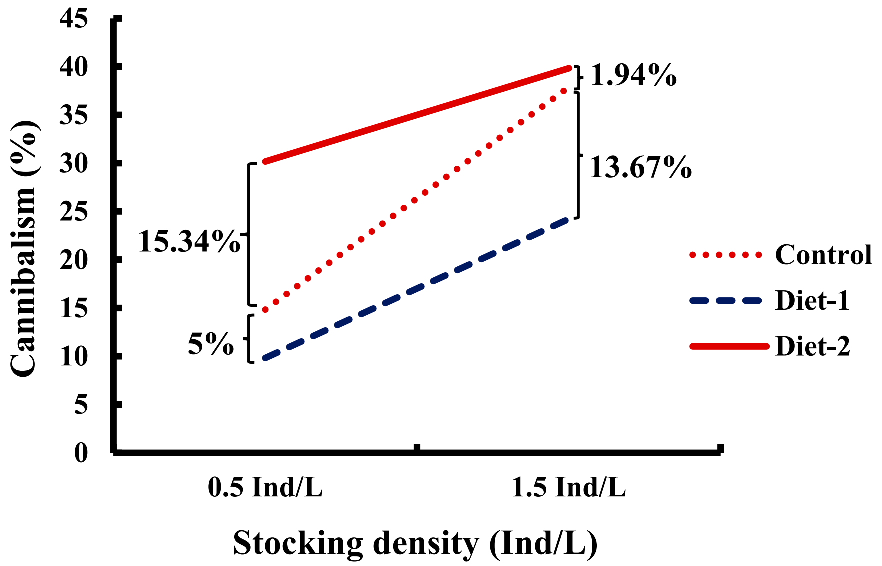

Figure 1. Cannibalism (%) of *Lates calcarifer* juveniles and their rational difference in percentage (%) at different dosages of L-tryptophan diets and two stocking densities.

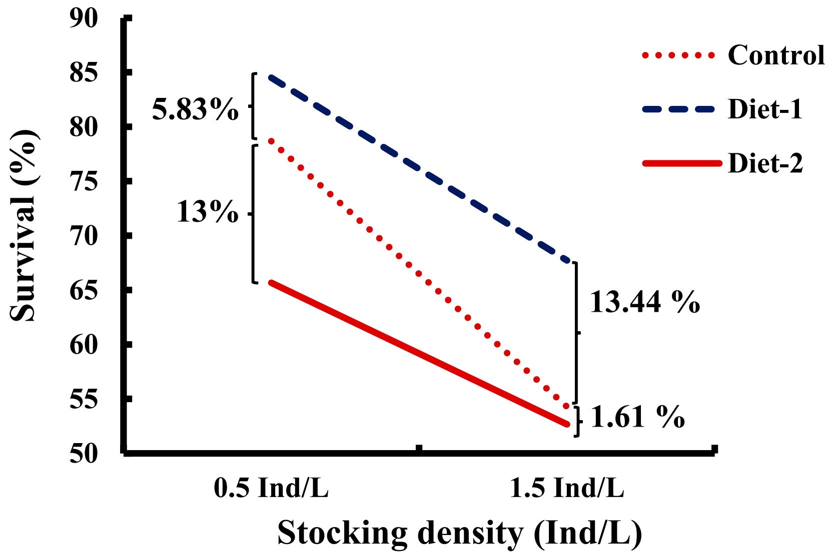

Figure 2. Survival rate (%) of *Lates calcarifer* juveniles and their rational difference in percentage (%) at different dosages of L-tryptophan diets and two stocking densities.

3.3. Biochemical Composition of Fish

Significantly higher lipid deposition was found in the muscles of fish fed with Diet 2 and at a 1.5 ind./L stocking density ($p < 0.001$) (Table 4). A two-way factorial ANOVA showed that the dosage of TRP in the experimental diets and stocking density had significant interaction effects on lipid deposition in the fish ($p < 0.001$). The results indicated that Diet 1 decreased lipid deposition by 0.03% points, and Diet 2 increased lipid deposition by 0.13% points when the stocking density was unchanged. The lipid deposition of both diets was also unchanged when the stocking density and lipid deposition increased by 0.14% points, keeping the TRP dosages unchanged. Specifically, the effect of Diet-1 on lipid deposition increased by 0.23% points more than the regular feed at the stocking density of 0.5 ind./L and decreased by 0.27% points at a higher stocking density (1.5 ind./L). Likewise, Diet 2 increased lipid deposition by 0.08% points at 0.5 ind./L and 0.19% at 1.5 ind./L stocking density (Figure 3). However, none of the variables significantly affected the moisture, ash, protein, and fiber content of fish muscle ($p > 0.05$).

Table 4. Muscle biochemical composition (% of wet weight) of Asian seabass juveniles with different experimental diets and stocking densities: full factorial ANOVA. (n = 18).

Component (%)	Mean ± SE					Interaction Effects (Two-Way Factorial *p*-Value)
	Experimental Diet			Stocking Density		
	Control	Diet-1	Diet-2	0.5 ind./L	1.5 ind./L	
Moisture	77.88 ± 0.25	77.82 ± 0.39	77.02 ± 0.26	77.36 ± 0.30	77.79 ± 0.23	0.164
Ash	1.28 ± 0.01	1.30 ± 0.03	1.33 ± 0.01	1.32 ± 0.02	1.29 ± 0.01	0.050
Lipid	0.44 ± 0.06 ^{a}	0.41 ± 0.05 ^{a}	0.57 ± 0.09 ^{b}	0.40 ± 0.04 ^{A}	0.54 ± 0.07 ^{B}	<0.001
Protein	18.13 ± 0.26	18.33 ± 0.29	18.59 ± 0.23	18.53 ± 0.26	18.17 ± 0.15	0.510
Fiber	0.05 ± 0.00	0.05 ± 0.00	0.07 ± 0.01	0.06 ± 0.01	0.05 ± 0.00	0.294

Note: Different superscripts (lower case for experimental diet and upper case for different stocking densities) within a row indicate significant differences in means based on Tukey's HSD test at a 5% significance level.

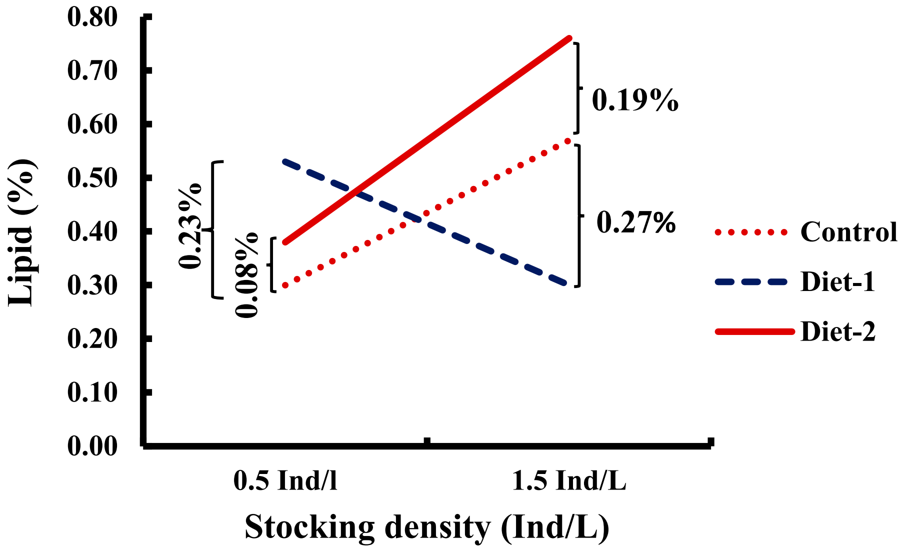

Figure 3. Lipid (%) in body muscle of *Lates calcarifer* juveniles and the difference in percentage (%) at different dosages of L-tryptophan diets and two stocking densities.

4. Discussion

All the water quality parameters recorded throughout the experiment, including water temperature, DO, pH, ammonia–nitrogen, and nitrite–nitrogen, were within the acceptable range for brackish water farming of Asian seabass [1].

TRP-supplemented diets significantly influenced the growth parameters of the Asian seabass in the present study. The total length (TLf), body weight (BWf), weight gain (WG), and specific growth rate (SGR) of the fish supplemented with dietary TRP at a higher percentage (1.50%) were significantly greater. The literature on this subject reports conflicting findings. In contrast to our results, Król and Zakęś [21] observed that the dietary supplementation of TRP did not affect the growth of pikeperch *Sander lucioperca* post-fry. Hseu et al. [24] similarly observed decreased growth rates in juvenile grouper *Epinephelus coioides* fed a TRP-supplemented diet, possibly due to a decreased appetite.

On the contrary, Coloso et al. [28] found that Asian seabass juveniles fed diets low in tryptophan gained less weight and had a lower mean percentage of final body weight than those fed diets high in tryptophan during a 12-week experiment. Again, Kumar et al. [22] reported that a TRP-supplemented diet at 1.50% resulted in greater weight gain for the Asian seabass juveniles following 45 days of nursery rearing in RAS. These two findings corroborate the current study on Asian seabass and indicate that TRP positively affected the growth performance of Asian seabass juveniles.

There was no significant difference in the growth of the juvenile Asian seabass between the control diet and Diet 1 in our study, while in Diet 2, the TL, BW, WG, and SGR were significantly higher. However, lower FCR and higher PER were observed in fish fed with Diet 1. Diet 1 and stocking density exhibited a strong synergy in regulating fish yield, as indicated by the interaction effect, signifying better feed utilization with Diet 1 than with the other diets, apparently without impairing appetite or growth. The result of the present experiment is consistent with prior research on Asian seabass [22,28]. Additionally, negative results indicating increased FCR due to TRP supplementation were observed in rainbow trout, *Oncorhynchus mykiss*, and European seabass, *Dicentrarchus labrax* [32,42]. Nonetheless, a lower FCR could be attributed to reduced feed intake due to increased brain serotonergic activity, as previously found in mammals and birds [43,44]. Reduced food intake was also observed in goldfish *Carassius auratus* when serotonin levels (5-HT) were elevated [31].

While CVs for length did not differ significantly by dietary TRP intake in the present experiment, CVs for weight did. This implies that dietary TRP had no effect on the heterogeneity in length but did influence weight heterogeneity. The heterogeneity in weight contrasts with the findings reported by Król and Zakęś [21] and Kumar et al. [22] on pikeperch and Asian seabass, respectively. Moreover, size heterogeneity and TPR intake varied by stocking density, as supported by the significant interaction effects. All the growth parameters showed improved values with increased stocking density. The CVs for length and weight were considerably greater when the stocking density was increased. A faster growth rate in higher stocking density was reported in juvenile perch *Perca fluviatilis* [45].

The intensity of cannibalism was significantly affected by dietary TRP supplementation and stocking density in the present experiment. Several studies have reported that dietary TRP supplementation reduces cannibalism in fish [21,22,24]. In the present study, 1.00% dietary TRP supplementation (Diet 1) reduced cannibalism, whereas the inclusion of 1.50% TPR (Diet 2) increased cannibalism. Juvenile grouper-fed diets containing a TRP dosage ranging from 0.25 to 1.00% exhibited a significant reduction in cannibalism due to increased brain serotonin levels [24]. On the other hand, higher dosages (1.50%) of dietary TRP altered the growth rate of fish in the present study since cannibals consumed a high concentration of TRP. This may have triggered even more cannibalism, as the potential cannibals were not removed from the culture tank. Based on the interaction effect of diet and stocking density, a higher density may improve the effect of Diet 1 on cannibalism.

Cannibalism was exacerbated in the current experiment at the higher stocking density, perhaps due to an increased scope of cannibal–prey interactions [10]. Khan et al. [46] and Khan et al. [34] also obtained consistent results. Stress generated from higher stocking density has been shown to lower brain serotonin levels [15,16], although additional TRP does not appreciably suppress the aggressive behavior of cannibals [21].

In the present study, mortality was significantly associated with higher stocking density, which was attributed to increased cannibalism [21]. This finding is in line with previous studies on Asian seabass [34]; northern pike, *Esox lucius* [47]; pikeperch [48]; perch [37]; and African sharp-tooth catfish, *Clarias gariepinus* larvae [49]. A higher survival rate was observed in the fish fed with Diet 1 than in those fed with other diets. Kumar et al. [22] used a stocking density of 2 ind./L in the nursery culture of Asian seabass and obtained a maximum survival rate (39.80 $\pm$ 3.00%) using 1.00% supplementary TRP throughout the 45-day culture period in RAS. On the other hand, Coloso et al. [28] used a 0.06 ind./L stocking density to determine the optimal dosages of dietary TRP in the feed of Asian seabass over 12 weeks in a flow-through water system and found that 0.41% dietary tryptophan was required for normal growth and survival of the juvenile Asian seabass.

In the present investigation, the maximum survival (76.28 $\pm$ 2.88%) was attained at a 0.5 ind./L stocking density, while the survival was lowest (58.22 $\pm$ 2.49%) at 1.5 ind./L when feeding fish with the same diet. Khan et al. [46] also reported that a lower stocking density significantly improved the survival of Asian seabass in a 45-day RAS, maintaining the same feeding rate and frequency. Furthermore, significant interaction effects on survival hinted that the different dosages of TRP might increase survival by suppressing the aggressive behavior of Asian seabass at different stocking densities. Based on the interaction effect of diet and stocking density, a strong synergy was observed in survival, indicating that a higher density could improve the effect of Diet 1 on survival (Figure 2). However, in general, dietary TRP supplementation exhibited mitigation of invective behavior in various fish species, including rainbow trout [16], grouper *E. coioides* [24], Atlantic cod *Gadus morhua* [25], and pikeperch [21].

Except for lipid content, no significant differences in the muscle composition of fish were observed in the present study. Although there was no significant difference between the control diet and Diet 1, the lipid content of the fish muscle was significantly higher in Diet 2, which may be due to TRP being stored as a lipid in fish muscles. Increased lipid content in fish muscles was also observed at a higher stocking density. This could be due to the increased opportunity for protein intake via cannibalism associated with higher stocking densities. Significant interaction effects between the two factors on muscle lipid content were observed. This result shows that the higher stocking density improved the effect of Diet 2 on lipid deposition. In contrast, dietary TRP levels or stocking densities had no significant effect on the moisture, ash, muscle proteins, and fiber content of Asian seabass juveniles.

A single female Asian seabass (>120 cm TL) can produce up to 46 million eggs [1]; however, nursery survival of extensively reared seabass has remained highly unpredictable due to its increased cannibalistic behavior. Though size grading of fish and frequent ad libitum feeding is widely used to minimize population losses, these methods are laborious and costly. They can potentially increase mortality due to physical handling during grading and drastic deterioration of water quality due to excess feeding. The effects of dietary incorporation of TRP and different stocking densities on nursery growth performance, cannibalism, and survival of Asian seabass were investigated in this study over 45 days in a recirculating system. The results indicated that the dietary incorporation of TRP (TRP) at 1.00% and a higher stocking density significantly improved the nursery growth of Asian seabass. Adding 1.00% TRP to the diet at a stocking density of 0.5 ind./L dramatically reduced cannibalism and enhanced survival rate without affecting feed consumption.

5. Conclusions

This study demonstrated that a diet supplied with 1.5% L-tryptophan and a stocking density of 1.5 ind./L resulted in superior growth performance, while a diet supplied with 1.0% L-tryptophan and a stocking density of 0.5 ind./L showed advantages in terms of feed utilization efficiency, reduced cannibalism, higher survival rate, and lower lipid deposition of Asian seabass in a RAS system. The interaction effects between TRP dosage and stocking density indicate the importance of considering these factors together for optimizing the growth and overall performance of juvenile Asian seabass in a recirculating aquaculture system. Further research is needed on the turnover of brain serotonin levels in fish fed with different dosages of TRP-supplemented diets at various stocking densities, which was not examined in this study.

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Data Availability Statement: The author declares that all data supporting the findings of this study are included within the article. The datasets of the current study are available upon reasonable request.

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