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Implications of Certain Biochemical Metabolites and Meta-analysis on the Fish *Liza parsia* (Hamilton, 1822) with Isopod Parasite *Norileca indica*

Babu K.[1] and Ravichandran S.[2,3]*

[1]P.G and Research Department of Zoology, Government Arts College (Autonomous), Karur 639 005, Tamil Nadu, India
[2]Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai 608 502, Tamil Nadu, India
[3]Government Thirumagal Mills College, Gudiyattam, Vellore, India

**Corresponding Author*

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Abstract: *Liza parsia* (Goldspot Mullet) is an important fishery resource in estuarine areas. This fish is in high demand among the local consumers due to its affordability in price and deliciousness. Unfortunately, this fish population is also highly affected by crustacean isopod parasites. One of the most important parasites is *Nerolica indica* which causes economic loss to fishermen. The mechanical damages to parasitized fish and secondary biochemical metabolites in the diseased fish will cause serious complications to consumers. The obligate isopod ectoparasites vary in size, and many of them continue their complete or partial life cycle in the host and affect the host's anatomy and physiology. The isopod parasites attach to the body surface, buccal cavity, and branchial cavity. These surface-attached parasites scratch the skin and feed on the blood; buccal cavity parasites often cut the tongue and replace it; the brachial cavity parasites harbour between the gills and make a serious effect on fish respiration. The affected fish loses its weight and body fluid continuously and eventually dies. The aim of this study was primarily to assess the biochemical variations between healthy and parasite-infested fish. A significant reduction in total proteins, glucose, and cholesterol levels were observed in the infected fish. The monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) of parasitized fish were also significantly reduced when compared to non-parasitized fish. The objective of this study was to analyse these biochemical metabolite's variations and critically assess the metabolites roles in the physiology and biochemistry of the infected fish.

Keywords: Estuary, Ectoparasite, Glucose, Squalene, Cholesterol, Monounsaturated fatty acid, Polyunsaturated fatty acid

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Introduction

It is known that most of the living organisms are endowed with a symbiotic association which is differently designated as mutualism, commensalism, parasitism, exploitation, etc. (de Bary, 1879). These parasitic associations, unlike other pathogenic bacteria and viruses that kill the host organism, bring about a dynamic host-parasite interrelationship. It is advantageous to both the host and the obligate parasite since the death of the host will make the survival of the parasite differently and similarly, the parasites also thrive with a minimum threshold load of infection and enable the host to survive (Noble *et al.*, 1989). Isopods are crustaceans that exist both in terrestrial and aquatic habitats and also live as independent organisms or obligatory parasites on some host species of fish (Rameshkumar and Ravichandran, 2014). It is quite common that fluctuation of immunity depression and reduction of vital biochemical metabolites are due to bearable and minimal parasitisation. However, the magnitude of high-level parasitisation will cause a great loss to host health and reduce marketability if the host is a high priority food species for man. As the fish are endowed with certain biochemical parameters which help to bring about health to the human population, these biochemical parameters are of interest to analyze in fish lodged with parasites.

The isopod parasite *Norileca indica* represents the obligatory parasite of several pelagic marine species of fish belonging to the various 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; G. Cuvier, 1817, Pleuronectidae; G. Cuvier, 1816, Scombridae; Rafinesque, 1815 and Haemulidae; T. N. Gill, 1885 (Trilles *et al.*, 2013; Ravichandran *et al.*, 2019). Parasitisation causes morbidity and mortality in host animals, as well as significant economic losses in the fishing industry (Aneesh *et al.*, 2017). In recent years, awareness about the consumption of fish towards preventing certain human diseases has been realised. Several publications on the chemical composition of fish have documented the beneficiary aspects of the above chemical compounds mentioned per se (Khalili and Samples, 2018; Balami *et al.*, 2019). In this context, the omega-3 and omega-6 essential fatty acids, glucose, cholesterol, and squalene (Sumi *et al.*, 2018) are important parameters to assess both for the health of the fish and the health of the human population. Till now, no detailed studies were reported on the comparison of the lipid profiles of isopod parasite-infected and uninfected fish. However, major biochemical parameters were studied (Rajaram *et al.*, 2018). In the present study, the various fatty acid compounds, glucose, cholesterol, and squalene have been analysed in the non-parasitized fish *Liza parsia* and also in their parasitized counterparts.

Materials and Methods

Parasitized and non-parasitized fish *Liza parsia* (Hamilton, 1822) belonging to family Mugilidae, Jarocki, 1822 were collected from Vellar estuary (Lat.11°29 N and Long 79°46 E), Parangipettai, Tamil Nadu, Southeast coast of India.

Parasitological Examination:

The parasitological examination was carried out after capturing the fish from the estuary and identifying the external parasites on the buccal, branchial, and body surfaces of the parasitized fish. Attached isopod parasites were removed from the hosts carefully without damaging appendages and organs and preserved in 70% ethanol. Preserved isopod parasites were brought to the laboratory for the identification based on literature data (Trilles, 1969, 1994; Trilles *et al.*, 2011; Ravichandran *et al.*, 2011, 2019). The number of fish were 20 and the average fish sizes were 151-200 g, with 10-12 cm , and 200–250 g, with 11-15 cm.

Biochemical analysis:

The Biochemical estimation of glucose, total protein, and cholesterol were determined by

following standard methods (Folin and Wu,1920; Fawcette and Scott, 1960; Henry, 1960; Annino, 1976). Estimation of glucose in whole blood was done by the portable blood glucose analyzer (coenzyme (PQQ) electron acceptor method ACCU-Chek Active, Roche Diagnostics GmbH, and Mannheim, Germany). Estimation of total protein and cholesterol was done from the fresh blood sample collected from the parasitized and non-parasitized fish and centrifuged without EDTA for 10 min at 3000 rpm. The commercial kits (BioSystem, Barcelona, Spain- BIURET method for protein, Cholesterol Oxidase/Peroxidase for Cholesterol-Modified Abell-Kendall method) (Abell *et al.*, 1952) were used.

Fatty acids:

The fatty acid compositions of *P. parsia* were determined by Capillary Gas Chromatography (CGC) technique. For the determination of fatty acid composition, the fat samples were converted to their corresponding methyl esters by BF3-methanol esterification by the AOCS official method Ce-2-66 (AOCS, 1972-Helrich method). Therefore, fat samples were first hydrolyzed for 10 min adding the specified amount of 0.5 N methanolic sodium hydroxide and then fatty acids were methylated using BF3-methanol reagent. The obtained fatty acid methyl esters were dried with anhydrous sodium sulfate and stored under nitrogen in Teflon-capped vials at –20°C until analyzed (Helrich, 1990).

The fatty acid methyl esters were quantified by gas-liquid chromatography method using a capillary column, Ultra 2 (25 μm 0.32 μm 0.52 μm film thickness of 5% diphenyl and 95% dimethyl polysiloxane) and flame-ionization detector in HP-5890 series II gas chromatograph (Hewlett Packard, Waldron, Germany). Nitrogen was used as the carrier gas at a flow rate of 1.72 ml/min. Air and hydrogen flow rates were 400 and 30 ml/min, respectively. The detector and injector temperatures were chosen as 280 °C and 225 °C, respectively. The oven temperature was set to 150 °C for 5 min and heated to 225 °C with a heating rate of 5 °C/min and maintained at this temperature for 30 min. Peaks were identified by comparing the retention times with those of a mixture of standard methyl esters (Supelco 37 FAME Component, Cat. Number: 4-7885). Menhaden oil fatty acid methyl esters (Sigma Chemical Co. Ltd., Poole, UK) were also used as a secondary standard. All of the other chemicals used in the experiments were analytical grade (Merck, Darmstadt, Germany). The liver oil of both parasitized and non-parasitized *P. parsia* was collected and analyzed by AOAC (1975), specific gravity by Abbe's refractometer at 27±1 °C for squalene; 1 kg of raw visceral fat used and identified by TLC n-butanol: acetic acid: water (3:2:2) (Bernard and Sherma, 1982) and estimated by using Iatroscan TH-10 TLC/Analyser, MarkIII (Iatron Labs. Inc. Tokyo, Japan) with a flame ionization detector with 0.3 cm/sec scanning speed.

Statistical Analysis:

The biochemical parameters variation of both parasitized and non-parasitized fish was statistically tested with unpaired student's t test. Mean and standard error (SEM) were estimated for each parameter. All these statistical analyses were analysed by using the statistical software Prism v. 4.00 (Graphpad Software Ldt., USA, 2003).

Results

The biochemical parameters of parasitized *P. parsia* showed a significant reduction compared to non-parasitized samples. The total proteins were reduced to 2.08 ±0.051 from 3.43 ±0.10 (mg/dl). The glucose level was significantly reduced to 68.50 ±1.72 from 82.75±1.51 (mg/dl). The cholesterol level decreased to 168.7±2.45 from 192.6 ±3.16 (mg/dl). Squalene content was not significantly reduced, it was reduced to 47.2±0.18 from 47.3±0.14 (mg/100 g) (Table 1).

In this study, the non-parasitized and parasitized *P. parsia* were compared for the total proteins, glucose, cholesterol and fatty acid metabolites. The total proteins, glucose, cholesterol metabolites levels were reduced

Table 1: Biochemical parameters of the fish *Liza parsia* muscles: non-parasitized and parasitized with isopod *Norileca indica*

S. No.	**Biochemical parameters**	**Not parasitized**	**Parasitized**
1.	Total proteins (mg/dl)	3.43 ±0.10	2.08 ±0.051
2.	Glucose (mg/ dl)	82.75 ±1.51	68.50 ±1.72
3.	Cholesterol (mg/dl)	192.6 ±3.16	168.7±2.45
4.	Squalene (mg/kg visceral fat)	47.3±0.14	47.2±0.18

Values are mean ± SE (n=20), P< 0.05

Table 2: Comparison of the fatty acid composition (% of total fatty acids) of *Liza parsia* muscles that were not parasitized and parasitized with the isopod *Norileca indica*

S. No.	**Fatty Acid Components (%)**	**Non- parasitized**	**Parasitized**
1.	C 14:0 Myristic acid-s	3.1 ±0.3	3.0± 0.1
2	C 15:0 Pentadecylic acid-s	0.8±0.1	0.4±0.3
3.	C 15:1 Pentadeceonoic	0.4±0.3	0.4±0.1
4.	C16:0 Palmitic acd	18.6±1.2	15.2±1.0
5.	C 16 : 2 Hexadecadienoic Acid	1.5 ±0.3	1.4±0.1
6.	C 16 : 4 Hexadecadienoic Acid	9.6±0.5	5.6±0.7
7.	C 17:0 Heptadecanoic	1.8±0.2	1.2±0.3
8.	C 17:1 Heptadecenoic	0.1±0.1	0.1±0.2
9.	C 18:0 Oleic acid	1.9± 0.4	1.2±0.5
10.	C 18 : 1 Elaidic	9.6± 1.4	6.8±1.7
11.	C 18:2 Linoleic	1.8± 1.2	1.4±1.0
12.	C 18 : 3 Alba linolenic	0.5± 0.7	0.2±0.3
13.	C 18 : 4 Stearidonic acid	10.3± 1.6	8.2±1.4
14	C 20 : 2 Eicosadienoic acid	0.8 ±0.3	0.6±0.1
15	C 20 : 3 Dihomo-γ-linolenic acid	0.4 ±0.2	0.1±0.3
16	C 20 : 4 n-6 Arachidonic	1.7±0.6	1.4±0.4
17	C 20 : 5 n-3 Eicosapentaenoic acid	0.5±0.1	0.1±0.2
18	C 22 : 2 Docosadienoic acid	1.6 ±0.5	1.8±0.4
19	C 22 :6 n-3 Docosahexaenoic acid	1.8±0.1	1.6±0.3
20	ΣMUFA(Monounsaturated FA)	23.8 ±0.5	25.2±0.7
21	ΣPUFA (Polyunsaturated FA)	35.2±1.3	32.1±1.0
22	n-3 Fatty Acids (ω−3 fatty acid)	1.8±0.2	1.2±0.4
23	Saturated	28.0±0.4	25.4±0.2

Values are mean ± SE (n=20), P< 0.05

significantly in parasitized fish as compared to non-parasitized fish. However, the Squalene showed no difference in the two sets of fish (non-parasitized vs parasitized). The fatty acids comprised saturated fatty acid, unsaturated fatty acid, MUFA, PUFA and Squalene. The percentage of saturated fatty acid namely Pentadecyllic acid, Palmitic acid, Heptadecanoicacid, Oleic acids and n-3 fatty acids (omega-3 fatty acid) were reduced significantly in parasitized fish (Table 2) as compared to control (non-parasitized). However, the Myristic acid is not reduced significantly and differed by only 0.1% when compared to control. The unsaturated fatty acids namely Hexadecadieonic acid, Linoleic acid, alpha Lenolenic acid, Eicosadienoic acid, Arachidonic acid and Eicosapentaenoic acid were not reduced significantly in parasitized fish as compared to non-parasitized fish. However, the Pentadecanoic acid level has not changed. The MUFA and PUFA of parasitized fish were also significantly reduced when compared to non-parasitized fish.

Discussion

The general metabolism of fish required major bimolecular substances like carbohydrate, protein and lipid. According to Paul *et al*. (2015) aquatic animals' fatty acid profile is influenced by various

internal factors such as species, sex, age, and size; and external factors like diet, salinity, temperature, habitat, and rearing area. In the saturated fatty acids profile, the palmitic acid level is very high i.e. 18.6% in parasitized fish and it resembles with the previous records (Kaya *et al.*, 2008) and it is involved in the key role of energy providing fatty acid metabolism in all aquatic and terrestrial organism Mohanty *et al.*, 2016)**.** The arachidonic acid was low in parasitized fish due to over-infection and wounds in the epidermis that are created by parasites on the skin (Pompeia *et al.*, 2002). Arachidonic acid (C20:4 n-6) is involved as the precursor for the synthesis of prostaglandin (PG) and its derivatives such as PGF2a, PGE2, and PGI2, thromboxane and leukotrienes are three classes of arachidonic acid derivatives collectively called eicosanoids and some eicosanoids is obtained from the plasma membrane (Robert and Carroll, 2007). The prostaglandin (PG) is very essential for synthesis, proliferation, differentiation and development of the myoblasts (Korotkova and Lundberg, 2014) and involved in the acute inflammatory response in the infected organisms. The arachidonic acid derived metabolite lopoxin A4 has the notable wound and lesion healing capability during the skin cells rejuvenation of mammals (Börgeson *et al.*, 2012). The n-6 and n-3 PUFA are involved in the important structural biomolecules such as phospholipids in cell membranes, and key molecules for several physiological functions (Baie and Sheikh, 2000). The level of oleic acid (1.9 %) in non-parasitized fish is in agreement with the earlier reports (Swapna *et al.*, 2010; Jakhar *et al.*, 2012). Total protein, glucose, and lipid biochemical contents of host were reduced when compared with non-parasitized; these results correlated with the host infected with piscian tape worm Senga (Bhure *et al.*, 2011), tape worm *Postgangesia armata* infected fish (Dawood *et al.*, 2020). Similar result has been observed in isopod infected fish in the present investigation.

Blood glucose represents the immediate energy source in fish and liver involved in the regulative process to maintain the glucose level (Thau *et al.*, 2021). As the host of fish is living a pelagic life, the water currents in the marine environment may prompt their continuous muscular activity by swimming. The Client Fish (*Scolopsis bilineatus* Bloch, 1793) after 30 min of artificial exposure with the gnathiid isopods (*Gnathia aureamaculosa*) showed elevated blood cortisol hormone level. This acute parasitic stress causes hormone stress. Client Fish (*Scolopsis bilineatus*) has hypermuscular activity with a high level of cortisol level due to the infection of gnathiid isopods (*Gnathia aureamaculosa*) (Enes *et al.*, 2009). Cortisol induces hyperglycemia through gluconeogenesis and glycogen in the liver (Baynes, 2009). Hypermuscular activity caused insufficient supply of oxygen to tissues and the consequent mobilization of tissue glycogen in blood sugar. Since parasitisation in the fish represent a biotic association to the host-parasite interrelationship which may have induced the stress. In the present study, the blood sugar in the parasitized fish has significantly reduced as compared to non-parasitized fish. The parasitic micro environmental niche being the gill area of the fish and in direct contact with host blood circulation may not be unexpected, so the parasite would have derived the blood sugar of the host for their metabolic needs and existence.

The total proteins may represent both the soluble and insoluble and constitute the sparing energy source next to carbohydrate (Nellaiappan and Ramalingam, 1980). It decreased in the parasitized form which suggests that the protein may act as an extra energy source for the parasite. The considerable high-level presence of protein, carbohydrate and lipid content in non-parasitized fish was reported when compared to infected fish (Radhakrishnan and Nair, 1983). Cholesterol being a saturated lipid and multifarious functions and the standard level may indicate the health of the fish. The steroid hormone cortisol is synthesized in kidney by using cholesterol. This gnathiid load directly correlates with the cortisol level in the blood (Enes *et al.*, 2009). Trauma and stressful events for a prolonged period also increase the blood cortisol (Smith *et al.*, 2009).

Hence, in the parasitized forms cholesterol level decreased. This implies that the parasitized forms of the above hosts could have affected the integrated cellular metabolism of fish. The chronic utilization of cholesterol and blood sugar by the parasite combined with other environmental stress such as temperature, salinity acidity, alkalinity, pH, water conductivity, dissolved substances etc. may affect the health of the fish. The PUFA content of parasitized fish in the present study was noticed slightly higher than non-parasitized and it is in conformity with the study of marine *Sardina pilchardus* fish infected with copepod *Peroderma cylindricum* Heller, 1865 (Khaoula *et al.*, 2017), and blood sucking parasitized fish such as gar fish (*Belone belone,* Belonidae) (Rafika *et al.*, 2013) and other freshwater fish (Kenari *et al.*, 2009; Swapna *et al.*, 2010; Jakhar *et al.*, 2012). The PUFA levels in fish are directly related to increased vitamin E requirements in fish (Cowey *et al.*, 1985; Nassr-Allah *et al.*, 2012).

Vitamin E is one of the fat-soluble tocopherol antioxidant lipid compound which scavenging the free radicals. Free radicals are reactive oxygen species that causes the oxidative stress when the cells are exposed to the adverse environment and pathological states (Tappel, 1962; Parker, 1989; Pham-Huy *et al.*, 2008; Lobo *et al.*, 2010). Oxidative stress is responsible for cardiovascular disease, neurological disease, nephropathy, ocular disease, and inflammation and other co-morbidities. In all organisms including fish, the n-3 and n-6 fatty acids remain in dynamic balance and in a fixed ratio. When this balance is upset and tilt up, balance towards the n-6 category of essential fatty acid may bring ill effects to the organism, since they are acting as pro-inflammatory compounds. In this context our results suggested that parasitization affected the beneficial fatty acids. Hence, it may be expected that heavy infection may lead to the preponderance of the n-6 categories in the food species of fish. Considering the impact of environmental contamination alongside parasitisation, the pollutants may become super stress in the parasitized infected species and also these super stressor pollutant substances may make adverse effect to a consumer when such food species become the table menu of our diet. The disproportionate levels of essential fatty acid may induce inflammatory diseases to human population. In this study, in both parasitized and non-parasitized fish squalene amount was not significantly reduced. The squalene is an inevitable substance for the human welfare product synthesis and essential for normal biochemical, physiological and wellness of effective immunological mechanisms. Apart from the abundance in fish body, the squalene rich fish food helps us to mitigate health-related, issues. Squalene is a polyunsaturated hydrocarbon abundantly present in liver ((Hadinoto *et al.*, 2020) and it act as a free radical scavenging agent (Saint-Leger *et al.*, 1986; Danping *et al.*, 2021). The oxidative stress caused by the parasitic infestation co-morbidities are effectively challenged by the antioxidant property of lipophilic squalene which pass through the lipophilic abundant lipid bilayer membrane and protect the molecules such as lipids, proteins, DNA and cellular organelle damage (Hauβ *et al.*, 2002). Squalene is exploited and used in nutraceutical and pharmaceutical products production such as antioxidant, drug carrier, detoxifier, skin hydrating, protect from coronary artery diseases and aid to eliminate xenobiotic substances through bile secretion process etc. (Gohil *et al.*, 2019) .

A perusal of literature revealed that Squalene is an anticancer compound which provide vital anti-cancer properties, antioxidants activity and apoptosis of neoplastic cells (Lewkowicz *et al.*, 2006) and primarily used in supportive therapy for many types of cancers. Low incidence of cancer in Mediterranean people is due to the Squalene rich diets such as fish and olive oils. Thus, squalene used to load with RNA to deliver into target cells in order to maintain safety and non-toxicity to host cells (Güneş, 2013). In many clinical industries such as Amyris, Pfizer and Moderna; semi-synthetic Squalene lipid nano-particles are used as potent adjuvant in the production of yeast vaccines to human diseases

(Danping *et al.*, 2021), this may lead to the production of Zika virus and COVID-19 vaccines. This squalene adjuvant-based cell targeting mechanism research is still undergoing for the treatment of cancers in order to enhancing efficacy of anti-cancer drug.Deficiencies of above-mentioned supplementary elements in the parasitized fish intake results in specific inflammatory diseases. Inflammation is caused by the deficiency of mineral such as magnesium (Nielsen, 2018) and arachidonic acid in the diet and /or anti-inflammatory supplements suppress inflammation.

Chronic inflammation is the stepping stone for all chronic diseases. It is also a switching process and driver for the cancer diseases. Thus, fish food represents an important source for all important minerals and trace elements. It can be termed as anti-inflammatory supplement diet. In contrast to fish foods, the animal protein and dairy products are toxic to the body and are responsible for various ailments in human.

Conclusion

The infestation of isopod parasite *Nerolica indica* in *Liza parsia* reduced the quality and flavour of fish. This was mainly due to all important biochemical metabolites, amino acids, minerals and trace elements which are required for the normal growth and metabolism of the fish.

References

Abell LL, Levy BB, Brodie BB and Kendall FE. (1952) A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity. J Biol Chem. 195: 357-366.

Aneesh PT, Helna AK and Trilles JP. (2017) Occurrence and redescription of *Anilocra leptosoma* Bleeker, 1857 (Crustacea: Isopoda: Cymothoidae) parasitizing the clupeid fish *Tenualosa toli* (Valenciennes) from the Arabian Sea, India. Marine Biodiver. 49: 443-450.

Annino JS. (2003) Clinical chemistry, principles and procedures. Little Brown, Boston.

AOAC (1975) Official methods of analysis. (ed.) Horwitz W., 12th edition. Association of Official Analytical Chemists, Washington.

Baie SH and Sheikh KA. (2000) The wound healing properties of *Channa striatus*-cetrimide cream - tensile strength measurement. J Ethnopharmacol 71: 93-100.

Balami S, Sharma A and Karn R. (2019) Significance of nutritional value of fish for human health. Malaysian J Halal Res. 2(2): 32-34.

Baynes J and Dominiczak M. (2009) Medical biochemistry. Mosby Elsevier, ISBN 978-0-323-05371-6.

Bernard F and Sherma J. (1982) Applications of thin layer chromatography to different compound types. Marcel Dekker Inc. Newyork, pp. 212.

Bhure DB, Nanware SS and Kardile SP. (2011) Studies on biochemical profile of piscian tapeworm, *Tylocephalum*. Asian J Anim Sci. 6(2): 172-174.

Börgeson E, McGillicuddy FC, Harford KA, Corrigan N, Higgins DF, Maderna P, Roche HM and Godson C. (2012) Lipoxin A4 attenuates adipose inflammation. FASEB J. 26(10): 4287-4294.

Cowey C, Degener E, Tacon A, Youngson A and Bell J. (1984) The effect of vitamin E and oxidized fish oil on the nutrition of rainbow trout (*Salmo gairdneri*) grown at natural, varying water temperatures. British J Nutrition 51(3): 443-451.

Dawood MH, Al-Kallak SN and Al-Niaeemi BH. (2020). Detection of Alkaline phosphatase activity and protein content, isolated from *Bothriocephalus acheilognathi* tissues, A parasitic cestoda of Barbusesocinus fish (Heckel, 1843). 2nd AI-Noor International Conference for Science and Technology, Baku, Azerbaijan, pp. 132-138.

de Bary A. (1879) Die Erscheinung der Symbiose. Strassburg : Verlag von Karl J. Trübner.

Danping W, Wean SC, Nur A, Muhammad F and Kunn H. (2021) Selecting optimal pharmaceutical excipient formulation from life cycle assessment perspectives: A case study on ibuprofen tablet formulations. J Cleaner Prod. 292: 126074.

Enes P, Panserat S, Kaushik S and Oliva-Teles A. (2009) Nutritional regulation of hepatic glucose metabolism in fish. Fish Physiol Biochem. 35: 519-539.

Fawcette JK and Scott JE. (1960) Practical clinical biochemistry, 4th edition, Arnold Harold Varley, India.

Folin O and Wu H. (1920) Span diagnostic kits. J Biol Chem. 9: 341-367.

Gohil N, Bhattacharjee G, Khambhati K, Braddick D and Singh V. (2019) Engineering strategies in microorganisms for the enhanced production of squalene: advances, challenges and opportunities. Front Bioeng Biotechnol. 7: 50.

Güneş FE. (2013) Medical use of squalene as a natural

antioxidant. J Marmara Univ Inst Hlth Sci. 3(4): 220-228.

Hadinoto S, Idrus S, Smith H, Sumarsana and Radiena M SY. (2020) Characteristics squalene of Smallfin Gulper Shark (*Centrophorus moluccensis*) livers from Aru Islands, Mollucas, Indonesia. J Physics: Conf. Series 1463: 012021.

Hauβ T, Dante S, Dencher NA and Haines T. (2002) Squalene is in the midplane of lipid bilayer: implications for its function as a proton permeability barrier. Biochim Biophys Acta 1556: 149-154.

Helrich K. (1990) Official methods of analysis. 15th Edn., Association of Official Analytical Chemists, Virginia, USA.

Henry RJ. (1968) Clinical chemistry, principles and techniques. Harper and Row, New York. pp. 664-666.

Jakhar JK, Pal AK, Reddy AD, Sahu NP, Venkateshwarlu G and Vardia HK. (2012) Fatty acid composition of some selected Indian fish. African J Basic Appl Sci. 4(5): 155-160.

Kaya Y, Turan H and Erdem ME. (2008) Fatty acid and amino acid composition of raw and hot smoked sturgeon (*Huso huso*, L. 1758). Int J Food Sci Nutrition 59(7-8): 635-642.

Kenari AA, Regenstein JM, Rezai M, Tahergorabi R, Nazari RM, Mogaddasi M and Kaboli SA. (2009) Amino acid and fatty acid composition of cultured Beluga (*Huso huso*) of different ages. J Aquat Food Prod Technol. 18: 245-265.

Khalili TS and Sampels S. (2018) Nutritional value of fish: lipids, proteins, vitamins, and minerals. Rev Fish Sci Aquacult. 26(2): 243-253.

Khaoula T, Tarek H, Mohamed G, Ameur C and Mhamed EC. (2017) The parasitic copepod *Peroderma cylindricum* Heller, 1865 (Copepoda: Pennellidae) and its host *Sardina pilchardus* (Walbaum, 1792): trophic relationships as revealed by fatty acid profiles. J Crustacean Biol. 37(4): 453-457.

Korotkova M and Lundberg IE. (2014) The skeletal muscle arachidonic acid cascade in health and inflammatory disease. Nat Rev Rheumatol. 10(5): 295-303.

Lewkowicz P, Lewkowicz N, Sasiak A and Tchórzewski H. (2006) Lipopolysaccharide-activated CD4+CD25+ T regulatory cells inhibit neutrophil function and promote their apoptosis and death. J Immunol. 177(10): 7155-7163.

Lobo V, Patil A, Phatak A and Chandra N. (2010) Free radicals, antioxidants and functional foods: Impact on human health. Pharmacog Rev. 4(8): 118-126.

Mohanty BP, Ganguly S, Mahanty A, Sankar TV, Anandan R, Chakraborty K, Paul BN, Sarma D, Syama Dayal J, Venkateshwarlu G, Mathew S, Asha KK, Karunakaran D, Mitra T, Chanda S, Shahi N, Das P, Das P, Akhtar MS, Vijayagopal P and Sridhar N. (2016) DHA and EPA Content and Fatty Acid Profile of 39 Food Fishes from India. Biomed Res Int. 2016: 4027437.

Nassr-Allah HAH, Shabi FA and Mukhtar AK. (2012) Dietary vitamin E requirement for maximizing the growth, conversion efficiency, biochemical composition and haematological status of fingerling *Channa punctatus*. Aquacult Res. 43(2): 226-238.

Nellaiappan K and Ramalingam K. (1980) Prophenoloxidase and its activation in *Paraplerurus sauridae* (Digenea:Hemiuridae). Molec Biochem Parasitol. 2(2): 109-112.

Nielsen FH. (2018) Magnesium deficiency and increased inflammation: current perspectives. J Inflamm Res. 11: 25-34.

Noble ER, Noble GA, Schad GA and MacInnes AJ. (1989) Parasitology: the biology of animal parasites. Philadelphia, PA: Lea and Febiger.

Parker RS. (1989) Dietary and biochemical aspects of vitamin E. In: Advances in Food and Nutrition Research, (ed.) Kinsella J.E., Academic Press, San Diego, CA, pp. 157-232.

Paul BN, Chanda S, Sridhar N, Saha GS and Giri SS. (2015) Fatty acid profile of indian major carp. Indian J Anim Nutr. 32(2): 221-226.

Pham-Huy LA, He H and Pham-Huy C. (2008) Free radicals, antioxidants in disease and health. Int J Biomed Sci. 4(2): 89-96.

Pompeia C, Freitas JS, Kim JS, Zyngier SB and Curi R. (2002) Arachidonic acid cytotoxicity in leukocytes: implications of oxidative stress and eicosanoid synthesis. Biol Cell 94(4-5): 251-265.

Radhakrishnan S and Nair NB. (1983) Nature of crustacean infestation of fish along the south-west coast of India; distribution, mode of attachment to the host tissue and incidence and intensity of infestation. Acta Ichthyologica Piscatoria 13(2): 93-115

Rafika FB, Mohamed ABS, Hassen M and M'hamed EC. (2013) Impact of a blood-sucking parasite on the chemical composition of fatty acids in the white muscle of garfish (*Belone belone*, Belonidae) from Tunisian coasts (Central Mediterranean). African J Biotechnol. 12(44): 6335-6339.

Rajaram R, Rakesh Kumar K, Vinothkumar S and Metillo EB. (2018) Prevalence of cymothoid isopods (Crustacea, Isopoda) and proximate analysis of parasites and their host fish, South eastern India. J Parasitic Dis. 42(2): 259-268.

Rameshkumar G and Ravichandran S. (2014) Problems caused by isopod parasites in commercial fish. J Parasitic Dis. 38(1): 138-141.

Ravichandran S, Rameshkumar G and Trilles JP. (2011) New records of two parasitic cymothoids from Indian fish. J Parasitic Dis 35(2): 232-234.

Ravichandran S, Vigneshwaran P and Rameshkumar G. (2019) A taxonomic review of the fish parasitic isopod family Cymothoidae Leach, 1818 (Crustacea: Isopoda Cymothooidea) of India. Zootaxa 4622(1): 1-99.

Robert GC. (2007) Prostaglandins, thromboxanes and Leukotrienes, Elsevier's Integrated Physiol. 2007: 157-176.

Saint-Leger D, Bague A, Cohen E and Chivot M. (1986) Possible role for squalene in the pathogenisis of acne. I. In vitro study of squalene oxidation. Br J Dermatol. 114: 535-542.

Smith JL, Gropper SA and Groff JL (2009) Advanced nutrition and human metabolism. Belmont, CA: Wadsworth Cengage Learning. pp. 247. ISBN 978-0-495-11657-8.

Sumi ES, Anandan R, Rajesh R, Ravishankar CN and Suseela M. (2018) Nutraceutical and therapeutic applications of squalene. Fish Technol. 55(4): 229- 237.

Swapna HC, Kumar RA, Bhaskar N and Sachindra NM. (2010) Lipid classes and fatty acid profile of selected Indian freshwater fish. J Food Sci Technol. 47(4): 394-400.

Tappel AL. (1962) Vitamin E as the biological lipid antioxidant. Vitam Horm. 20: 493-510.

Thau L, Gandhi J and Sharma S. (2021) Physiology, Cortisol. https://pubmed.ncbi.nlm.nih.gov/30855827/.

Trilles JP. (1969) Recherches sur les IsopodesCymothoidae des côtesFrançaises. Aperçugénéral et comparatif sur la bionomie et lasexualité de cescrustacé, Bulletin de la Société Zoologiquede France 94: 433-445.

Trilles JP. (1994) Les Cymothoidae (Crustacea, Isopoda) du monde. Studia Marina 21/22: 5-288.

Trilles JP, Rameshkumar G and Ravichandran S. (2013) Nerocila species (Crustacea,Isopoda, Cymothoidae) from Indian marine fish. Parasitol Res. 112(3): 1273-1286.

Trilles JP, Ravichandran S and Rameshkumar G. (2011) A checklist of the Cymothoidae (Crustacea, Isopoda) recorded from Indian fish. Acta Parasitol. 56(4): 446-459.