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RESEARCH PAPER

## FATTY ACID PROFILE OF FRESHWATER SNAIL *Pilaglobosa* (SWAINSON, 1822)

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Publication date: 12.12.2024

### ARTICLE INFO

**Keywords:**

*Fatty acid,*  
*Omega,*  
*Pilaglobosa*

### ABSTRACT

*Pilaglobosa*, the Indian apple snail, is crucial in freshwater and grassland ecosystems. *P.globosa* exhibits a broad geographical distribution. *P.globosa* is considered the most cost-effective source of nutrients for indigenous populations and impoverished rural inhabitants. The fat of snails, similar to fat found in other animals, consists of three types of fatty acids: saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA). Ensuring an adequate intake of essential unsaturated fatty acids, namely polyunsaturated fatty acids are of utmost importance for a balanced diet. The fatty acid contents of *P.globosa* meat collected from the Chalanbeel region were analyzed. The gas chromatography method was used to determine the profiles of fatty acids. A proximate study reveals that snails possess a low fat level of 3.10% and a high protein content of 40.87%. The primary fatty acids were detected C14:0 (3.78%), C16:0 (16.85%), C17:0 (0.81%), C18:0 (5.88%), C20:0 (0.20%), and C24:0 (2.79%). The lipid composition consisted of 30.31% saturated fatty acids, 26.1% monounsaturated fatty acids, and 33.58% polyunsaturated fatty acids. EPA and DHA levels of *P. globosa* were 2.27% and 1.28%, respectively. The study's findings indicate that snails are a rich source of omega fatty acids.

Received 27 June 2024, Revised Received 31 October 2024, Accepted 14 November 2024

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## 1. Introduction

Snail flesh holds a prominent position due to its high nutritional content. Its chemical composition reveals its characteristics. Snail meat possesses a distinct advantage over other varieties of meat due to its composition, in addition to its favorable sensory features. Fat is a crucial macronutrient significant in delivering energy, with a high energy density of 9 kilocalories per gram. It is also the body's most efficient form of energy storage (Trumbo et al., 2002). The meat of *P. globosa* contains 3.10% fat content. Aquatic food webs are the primary suppliers of n-3 PUFAs (Gladysheva et al., 2012). They have a crucial function in biological processes and are among the most significant chemicals exchanged across the interface between plants and animals in aquatic food webs.

The families of  $\Omega$ -3 and  $\Omega$ -6 polyunsaturated fatty acids play a crucial role in vital physiological processes. Marine environments serve as their primary supply. Phytoplankton, the main source of organic matter and polyunsaturated fatty acids transport these fatty acids to consumers through food webs. The significance of fatty acids in aquatic environments mostly revolves around polyunsaturated fatty acids, specifically eicosapentaenoic acid (EPA, 20:5n-3), docosahexaenoic acid (DHA, 22:6n-3), and to a lesser degree, arachidonic acid (ARA, 20:4n-3). These fatty acids play a crucial role in maintaining human health. They originate from two physiologically different families of  $\Omega$ -3 and  $\Omega$ -6 fatty acids. The metabolic precursor for eicosapentaenoic acid and docosahexaenoic acid is  $\alpha$ -linolenic acid (ALA, 18:3n-3), while arachidonic acid is derived from linoleic acid (LA, 18:2n-6). It is widely known that animals and humans cannot produce both  $\Omega$ -3 and  $\Omega$ -6 PUFAs from scratch. However, they are necessary for regular development, growth, and optimal health. Humans can generate these substances internally, but the pace at which they are naturally synthesized is insufficient to meet the body's physiological needs. Hence,  $\Omega$ -3 and  $\Omega$ -6 PUFAs are crucial for vital physiological activities and must be obtained through dietary means. The positive impacts of  $\Omega$ -3 and  $\Omega$ -6 PUFA

supplementation in diets have been extensively confirmed for humans and aquatic animals (Stefanie et al., 2017; Endo and Arita, 2016). PUFAs have been attributed with numerous advantageous cardiovascular benefits, such as hypolipidemic, antithrombotic, antihypertensive, anti-inflammatory, and antiarrhythmic qualities, and the ability to lower blood pressure (Zhukova and Novgorodtseva, 2010). The efficacy of n-3 PUFAs in preventing cardiovascular diseases (CVD) relies on many molecular pathways, such as altering the structure of cell membranes (Witte et al., 2014; Novgorodtseva et al., 2013). Supplementing the diet with n-3 PUFAs has beneficial effects on the structure and function of the brain in elderly persons who are in good health (Kang et al., 2020). Moreover, there is no established correlation between fat consumption and a heightened susceptibility to stroke, colorectal, and ovarian malignancies (Yamine et al., 2020; Kim and Park, 2018; Metcalfe et al., 1996). However, it has been observed that high fat intake is associated with an elevated risk of breast cancer (Polak-Juszczak and Komar-Szymezak, 2009).

The primary objective of this work was to qualitatively analyze the fatty acid composition of *P. globosa*. Additionally, it seeks to compare the fatty acid composition in the dry flesh of freshwater crab *Paratelphusa lamellifrons* and bighead carp *Aristichthys nobilis* across various zones.

## Material and Method

### Sample collection and preparation

In March, the *Pilaglobosa* freshwater snails were obtained from fishermen who used nets to catch fish in the Chalan Beel of Natore district. The gathered snails were cleansed using running tap water to eliminate any attached substances. Subsequently, the meat and shell were detached and subjected to drying in an electric oven at a temperature of 70°C for approximately 60 hours. The dried snail samples were pulverized into a powdered form using a mortar and pestle and then stored in a refrigerator until they were analyzed for their lipid and fatty acid contents.

Analysis of fatty acids the fatty acid contents of the total lipid from the whole snail were estimated using the chloroform: methanol (2:1, v/v; with BHT 0.1 mg/100 g) extraction method (Folchet al., 1957). The process of extracting fats and preparing fatty acids methyl esters followed the procedure described by Metcalfe et al., 1996. Fatty acids underwent esterification after lipid extraction by the Soxhlet process and subsequent saponification. The FAMES were ultimately removed by utilizing methanol. The FAMES were examined using a Shimadzu gas chromatograph (GC-2010) that was fitted with a polar capillary column (SPTM-2560) of 75 meters in length, 0.18 millimeters in internal diameter, and with a film thickness of 0.14 microns. The oven temperature was set at 180°C and held for 45 minutes. It then increased at a rate of 4°C per minute until reaching 240°C, where it was maintained for 15 minutes. A 1 ml/min flow rate was maintained using nitrogen as the carrier gas. The temperature of the injection port and the flame ionization detector was kept at 250°C. The identification was achieved through the comparison of retention times with those of genuine standards.

## Results and Discussion

Researchers have hypothesized the microalgal diet of several marine animals by identifying specific fatty acids, known as "marker" fatty acids, present in their tissues. The  $\Omega$ -3 and  $\Omega$ -6 polyunsaturated fatty acid families are crucial in vital physiological processes. Marine environments serve as their primary supply. Phytoplankton, as the main source of organic matter and polyunsaturated fatty acids, transport these fatty acids to consumers through food webs. A total of 22 distinct fatty acid types were identified in the lipid composition of *P. globosa*. The most significant proportion of fatty acids was polyunsaturated fatty acids at 33.58%, followed by saturated fatty acids at 30.31%. Monounsaturated fatty acids had the lowest proportion at 26.1%. The primary saturated fatty acids present in the sample were myristic acid (C14:0) at a concentration of 3.78%, palmitic acid (C16:0) at 16.85%, heptadecanoic acid (C17:0) at 0.81%, stearic acid (C18:0) at 5.88%, arachidic acid (C20:0) at 0.20%, and lignoceric acid (C24:0) at 2.79%. Among these, palmitic acid was the most abundant saturated fatty acid, while arachidic acid had the lowest concentration. The GC-FID analysis of the fatty acid content of the freshwater snail *P. globosa* is presented in Table 1, while Figure 1 provides a graphical representation of the data.

Table 1: Fatty acid composition of *P.globosa*'s lipid (% of total fatty acid):

Type of fatty acid	Name of fatty acid	C: D	%
Saturated fatty acid (SFA)	Myristic acid	C14:0	3.78
	Palmitic acid	C16:0	16.85
	Heptadecanoic acid	C17:0	0.81
	Stearic acid	C18:0	5.88
	Arachidic acid	C20:0	0.20
	Lignoceric acid	C24:0	2.79
Total=			30.31
Mono unsaturated fatty acid (MUFA)	Myristoleic acid	C14:1( $\Omega$ -5)	0.70
	Palmitoleic acid	C16:1( $\Omega$ -7)	3.58
	Heptadecenoic acid	C17:1	0.48
	Trans Oleic acid	C18:1( $\Omega$ -9)	0.23
	Cis-9- Oleic acid	C18:1( $\Omega$ -9)	11.49
	Vaccenic acid	C18:1( $\Omega$ -7)	1.70
	Eicosenoic acid	C20:1( $\Omega$ -12)	7.05
	Nervonic acid	C24:1( $\Omega$ -9)	0.87
Total=			26.1
Poly unsaturated fatty acid (PUFA)	Trans Linoleic acid	C18:2( $\Omega$ -6)	0.99
	Linoleic acid	C18:2( $\Omega$ -6)	16.48
	Alpha Linoleic acid	C18:3( $\Omega$ -3)	1.99
	Eicosadienoic acid	C20:2( $\Omega$ -9)	2.98
	Eicosatrienoic acid	C20:3( $\Omega$ -3)	1.03
	Arachidonic acid	C20:4( $\Omega$ -3)	6.56
	Eicosapentaenoic acid (EPA)	C20:5( $\Omega$ -3)	2.27
	Docosahexaenoic acid (DHA)	C22:6( $\Omega$ -3)	1.28
Total=			33.58

Out of the 22 fatty acids shown in Table 1, six saturated fatty acids and sixteen unsaturated fatty acids. Eight of the unsaturated fatty acids are monounsaturated fatty acids, making up 26.1% of the total lipid. The remaining eight unsaturated fatty acids are highly unsaturated. The data from Table 1 revealed that polyunsaturated fatty acid was the most abundant component among the studied fatty acids, including saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids. The

quantities of these fatty acids were observed in the following sequence: The composition of polyunsaturated fatty acids in the given sample is as follows: linoleic acid (16.48%), arachidonic acid (6.56%), eicosadienoic acid (2.98%), eicosapentaenoic acid (2.27%), alphas linoleic acid (1.99%), docosahexaenoic acid (1.28%), eicosatrienoic acid (1.03%), and trans linoleic acid (0.99%). Therefore, linoleic acid is the predominant PUFA in this sample. During the fatty acid analysis in this investigation, a component with a detection rate of 9.98% was not found.

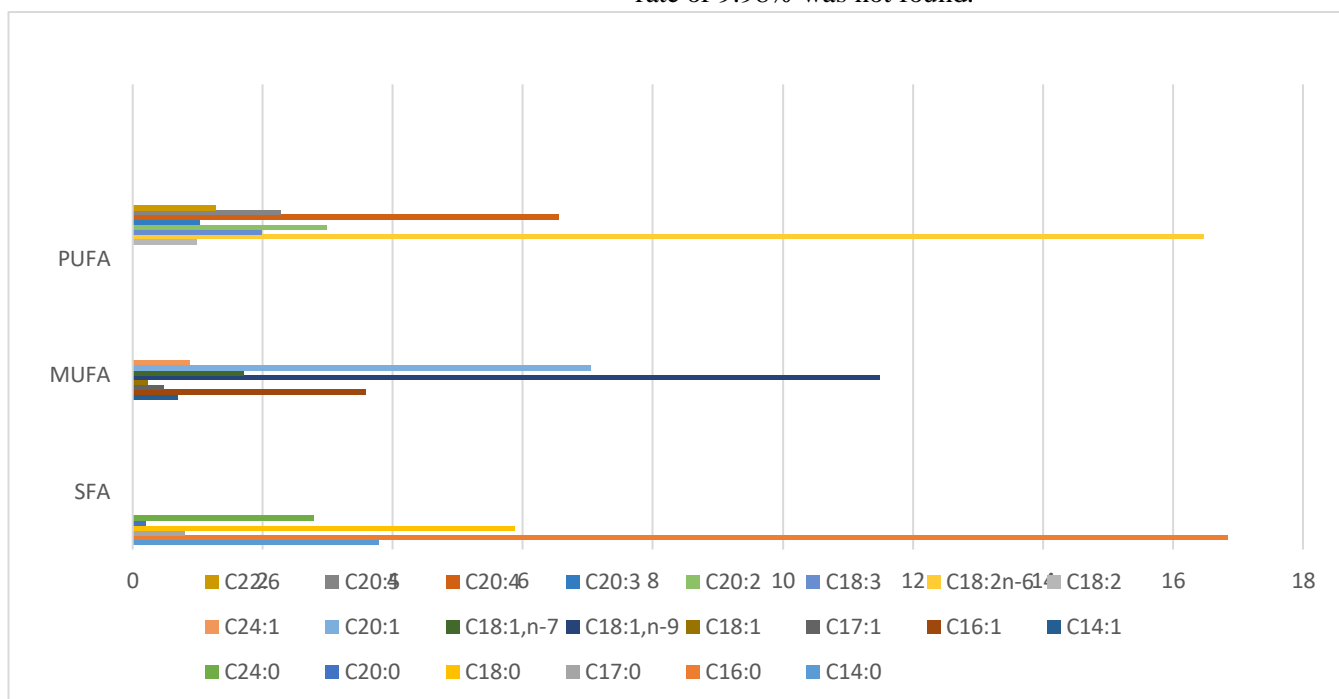


figure 1: Fatty acid composition of Pilaglobosa's lipid.

The results indicate a diverse distribution of fatty acids throughout the various categories. The primary fatty acids in the Saturated fatty acid category were myristic acid (C14:0) and palmitic acid (C16:0). Cis-9-Oleic acid (C18:1( $\Omega$ -9)) exhibited the highest proportion within the Monounsaturated fatty acid group. The most prevalent Polyunsaturated Fatty Acids were Linoleic acid (C18:2( $\Omega$ -6)) and Arachidonic acid (C20:4( $\Omega$ -3)).

The ratio of polyunsaturated fatty acids  $\Omega$ -6 to  $\Omega$ -3 and PUFA to saturated fatty acids is

crucial for maintaining optimal health. Nutritionists stress the importance of keeping a low ratio of omega-6 to omega-3 in diets to avoid the development of arteriosclerosis (Schubert et al., 2007). Values exceeding the maximum threshold can have detrimental effects on health and could contribute to the development of cardiovascular illnesses (Reidlinger et al., 2013). The UK Department of Health advises a maximum n-6 to n-3 ratio of 4.0, as stated in reference (Calder, 2018). The fish evaluated in the current investigation had a ratio of  $\Omega$ -6 to  $\Omega$ -3 of 1.33%, below the recommended maximum dietary ratio of 4.0 (Table 2).

Table 2: Fatty acid ratio (%) in edible part of *P.globosa*.

Fat content	%
PUFA/SFA	1.108
$\sum n6$	17.47
$\sum n3$	13.13
$\sum n6/\sum n3$	1.330
$\sum n5$	0.70
$\sum n7$	5.28
$\sum n9$	15.57
$\sum n12$	7.05

The inclusion of n-3 PUFAs in the diet has beneficial effects on the structure and function of the brain in elderly persons who are in good health (Miles and Calder, 2017). Eicosapentaenoic acid and docosahexaenoic acid can partially suppress inflammatory responses, such as leukocyte chemotaxis, expression of adhesion molecules, interactions between leukocytes and endothelial cells, production of inflammatory cytokines, and responsiveness of T cells (Klingenberg and Hansson, 2009). Insufficient consumption of EPA and DHA in one's diet is linked to heightened

inflammatory processes, overall cardiovascular health issues, and an elevated risk of developing Alzheimer's disease. It is also connected with inadequate fetal development, affecting neuronal, retinal, and immunological function (Jelińska, 2005; Kolanowski and Swiderski, 1997). The study found that the snail has EPA and DHA levels of 2.27% and 1.28%, respectively, while the crab has levels of 1.36% and 0.49%, and the carp fish has levels of 2.92% and 2.29%. Figure 2 demonstrates that the ratios of PUFA/SFA and n6/n3 exhibit a consistent order: snail > crab > carp.

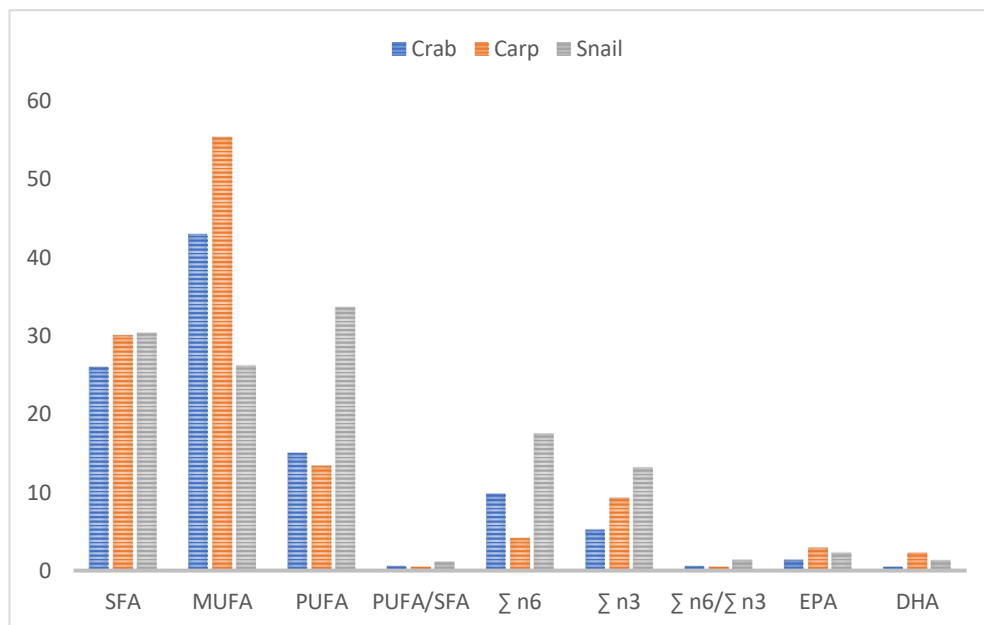


Figure 2: Comparing fatty acid of Crab, Carp and Snail.

The metabolic precursor of eicosapentaenoic acid and docosahexaenoic acid is  $\alpha$ -linolenic acid (ALA, 18:3n-3), while linoleic acid (LA, 18:2n-6) serves as the metabolic predecessor of arachidonic acid. Given the competition between these two acids for the enzymes responsible for the production of LA and ALA metabolites, it is crucial to consume a diet with a balanced ratio of LA to ALA. The optimal ratio of n6 to n3 unsaturated fatty acids is 4:1, and these fatty acids should constitute approximately one-third of the daily fat intake 22-25 grams (Achremowicz and Szary-Sworst, 2005). According to dietary standards, it is recommended that the ratio of saturated to unsaturated acids should be 0.45:1 or below. Lowering the saturated fatty acid term is typically considered beneficial in this context (WHO, 2023). Fat serves several crucial

physiological roles, such as improving the taste of foods and facilitating the uptake of fat-soluble vitamins (Islam et al., 2017; Pyz-Łukasik and Kowalczyk-Pecka, 2017).

Nevertheless, the World Health Organization (WHO) advises that the consumption of dietary fat should not surpass 30% of the overall caloric intake. Several public health organizations stress the need to follow the recommended daily intake levels of dietary fat. As to the Institute of Medicine's recommendations, adults should limit their overall fat intake to no more than 35% of their total caloric intake. The daily fatty acid composition of snail meat is comparable to that of crab and carp fish (Figure 3). Snail meat can have significant nutritional content and can be a valuable addition to a non-vegetarian diet.

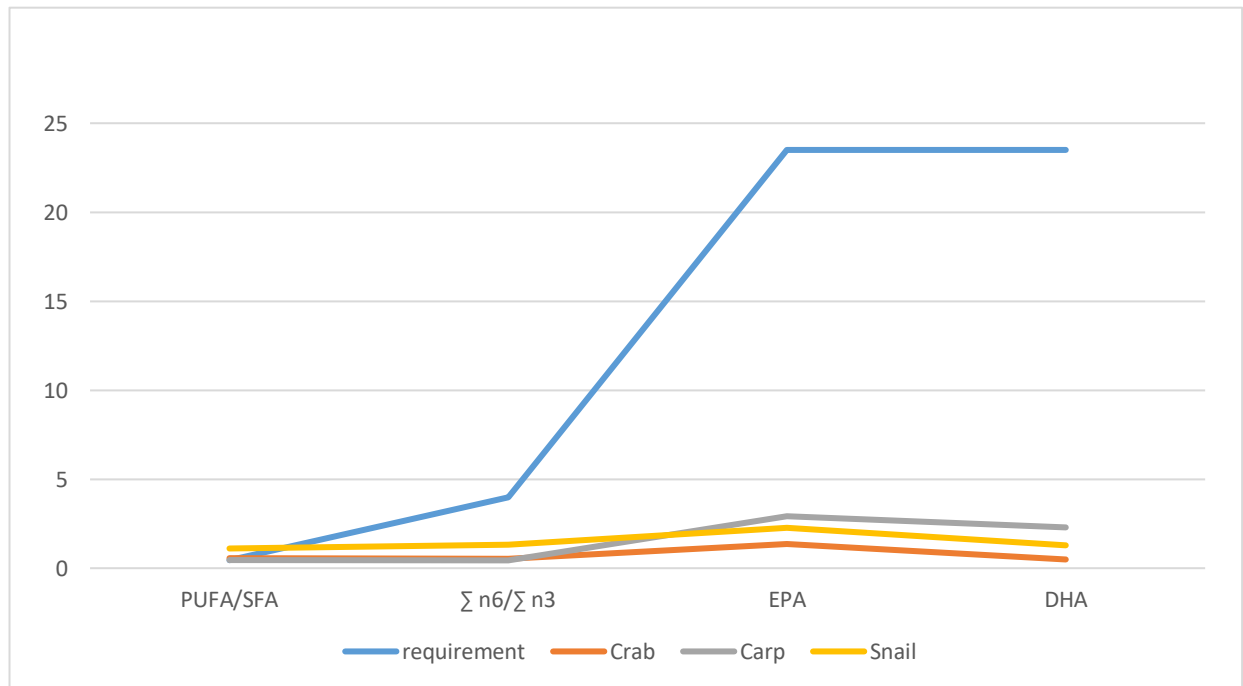


Figure 3: Comparing daily intake limit of crab, carp fish and snail.

## Conclusion

It may be inferred that both the muscle tissue and fat of the freshwater snail *P.globosa* from Chalan Beel are excellent sources of proteins and polyunsaturated fatty acids. The results unambiguously demonstrate that freshwater snails are a very nutritious diet and can serve as a terrific supply of fatty acids, particularly EPA and DHA, crucial for infants' optimal growth and development. Polyunsaturated fatty acids, particularly eicosapentaenoic acid and docosahexaenoic acid, are essential for the well-being and continued existence of aquatic creatures. *Pilglobosa* fatty acids have crucial functions in marine ecology and trophic food webs, serving as significant sources of vital polyunsaturated fatty acids that significantly affect human health.

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