# Nutritional and lipid profiles of the dorsal and ventral muscles of wild pirarucu

Carlos Andre Amaringo Cortegano[(1)], Leandro Cesar de Godoy[(2)], Maria Eugênia Petenuci[(3)], Jesuí Vergílio Visentainer[(3)], Elizabeth Gusmão Affonso[(1,4)] and Ligia Uribe Gonçalves[(1,4)]

[(1)]Universidade Nilton Lins, Programa de Pós-Graduação em Aquicultura, Avenida Professor Nilton Lins, nº 3259, Parque das Laranjeiras, CEP 69058-030 Manaus, AM, Brazil. E-mail: carlosand8@hotmail.com [(2)]Universidade Federal do Rio Grande do Sul, Departamento de Zootecnia, Avenida Bento Gonçalves, nº 7.712, CEP 91540-000 Porto Alegre, RS, Brazil. E-mail: leandro.godoy@ufrgs.br [(3)]Universidade Estadual de Maringá, Departamento de Química, Avenida Colombo, nº 5.790, CEP 87020-900 Maringá, PR, Brazil. E-mail: petenuci@ibest.com.br, jvvisentainer@uem.br [(4)]Instituto Nacional de Pesquisas da Amazônia, Avenida André Araújo, nº 2.936, CEP 69060-001 Manaus, AM, Brazil. E-mail: pgusmao@inpa.gov.br, ligia.goncalves@inpa.gov.br

Abstract – The objective of this work was to analyze the proximate and fatty acid composition of the dorsal and ventral muscles of wild pirarucu (*Arapaima gigas*) captured from a Brazilian Amazonian lake. Dorsal and ventral muscles were dissected out, freeze-dried, vacuum-packed, and had the proximate and fatty acid composition analyzed. Ash, total proteins, and lipids were inversely proportional to moisture and had higher levels in the ventral muscles. Twenty-seven fatty acids were quantified in both muscles without significant differences between them, except for the heneicosylic, palmitoleic, γ-linolenic, and dihomo-γ-linolenic acids. Saturated and monounsaturated fatty acids were predominant in both muscles. The eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids were quantitatively similar: 9.25 (dorsal) to 10.14 (ventral) and 8.50 (dorsal) to 10.63 (ventral) mg $g^{-1}$ of total lipids, respectively. The EPA+DHA content of the dorsal and ventral muscles were 113.25 and 165.78 mg 100 $g^{-1}$, respectively. The ratios of polyunsaturated/saturated (0.54 and 0.59 for the dorsal and ventral muscles, respectively), n-3/n-6 (0.20 and 0.21), and hypocholesterolemic/hypercholesterolemic fatty acids (1.41 and 1.45) ratios, as well as the atherogenicity (0.59 and 0.53) and thrombogenicity (1.02 and 0.94) indices, indicate that pirarucu muscle is a good dietary source of EPA+DHA, and its nutritional lipid quality can be beneficial for human health.

Index terms: *Arapaima gigas*, fatty acids, flesh lipid quality, n-3 Hufa, n-3/n-6, nutraceutical fish.

# Perfis nutricionais e lipídicos dos músculos dorsal e ventral em pirarucus selvagens

Resumo – O objetivo deste trabalho foi avaliar a composição proximal e de ácidos graxos dos músculos dorsal e ventral em pirarucus (*Arapaima gigas*) selvagens, capturados em um lago amazônico brasileiro. Os músculos dorsal e ventral foram dissecados, liofilizados, embalados a vácuo, e sua composição proximal e de ácidos graxos foram analisadas. Cinzas, proteínas totais e lipídeos foram inversamente proporcionais à umidade e apresentaram teores maiores no músculo ventral. Foram quantificados 27 ácidos graxos em ambos os músculos sem diferença significativa entre eles, exceto para os ácidos heneicosílico, palmitoleico, γ-linolênico e dihomo-γ-linolênico. Ácidos graxos saturados e monoinsaturados predominaram em ambos os músculos. O ácido eicosapentaenoico (EPA) e o docosahexaenoico (DHA) foram quantitativamente similares 9,25 (dorsal) a 10,14 (ventral) e 8,50 (dorsal) a 10,63 (ventral) mg $g^{-1}$ do total de lipídeos, respectivamente. O conteúdo de EPA+DHA nos músculos dorsal e ventral foi 113,25 e 165,78 mg 100 $g^{-1}$, respectivamente. A proporção de ácidos graxos poli-insaturados/saturados (0,54 e 0,59 para os músculos dorsal e ventral, respectivamente), n-3/n-6 (0,20 e 0,21) e hipocolesterolêmicos/hipercolesterolêmicos (1,41 e 1,45), assim como os índices de aterogenicidade (0,59 e 0,53) e trombogenicidade (1,02 e 0,94), indicam que o músculo do pirarucu pode ser considerado uma boa fonte dietética de EPA+DHA, e a qualidade nutricional de seus lipídios pode ser benéfica para a saúde humana.

Termos para indexação: *Arapaima gigas*, ácidos graxos, qualidade lipídica da carne, Hufa n-3, n-3/n-6, peixe nutracêutico.

## Introduction

Pirarucu (*Arapaima gigas*) (Schinz, 1822) is the world's largest scaled freshwater fish, which is native to the Amazon basin and belongs to the most basal branch of extant Teleostei. This is a carnivorous fish reaching sizes of 3 m in length and weighting up to 200


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kilograms (Fontenele, 1948). *Arapaima gigas* has an obligatory air-breathing system, moving to the water surface to breathe every 5 to 15 minutes (Fontenele, 1948), which makes it easy its identification and capture by fishermen. It is an emblematic species of the Amazonian ichthyofauna that has become very uncommon in the wild due to the overfishing in the last decades (Cavole et al., 2015), except in some natural reserves such as Pacaya-Samiria, in Peru, and Mamiraua, in Brazil (Núñez et al., 2011). Nowadays, *A. gigas* is in the Appendix II of the Convention on International Trade of Endangered Species of Wild Fauna and Flora, which means that its trade is controlled as a strategy to preserve the fish stocks (Cites, 2015). In Brazil, its fishing is regulated and only allowed under a community-based sustainable management system in protected areas during the dry season (Cavole et al., 2015).

Due to its large size, *A. gigas* represents a potential food resource not only for national trade in Amazonian regions, but also for international trade. In fact, this species has already been introduced in the United States (Hill & Lawson, 2015; Lawson et al., 2015), China, Cuba, Mexico, Philippines, Singapore and Thailand (FAO, 2012) as a promising alternative for food security. For local commercialization, Brazilian sellers have cut the fish and obtained the ventral and dorsal parts. These cuts have been sold at the market for different prices, US$ 3.75–5.00 per kg for ventral muscle and US$ 6.25–7.00 per kg for dorsal muscle. Nevertheless, little is known about the flesh nutritional value of wild *A. gigas*, there is no scientific information regarding its fatty acid composition and whether there are differences between dorsal and ventral muscles.

Interest for studies regarding the nutritional and lipid value in foods has increased with particular focus on the highly unsaturated fatty acids (Hufa) of n-3 series, such as eicosapentaenoic (EPA; 20:5n-3) and docosahexaenoic (DHA; 22:6n-3) acids, due to their health benefits throughout human life (Inhamuns et al., 2009; Jabeen & Chaudhry, 2011; Swanson et al., 2012). EPA and DHA are associated with important physiological functions, such as the proper fetal development including neuronal, retinal, and immune functions, as well as the prevention of cardiovascular problems and Alzheimer's disease (Swanson et al., 2012). Since these fatty acids are primarily obtained by diet, the World Health Organization recommends the minimum consumption of 200 mg of EPA+DHA per day (WHO, 2015), and fish are considered to be one of the main natural sources of n-3 Hufa for human diets (Simopoulos, 2008; Inhamuns et al., 2009).

The objective of this work was to analyze the proximate and fatty acid composition of dorsal and ventral muscles of wild *A. gigas*, captured from a Brazilian Amazonian lake.

## Materials and Methods

This study has been approved by the ethical committee of animal experimentation and research of the Instituto Nacional de Pesquisas da Amazônia (Inpa), municipality of Manaus, state of Amazonas, Brazil (Protocol number 002/2015).

Six wild fish (48.80±14.34 kg; 1.70±0.14 m) were collected in the community of Ilha da Paciência (3°22'S, 60°12'W), located in municipality of Iranduba, state of Amazonas, Brazil. Fish were captured using a dragnet and immediately slaughtered by medullary transection. Dorsal and ventral muscles were dissected out and kept in ice during the boat-land transportation to Inpa facilities, and were immediately stored in a freezer at -80°C. Afterwards, samples were freeze-dried, vacuum packed and sent to the chemistry department of the Universidade Estadual de Maringá for proximate and fatty acid composition analyses.

Moisture, ash and total protein in dorsal and ventral muscles were determined according to the procedures described by Horwitz (2005), and total lipids were determined by the Bligh & Dyer (1959) method.

Fatty acid methyl esters were prepared by the method proposed by Santos Júnior et al. (2014). Methyl esters were separated by gas chromatography using a Thermo Scientific Trace Ultra Gas Chromatographer (Thermo Scientific, Waltham, MA, USA), fitted with a flame ionization detector (FID) and a fused-silica capillary column (100 m × 0.25 mm i.d., 0.25 µm cyanopropyl CP-7420 select Fame). The operation parameters were as follows: detector temperature, 240°C; injection port temperature, 230°C; column temperature, 165°C for 18 minutes, programmed to increase at 4°C $min^{-1}$ up to 235°C, with final holding time of 14.5 minutes; carrier gas, hydrogen at 1.2 mL $min^{-1}$; nitrogen was used as the makeup gas at 30 mL $min^{-1}$; and there was split injection at 1/80 ratio. For identification, the retention times of the fatty acids were compared to those of standard methyl esters (Sigma-Aldrich, St. Louis, MO, USA). Retention times and peak area percentages were automatically computed by a Software Chronquest 5.0. Quantification of fatty acids (mg $g^{-1}$ of total lipids) was performed using tricosanoic acid methyl ester (Sigma-Aldrich, USA) as an internal standard (23:0) (Joseph & Ackman, 1992). Theoretical FID correction factor values were used to obtain concentration values of fatty acids in mg $g^{-1}$ of total lipids (Visentainer, 2012) by using the equation below: $FA = [(AX \times WIS \times CFX)/ (AIS \times CFAE \times WX)]$; in which FA is fatty acid in mg $g^{-1}$ of total lipids, AX is the peak area (fatty acids), WIS is the weight (mg) of standard, CFX is the theoretical correction factor, AIS is the peak area standard (23:0), CFAE is the conversion factor necessary to express results as mg of fatty acid rather than as methyl ester, and WX is the sample weight (g).

The nutritional quality of the lipid fraction of dorsal and ventral muscles of wild *A. gigas* was estimated by three indexes using the equations below: atherogenicity index (AI) (Ulbricht & Southgate, 1991): $AI = [12{:}0 + (4 \times 14{:}0) + 16{:}0]/\sum Mufa + \sum n\text{-}6 + \sum n\text{-}3$, in which Mufa is monounsaturated FA; thrombogenicity index (TI) (Ulbricht & Southgate, 1991): $TI = (14{:}0 + 16{:}0 + 18{:}0)/ [0.5 \times (\sum Mufa + \sum n\text{-}6)] + (3 \times \sum n\text{-}3) + (\sum n - 3/ \sum n - 6)$; hypocholesterolemic and hypercholesterolemic fatty acid ratio (H/H) (Santos-Silva et al., 2002): $H/H = (18{:}1n\text{-}9 + 18{:}2n\text{-}6 + 18{:}3n\text{-}3 + 20{:}4n\text{-}6 + 20{:}5n\text{-}3 + 22{:}5n\text{-}3 + 22{:}6n\text{-}3)/ (14{:}0 + 16{:}0)$.

For data analysis, proximate and fatty acid analyses of dorsal and ventral muscle were determined in triplicate for each fish. Proximate values were correlated between them by using Pearson's correlation at 5% probability. Means of proximate parameters, fatty acid composition and nutritional quality indexes of dorsal and ventral muscles were compared by student's t-test, at 5% probability. Data were processed using SigmaPlot 11.0 software.

## Results and Discussion

The content of ash, total proteins and lipids was inversely proportional to moisture content and higher in ventral muscles of wild *A. gigas* (Table 1). Regarding the total lipid content found in both muscles, *A. gigas* is considered a medium fat fish (4.00 to 8.00% body fat) in accordance with the classification of Ackman (1989). Moreover, this study reported higher lipid content in ventral muscles of wild *A. gigas*. In fact, it is known that ventral areas usually contain more lipids than the dorsal area in fishes (Thammapat et al., 2010).

Despite significant differences between the lipid contents, the fatty acid compositions in both muscles were quantitatively similar, except for heneicosylic (21:0), palmitoleic (16:1), γ-linolenic (18:3n-6) and dihomo-γ-linolenic (20:3n-6) acids (Table 2). In these sense, 27 fatty acids were quantified in both muscles, with predominance of saturated (SFA) and monounsaturated (Mufa) fatty acids, in a range from 295.32±27.55 to 308.67±28.58 (SFA), and from 242.24±28.49 to 248.88±28.84 (Mufa) (mg $g^{-1}$ of total lipids) in dorsal and ventral muscles, respectively, without quantitative difference between them. Polyunsaturated fatty acids (Pufa) were lower than SFA and Mufa in the two analyzed muscles, varying from 158.22±11.93 to 183.18±16.29 (mg $g^{-1}$ of total lipids) in dorsal and ventral muscles, respectively, being higher in the ventral part. A similar predominance pattern has been observed in some Amazonian fish, such as the tucunaré (*Cichla* sp.) in dry season (Inhamuns et al., 2009); mapará (*Hypophthalmus* sp.) (Inhamuns & Franco, 2001); and spotted sorubim (*Pseudoplatystoma corruscans*) (Tanamati et al., 2009); as well as in wild freshwater fish from other regions (Jabeen & Chaudhry, 2011).

The predominant fatty acids in both dorsal and ventral muscles were palmitic (16:0; 180.03±34.05 to 185.38±15.21 mg $g^{-1}$ of total lipids), oleic (18:1n-9; 139.00±37.02 to 158.69±29.56 mg $g^{-1}$ of total lipids), linoleic (18:2n-6; 78.44±11.82 to 86.02±14.51 mg $g^{-1}$ of total lipids) and stearic (18:0; 73.72±14.02 to 87.94±11.63 mg $g^{-1}$ of total lipids) acids. These fatty acids have mainly been found in other freshwater species, with higher predominance of

**Table 1.** Proximate composition (% in wet weight basis) of dorsal and ventral muscle of wild *Arapaima gigas* from Brazilian Amazon[(1)].

| Composition | Dorsal muscle | Ventral muscle |
|---|---|---|
| Moisture | 79.52±0.64a | 76.07±1.57b |
| Ash | 0.59±0.04a | 0.74±0.08b |
| Total proteins | 13.09±0.56a | 14.50±1.33b |
| Total lipids | 6.38±0.45a | 8.26±0.49b |

[(1)]Means followed by equal letters within rows, do not differ, by Student's t-test, at 5% probability. Results are shown as means±standard deviations of triplicate analyses per fish (n = 6).

palmitic and oleic acids (Table 2), as observed in mapará, *C. carpio*, *L. rohita*, and *O. mossambicus* (Jabeen & Chaudhry, 2011).

All the quantified Pufa showed quite similar values in both dorsal and ventral muscles of wild *A. gigas*, except for linoleic acid, which was higher than the other Pufa and is considered one of the essential fatty acids for freshwater fish (NRC, 2011). Probably, the highest proportion of linoleic acid in both muscles comes from the fish diet, since *A. gigas* is a carnivorous fish showing a preference for fishes of the Loricariidae family, which are mainly herbivorous and planctophagic (Fontenele, 1948). Actually, the fatty acid composition is strongly influenced by the age, feeding, reproductive cycle, season, salinity, geographical location and biology of the fish species. Therefore, different results can be found even in the same species under different conditions (Inhamuns et al., 2009; Jabeen & Chaudhry, 2011).

**Table 2.** Fatty acid composition (mg $g^{-1}$ of total lipids) of dorsal and ventral muscle of wild *Arapaima gigas* from Brazilian Amazon[(1)].

| Fatty acid/acid | Dorsal muscle | Ventral muscle |
|---|---|---|
| 12:0/lauric | 1.27±0.43 | 1.86±0.95 |
| 13:0/tridecanoic | 1.16±0.66 | 1.41±0.58 |
| 14:0/myristic | 12.10±1.75 | 11.27±2.24 |
| 15:0/pentadecanoic | 2.85±1.08 | 2.77±1.03 |
| 16:0/palmitic | 185.38±15.21 | 180.03±34.05 |
| 17:0/margaric | 2.08±0.88 | 2.05±0.74 |
| 18:0/stearic | 73.72±14.02 | 87.94±11.63 |
| 20:0/arachidic | 1.42±0.40 | 1.05±0.37 |
| 21:0/heneicosanoic | 2.11±0.18a | 0.89±0.08b |
| 22:0/behenic | 7.20±3.05 | 10.19±3.34 |
| 24:0/lignoceric | 6.04±3.20 | 9.21±3.07 |
| ∑SFA | 295.32±27.55 | 308.67±28.58 |
| 14:1/myristoleic | 4.21±1.26 | 4.36±1.29 |
| 15:1/pentadecenoic | 2.90±0.47 | 2.49±0.70 |
| 16:1/palmitoleic | 54.47±14.99a | 78.44±21.27b |
| 17:1/heptadecenoic | 2.31±0.59 | 2.49±1.21 |
| 18:1n-9/oleic | 158.69±29.56 | 139.00±37.02 |
| 20:1n-9/eicosenoic | 10.88±4.37 | 9.86±1.73 |
| 24:1n-9/nervonic | 8.79±4.50 | 12.24±2.63 |
| ∑Mufa | 242.24±28.49 | 248.88±28.84 |
| 18:2n-6/linoleic | 78.44±11.82 | 86.02±14.51 |
| 18:3n-6/gamma-linolenic | 9.62±3.65a | 16.06±2.52b |
| 18:3n-3/alpha-linolenic | 8.12±1.91 | 11.12±2.74 |
| 20:2n-6/eicosadienoic | 8.08±1.88 | 9.66±1.79 |
| 20:3n-6/eicosatrienoic | 12.99±4.55a | 7.49±2.90b |
| 20:4n-6/arachidonic | 14.67±3.20 | 20.91±6.81 |
| 20:5n-3/eicosapentaenoic | 9.25±4.73 | 10.14±2.90 |
| 22:4n-6/docosatetraenoic | 8.54±4.03 | 11.17±5.19 |
| 22:6n-3/docosahexaenoic | 8.50±1.25 | 10.63±2.56 |
| ∑Pufa | 158.22±11.93a | 183.18±16.29b |
| ∑n-6 | 132.35±13.08a | 151.30±16.12b |
| ∑n-3 | 25.87±6.15 | 31.89±5.84 |
| EPA+DHA | 17.75±4.91 | 20.77±5.37 |
| ∑Fatty acids | 695.79±47.15 | 740.73±31.75 |

[(1)]Means followed by equal letters within rows, do not differ, by Student's t-test, at 5% probability. SFA, saturated fatty acids; Mufa, monounsaturated fatty acids; Pufa, polyunsaturated fatty acids; EPA, eicosapentaenoic acid; and DHA, docosahexaenoic acid. Results are shown as means±standard deviations of triplicate analyses per fish (n = 6).

The amounts of EPA and DHA were similar between dorsal and ventral muscles – 9.25±4.73 (dorsal) to 10.14±2.90 (ventral); 8.50±1.25 (dorsal) to 10.63±2.56 (ventral) mg $g^{-1}$ of total lipids, respectively. The n-6 fatty acids showed higher proportions than the n-3 fatty acids in both dorsal and ventral muscles, especially linoleic and arachidonic – 20:4n-6; 14.67±3.20 (dorsal) to 20.91±6.81 (ventral) mg $g^{-1}$ of total lipids – acids (Table 2). The proportion of EPA in both dorsal and ventral muscles of wild *A. gigas* (Table 2) was higher in comparison with other Amazonian fishes, such as the matrinxã (*Brycon cephalus*) (5.20–8.10 mg $g^{-1}$ of total lipids), tambaqui (*Colossoma macropomum*) (4.70 mg $g^{-1}$ of total lipids) and *Cichla* sp. (6.00–9.00 mg $g^{-1}$ of total lipids) (Almeida et al., 2008, 2009; Inhamuns et al., 2009), although the DHA in *A. gigas* was lower when compared to the above mentioned fish.

The sum of EPA and DHA in *A. gigas* reached 113.25 mg and 165.78 mg in 100 g of dorsal and ventral muscles, respectively. It means that a person should eat at least 120.64 g of ventral muscle or 176.60 g of dorsal muscle to achieve the minimum EPA+DHA amount of 200 mg per day as recommended by the World Health Organization (WHO, 2015). That amount of flesh is relatively small in comparison to the size that *A. gigas* could reach and considering its availability in the Amazonian market.

The Pufa/SFA, n-3/n-6 and H/H ratios, as well as the AI and TI indexes, indicate that wild *A. gigas* flesh could be beneficial for human health. The Pufa/SFA ratio in *A. gigas* (Table 3) is higher than the value (0.45) recommended by the Department of Health and Social Security of the United Kingdom (DHSS, 1984), which considers diets with a Pufa/SFA ratio below 0.45 as not healthy due to their potential in inducing cholesterol increase in the blood.

The n-6 series fatty acids showed higher proportions than the n-3 in both dorsal and ventral muscles, especially linoleic and arachidonic acids – 20:4n-6; 14.67±3.20 (arachidonic, dorsal) to 20.91±6.81 (arachidonic, ventral) mg $g^{-1}$ of total lipids. The n-3/n-6 ratios in *A. gigas* were not quantitatively different between muscles. However, these data are lower when compared with the ones of other Amazonian fish, such as the *Cichla* sp. (0.66–0.98) (Inhamuns et al., 2009), *C. macropomum* (0.41) (Almeida et al., 2008) and *Hypophthalmus* sp. (1.5–1.6) (Inhamuns & Franco, 2001). In those fish, all the n-3/n-6 ratios were affected by the high proportion of DHA in their muscles. Nevertheless, nutritionists believe that the n-3/n-6 ratio should be at least 0.20 (Simopoulos, 2008) to provide nutraceutical benefits for human health, and the *A. gigas* ratio was into that limit.

The hypocholesterolemic and hypercholesterolemic fatty acid ratio (H/H) considers the specific effects of fatty acids on cholesterol metabolism, and higher H/H values are considered beneficial for human health. The obtained H/H values of 1.41±0.20 and 1.45±0.42 for dorsal and ventral muscles, respectively are higher than those found in other commercially important freshwater fish (Table 3), such as the flatwhiskered catfish (*Pinirampus pirinampu*) (1.14) (Ramos-Filho et al., 2010), *Hypophthalmus* sp. (0.75) (Inhamuns & Franco, 2001), pirapitinga (*Piaractus brachypomus*) (1.29) and sorubim *Pseudoplatystoma fasciatum* (0.52) (Perea et al., 2008).

The atherogenicity (AI) and thrombogenicity (TI) indexes indicate the potential to stimulate platelet aggregation. Thus, lower AI and TI values have beneficial effects in preventing coronary artery diseases (Ulbricht & Southgate, 1991). Both dorsal and ventral muscles showed lower AI (0.59±0.08 and 0.53±0.09) and TI (1.02±0.16 and 0.94±0.14) values (Table 3) when comparing to other freshwater fish, such as the *P. pirinampu* (AI = 0.79; TI = 1.18), jaú (*Paulicea luetkeni*) (AI = 0.62; TI = 1.00) (Ramos-Filho et al., 2010), and *P. brachypomus* (AI = 1.10; TI = 1.49) (Perea et al., 2008), indicating that *A. gigas* flesh could be beneficial for human health.

**Table 3.** Nutritional quality indexes of the lipid fraction of dorsal and ventral muscle of wild *Arapaima gigas* from Brazilian Amazon(1).

| Lipid fraction | Dorsal muscle | Ventral muscle |
|---|---|---|
| Pufa/SFA | 0.54±0.07a | 0.59±0.11a |
| n-3/n-6 | 0.20±0.06a | 0.21±0.05a |
| H/H | 1.41±0.20a | 1.45±0.42a |
| Atherogenicity index (AI) | 0.59±0.08a | 0.53±0.09a |
| Thrombogenicity index (TI) | 1.02±0.16a | 0.94±0.14a |

(1)Means followed by equal letters within rows do not differ, by Student's t-test, at 5% probability. Pufa/SFA, total polyunsaturated fatty acids/total saturated fatty acids ratio; n-3/n-6, total n-3 fatty acids/total n-6 fatty acids ratio; H/H, hypocholesterolemic and hypercholesterolemic fatty acid ratio. Results are shown as means ± standard deviations of triplicate analyses per fish (n = 6).

## Conclusions

1. Dorsal and ventral muscles of wild Pirarucu are a good dietary source of eicosapentaenoic and docosahexaenoic acids (EPA+DHA), highly unsaturated fatty acids.
2. Both dorsal and ventral muscles show lower atherogenicity and thrombogenicity indexes, which indicate beneficial effects in preventing coronary artery diseases.
3. The lipids nutritional quality of Pirarucu flesh can be beneficial for human health.

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