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The Meta-Analysis of Aminoacids and Minerals in the Fish *Liza parsia* (Hamilton 1822) Parasitized with the Isopod, *Norileca indica*

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Abstract The aim of the present investigation was to study the effect of parasite infestation of the crustacean isopod, *Norileca indica* in the fish, *Liza parsia* (Goldspot Mullet). The crustacean parasitic isopods affect the economically important fishes. Due to the infection, the host loses its weight, growth, and physiological inactivity, which eventually leads to death. Important biochemical parameters such as aminoacid profile, elements, and minerals were estimated. Arginine, isoleucine, methionine, leucine, and valine were found to be reduced by 0.5, 0.8, 0.6, and 0.7% in the parasitized fish. Significant reductions in lysine and phenylalanine by 1.2 and 1.6% respectively were noticed in the parasitized fish. No change in the tryptophan level was noticed. The aspartic acid and glutamine were also significantly reduced by 1.4 and 2.3 Wt/% respectively. The minerals like phosphorous and iron have increased significantly by 7.27 and 0.03 mg/100 gm respectively in parasitized fishes, whereas the minerals such as calcium, potassium, sodium and magnesium levels were found to be reduced in the parasitized fishes. No significant difference in manganese was observed. The amino acid and mineral content alterations observed in the infested fishes are reflecting, anaemia, tissue damage and disturbances in physiological homeostasis of fish physiology and biochemistry. Parasite infestation leads to the poor quality and flavour of the fish.

Keywords *Liza parsia*, Crustacean Isopod, Phenylalanine, Tyrosine, Tryptophan, Aspartic Acid, Glutamine Phosphorous, Magnesium, Anaemia

1. Introduction

Parasitisation causes morbidity and mortality to host animals and notable heavy economic loss to the fishing industry [1] due to consumer rejection of parasitized fish dishes on the table menu. Isopods are the crustaceans that exist in both aquatic and terrestrial life forms. Aquatic isopods are categorized into three major groups namely Cymothoids, Epicardians, and Gnathiids. The family Cymothoidae is the largest family, having several genera and species. However, the other families such as Sphaeromatidae, Cirolanidae and Bopyridae also possess larger diversity next to Cymothoidae [2,3]. Marine isopod-infected fishes showed significant variation in amino acids and minerals content. The infected fishes may lead to reflect anaemia and tissue damage caused by parasite attack and make a path for the invasion of pathogenic microorganisms. These Cymothoids, Epicardians and Gnathiids parasitize on most of the commercially important fish families such as Mugilidae

(Jarocki, 1822); Atherinidae (Risso, 1827); Serranidae (Swainson, 1839); Carangidae (Rafinesque, 1815); Sciaenidae (Cuvier, 1829); Embiotocidae (Agassiz, 1853); Bothidae (Smitt, 1892), Clupeidae (Cuvier, 1817); Pleuronectidae (Cuvier, 1816); Scombridae (Rafinesque, 1815) and Haemulidae (Gill, 1885). Parasitic isopods cling to host organs or on the skin's surface tightly with seven pairs of legs and pierce the muscle with the help of sharply curved hook-like dactyli. The mouth cone is highly adapted for piercing and sucking types of nutrition. The host animals cannot usually escape after the isopod attachment and infestation until the death of the parasite.

Cymothoid isopods are mostly attached to specific sites on the host viz., the skin surface, gills, and buccal cavity. Buccal cavity attack eventually leads to the replacement of the host tongue. The attachment besides to fins for getting better protection, nourishment and good contact with the external environment than other sites of attachment. It partially invades the host tissue and exploits the vital resources from the host's body fluid, such as blood in the most effective manner for its growth; resulting in serious physical and physiological damages, such as reduced growth, impaired reproduction, behavioural alterations, and in extreme cases, it leads to death [2]. Biochemical analysis of amino acids, minerals, and health status had been done on parasitized fishes during the adverse stress condition [4]. The effects of parasites on fishes include nutrient devaluation [5], alteration of biology and behaviour [6], lowering of immune competency, induction of blindness, morbidity, mortality, growth and fecundity reduction [7] and physical injuries impact depending on the parasitic types and numbers on the host [8]. Numerous studies on the biology, ecology, and parasites of the Vellar estuary's fishes have been published [9,10,11,12,13]. However, no study has been done on the differences between the parasitized and non-parasitized fishes of the Vellar estuary with special reference to variations in the quantity of amino acids, elements, and minerals.

2. Materials and Methods

Vellar Estuary

Healthy and parasitized marine teleost fish, *Liza parsia* were collected from Vellar estuary (Lat.11°29 N and Long 79°46 E), Parangipettai on the southeast coast of Tamil Nadu, India.

Parasitological Examination

The parasitological examination was carried out for the identification of external parasites on the buccal cavity, fin and all surfaces of the body. The tissue, liver and blood samples were collected immediately at the fresh sub-lethal condition of both non-parasitized and parasitized fishes for various analysis. Fish that attained the rigor mortis were discarded and all isopod parasites were removed from hosts. Isolated parasites were stored in 70% ethanol and brought to the laboratory for identification based on literature data [14,15,16,17]. The sample size of the fish was 20 and the weight of the fish was in the range of 151-200 g, with 10-12 cm, and 200 –250 g, with 11-15 cm. Tissue samples collected from infected and non-infected areas of both parasitized and non-parasitized fish were used in the quantification of amino acids, elements and mineral.

Amino Acid Analysis

Briefly, 100g of homogenized tissue was taken in an Eppendorf tube. 250 µL of methanol and 125 µL of chloroform were added. The tube was stored in a dark place for one hour and it was shaken in a vortex at 15 min intervals during the incubation. After the incubation 380 µL of chloroform and 90 µL of KCl 0.2 M were added then it was shaken in a vortex next it was centrifuged for 10 min at 15000 rpm at 4 °C. The hydrophilic phase was decanted into another vial and dried by using a nitrogen stream. 50 µL of methoxamine pyridine solution (104 mg/L) was added to the vial and the solution was incubated in a dark place for 17 h to obtain derivatization. Before the gas-chromatography analysis, 100 µL of MSTFA (N-Methyl-N-(trimethyl silyl trifluoro acetamide) was added to the vial, followed by a shake of 3 min in a vortex and kept for 1 h in a dark place. 600 µL of dodecanone solution (20 mg/L) in hexane (IS) was added to the vial.

Samples were injected (1 µL) without splitting or gap into a 6850 gas-chromatograph coupled with a 5973 mass-spectrometer (Agilent Technologies, Santa Clara, CA, USA). The injector temperature was maintained at 200 °C, and the gas flow rate was 1 mL/min. The column was a DB5-MS capillary fused silica column (0.25 µm, 30 mx 0.25 mm ID; J&W Scientific, Folsom, CA, USA). The initial temperature maintains at 50 °C (10 min), then it increased to 300 °C gradually at 1 min interval time and kept for 10 min. Ionization occurred at 70 eV in EI+, over m/z 50–550 mass range. Identification of free amino acids was done by using 20 Amino Acids Kit (19 L amino acids + Glycine) kits containing all standard L-amino acids and the NIST08 mass spectra library

The collected blood serum sample of parasitized and non-parasitized fishes used directly with prescribed dilution and analyzed in Flame atomic absorption spectrometry (FAAS) Major elements (Na, K, Ca, Mg) determined by direct FAAS after 1:50dilution with distilled water in Graphite furnace flame. Ca and Mg were mixed with 1% sodium EDTA, 0.5% lanthanum in hydrochloric acid added to suppress phosphate interference in the air-acetylene flame. For Minor Trace elements Fe, Cu and Zn were determined directly by FAAS in the Graphite furnace flame. Zn and Mn were determined by mixing aqueous standards using 1:5 and 1:2 diluted serum with distilled water. Different Spectrum

wavelength used for the appropriate element analysis Viz; (nm) Ca-422.7, P-213.6, K-766.5, Na-589.0, Fe-248.3, Mg-285.2, Mn-280.1, Zn- 213.8, Cu-324.7, I-304.0. (Model-Atomic absorption spectrometer contrAA 800)

The ash content was obtained by using the AOAC Official Method of analysis. 2 gm of tissue sample from the same place of both infected and non-infected fishes were kept in the two different Silica crucibles; the weighed silica crucible was placed over an electric burner. Crucible in a muffle furnace heated up to 600°C for 2 hr, then crucible removed from the burner and kept in desiccators for cooling them to room temperature.

Ash content was calculated using the following formulae:

Calculation Ash content (%) = (Z – X /Y – X) x 100 Weight of empty crucible - X g Weight of crucible + sample - Y g after complete ashing, Weight of crucible + ash - Z g [18].

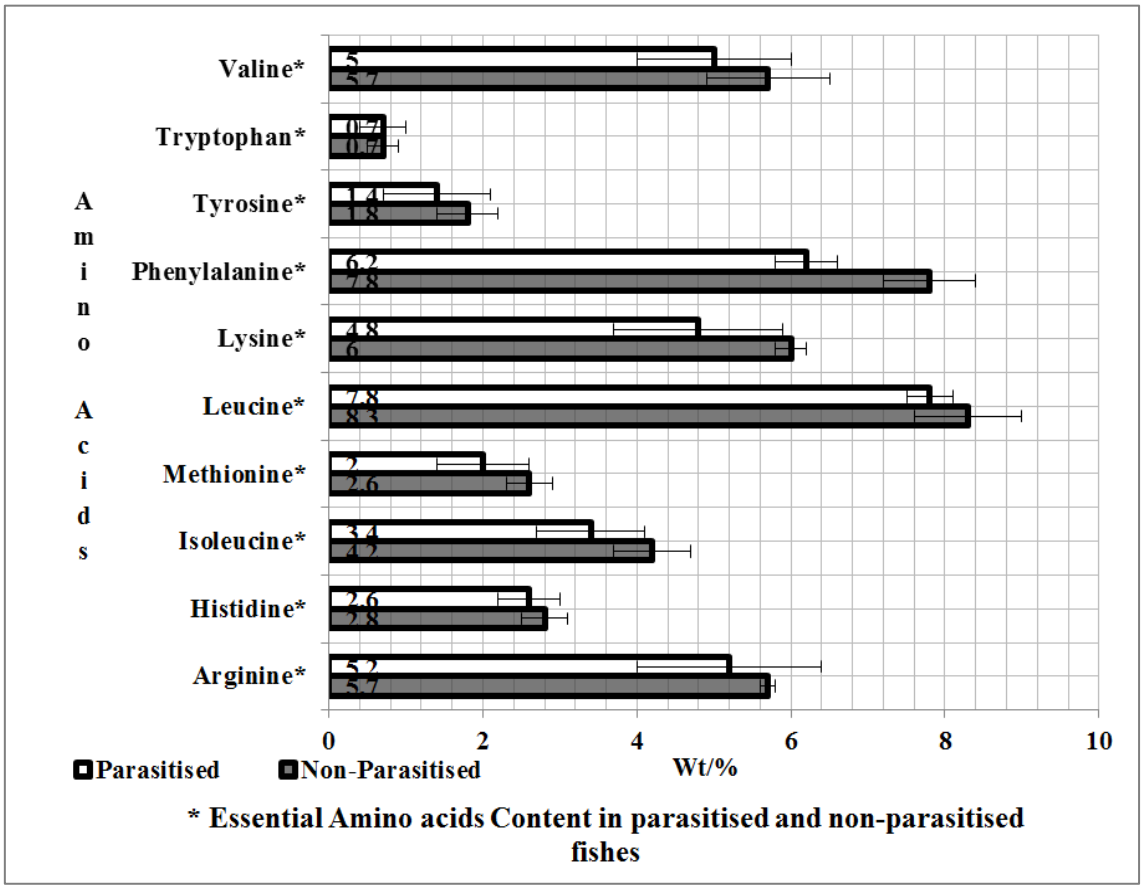
Moisture Content Analysis

The wet tissue sample was collected from both infected and non-infected fish. The weighed samples were placed in two different porcelain cups and covered with lid and kept in a microwave oven for 24 h at 105°C. Then it was weighed for moisture calculation. Moisture content was calculated using the following method: Moisture content (%) = W2 - W3 x 100 W2-W1 where, W1 = weight of porcelain cup with lid; W2 = weight of porcelain cup with lid and sample before drying; and W3 = weight porcelain cup with lid and sample after drying [18].

Statistical Analysis

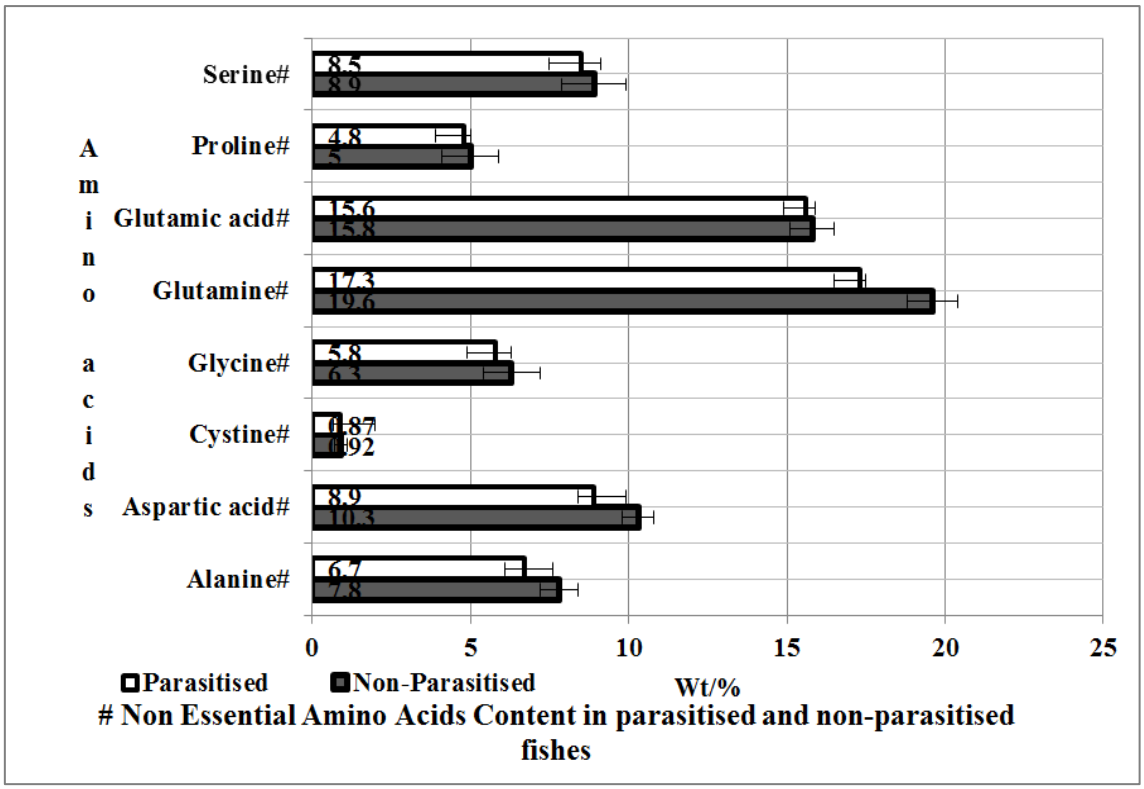
Differences in Aminoacids, minerals and element parameters between the parasitized and non-parasitized fishes were statistically analyzed by Unpaired T-test. The mean and standard error (SEM) were calculated for each parameter. All the statistical analyses were computed by using the Prism v. 4.00 statistical software (GraphPad Software Ldt., USA, 2003).

3. Results



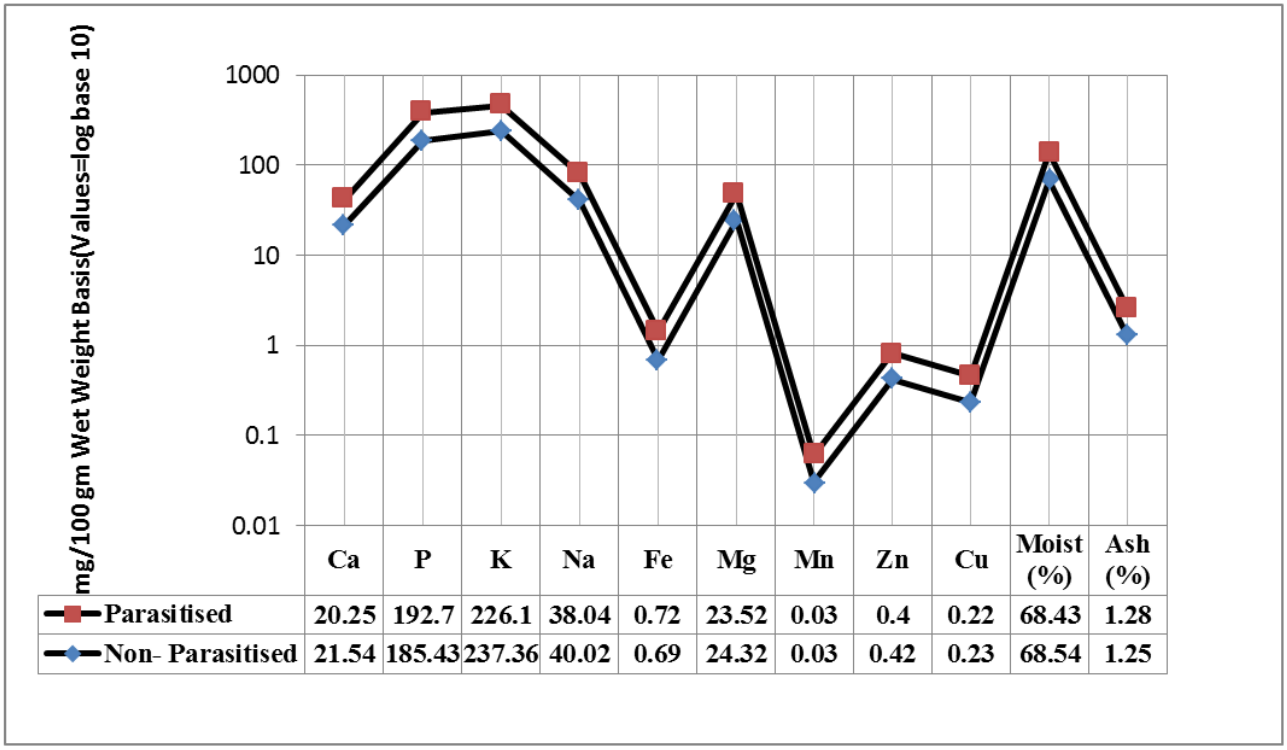
*EAA-Essential amino acid, # NEAA- Non-essential amino acid, All values are expressed as a percentage (%) of total amino acids.

Figure 1. Comparison of Free Essential amino acid composition in non-parasitized and parasitized fish *Liza parsia* with isopod *Norileca indica* (wt %) (wt Values are mean ± SE (n=20), P< Value=0.05



*EAA-Essential amino acid, # NEAA- Non-essential amino acid, All values are expressed as a percentage (%) of total amino acids.

Figure 2. Comparison of Free Non- Essential amino acid composition in non-parasitized and parasitized fish *Liza parsia* with isopod *Norileca indica* (wt Values are mean ± SE (n=20), P< Value=0.05



	Ca	P	K	Na	Fe	Mg	Mn	Zn	Cu	Moist (%)	Ash (%)
Parasitised	20.25	192.7	226.1	38.04	0.72	23.52	0.03	0.4	0.22	68.43	1.28
Non- Parasitised	21.54	185.43	237.36	40.02	0.69	24.32	0.03	0.42	0.23	68.54	1.25

Figure 3. Comparative Mineral composition of non-parasitized and parasitized fish *Liza parsia* with isopod *Norileca indica* (wt %) * (wt Values are mean ± SE (n=20), P< Value=0.005

In the present study, the changes in the composition of amino acids, elements and minerals between the *L. parsia* parasitized with the isopod *N. indica* and non-parasitized fishes were analyzed. The results revealed the following: No significant variation in the essential amino acids histidine and tyrosine which were reduced by 0.2 and 0.4 Wt/% respectively in parasitized fish. Similarly, Arginine, isoleucine, methionine, leucine and valine were also found to be reduced by 0.5, 0.8, 0.6, 0.5, 0.7 Wt/% respectively in parasitized fish. However, the lysine and phenylalanine were found to be reduced significantly by 1.2 and 1.6 Wt/% respectively in parasitized fish. Among essential amino acids, the tryptophan level remained the same in both parasitized and non-parasitized fish.

The non-essential amino acids both glutamic acid and proline were observed to be reduced by 0.2 Wt/%; serine by 0.4 Wt/%; glycine and cystine by 0.5 Wt/% and alanine by 0.9 Wt/%. The aspartic acid and glutamine were reported to be significantly reduced by 1.4 and 2.3 Wt/%.Among the amino acid profile lysine, phenylalanine, and aspartic acid were significantly reduced by more than 1 Wt/% and glutamine was reduced to the highly significant level of 2.3 Wt/% in *N. indica* parasitized fishes as compared to non-parasitized fishes in the present comparative analysis.

In the present study the quantity of macro elements such as potassium, and phosphorus were 237.36 and 185.43mg/100gm wet weight respectively which were found to be very high. Among the other macro elements, Sodium and calcium were estimated as 40.02 and 21.54 in non-parasitized fishes and 38.04 and 20.25 mg/100gm in parasitized fishes respectively. The micro elements such as iron, magnesium and manganese were 0.69, 24.32 and 0.03 mg/100gm, respectively, in the wet weight of non-parasitized fish.

Mineral composition showed a decreased trend in parasitized fishes when compared with non-parasitized fish except for phosphorus and iron. The C, K, Na, Mg, Zn, and Cu have been found to be reduced with a difference of 1.29, 11.26, 1.98, 0.8, 0.02, 0.01mg/100 g respectively from the total weight of 21.54, 237.36, 40.02, 0.69, 24.32, 0.42, 0.23 mg/100 g wet weight. However, the minerals P and iron have increased significantly by 7.27 and 0.03 mg/100gm from185.43, and 0.69 mg/100 g wet weight in *N. indica* parasitized fishes. The other minerals such as calcium, potassium, sodium and magnesium levels were reduced in parasitized fishes. There was no difference in the manganese level of both sets of fish. The moisture content ranged between 68.54 and 68.43% with a mean of 68.49% between parasitized non parasitized fishes. The ash content ranged between 1.25% and 1.28%, and the mean crude ash in both sets of fish was 1.27% (%).

4. Discussion

Amino acids are mainly obtained from proteins in diet and the quality of dietary protein is assessed from essential to nonessential amino acid ratio. High-quality proteins are readily digestible and contain the dietary essential amino acids (EAA) in quantities that correspond to human requirements [19]. Fishes are the only animals that have abundant, high-quality, low-cost, easily digestive, and quickly available amino acids to the non-vegetarian. [20].

In the present study, the differences in the quantity of amino acids and minerals of non-parasitized fish *L. parsia* and fish parasitized with *N. indica* were analyzed. Among all the essential amino acids estimated, the quantity of all amino acids except tryptophan was found to be decreased in the parasitized fish. Tryptophan remained almost the same in both fish. Statistical tool 't'test revealed that a significant decrease in the quantity of phenylalanine and lysine was observed in the parasitized fish when compared with that of the non-parasitized fish. Similarly, the quantity of all non-essential amino acids except cystine also decreased in parasitized fish than that of non-parasitized fish. No change was observed in the quantity of cystine in both parasitized and non-parasitized fish. Glutamine and aspartic acid were observed to be decreased in the parasitized fish than that in the non-parasitized fish. Though *N. indica* is a branchial parasite causing branchial cavity and gills of the fish, it reduces the respiratory metabolism [21] leading to a decrease in movement and other physiological activities. The decrease in the quantity of all aminoacids might be correlated with the decreased rate of digestibility, absorption and assimilation due to the infestation of the isopod parasite. This view could be supported by the report of Sitjà-Bobadilla *et al.,* [22] that the intestinal myxozoan parasite *Entero myxumleei* dwells between gut epithelial cells and causes severe enteritis in gilthead sea bream (*Sparusaurata*), anorexia, cachexia, growth impairment, they affect the marketability and increased the mortality**.** In contrary with the present report, the free amino acid content showed an increase due to the effect of parasitic infection in the tissues of *Catla catla* and *Labeo* sp. [23].

Most amino acids such as glutamic acid, aspartic acid, alanine, and glycine are important because they are determining the taste and flavour [24]. The high content of aspartic acid, glutamic acid, lysine, arginine, and leucine is a good source of proteins [25,26]. The aminoacids such as proline, arginine, lysine, alanine, histidine, glutamic acid, and taurine are the rich source of nitrogenous compounds [24,25,26]. The delicious nature of fish muscle tissue is due to the presence of plenty of amino acids. Amino acids provide tissue healing and growth [27]. Since the quantity of all aminoacids in parasitized fish in this study was lowered than that of the non-parasitized fish, the flavour and quality of parasitized fish would be very low. Lysine is an EAA that is extensively required for optimal growth and its deficiency leads to immunodeficiency [28]. So the lowering of lysine in parasitized fish might lead to immunodeficiency.

Glycine is involved in the skin collagen synthesis, and promotes the tissue re-growth through tissue healing by combining with other essential amino acids such as alanine; Leucine promotes the healing of bones, skin and muscle tissue;

A significant reduction in glutamine of the parasitized fish might lead to the poor growth of the fish. This can be ascertained by the following facts. Glutamine is essential for cell signalling. The deficiency of glutamine leads to intestinal atrophy. Glutamate plays a role in the intestinal health of fish by providing enterocyte development. The intestine and gills are the essential organs for the absorption of all nutrients in digested food, the glutamine deficiency may affect the absorption of nutrients in the alimentary canal in animals [30]. Glutamine is an important abundant amino acid nearly 60% of the constituents of skeletal muscle [31] and is essential for the proper functioning of many organ systems [32].

Arginine is involved in transporting the ammonia (nitrogen) from muscle to the splanchnic area and immune system [33] and an antioxidant response was reported by Coutinho *et al.,* [34]. However, the isopods down-regulated the host nucleotide metabolism by secreting the unknown molecules to degrade the nucleotide and prohibit the homeostasis and inflammatory response [35]. Though glutamine is being reduced in the parasitized forms it is not below the threshold level to affect the cellular metabolism of the fish. Gnathiid isopods *(Gnathiaaureus maculosa*) artificial infection on client Fish (*Scolopsis bilineatus*) in laboratory condition exhibits the elevated blood cortisol hormone level [36,37] in the host fish and induced gluconeogenesis through glucogenic amino acids such as alanine, histidine, glutamine, and proline were utilized in the TCA cycle pathway to generate the energy. The NEAA Aspartic acid is synthesized from Oxaloacetic acid through the transamination process. It is involved in the formation of urea by donating the amine group in the ornithine cycle, it donates one nitrogen atom for the synthesis of inosine which is the precursor for the purine metabolism and acts as a hydrogen acceptor in ATP synthase [29]. May such a crucially important role have led to the high utilization of aspartate in parasitized fishes.

Arginine and phenylalanine increase the GTP and regulate the Nitric oxide (NO) synthesis. This NO pathway is regulated by several other amino acids such as lysine, glutamic acid, glutamine, proline and homo cysteine which are exerting their physiological and pathological effects in fishes. In this study, the above-mentioned amino acids showed a reduced level in parasitized fishes.

Tryptophan is mainly required for the neurological and has less role in immunological functions [38] such as antitoxic, anti-inflammatory agents, modulating fish behaviour, and stress responses through multiple metabolites including serotonin and 5HT-melatonin [39]. Two-fold level supplementation of tryptophan may require for stress fish for the increased disease resistance and unaffected stress-free fishes required for compromised immune status and disease resistance [40]. In our study, both fishes exhibit the same level of tryptophan might be due to increased disease resistance in infected fish and compromised immune conditions, and disease resistance in isopod-free fishes. Both ash and moisture results are less agreed with Osibona *et al.,* [41].

In this study, reduced levels of calcium and phosphorous were observed in parasitized fish. This is similar to the report of Heagney Elizabeth *et al.,* [42] that *Trachurus novaezelandiae* infected with an ectoparasite blood-feeding isopod *Ceratothoa sp.* exhibited a reduced concentration of Calcium, and magnesium when compared with the uninfected fish group. Since both calcium and phosphorus are essential for skeleton formation might lead to poor growth of bones and thus reduced activity. The lower amount of calcium and phosphorus in the parasitized form may be due to leaching out of the bones and their consequent seepage to the blood plasma and hemolymph to meet out the stress caused by the parasite.

In the present study, the trace elements copper showed no significant deviation in the parasitized fishes compared to the control. Copper (Cu) and iron (Fe) and Isoleucine are essential for the formation of haemoglobin [43]. The reduced Cu level in the fish led to anemia, reduced growth, and enzyme activities such as liver Cu, Zn, superoxide dismutase [SOD], heart cytochrome c-oxidase, spinal cord, cranial malformations, decreased length, delayed yolk absorption, edema, low pigmentation cataract formationetc., [44]. The shrimp Palaemon, *Tesargentinus nobili* superoxide dismutase [SOD] activity had highly reduced with the infection of isopod *Probopyrus ringueleti* and no reduced activity was observed in the other enzymes such as catalase [CAT], and glutathione peroxidase [GPx] [45]. Ceruloplasmin Copper serves as a prosthetic part of several coenzyme systems such as cytochrome oxidase and tyrosinase levels have been reduced in 14 freshwater and marine water fishes due to Cu deficiency feeds [46]. Copper has got synergistic function with certain amino acid-like glutamine and glutamate. Copper is an essential part of the copper/zinc superoxide dismutase, a key enzyme in the defense mechanism against reactive oxygen species [ROS] which allows it to act as a free-radical scavenger. Such anti-oxidative properties can prove as crucial necessities at times of high stress [43]. It may have differences in copper levels and glutamate levels between the parasitized and non-parasitized fishes.

Numerous aspects of cellular metabolism are zinc-dependent. A decrease of zinc in parasitized fish might cause the loss of appetite, growth reduction, and skin and immunological abnormalities (National Research Council Recommended dietary allowances, 1989). In the light of the above information, the results of the present study construe that all three substances were affected by the isopod parasite over the gill of the fish host.

The iron content was reduced in parasitized fishes. The iron level has been reduced in the host according to the parasite population. Iron is the microelement responsible

for the synthesis of haematological related proteins such as hemeprotein, hemoglobin and cell respiratory physiology enzymes such as NADHase and succinate dehydrogenase. Isopod Parasitic infection and continuous absorption of blood might have resulted in the reduced volume of blood and haematocrit as reported by Jones and Grutter [47]. The magnesium (Mg) level was not significantly reduced. The Mg deficiency leads to the cause of inflammation in fish [48].

5. Conclusions

The infestation of isopod parasite *Norileca indica* in Goldspot Mullet, *Liza parsia* reduced the quality and flavour of fish. This was mainly due to the lowering of all amino acids and minerals which are required for the normal growth and metabolism of the fish.

Compliance with Ethical Standards

In this study, no live animals were used. According to the IUCN, *Liza parsia* is the least concerning fish in India, there is no such permission required for this type of commercial fish used to conduct estimations or assays. All the parasitized and non-parasitized fish were procured a couple of hours after the fishing boat and initiated the above estimations and assays in very fresh condition without rigour mortis.

Funding for This Work

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Conflict of Interest

No conflict of Interest with anybody else.

Authors' Contribution Statement

The concept of the study was obtained from the first author's doctoral degree. The Author collected the sample directly from the Vellar fishing area, and biochemical analysis of the sample has been done in CAS in Parangipettai, Annamalai University research Lab. Chart and table have been done in MS office package. The Corresponding author has guided the materials methods and discussion work. Dr. K. Ramalingam, Former Scientist, Mediclone biotech, and Dr. A. Karthikeyan, Associate Professor and Head, PG & Research Department of Zoology, Government Arts College (Autonomous), Karur helped with the discussion and proof-reading.

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