RESEARCH ARTICLE Indian Journal of Animal Research, Volume 59 Issue 8: 1355-1362 (August 2025)

Effect of Selected Nutraceuticals on Growth and Metabolism of *Penaeus vannamei* Juveniles Reared in Inland Saline Water

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10.18805/IJAR.B-5587

ABSTRACT

Background: The objective of this study is to evaluate the individual and combinatorial effects of lipid-utilizing nutraceuticals (L-carnitine, taurine and quercetin) on growth, protein and carbohydrate metabolic enzymes in *P. vannamei* juveniles reared in ISW of 10 ppt salinity.

Methods: A 60-day experiment was conducted to evaluate the individual and combinatorial effects of L-carnitine, taurine and quercetin on the growth and metabolism of *P. vannamei* juveniles. The shrimps were fed with eight experimental diets, *viz*. Control, T_C, T_T, T_Q, T_{C+Q}, T_{C+T}, T_{T+Q} and T_{C+T+Q} to evaluate their effect.

Result: All the nutraceutical-fed groups exhibited significantly higher growth, feed utilization, AST and ALT activities than the control group. Among the nutraceutical fed groups, T_{C+T} and T_{C+T+Q} treatment groups exhibited higher growth, AST, ALT and lower LDH and MDH activities than other treatment groups.

Key words: Inland saline water, Nutraceuticals, *Penaeus vannamei*.

INTRODUCTION

The global population has been growing at a steady rate of 1.05% per year in the last decade and it has created tremendous pressure on all food-producing sectors to meet global demands. Aquaculture has been the fastest-growing animal food-producing sector, contributing about 20% of animal protein needs worldwide (Raghuvaran *et al*., 2024). However, the stagnation in capture fisheries has increased the demand for aquaculture, as it must produce an additional 47.5 million tonnes of fish by 2050. This high demand can be fulfilled only by the expansion of aquaculture resources. However, the non-availability of sufficient land and water sources has made vertical expansion impossible and hence, the horizontal expansion of available resources is the only viable option left. One such option with potential is using salt-affected inland areas for aquaculture purposes to enhance food security. These saline lands are unproductive and unsuitable for other agricultural activities (Jana *et al*., 2021). Using these wastelands not only improves food security but also generates additional income. Hence, the culture of euryhaline fish species in ISW has become popular among farmers globally (Roy *et al*., 2010). Among the crustaceans, *Penaeus vannamei* has become a candidate species for aquaculture due to its faster growth rate and wide tolerance to salinity (Chuphal *et al*., 2021; Raghuvaran *et al*., 2023). However, the imbalance of ions in ISW affects growth and immunity and increases the energy demands of the cultured species (Singha *et al*., 2021; Thirunavukkarasar *et al*., 2022).

Feed alone contributes 50-60% of the total operational expenses of aquaculture (Raghuvaran *et al*., 2024; Dheeran *et al*., 2025). Among dietary macronutrients, protein is the costliest and is vital in fish growth. The protein and lipid requirement of *P. vannamei* in ISW is 35.6% (Nanda, 2019) and 5.13% (Chuphal *et al*., 2021), respectively, at 10 ppt ambient salinity. This higher protein demand increases the production cost and is detrimental to sensitive culture systems like ISW, as this increases the release of nitrogenous wastes into the system. Hence, using low protein high energy diets is a *via*ble alternative option in ISW. The protein-sparing effect of lipids can be better utilized by increasing the dietary lipid content in fish feed. But, the inclusion of high lipids in the diet results in excessive fat deposition in the body tissues and ultimately affects the health of the cultured organisms (Bhusare *et al*., 2023; Mai *et al*., 2025). In this regard, earlier studies conducted found that lipid-utilizing nutraceuticals such as L-carnitine (Raghuvaran *et al*., 2023), Taurine (Bhavatharaniya, 2021) and Quercetin (Unpublished data) have been found to improve growth and lipid utilization in *P. vannamei* reared in ISW. However, the information on the combinatorial effect of these nutraceuticals in *P. vannamei* is still lacking.

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How to cite this article: Raghuvaran, N., Sahu, N.P., Sardar, P., varghese, T., Shamna, N., Rani, B., Bhuvaneshwaran, T. and Revathi, A. (2025). Effect of Selected Nutraceuticals on Growth and Metabolism of *Penaeus vannamei* Juveniles Reared in Inland Saline Water. *Indian Journal of Animal Research*. **59(8):** 1355-1362. doi: 10.18805/IJAR.B-5587.

Submitted: 22-03-2025 **Accepted:** 28-04-2025 **Online:** 23-05-2025

Implementing the ideal protein concept has been found to reduce protein requirement from 35.16 to 32.09% without compromising the growth of *P. vannamei* reared in ISW (Maiti, 2022). Hence, this study was conducted to evaluate the effect of selected nutraceuticals (L-carnitine, Taurine and Quercetin) and limiting amino acids (Lysine and Methionine) on the growth and metabolic responses of *P. vannamei* reared in ISW of 10 ppt salinity.

MATERIALS AND METHODS

Ethical statement

The ethical committee of ICAR-Central Institute of Fisheries Education, Mumbai, India, approved this study.

Formulation and preparation of experimental diets

Eight isonitrogenous (32% CP), isolipidic (6%) and isocaloric (384 Kcal digestible energy, DE/100 g) practical diets were supplemented with three different nutraceuticals (L-carnitine, taurine and quercetin) to formulate (Table 1) and prepare eight experimental diets, *viz*. Control, optimum dietary protein (32%) and lipid level (6%) with limiting amino acids and no supplementation of nutraceuticals; T_C, Control diet supplemented with 0.05% L-carnitine; T_T, Control diet supplemented with 0.5% Taurine; T_Q, Control diet supplemented with 0.04% Quercetin; T_{C+Q}, Control diet supplemented with L-Carnitine (50% dose) + Quercetin (50% dose); T_{C+T}, Control diet supplemented with L-Carnitine (50% dose) + Taurine (50% dose); T_{T+Q}, Control diet supplemented with Quercetin (50% dose) + Taurine (50% dose); T_{C+T+Q}, Control diet supplemented with L-carnitine +Taurine+ Quercetin (33.3% dose each). All ground ingredients, except for feed additives, BHT and oils, were uniformly mixed and water was added as needed to form a dough. This dough was placed in a heat-resistant plastic bag and steam-cooked for 25 minutes before being cooled. The remaining ingredients were then thoroughly blended into the dough. Subsequently, the dough was pressed through a pelletizer (Uniextrude-single screw extruder; S. B. Panchal and Co. Mumbai, India) fitted with a perforated die of 1 mm diameter to make the pellets of the same diameter and initially air-dried at room temperature, then dried at 40°C until the moisture content was reduced to approximately 10%. The dried pellets were then crushed into smaller sizes and stored at 4°C until use for feeding.

Experimental setup, design and feeding trial

The experiment was conducted for 60 days (July-September, 2023) in an ISW pond (10 ppt) of the Baniyani shrimp farm, ICAR-CIFE Rohtak Centre, Haryana. To conduct the feeding trial, 24 hapas (2.5 m × 2 m × 1 m) were installed in an optimally manured and fertilized ISW (10 ppt salinity) pond fortified with murate of potash. Juveniles of *P. vannamei* were stocked in hapas at the rate of 80 per hapa. The experiment followed a completely randomized design with eight experimental groups. The shrimps of all experimental groups were fed to satiation level four times (8.00, 12.00, 16.00 and 20.00 h) daily with respective diets. The hapas were cleaned every two days to remove the uneaten feed and algal debris to achieve uniform water circulation. Every fortnight, the shrimp weight was measured carefully to adjust the satiation level of feeding. The ISW quality parameters were analyzed daily according to APHA (2018). All the water quality parameters were within the optimum range during the entire experimental period of 60 days (Table 2).

Sampling

The shrimps' initial and final live weight was measured using an electronic balance before commencement and after completion of the trial. The shrimps were kept unfed overnight before measuring the final body weight (for 22.00 h). Three shrimps from each replicate hapa were randomly collected, anaesthetized with ice-chilled water (Jana *et al*., 2021) and the hepatopancreas and muscle tissues were dissected in an ice-chilled condition. Tissues were then put into ice-cold phosphate buffer (0.025 M) solution and tissue homogenates (5%, w/v) were prepared using a mechanical homogenizer (REMI Equipments, India) for performing different enzyme assays. The entire process, starting from tissue collection, was carried out meticulously while maintaining a cold chain to preserve enzymatic activity. The tissue homogenates were centrifuged at 5000 rpm for 10 minutes at 4°C using a refrigerated centrifuge. The resulting supernatant was transferred into 2 ml Eppendorf tubes and stored at -20°C for further analysis.

Growth and nutrient utilization

The growth and nutrient utilization parameters were calculated using the following formulae:

Weight gain (g) = Final wet weight (g) - Initial wet weight (g)

The following formula calculates the metabolic growth rate of fish:

$$\text{MGR (g/kg}^{0.8}\text{ /day)} = \frac{\text{Wet weight gain (g)}}{[\{(\text{IWW}/1000)^{0.8} \times (\text{FWW}/1000)^{0.8}\} \times 2] \times \text{ED}}$$

Where,
IWW = Initial wet weight (g).
FWW = Final wet weight (g).
ED = Experimental duration (days).

$$\text{TGC} = \frac{\{\text{Final wet weight (g)}\}^{1/3} - \{\text{Initial wet weight (g)}\}^{1/3}}{\text{Experiment duration in days} \times \text{Water temperature (°C)}}$$

$$\text{Feed efficiency ratio (FER)} = \frac{\text{Net weight gain (in g on wet weight basis)}}{\text{Feed consumed (in g on dry weight basis)}}$$

Enzyme assays

Tissue homogenate preparation

A 5% (w/v) tissue homogenate was prepared in an ice-cold phosphate buffer (0.025 M KH_2PO_4, 0.025 M $Na_2PO_4 \cdot 12H_2O$, pH 7.5) using a mechanical tissue homogenizer (REMI Equipments, India). All steps were conducted under strict cold chain conditions from the tissue collection stage onward to maintain enzymatic activity. Using a cooling centrifuge, the homogenates were centrifuged at 5000 rpm for 10 minutes at 4°C. The resulting supernatant was transferred into 2 ml Eppendorf tubes and stored at -20°C.

Protein metabolic enzyme assays

The activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in muscle and hepatopancreas tissues were measured using the method described by Wooten (1964). The substrates α-ketoglutarate and DL-

Table 1: Formulation and proximate composition of experimental diets fed to experimental *Penaeus vannamei* reared in inland saline water at 10 ppt salinity for the period of 60 days.

Ingredients (%)	Diets/Treatments[1]							
	Control	T_C	T_T	T_Q	T_{C+Q}	T_{C+T}	T_{T+Q}	T_{C+T+Q}
Fish meal	150.00	150.00	150.00	150.00	150.00	150.00	150.00	150.00
Shrimp meal	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00
SBM	150.00	150.00	150.00	150.00	150.00	150.00	150.00	150.00
GNOC	140.00	140.00	140.00	140.00	140.00	140.00	140.00	140.00
Maize flour	140.00	140.00	140.00	140.00	140.00	140.00	140.00	140.00
DORB	130.00	130.00	130.00	130.00	130.00	130.00	130.00	130.00
Wheat flour	130.00	130.00	130.00	130.00	130.00	130.00	130.00	130.00
Cellulose	28.10	27.40	22.90	27.50	27.40	25.10	25.20	25.90
Cod liver oil	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Sunflower oil	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Vit-Min mix	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
CMC	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
BHT4	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Betaine	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Choline chloride	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Stay C	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Cholesterol	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Lecithin	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Vit E	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Limiting AA	12.20	12.20	12.20	12.20	12.20	12.20	12.20	12.20
L-carnitine	0.00	0.50	0.00	0.00	0.25	0.25	0.00	0.16
Taurine	0.00	0.00	5.00	0.00	0.00	2.5	2.5	1.66
Quercetin	0.00	0.00	0.00	0.4	0.2	0.00	0.2	0.13
Total	1000	1000	1000	1000	1000	1000	1000	1000
Proximate composition (on dry matter basis)								
Dry matter (%)	90.50	90.20	90.20	90.60	90.60	90.30	90.50	90.30
Crude protein (%)	32.21	32.19	32.15	32.20	32.27	32.17	32.16	32.21
Ether extract (%)	6.14	6.12	6.15	6.14	6.13	6.12	6.11	6.21
Crude fibre (%)	4.52	4.51	4.51	4.52	4.52	4.50	4.52	4.53
Total ash (%)	7.06	7.10	7.14	7.14	7.12	7.08	7.10	7.10
Nitrogen free extract (%)	50.06	50.06	50.02	49.98	49.95	50.12	50.09	49.94
DE[2] (Kcal/100 g)	384.38	384.18	384.13	384.05	384.09	384.25	384.10	384.53
P/E[3] (mg Crude protein/Kcal DE)	83.81	83.79	83.70	83.85	84.01	83.73	83.76	83.76

[1]Control, optimum dietary protein (32%) and lipid level (6%) with limiting amino acids and no supplementation of nutraceutical; T_C: Control diet supplemented with 0.05% L-carnitine; T_T: Control diet supplemented with 0.5% Taurine; T_Q: Control diet supplemented with 0.04% Quercetin; T_{C+Q}: Control diet supplemented with L-Carnitine (50% dose) + Quercetin (50% dose); T_{C+T}: Control diet supplemented with L-Carnitine (50% dose) + Taurine (50% dose); T_{T+Q}: Control diet supplemented with Quercetin (50% dose) + Taurine (50% dose); T_{C+T+Q}: Control diet supplemented with L-carnitine +Taurine+ Quercetin (33.3% dose each; 7DE, digestible energy; 8P/E, protein to energy ratio.

aspartic acid were used for AST, while DL-alanine replaced DL-aspartic acid for the ALT assay. The optical density (OD) was measured at 540 nm relative to a blank.

Table 2: Physico chemical parameters of ISW Pond water for 60 days.

Parameters	Values in ranges
Temperature (°C)	27.56-30.98
pH	8.06-8.41
Salinity (g/L)	9.59-10.71
DO (mg/L)	6.32-6.75
Free CO_2 (mg/L)	0.01-0.02
Alkalinity (mg/L)	282.75-305.42
Hardness (mg/L)	2912-3085
TA-N (mg/L)	Nil
$NO^{2-}N$ (mg/L)	Nil
$NO^{3-}N$ (mg/L)	Nil
Ca^{2+} (mg/L)	235.65-242.43
Mg^{2+} (mg/L)	522.34-529.21
K^{+} (mg/L)	61.82-67.40
Osmolality (mOsmo/kg)	242.38-244.52

Carbohydrate metabolic enzyme assays

The methodology of Wroblewski and Ladue (1955) was used to assess the lactate dehydrogenase (LDH) activity. Similarly, malate dehydrogenase (MDH) activity in hepatopancreas and muscle tissues was evaluated using the illustrated method of Ochoa (1955).

Statistical analysis

The data were analyzed using one-way ANOVA in SPSS software (version 22.0) to assess the overall effects of the means, following verification of normality and homogeneity of variance with Levene's test. A 5% probability level ($p<0.05$) significant difference between the means was observed using Duncan's multiple range test (DMRT) with post hoc analysis. The data analysis was displayed as mean ± standard error (SE).

RESULTS AND DISCUSSION

Growth and nutrient utilization

The growth of animals, including fish, involves both muscle hypertrophy and hyperplasia, which are affected by the

Table 3: Growth and Nutrient utilization of *P. vannamei* juveniles fed with lipid-utilizing nutraceuticals reared in ISW.

Treatments[1]	IBW[2]	FBW[3]	WG[4]	MGR[5]	TGC[6]	FER[7]
C	3.17±0.02	14.53^{d}±0.12	11.35^{d}±0.15	8.62^{d}±0.08	0.20^{d}±0.01	0.66^{d}±0.01
T_C	3.17±0.01	16.02^{bc}±0.38	12.85^{bc}±0.40	9.17^{bc}±0.16	0.22^{bc}±0.01	0.69^{cd}±0.01
T_T	3.19±0.01	16.16^{bc}±0.33	12.96^{bc}±0.32	9.20^{bc}±0.10	0.23^{bc}±0.01	0.70^{b}±0.01
T_Q	3.19±0.01	15.53^{c}±0.20	12.34^{c}±0.21	8.97^{c}±0.08	0.22^{c}±0.01	0.68^{c}±0.01
T_{C+Q}	3.16±0.01	16.57^{b}±0.14	13.41^{b}±0.13	9.38^{b}±0.04	0.23^{b}±0.02	0.69^{bc}±0.01
T_{C+T}	3.18±0.02	18.04^{a}±0.15	14.85^{a}±0.16	9.83^{a}±0.06	0.26^{a}±0.01	0.75^{a}±0.01
T_{T+Q}	3.17±0.01	16.58^{b}±0.09	13.41^{b}±0.11	9.38^{b}±0.05	0.23^{b}±0.01	0.69^{bc}±0.01
T_{C+T+Q}	3.15±0.01	18.33^{a}±0.13	15.18^{a}±0.13	9.97^{a}±0.04	0.27^{a}±0.01	0.73^{a}±0.01
P value	<0.001	<0.001	<0.001	0.001	<0.001	<0.001

Data are expressed as mean±SE (n = 3); Mean values in the same column with different superscripts vary significantly ($p<0.05$); [2]IBW- Initial body weight; [3]FBW- Final body weight; [4]WG- Weight gain; [5]MGR- Metabolic growth rate; [6]TGC- Thermal growth coefficient; [7]FER- Feed efficiency ratio.

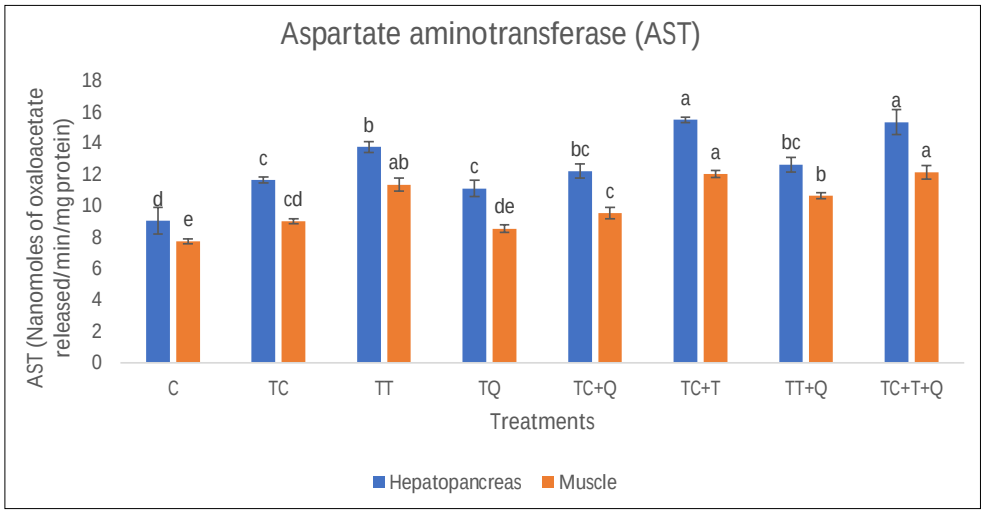


Fig 1: Aspartate aminotransferase (AST) activity of hepatopancreas and muscle tissues.

nutrients in their diet. Fish, including crustaceans, prefer protein over lipids and carbohydrates as the primary energy source. Protein being the costliest nutrient among others not only increases the feed cost but also results in the pollution of the culture environment *via* excretion of ammonia (Talukdar *et al.*, 2021; Jana *et al.*, 2022; Chuphal *et al.*, 2024). Thus, using low-protein, high-energy diets can be a suitable dietary intervention strategy to improve production without compromising growth (Raghuvaran *et al.*, 2023). Higher lipid utilization can be enhanced by using lipid utilizing nutraceuticals. Earlier studies evaluating individual effects of lipid-utilizing nutraceuticals such as taurine (Bhavatharaniya, 2021; Jana, 2022), L-carnitine (Raghuvaran *et al.*, 2023) and Quercetin (Unpublished data) found that nutrient utilization can be improved without compromising growth. However, the information on the combinatorial effect of these nutraceuticals is still lacking. Hence, this study was conducted with this backdrop to assess the synergistic effect of these nutraceuticals.

The growth and nutrient utilization parameters are given in Table 3. In the present study, the growth and nutrient utilization parameters, such as WG, MGR, TGC and FER, were significantly improved in all the nutraceutical-supplemented groups compared to the control group without nutraceuticals. Among the nutraceutical supplemented groups, treatment groups T_{C+T} (50% dose of L-carnitine and taurine) and T_{C+T+Q} (33.3% of L-carnitine, taurine and quercetin) exhibited significantly ($p<0.05$) the highest WG, MGR, TGC and FER. Fish fed with nutraceuticals in combination could exhibit better growth due to their synergistic effects (Jin *et al.*, 2019). Similarly, 0.5% dietary taurine (Bhavatharaniya, 2021) or 0.05% L-carnitine (Raghuvaran *et al.*, 2023) or 0.04% quercetin (Unpublished data) improved the growth performance of *P. vannamei* reared in ISW, which had been reported in earlier studies. Additionally, dietary taurine (To *et al.*, 2021; Shi *et al.*, 2023; Mai *et al.*, 2025), L-carnitine (Raghuvaran *et al.*, 2023) and quercetin (Chen *et al.*, 2023) mediated enhanced growth

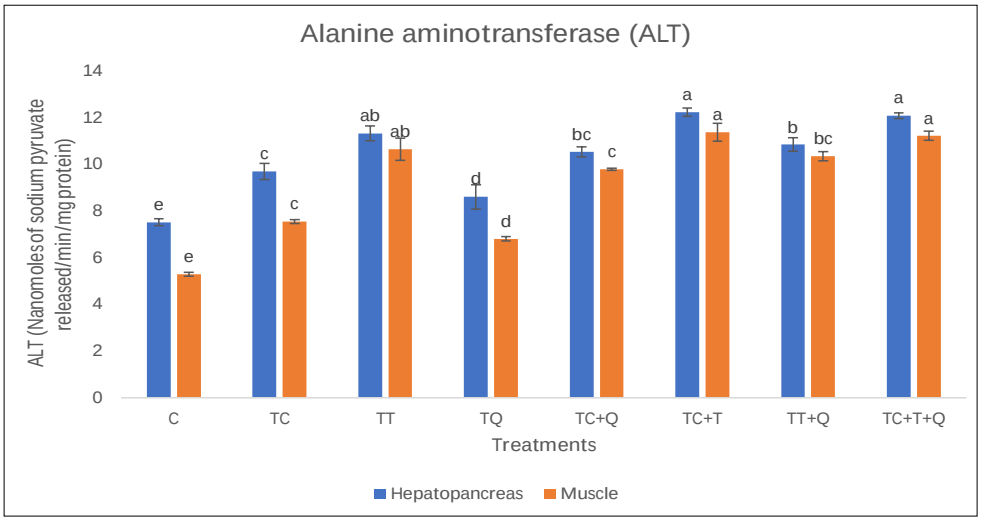


Fig 2: Alanine aminotransferase (ALT) activity of hepatopancreas and muscle tissues.

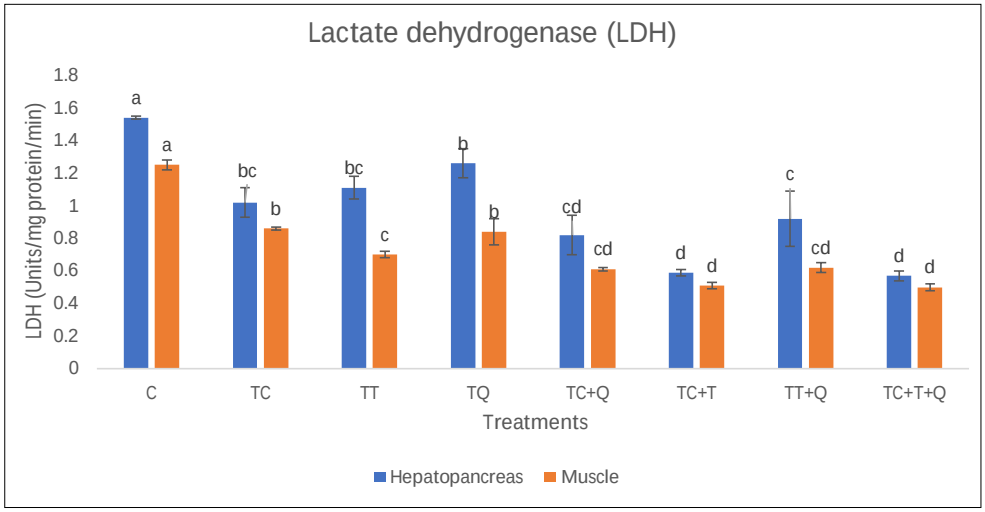


Fig 3: Lactate dehydrogenase activity of hepatopancreas and muscle tissues.

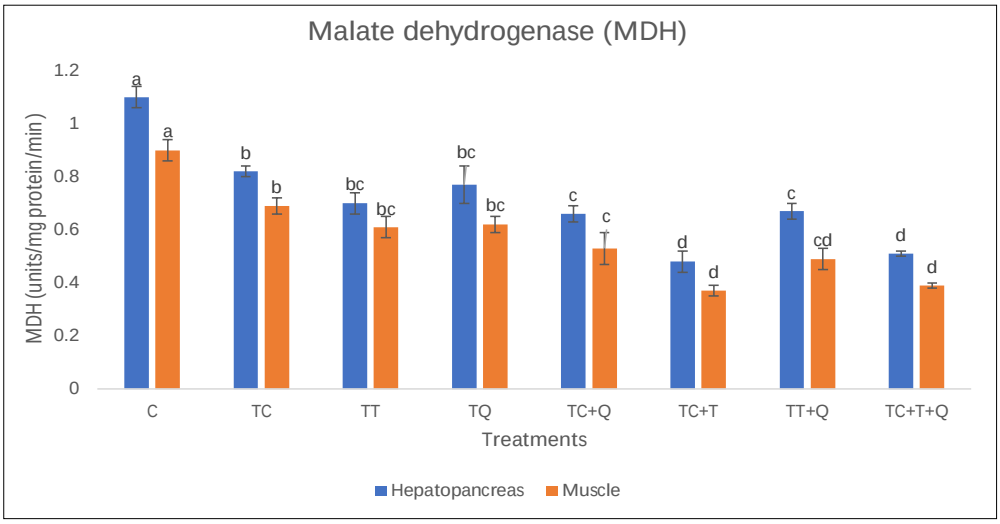


Fig 4: Malate dehydrogenase activity of hepatopancreas and muscle tissues.

performance have been reported in *P. vannamei*. Our results corroborated the findings of Jana (2022), who also found that taurine and L-carnitine had a synergistic effect and performed better than their counterparts. Taurine facilitated lipid digestion and L-carnitine further transported the fatty acids in mitochondrial β-oxidation to satiate the energy demand of shrimp. Das (2024) reported similar findings, stating that feeding a combination of glycerol monolaurate and leonardite humic acid improved growth performance and lipid utilization.

Protein metabolic enzymes activity

Protein metabolic enzymes, such as AST and ALT, are crucial for transamination reactions, which facilitate the synthesis of new amino acids from existing ones (Knox and Greengard, 1965). In energy-sufficient conditions, these enzymes support the synthesis and accumulation of body proteins, contributing to growth. Conversely, they are involved in the oxidative degradation of proteins to generate energy during energy deprivation, as observed in various animals, including shrimp (Jiang *et al*., 2015; De Silva and Anderson, 1995). The supplementation of 0.05% L-carnitine and 0.5% taurine notably increased AST and ALT activities in *P. vannamei* juveniles reared in ISW (Raghuvaran, 2021; Bhavatharaniya, 2021). In our study, significantly ($p<0.05$), the highest AST and ALT activities in hepatopancreas and muscle were exhibited in treatment groups T_{C+T} and T_{C+T+Q} (Fig 1,2). The AST and ALT activities were non-significant ($p>0.05$) among treatment groups T_C, T_T, T_Q, T_{C+Q} and T_{T+Q}. Individually, taurine and L-carnitine exhibited higher muscle AST and ALT activities than quercetin. However, all the nutraceutical groups showed significantly higher ($p<0.05$) activities than the control group without nutraceuticals.

Carbohydrate metabolic enzyme activity

LDH and MDH are enzymes involved in carbohydrate metabolism (Das, 2024; Paswan *et al*., 2025). Their activities increase in animals, including fish, under oxidative stress. During this condition, cytosolic LDH catalyzes the conversion of pyruvate into lactate through anaerobic glycolysis. The lactate is then converted into glucose in the liver *via* the Cori cycle and transported to muscles to sustain energy levels. Meanwhile, under oxidative stress-induced anaerobic conditions, mitochondrial MDH in the TCA cycle reduces oxaloacetate to malate. This malate moves to the cytoplasm, contributing to glucose synthesis through gluconeogenesis, ensuring a continuous energy supply. Due to their roles in stress response, LDH and MDH are considered biomarkers of oxidative stress (Raghuvaran *et al*., 2024; Mai *et al*., 2025). In the present study, significantly ($p<0.05$) higher LDH and MDH activities were expressed in the hepatopancreas and muscle of the control group (Fig 3, 4). All the experimental groups, except the control group, fed without nutraceuticals, exhibited significantly ($p<0.05$) lower LDH and MDH activities, indicating the stress-mitigating effect of the nutraceuticals. Both the T_{C+T} and T_{C+T+Q} groups exhibited significantly ($p<0.05$) lower MDH activity in hepatopancreas and muscle. However, in the case of LDH, similar activity was expressed among treatment groups T_{C+T}, T_{C+T+Q}, T_{C+Q} and T_{T+Q}.

Limitations

Although this study was conducted in hapas installed in ponds, a full-fledged feeding trial in ponds can be done in the future to validate the present findings.

CONCLUSION

In conclusion, all the nutraceuticals-fed groups exhibited improved growth and reduced stress compared to the control group. Among the nutraceutical-fed groups, both treatment groups, T_{C+T} and T_{C+T+Q}, showed higher growth, AST and ALT activities than other treatments. Similarly, lower LDH and MDH activities were found in the same treatment groups, indicating reduced stress compared to

the control group. This study reveals that the taurine and L-carnitine combination is better than other nutraceutical combinations. The taurine and L-carnitine combination synergized, resulting in better nutrient utilization and reduced stress. This information will help formulate a low-cost, eco-friendly diet for *P. vannamei* reared in an ISW of 10 ppt salinity.

Conflict of Interest

The authors declare that they have no conflict of interest to disclose.

REFERENCES

APHA. (2018). Standard Methods for Examination of Water and Wastewater, 24th edn. American Public Health Association, Washington. DC.

Bhavatharaniya, U. (2021). Evaluation of the low protein and high energy diets supplemented with taurine on growth and immunity of *Penaeus vannamei* (Boone, 1931) juveniles reared in inland saline water, M.F.Sc. Dissertation, ICAR-Central Institute of Fisheries Education (University under section 3 of UGC Act 1956), Panch Marg, Off Yari Road, Versova (W), Mumbai, India. ISBN: 978-93-924 33-20-7.

Bhusare, S., Sardar, P., Sahu, N., Shamna, N., Kumar, Paul, M., Jana, P., Raghuvaran, N. and Bhavatharaniya, U. (2023). Bile acid improves growth, lipid utilization and antioxidative status of genetically improved farmed tilapia (*Oreochromis niloticus*) fed with varying protein-lipid diets reared in inland saline water. *Animal Feed Science and Technology*. **303:** 115677. https://doi.org/10.1016/j.anifeedsci.2023.115677.

Chen, Z., Fan, D., Pan, L., Su, C., Ding, Y. and Lu, M. (2023). Study of effects of dietary quercetin (Que) on growth performance and disease resistance mechanism of *Litopenaeus vannamei. Aquaculture*. **563:** 738887. https://doi.org/10.1016/j.aquaculture.2022.738887.

Chuphal, N., Sardar, P., Sahu, N.P., Shamna, N., Harikrishna, V., Maiti, M.K., Mannur, V.S., Jana, P., Paul, M. and Krishna, G. (2021). Effects of graded dietary lipid levels on growth, feed utilization, body composition and metabolism in juvenile white leg shrimp, *Penaeus vannamei* (Boone, 1931) reared in inland saline water of 10 g/L salinity. *Aquaculture Nutrition*. **27(6):** 1811-1824. https://doi.org/10.1111/anu.13319.

Chuphal, N., Sardar, P., Sahu, N.P., Shamna, N., Krishnan, S., Varghese, T., Malik, M.A., Maiti, M.K. and Phulia, V. (2024). Optimal level of dietary arginine enhances growth performance, haemato-biochemical status, metabolic Responses and growth-related gene expression of GIFT juveniles reared in inland saline water. *Aquaculture*. **592:** 741219. https://doi.org/10.1016/j.aquaculture.2024.741219.

Das (2024). Evaluation of glycerol monolaurate and leonardite humic acid in low protein high energy diet of *Penaeus vannamei* (Boone, 1931) juveniles reared in inland saline water. Ph.D. Thesis, ICAR-Central Institute of Fisheries Education (University under section 3 of UGC Act 1956), Panch Marg, Off Yari Road, Versova (W), Mumbai. India-400 061. ISBN: 978-81-970702-4-2.

De Silva, S.S. and Anderson, T.A. (1995). Fish Nutrition in Aquaculture. Chapman and Hall, Springer, London. ISBN: 978-0-412-55030-0.

Dheeran, P., Verma, A.K., Krishnan, S., Krishnani, K.K., Hittinahalli, C.M., Campal, E.I. and Annadurai, R. (2025). Sensory attributes of *Channa striata* through dietary supplementation of selenium and taurine in inland saline aquaculture. *Indian Journal of Animal Research*. **59(5):** 885-891. doi: 10.18805/IJAR.B-5566.

Jana, P., Sahu, N.P, Sardar, P., Shamna, N., Varghese, T., Dharmendra Deo, A., Harikrishna, V., Paul, M., Panmei, H., Gupta, G. and Nanda, C. (2021). Dietary protein requirement of white shrimp, *Penaeus vannamei* (Boone, 1931) juveniles, reared in inland ground water of medium salinity. *Aquaculture Research*. **52(6):** 2501-2517. https://doi.org/10.1111/are.15100.

Jana, P., Sahu, N.P., Sardar, P., Varghese, T., Deo, A.D., Shamna, N., Harikrishna, V., Paul, M., Chuphal, N. and Krishna, G. (2022). Dietary lipid requirement of juvenile white leg shrimp, *Penaeus vannamei* (Boone, 1931) reared in inland ground saline water of 15 ppt. *Aquaculture Research*. **53(15):** 5270-5286. https://doi.org/10.1111/are.16012.

Jana (2022). Dietary intervention for improving growth and immunophysiological responses in *Penaeus vannamei* (boone, 1931) juveniles reared in inland saline water. Ph.D. Thesis, ICAR-Central Institute of Fisheries Education (University under section 3 of UGC Act 1956), Panch Marg, Off Yari Road, Versova (W), Mumbai, India. ISBN: 978-93-95650-18-2.

Jiang, Y., Fan, X., Wang, Y., Tan, H., Chen, P., Zeng, H., Huang, M. and Bi, H. (2015). Hepato-protective effects of six *Schisandra lignans* on acetaminophen-induced liver injury are partially associated with the inhibition of CYP-mediated bioactivation. *Chemico-biological Interactions*. **231:** 83-89. https://doi.org/10.1016/j.cbi.2015.02.022.

Jin, M., Pan, T., Cheng, X., Zhu, T.T., Sun, P., Zhou, F., Ding, X. and Zhou, Q. (2019). Effects of supplemental dietary L-carnitine and bile acids on growth performance, antioxidant and immune ability, histopathological changes and inflammatory response in juvenile black seabream (*Acanthopagrus schlegelii*) fed high-fat diet. *Aquaculture*. **504:** 199-209. https://doi.org/10.1016/j.aquaculture.2019.01.063.

Knox, W.E. and Greengard, O. (1965). The regulation of some enzymes of nitrogen metabolism-an introduction to enzyme physiology. *Advances in Enzyme Regulation*. **3:** 247-313. https://doi.org/10.1016/0065-2571(65)90059-2.

Mai, H., Li, Y., Song, Z., Zeng, Y., Lin, P., Sun, Z., Mai, K., Tan, B. and Ye, C. (2025). Effect of taurine on growth and immune response of Pacific white shrimp (*Litopenaeus vannamei*) cultured at different temperatures. *Aquaculture*. **594:** 741393. https://doi.org/10.1016/j.aquaculture.2024.741393.

Maiti. (2022). Optimisation of dietary crude protein based on ideal protein concept for *Penaeus vannamei* (Boone, 1931) juveniles reared in inland saline water. Ph.D. Thesis, ICAR-Central Institute of Fisheries Education (University under section 3 of UGC Act 1956), Panch Marg, Off Yari Road, Versova (W), Mumbai, India ISBN: 978-93-95650-61-8.

Nanda (2019). Studies on dietary protein requirement of *Litopenaeus vannamei* (Boone, 1931) juveniles reared in inland saline water. M.F.Sc. Dissertation, ICAR-Central Institute of Fisheries Education (University under section 3 of UGC Act 1956), Panch Marg, Off Yari Road, Versova (W), Mumbai, India-400 061.

Ochoa, S. (1955). [123] Malic Dehydrogenase from Pig Heart: l-malate + $DPN^+ \leftrightarrows$ Oxalacetate + DPNH + H^+. https://doi.org/10.1016/0076-6879(55)01128-2.

Paswan, V.K., Sudhagar, A., Lingam, R.S.S., Sangavi, S., Singh, D., Kumar, R. and Katira, N.N. (2025). Effects of salinity and Ca^{2+}: Mg^{2+} ratio on growth performance and survival of *Penaeus vannamei* (Boone, 1931) reared in inland saline ground water. *Indian Journal of Animal Research*. **59(1):** 163-167. doi: 10.18805/IJAR.B-5304.

Raghuvaran, N. (2021). Evaluation of the low protein and high energy diets supplemented with L-carnitine on growth and immunity of *Penaeus vannamei* (Boone, 1931) juveniles reared in inland saline water, M.F.Sc. Dissertation, ICAR-Central Institute of Fisheries Education (University under section 3 of UGC Act 1956), Panch Marg, Off Yari Road, Versova (W), Mumbai, India-400 061. ISBN: 978-93-92433-19-1.

Raghuvaran, N., Sardar, P., Sahu, N.P., Shamna, N., Jana, P., Paul, M., Bhusare, S. and Bhavatharaniya, U. (2023). Effect of L-carnitine supplemented diets with varying protein and lipid levels on growth, body composition, antioxidant status and physio-metabolic changes of white shrimp, *Penaeus vannamei* juveniles reared in inland saline water. *Animal Feed Science and Technology*. **296:** 115548. https://doi.org/10.1016/j.anifeedsci.2022.115548.

Raghuvaran, N., Varghese, T., Jana, P., RJ, A.B., Sudarshan, S., Alrashdi, Y.B.A., Ibrahim, A.E. and El Deeb, S. (2024). Current status and global research trend patterns of insect meal in aquaculture from scientometric perspective: (2013-2022). *Aquaculture Nutrition*. **22:** 5466604. https://doi.org/10.1155/2024/5466604.

Roy, L.A., Davis, D.A., Saoud, I.P., Boyd, C.A., Pine, H.J. and Boyd, C.E. (2010). Shrimp culture in inland low salinity waters. *Reviews in Aquaculture*. **2(4):** 191-208. https://doi.org/10.1111/j.1753-5131.2010.01036.x.

Shi, M., Yao, X., Qu, K., Liu, Y., Tan, B. and Xie, S. (2023). Effects of taurine supplementation in low fishmeal diet on growth, immunity and intestinal health of *Litopenaeus vannamei*. *Aquaculture Reports*. **32:** 101713. https://doi.org/10.1016/j.aqrep.2023.101713.

Singha, K.P., Shamna, N., Sahu, N.P., Sardar, P., Harikrishna, V., Thirunavuk-karasar, R., Chowdhury, D.K., Maiti, M.K. and Krishna, G. (2021). Optimum dietary crude protein for culture of genetically improved farmed tilapia (GIFT), *Oreochromis niloticus* (Linnaeus, 1758) juveniles in low inland saline water: Effects on growth, metabolism and gene expression. *Animal Feed Science and Technology*. **271:** 114713. https://doi.org/10.1016/j.anifeedsci.2020.114713.

Talukdar, A., Dharmendra Deo, A., Prasad Sahu, N., Sardar, P., Aklakur, M., Harikrishna, V., Prakash, S., Shamna, N. and Jana, P. (2021). Effects of different levels of dietary protein on the growth performance, nutrient utilization, digestive enzymes and physiological status of white shrimp, *Litopenaeus vannamei* juveniles reared in inland saline water. *Aquaculture Nutrition*. **27(1):** 77-90. https://doi.org/10.1111/anu.13166.

Thirunavukkarasar, R., Kumar, P., Sardar, P., Sahu, N.P., Harikrishna, V., Singha, K.P., Shamna, N., Jacob, J. and Krishna, G. (2022). Protein-sparing effect of dietary lipid: Changes in growth, nutrient utilization, digestion and IGF-I and IGFBP-I expression of genetically improved farmed tilapia (GIFT), reared in inland ground saline water. *Animal Feed Science and Technology*. **284:** 115150. https://doi.org/10.1016/j.anifeedsci.2021.115150.

To, V.A., Liou, C.H. and Yang, S.D. (2021). Can dietary with a taurine supplement improve lipid utilization, growth performance, haemolymph parameters and immune responses of white shrimp (*Litopenaeus vannamei*). *Aquaculture Research*. **52(12):** 6612-6625. https://doi.org/10.1111/are.15532.

Wooten, I.D.P. (1964). Microanalysis in medical biochemistry. J and A Churchill Ltd, London. pp. 101-103. https://www.cabidigitallibrary.org/doi/full/10.5555/19651405102.

Wrobleiuski, F. and Laude, J.S. (1955). LDH Activity in Blood. *Proceedings of the Society for Experimental Biology and Medicine*. **90:** 210-213.