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COMPOSIÇÃO LIPÍDICA DE PEIXES DE EXTRATIVISMO E DE CATIVEIRO

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Tese apresentada ao programa de Pós Graduação em Ciência de Alimentos da Universidade Estadual de Maringá, como parte dos requisitos para obtenção do título de doutor em Ciência de Alimentos.

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Orientador

Prof. Dr. Jesuí Vergilio Visentainer

BIOGRAFIA

MARIA EUGÊNIA PETENUCI nasceu em 05 de maio de 1984, na cidade de Maringá – PR. Estudou em escolas públicas e ingressou a Universidade em 2001 para cursar Engenharia de Alimentos. Finalizou a graduação em fevereiro de 2007, obtendo o grau de bacharel em Engenharia de Alimentos pela Universidade Estadual de Maringá. Atuou na indústria alimentícia por seis (6) anos. Em 2010 iniciou as atividades do mestrado como aluna especial na Universidade Federal da Grande Dourados, concomitantemente com o trabalho. Em 2011, ingressou no Mestrado em Ciência e Tecnologia Ambiental como aluna regular, obtendo o grau de mestre em 2013. Em 2014 iniciou as atividades no Programa de Pós-Graduação, nível de Doutorado, em Ciência de Alimentos pela Universidade Estadual de Maringá, sob orientação do Prof. Dr. Jesuí Vergilio Visentainer. Atualmente é servidora estadual na Universidade Estadual de Mato Grosso do Sul.

Dedico,

A minha família: Eugênia, Benedito, Ester, Bruno, Sheila, Benício, Giovana e Ricardo.

"Víver não cabe no Lattes".
Autor desconhecido

"Embora ninguém possa voltar atrás e fazer um novo começo, qualquer um pode começar agora e fazer um novo fím".
Chico Xavier

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APRESENTAÇÃO

Esta tese de doutorado está apresentada na forma de três artigos científicos:

1. Petenuci, M.E., Rocha, I.N.A., Sousa, S.C., Schneider, V.V.A., Costa, L.A.M., Visentainer, J.V. (2016), Seasonal variations in lipid content, fatty acid composition and nutritional profile of five freshwater fish from the Amazon basin. J. Am. Oil Chem. Soc. http://link.springer.com/article/10.1007/s11746-016-2884-8. (Aceite em anexo – ANEXO A).

2. Petenuci, M.E., Santos, V.J., Lopes, A.P., Montanher, P.F., Schneider, V.V.A., Matsushita, M., Santos Junior, O.O., Visentainer, J.V. Fatty acid composition of freshwater fish from central amazonia (*brycon sp.*) through four differents methods of quantification. Artigo enviado a resvista JBCS – Journal of the Brazilian Chemical Society em 23 de agosto de 2016, sob status de “under review”. (Comprovante de submissão em anexo – ANEXO B)

3. Petenuci, M. E., Schneider, V.V.A., Lopes, A. P., Gonçalves, R.M., Santos, V.J., Maldaner, L., Matsushita, M., Visentainer, J.V. Effect of sources of alpha-linolenic acid in diets for Nile tilapia on fatty acid composition of fish filet. Artigo enviado a revista Journal of Food Aquatic Product and Technology em 04 de julho de 2016, sob status de “under review”. (Comprovante de submissão em anexo – ANEXO C).

GENERAL ABSTRACT

INTRODUCTION. The beneficial effect of fish consumption is related to polyunsaturated fatty acids content, such as α-linolenic acid (ALA), eicosapentaenoic (EPA) and docosahexaenoic (DHA). These fatty acids have positive effects on human health, as indicated by several studies, reducing the risk factors of cardiovascular disease, hypertension and inflammatory diseases, among others. However, the fish fatty acids composition is not constant, varying depending on several factors such as seasonality, diet, life cycle, temperature and external factors. Therefore, the knowledge of fish fatty acids composition, especially the essential fatty acids, will provide a better understanding of the nutritional value of fish and allow diets and industrial processes be properly measured.

AIMS. This study aimed to evaluating the fatty acid composition of fish species in the Amazon Basin (native) and fish-farmed fish (Nile tilapia), quantify the essential fatty acids, evaluating the nutritional profile of lipid fraction and methods of fatty acids quantification. Moreover, evaluate the effect of seasonality and diet on fatty acids composition.

MATERIAL AND METHODS. Fish samples in the Amazon basin were collected respectively in Roraima-RR (02 ° 49 '12 "S, 60 ° 40' 23" W) and Mato Grosso - MT (13 ° 1 '59 "S, 55 ° 56 '38 "W). The first collection consisted of the following species: *Colossoma macropomum, Leporinus friderici, Prochilodus nigricans, Brachyplatystoma flavicans* and *Brachyplatystoma filamentosum*. The second collection of native fish consisted of the following species: *Brycon cephalus* and *Brycon microlepis*. The fish-farmed fish (*Oreochromis niloticus*) were provided by the Experimental Station UEM/CODAPAR and were collected in Floriano district of Maringá-PR. All samples were handled in accordance with animal welfare standards. The samples were processed; the filet was obtained, crushed, homogenized and stored in plastic bags at -18°C. Subsequently, the samples were characterized by moisture content and total lipids. The species *B. cephalus* and *B. microlepis* have their centesimal composition determined, and total lipids were used for further separation of lipid classes. The methyl esters were prepared and separated into gas chromatograph equipped with flame ionization detector and capillary column of cyanopropyl (100 m x i.d. 00:25, 12:25 micrometres CP-7420). Quantification of fatty acids was performed using the internal standard method with methyl tricosonoate (23:0). Samples of the second test had their fatty acid composition determined by four different methods: the area normalization, internal standard, alternative theoretical method and experimental alternative method. Data were statistically analyzed by analysis of variance (ANOVA), and the differences between means were determined by Tukey test at 5% probability. In some cases, it was applied Principal Component Analysis methodology (PCA), in order to assess the variables with the greatest impact on the results. For Nile tilapia were prepared three diets supplemented with 2.1% soybean oil (control), canola oil (TII) and chia oil (TIII). The fish were fed three different times: zero time, 15 days and 30 days.

RESULTS AND DISCUSSION. The five fish species from Branco River, Roraima, showed variation in lipid profile according to seasonality of periods of drought and flood. *B. flavicans* showed the greatest variation between the periods (6.75-15.43%), while *C. macropomum* showed no significant difference ($p>0.05$). The species *L. friderici, B. flavicans* and *B. filamentosum* showed reduction in total lipids during flood period, which could be related to the species characteristics, to *L. friderici* the flood period is

characterized by reproduction period, *i.e*, there is a large expenditure of energy for the formation of gametes needed at this time. For the species *B. filamentosum* and *B. flavicans* with carnivorous characteristics, at flood period higher energy expenditure is required in search of food in the flooded areas, covering therefore greater distances. A variation was also observed in fatty acids composition according to seasonal periods. In the drought period the content of saturated fatty acids (SFA) was higher and in the flood period, monounsaturated fatty acids (MUFA) and polyunsaturated (PUFA) showed the highest values. This increase in PUFA in the flood period is mainly related to the feeding of species, due to the abundance and diversity of food in the flooded areas, such as flowers, fruits, insects and seeds. These foods have high contents of precursors such as linoleic acid (LA, 18:2n6) and α-linolenic acid (ALA, 18:3n3), which through dessaturation and elongation processes synthetize the long chain PUFA, arachidonic (ARA, 20:4n-6), EPA (20:5n-3) and DHA (22:6n-3). The species *L. friderici* showed the highest content of ALA (14.86 mg g^{-1}), while *C. macropomum* showed the highest content of DHA*. P. nigricans* had the lowest content of ARA in both periods, while *B. flavicans* showed the highest ARA values in both periods, with 18.77 mg g^{-1} in drought period and 10.22 mg g^{-1} in flood period. The indices of atherogenicity and thrombogenicity showed significant differences ($p < 0.05$) between the seasonal periods for all species evaluated. The ratio HH (hypercholesterolemic/hypocholesterolemic) was higher in the flood period, with a significant difference ($p < 0.05$) between species and seasonal periods. The values ranged from 1.91 (*B. flavicans*) to 2.66 (*C. macropomum*). *Brycon* species showed similar proximate composition to other studies, however they were significant different ($p < 0.05$) from each other. The fatty acids composition was determined by four different methods: area normalization (MAN), internal standard (MIS), theoretical alternative method (MAT) and experimental alternative method (MAE). A significant difference ($p<0.05$) was observed between the methods employed and the species. MAN supplied information in percentage of relative area, which hinders their use in formulating diets, requiring accurate information. MIS, MAT and MAE provided the fatty acids composition of *B. cephalus* and *B. microlepis* in mass. *B. microlepis* had the highest content of ALA, while the sum of the fatty acids EPA and DHA was 104.37 mg $100g^{-1}$ and 117.89 mg $100g^{-1}$ to *B. cephalus* and *B. microlepis*, respectively. The diets formulated for the Nile tilapia feed showed similar composition, with no significant difference ($p>0.05$). Furthermore, the diets fatty acids composition showed that the treatment with chia oil (TIII) and canola oil (TII) have higher content of polyunsaturated fatty acids, in particular the n-3 series, than control treatment with soybean oil. The results showed a significant difference ($p<0.05$) between the treatments employed, and TII and TIII treatments incorporated higher amounts of PUFA and of long chain polyunsaturated fatty acids (LC-PUFA) than control (TI). These treatments provided respectively 33.16 mg g^{-1} and 58.96 mg g^{-1} of LNA. Compared to TI, this amount was higher around 30% for TII and 131% for TIII. At the end of 30 days of treatment, there was an increase of 97% in DHA content in tilapia fed with TIII and 91% in fed with TII. This increase is related to fish ability to synthesize long-chain fatty acids from ALA and LA precursors. PCA applied to the parameters: n-6, n-3, ALA, LA, DHA, ARA and the ratio n-6/n-3 showed that two main components explained 92.07% of data variance, promoting separation of treatments. The results showed that the fatty acids content of n-3 and n-6 influenced the separation of groups and consequently the results obtained.

CONCLUSIONS. Fish from the Amazon Basin (state of Roraima) have their lipid profile, the fatty acids composition and the nutritional profile of lipid fraction affected by seasonality. However, in both periods species showed excellent content of polyunsaturated fatty acids, particularly the essential and health-benefiting fatty acids. Regarding the method for fatty acids quantification of the two *Brycon* species from the

Central Amazon basin (Mato Grosso state), it was observed that the method with higher accuracy in the results was the internal standard method. Although alternative methods provided results in mass, generally they showed overestimated values, which can lead to erroneous information on diets and sizing process and product formulations. The Nile tilapia submitted to different treatments have their fatty acids composition influenced by the treatment received, therefore treatment with chia oil and canola promoted a greater incorporation of fatty acids of the n-3 series, beneficial to human health.

Key words: tilapia, Amazon basin, omega-3, fatty acids, native fish

RESUMO GERAL

INTRODUÇÃO. O efeito benéfico do consumo de peixes está relacionado, principalmente, ao seu conteúdo de ácidos graxos poli-insaturados e essenciais, como, α-linolênico (ALA), eicosapentaenoico (EPA) e docosaexaenoico (DHA). Esses ácidos graxos exercem efeitos positivos na saúde humana, como indicado por diversos estudos, reduzindo os fatores de risco de doenças cardiovasculares, hipertensão e doenças inflamatórias, entre outras. No entanto, a composição em ácidos graxos dos peixes não é constante, variando em função de diversos fatores, como sazonalidade, dieta, ciclo de vida, temperatura e fatores externos. Portanto, conhecer a composição em ácidos graxos dos peixes, em especial os ácidos graxos essenciais e conhecer, como esta varia em função dos fatores citados anteriormente, proporcionam um melhor entendimento do valor nutricional dos peixes e permite que dietas e processos industriais sejam mensurados adequadamente.

OBJETIVOS. O objetivo deste trabalho foi avaliar a composição em ácidos graxos de espécies de peixes da Bacia Amazônica (extrativismo) e de peixes de cativeiro (tilápia do Nilo), bem como quantificar os ácidos graxos essenciais, avaliando o perfil nutricional de qualidade lipídica e os métodos de quantificação de ácidos graxos. Além disso, avaliar o efeito da sazonalidade e da dieta sobre estes.

MATERIAL E METODOS. As amostras de peixes da Bacia Amazônica foram coletadas, respectivamente, em Roraima-RR (02° 49' 12"S, 60° 40' 23" O) e Mato Grosso – MT (13° 1′ 59″ S, 55° 56′ 38″ O), sendo que a primeira coleta consistiu das seguintes espécies: *Colossoma macropomum, Leporinus friderici, Prochilodus nigricans, Brachyplatystoma flavicans* e *Brachyplatystoma filamentosum*. A segunda coleta de peixes de extrativismo consistiu das seguintes espécies: Matrinxã (*Brycon cephalus*) e Piraputanga (*Brycon microlepis*). Os peixes de cativeiro (*Oreochromis niloticus*) foram fornecidos pela Estação Experimental UEM/Codapar e foram coletados na cidade no distrito de Floriano, Maringá-PR. Todas as amostras foram manuseadas de acordo com normas de bem estar animal. As amostras foram processadas, o filé foi obtido, triturado, homogeneizado e armazenado em embalagens plásticas a -18ºC. Posteriormente, as amostras foram caracterizadas através do teor de umidade e lipídios totais. As espécies *B. cephalus* e *B. microlepis* tiveram sua composição centesimal determinada e os lipídios totais foram utilizados para posterior separação de lipídios em classes. Os metil ésteres foram preparados e separados em cromatógrafo a gás equipado com detector de ionização de chama e coluna capilar de cianopropil (100 m x 0.25 d.i., 0.25 μm, CP-7420). A quantificação dos ácidos graxos foi realizada através do método de padronização interna com tricosonoato de metila (23:0). As amostras de peixes da segunda coleta tiveram sua composição em ácidos graxos determinadas por quatro métodos diferentes: normalização de área, padronização interna, método alternativo teórico e método alternativo experimental. Os dados foram analisados estatisticamente por meio da análise de variância (ANOVA), e as diferenças entre as médias foram determinadas pelo teste de Tukey a 5% de probabilidade. Em alguns casos, aplicou-se a metodologia de Análise de Componentes Principais (PCA), com intuito de avaliar as variáveis com maior impacto sobre os resultados. Para as tilápias do Nilo, foram preparadas três dietas suplementadas com 2.1% de óleo soja (TI - controle), óleo de canola (TII) e óleo de chia (TIII). Os peixes foram alimentados em três tempos diferentes: tempo zero, 15 dias e 30 dias.

RESULTADOS E DISCUSSÃO. As cinco espécies de peixes nativos da Bacia do Rio Branco, Roraima, apresentaram variação no perfil lipídico em função da sazonalidade dos períodos de cheia (vazante) e seco (jusante). *B. flavicans* apresentou a maior variação entre os períodos (6.75–15.43 %), enquanto *C. macropomum* não apresentou diferença significativa ($p>0.05$). As espécies *L. friderici, B. filamentosum* e *B. flavicans* apresentaram redução no teor de lipídios totais durante o período de vazante, o que esta relacionada às características das espécies, uma vez que para *L. friderici*, o período de vazante é caracterizado pelo período de reprodução, ou seja, há um grande gasto de energia para formação dos gametas necessários neste período. Para as espécies *B. filamentosum* e *B. flavicans*, com características de carnívoros, no período de vazante é necessário um maior gasto energético em busca de alimento nas áreas inundadas, percorrendo, portanto maiores distâncias. Em relação à composição em ácidos graxos também foi observado uma variação de acordo com os períodos sazonais, sendo que no período da seca o conteúdo de ácidos graxos saturados (AGS) foi maior e no período da cheia, os ácidos graxos monoinsaturados (AGMI) e os poli-insaturados (AGPI) apresentaram os maiores valores. Este aumento de AGPI no período da cheia está relacionado, principalmente, a alimentação das espécies neste período, em decorrência da grande abundância e diversidade de alimento nas áreas inundadas, como flores, frutos, insetos e sementes. Esses alimentos são ricos em precursores, como os ácidos graxos linoleico (LA, 18:2n6) e α-linolênico (ALA,18:3n3), que através de processos de dessaturaação e elongação dão origem aos AGPI de cadeia longa, araquidônico (ARA, 20:4n-6), EPA e DHA. A espécie *L. friderici* apresentou o maior conteúdo de ALA (14.86 mg g^{-1}), enquanto *C. macropomum* apresentou o maior conteúdo de DHA. *P. nigricans* apresentou o menor conteúdo de ARA em ambos os períodos, enquanto *B. flavicans* apresentou os maiores valores de ARA nos dois períodos, com 18.77 mg g^{-1} na seca e 22.10 mg g^{-1} na cheia. Os índices de aterogenicidade e trombogenicidade apresentaram diferença significativa ($p< 0.05$) entre os períodos sazonais para todas as espécies avaliadas. A razão HH (hipercolesterolêmico/hipocolesterolêmico) foi maior no período da cheia, com diferença significativa ($p<0.05$) entre as espécies e os períodos sazonais. Os valores variaram de 1.91 (*B. flavicans)* para 2.66 (*C. macropomum)*. As espécies de *Brycon* apresentaram composição centesimal semelhante a outros estudos, porém foram diferentes entre si significativamente ($p<0.05$). A composição em ácidos graxos foi determinada através de quatro métodos diferentes: normalização de área (MAN), padronização interna (MIS), método alternativo teórico (MAT) e método alternativo experimental (MAE). Observou-se diferença significativa ($P<0.05$) entre os métodos empregados e as espécies. MAN forneceu informações em porcentagem de área relativa, o que dificulta sua utilização na formulação de dietas, que necessitam de formações precisas. MIS, MAT e MAE forneceram informações em massa sobre a composição em ácidos graxos das espécies *B. cephalus* e *B. microlepis*. *B. microlepis* apresentou o maior conteúdo de ALA, enquanto o somatório dos ácidos graxos EPA e DHA foi de 104.37 mg $100g^{-1}$ e 117.89 mg $100g^{-1}$ para *B. cephalus* e *B. microlepis*, respectivamente. As rações formuladas para alimentação de tilápias do Nilo apresentaram composição centesimal semelhante, sem diferença significativa ($p>0.05$). Além disso, a composição de ácidos graxos das rações mostrou que os tratamentos com óleo de chia (TIII) e com óleo de canola (TII) apresentaram maiores conteúdos de ácidos graxos poli-insaturados, em especial os da serie n-3, que o tratamento controle (TI) com óleo de soja. Os resultados evidenciaram que houve diferença significativa ($p<0.05$) entre os tratamentos empregados, sendo que os tratamentos TII e TIII incorporaram maior quantidade de AGPI e de ácidos graxos poli-insaturados de cadeia muito longa (AGPI-CML) que o tratamento controle (TI). Estes tratamentos forneceram respectivamente 33.16 mg g^{-1} e 58.96 mg g^{-1} LNA. Em comparação com TI, essa quantidade foi superior em 30% para TII e 131% para TIII. No

final dos 30 dias de tratamento, houve um incremento de 97% no conteúdo de DHA nas tilápias alimentadas com TIII e 91% nas alimentadas com TII. Esse incremento esta relacionado a capacidade dos peixes em sintetizar ácidos graxos de cadeia longa a partir dos precursores ALA e LA. A ACP aplicada aos parâmetros: n-6, n-3, ALA, LA, DHA, ARA e a razão n-6/n-3, mostrou que duas componentes principais explicaram 92.07% da variância dos dados, promovendo separação dos tratamentos aplicados. Os resultados evidenciaram que os conteúdos de ácidos graxos n-3 e n-6 influenciaram na separação dos grupos e, consequentemente nos resultados obtidos.

CONCLUSÕES. Os peixes provenientes da Bacia Amazônica (Estado de Roraima) tiveram seu perfil lipídico, a composição em ácidos graxos e, consequentemente, o perfil de qualidade nutricional lipídica afetados pela sazonalidade. No entanto, em ambos os períodos as espécies estudadas apresentaram excelente conteúdo de ácidos graxos poli-insaturados, em especial, os ácidos graxos essenciais e tão benéficos. Em relação ao método de quantificação de ácidos graxos avaliados para as duas espécies de *Brycon* provenientes da região da bacia Amazônica Central (estado de Mato Grosso), observou-se que o método com maior acuracidade nos resultados foi o método de padronização interna. Os métodos alternativos, apesar de fornecerem resultados em massa, em geral, apresentaram valores superestimados, o que pode gerar informações errôneas em formulações de dietas e dimensionamento de processos e produtos. As tilápias do Nilo submetidas a diferentes tratamentos tiveram sua composição em ácidos graxos influenciada por estes, ou seja, os tratamentos com óleo de chia e canola promoveram uma maior incorporação dos ácidos graxos da série n-3, benéficos a saúde humana.

Palavras chaves: tilápia, bacia Amazônica, ômega-3, ácidos graxos, peixes nativos.

ARTIGO 1

Title

SEASONAL VARIATIONS IN LIPID CONTENT, FATTY ACID COMPOSITION AND NUTRITIONAL PROFILES OF FIVE FRESHWATER FISH FROM THE AMAZON BASIN

Authors

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Runnig Title

Fatty acid composition of five fish species from the Amazon Basin

KEY-WORDS: seasonal, nutritional quality index, PUFA, DHA, Amazon fish

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SEASONAL VARIATIONS IN LIPID CONTENT, FATTY ACID COMPOSITION AND NUTRITIONAL PROFILES OF FIVE FRESHWATER FISH FROM THE AMAZON BASIN

ABSTRACT

Fish from the Amazon Basin are affected by oscillations in the rivers' water volume, which influences the diet of animal species. This study was aimed at evaluating seasonal variations in lipid content, fatty acid composition and nutritional profiles of five fish species from the Amazon Basin. The lipid contents of all fish species were observed to be lower in flood periods than in drought periods; *B. flavicans* showed the largest variation (6.75–15.43 %) between these periods, while *C. macropomum* showed no significant difference ($p > 0.05$). The fatty acid composition in the five fish species varied throughout seasonal periods; saturated fatty acid (SFA) contents decreased in flood periods, whereas polyunsaturated fatty acid (PUFA) contents significantly ($p < 0.05$) increased for all the species in the same period. *L. friderici* showed the highest content of α-linolenic acid, (LNA, 14.86 mg g^{-1}) and *C. macropomum* presented the highest content of docosahexaenoic acid (DHA, 26.13 mg g^{-1}) in flood periods. *P. nigricans* showed the lowest content of arachidonic acid (AA) in both periods, while *B. flavicans* showed the greatest amount of AA, 18.77 mg g^{-1} in drought period and 22.10 mg g^{-1} in flood period. All the fish species presented favorable indices of nutritional quality of lipid fraction, suggesting that consumption of these species could be considered beneficial to human health.

KEY-WORDS: seasonal, nutritional quality index, PUFA, DHA, Amazon fish

INTRODUCTION

The Amazon Basin has the greatest diversity of freshwater fish in the world [1, 2]. Branco River, Roraima state, is located in the north of Brazil and is one of 19 tributaries in the Amazon Basin. According to Junk et al. [2], 1500 ichtyic species have been described in this region. As a result, fish consumption is common and some species are widely consumed, such as *Colossoma macropomum*, *Leporinus friderici*, *Prochilodus nigricans*, *Brachyplatystoma filamentosum* and *Brachyplatystoma flavicans*, which represented 24% of Brazilian continental capture of 233.972 tons in 2011 [3].

Colossoma macropomum, known as the tambaqui, belongs to *Characidae* family and inhabits main river channels and flood plains, moving into flooded forests during the flood season. *C. macropomum* is an herbivorous species, feeding on animal matter (insects, zooplankton and small fish) during drought periods [4] and fruits, nuts and seeds during flood periods [5]. Silva et al. [5] studied the stomach content of *C. macropomum* for nine months and observed a high frequency of fruits, seeds and nuts, mainly in flood periods. These authors identified 46 vegetal species in 21 families, classifying *C. macropomum* as a fruit and seed eater.

Leporinus friderici is from the *Anostomidae* family, and is regionally called aracu [4,6]. *L. friderici* is a very active species during the flood season. Santos [7] classified *L. friderici* as an omnivorous fish, feeding on a mixed diet of vegetal and animal matter, however they did not observe significant variations in diet related to age or season. In drought periods *L. friderici* diet is based on vegetal material (leaves and small branches), detritus and nymphs. During flood periods, their diet is enhanced with fruits, seeds, dipterous insects and arthropods, which are present in trees and wood dispersed in inundated areas. Some authors have characterized *L. Friderici* as an opportunistic species, since they feed of abundant material in each season [4,6,7].

Prochilodus nigricans, regionally called curimatã, is a fish from the *Prochilodontidae* family and occupies a wide variety of habitats in the Amazon Basin [6]. Is a detritivorous species, feeding on periphytic algae and microorganism, from organic matter, which is usually deposited in the back river and in flood plain lakes [8]. According to Goulding [1] as the water level retreats, *P. nigricans* is one of the first species to leave inundated forests and start migration to the main river channel.

Brachyplatystoma filamentosum and *Brachyplatystoma flavicans* belongs to the *Pimelodidae* family. *B. filamentosum* called piraíba (or filhote if smaller than 50 kg), is considered the largest predator of the Amazonian river channels. *B. flavicans*, known as dourada, is also a large riverine piscivore that occurs in the Amazon Basin, reaching at least 1.3 m in length [6,9]. Both species are piscivorous, feeding of a great variety of fish prey (around 17 species belonging to 11 families). According to Petrete Jr et al. [10], these species showed a high digestive efficiency, thus a high frequency of stomach emptying (94.34% for dourada and 87.9% for piraiba). The feeding frequency of these species vary according to level of the water, reaching a maximum when the river is drying out, which concentrates great amounts of fish species [11]. Garcia et al. [12] found 20 fish species in the stomach contents *Brachyplatystoma* species, most of them from Curimatidae and Characidae families (61.5%).

The beneficial effects of fish consumption on human health has been related, among other factors, to the content of n-3 fatty acids, especially α-linolenic acid (18:3n-3), eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3). The effect of these fatty acids is well documented in numerous studies [13-15], which have indicated that those fatty acids present health benefits such as reduction of risk factors associated with cardiovascular disease, hypertension, general inflammation, depression, asthma, psoriasis and, more recently, inflammatory bowel disease such as Crohn's disease and ulcerative colitis [15].

Notably, the fatty acids composition of fish is not constant, but is related to the life cycle, external factors, diet, temperature and seasonality [16]. Fish from the Amazon Basin are also influenced by oscillations in the river water volume, which determines the harvest and off-season periods. This seasonal variation influences the diet of animal species [2]. In flood periods there is an overflow of rivers, into large areas of forest, which allows many organisms occupy these places in search of food and shelter. The flooded forest is an important source of available food, since many tree species have their fruits, flowers, leaves and seeds carried by the water and there is also a great amount of organic matter deposited on flood plain areas [2,4,7]. The fruits and seeds are important energy sources for feeding fish. The food abundance in flood periods suggests that omnivorous, herbivorous and detritivorous fish species spend less energy searching for food, while piscivorous species search for food over a larger area, spending fat reserves [17]. In the drought period, as the water level retreats into the main river channel, the food diversity decreases for omnivorous and herbivorous species [4,7,8], while the food abundance and diversity increases to piscivorous species [9-12].

Therefore, this study was aimed at evaluating the seasonal variations in lipid content, fatty acid composition and nutritional profiles of the aforementioned fish species from the Amazon Basin of Roraima State, in the north of Brazil, since data on the fatty acid composition and nutritional aspect of those fish are limited, especially with distinction between seasons. Furthermore, this knowledge allows human diets to be formulated more precisely and processing procedures to add nutritional value and quality.

MATERIALS AND METHODS

Fish Samples

Five different fish species were caught from Branco River, Roraima State (02° 49' 12"S, 60° 40' 23" W), in the northern area of Brazil, in two different periods drought (July - December) and flood (January - June) periods. The species studied were *C. macropomum*, *L. friderici*, *P. nigricans*, *B. filamentosum* and *B. flavicans.* In each season, twenty individuals of similar size for each fish species were collected and kept on ice in polystyrene boxes until transferred in ice to the Laboratory of Organic Synthesis and Energy Research Center (NUPENERG), Federal University of Roraima.

The average weights of the five fish species are shown in Table 1. All fish samples were eviscerated, heads were removed and dorsal muscle tissue was obtained. Subsequently, skin and spines were removed from the muscle tissue. The muscle tissue of each species was ground in a food processor until formation of a homogeneous mixture. These samples were packaged, identified and kept frozen (-18 ºC) until analysis.

Materials

All chemicals used in this study were purchased from either Merck (Brazil) or Sigma-Aldrich (St. Louis, MO, USA) unless stated otherwise.

Analysis

The moisture content in fillets was determined as described by Cunniff [18] and the total lipid was determined using the method described by Bligh & Dyer [19]. Analyses were carried out in triplicate. Fat samples were packed in

amber bottles and stored at -18°C, transported to the Laboratory of Chromatography at the State University of Maringa, Parana State, where fatty acid composition analysis was performed.

Fatty acid methyl esters were prepared by the method proposed by Hartman and Lago [20] and modified by Maia and Rodriguez-Amaya [21]. Analyses were carried out in triplicate. Methyl esters were separated by gas chromatography using a Thermo 3300 gas chromatograph fitted with a flame ionization detector (FID) and a fused-silica CP-7420 (SELECT FAME) capillary column (100 m x 0.25 mm i.d. x 0.25 lm of cianopropilpolisiloxane). Operational parameters were as follows: detector temperature, 240ºC; injection port temperature, 230ºC; column temperature, 165ºC for 18 min, programmed to increase at 4ºC min^{-1} up to 235ºC, with final holding time of 14.5 min; carrier gas, hydrogen at 1.2 mL min^{-1}; nitrogen was used as the makeup gas at 30 mL min^{-1}; split injection at 1:80 ratio. For identification, the retention times of the fatty acids were compared to those of standard methyl esters (Sigma, St. Louis, MO, USA). Retention times and peaks area percentages were automatically computed by a Chronquest Software 5.0. Quantification of fatty acids (in mg g^{-1} of total lipids) was performed using tricosanoic acid methyl ester (Sigma) as an internal standard, as described by Visentainer [22].

Lipid Nutritional Quality Indices

The data from fatty acid composition analyses were used to determine the nutritional profile of the lipid fraction. Nutritional quality was assessed by three indices: index of atherogenicity (IA), index of thrombogenicity (IT) and hypocholesterolemic/hypercholesterolenic fatty acid ratio (HH).

IA indicates the relationship between the sum of the main saturated fatty acids and that of the main classes of unsaturated, the former being considered pro-atherogenic and the latter anti-atherogenic [23]. The following equation (Equation 1) was applied to calculate IA:

$$IA = \frac{[(4 \times 14:0)+16:0]}{MUFA+n-6+n-3} \qquad \text{Equation (1)}$$

IT is defined as the relationship between the pro-thrombogenetic (saturated) and the anti-thrombogenetic fatty acids (MUFAs, n-6 and n-3 fatty acids) [23]. The equation (2) was applied:

$$IT = \frac{(14:0+16:0+18:0)}{\left[(0.5 \times MUFA)+(0.5 \times n\text{-}6)+(3 \times n\text{-}3)+\left(\frac{n\text{-}3}{n\text{-}6}\right)\right]} \qquad \text{Equation (2)}$$

The HH ratio is related to cholesterol metabolism and was calculated according to Equation (3) [24]:

$$HH = \frac{[(18:1n-9+18:2n-6)+18:3n-3+20:5n-3+22:5n-3+22:6n-3)]}{(14:0+16:0)} \qquad \text{Equation (3)}$$

Statistical analysis

Results of analyses are presented as mean ± standard deviation (SD). All analyses were carried out in triplicate. Data were submitted to one-way analysis of variance (ANOVA) tests and means were compared by Tukey's test. The significance level used was 5% ($p < 0.05$). Data were processed using Statistica software, version 7.0 [25].

RESULTS AND DISCUSSION

Moisture and Total Lipids Contents

Total lipid content and moisture content of the studied fish species are showed in Table 1. Moisture content presented a significant difference ($p<0.05$) between fish species and between the drought and flood periods. The variations between seasonal periods have also been reported in studies of freshwater fish from the Amazon basin [26, 27] and of marine species [28]. Those studies showed the moisture content of fish increased with a decrease in lipid content, due to food availability and also due to the reproduction activities of fish species. This behavior was also noted in our study.

All species studied showed variations in total lipid content by season. *B. flavicans* presented the highest total lipid content (15.43%) in the drought period, whereas *C. macropomum* showed the highest level of lipid content (7.56%) in the flood period. These variations are related to differences in the diets of fish species during the different seasonal periods, since food availability changes both in quantity and quality as the water level oscillates. Therefore, many fish of the Amazon exhibit flexibility in their diets, as has been reported previously [1, 2, 4, 6].

C. macropomum showed no significant difference ($p>0.05$) between the seasonal periods, which was also observed by Almeida and Franco [29] with *C. macropomum* from Manaus, Amazonas State, Brazil, evaluated in drought and flood periods. Goulding [30] showed that during flood period 94% of the total volume of food ingested by *C. macropomum* fruits and seeds (from at least 13 different sources) and only 6% was of animal origin (fish and feces). During drought period, the reverse trend was observed: 90% of the total volume of food ingested was of animal origin (fish, zooplankton, mayfly larvae and cockroaches) while only 10% was fruits and seeds. Other researches confirmed this trend through evaluation of the digestive content from *C. macropomum* for nine months in Manaus [5]. They noted that the great amounts of fruits, nuts and seeds eaten by this species had high total lipid contents, characterizing them as energetic food. Some seed species such as seringa-barriguda (*Hevea spruceana*) and piranheira (*Piranhas trifoilata*) have, 43.7% and 40.7% total lipid content, respectively. Suggesting that the ability of *C. macropomum* to change its diet according to food (vegetal or animal) abundance allows the fat content to be maintained over seasonal variations [5, 8, 17].

L. friderici showed a decrease in total lipid content during the flood period. This behavior was not expected, since flood period are rich in foods such as fruits, seeds, flowers and insects, which *L. friderici* consume. However, the decrease in lipid content could be associated with the reproduction period, which also happens during the flood period. Santos [7] reported that drought season is an intense feeding season for *L. friderici* to accumulate fat for the reproduction period in the flood season, when the feeding activity decreases in this species. The same author also evaluated the maturation stage of three species of *Leporinus* during seasonal variations in Janauacá Lake, Manaus. He observed that the highest degree of gonadal maturation (during and after spawning) occurred in the flood season. Furthermore, studies with *Leporinus* species noted that the oocyte maturation and reproduction process promote depletion of organic reserves (adipose tissue) of those species [31]. Those studies also reported that gonadal maturation limits the peritoneal space, exerting a mechanical pressure on the gastrointestinal tract, which decreases the food intake.

Total lipids contents in *P. nigricans* muscle tissue increased in the flood period, which is associated with the high food availability in this season to detritivorous species. Floating macrophytes, roots, tree branches, leaves, fruits, etc. are, as emphasized before, the main sources of detritus in the Amazon basin and are deposited in flooded areas [6, 8, 32]. After spawning, this species migrates to flooded areas to feed, enhancing fat reserves. So, this period is

characterized as intense feed activity. As the water retreats, these species leave lakes in inundated areas to migrate to the main river and then upstream, spending energy and fat reserves [8, 32].

Piscivorous species, *B. flavicans* and *B. filamentosum*, showed a significant decrease in total lipid content between the seasonal periods, with higher values in the drought period. According to Val and Almeida-Val [17] piscivorous fish species present a reverse trend in lipid content than herbivorous and omnivorous species. After the flood period, the water retreats from the flooded areas, causing a significant increase in the density of the ichthyofauna, and thus, food for piscivorous species is plentiful and easy to procure. Similar results were observed by Luz-Agostinho et al. [11] with piscivorous fish form the upper Parana River Floodplain. Those authors observed that flooding periods negatively affected piscivorous fish, due to the high energy requirement to search for food dispersed in inundated lowlands of rivers, consuming fat reserves. This behavior explains the results presented in Table 1 for *B. flavicans* and *B. filamentosum*. Inhamuns and Franco [26] observed similar variations in the total lipid content in piscivorous species of *Cichla sp* in drought (2.1%) and flood (0.8%) periods. In studies of marine fish variations in total lipid content were also observed [16, 28].

In general, three of the five species studied showed a decrease in total lipid content in the flood period. These variations were associated with different causes such as food availability (piscivorous) and reproduction period (*L. friderici*). As reported by Nikolsky [33] the seasonal variations in fat content of fish are closely linked to diet and reproduction.

Fatty acids composition

Table 2 shows seasonal variations in the fatty acid composition of fish species. Twenty-six fatty acids were found, with a predominance of saturated fatty acid (SFA) and monounsaturated fatty acid (MUFA) in the drought period for all studied species. Polyunsaturated fatty acid (PUFA) showed higher values in the flood period than the drought period for all studied species. Among the species, the contents of SFA in *B. flavicans*, MUFA in *B. filamentosum* and PUFA in *C. macropomum* were found to be higher than others throughout seasonal periods. Fatty acids are source of metabolic energy in fish for growth, reproduction and movement, including migration [34,35].

A significant difference ($p<0.05$) was noted in SFA content for all species between drought and flood periods (Table 2). SFA contents in the flood period were, on average, 8.6% lower than in drought period, since SFA are primarily used as source or storage of energy due to their high caloric content [34]. So, the movement of herbivorous fish (*L. friderici*, *C. macropomum*) and detritivorous fish (*P. nigricans*) to inundated areas in flood period, when food availability is higher, may reduce SFA content during flood period. Fernandes [36] observed that many fish families (Curimatae, Characidae and Prochilodontidae) of the Amazon Basin performed many migrations from the main river channel to the floodplains and vice-versa during the seasonal variations in the river water level. The same author also noted that at beginning of flood period some specimens were found to exhibit reproductive maturity, indicating a migration associated with the reproductive period. According to Tocher [35] the reproductive period is very energy intensive, since the production of very large numbers of gametes, particularly eggs, occurs during this relatively short period.

The species *B. flavicans* and *B. filamentosum* showed the highest contents of SFA in both drought and flood periods, which may be associated with the diet of these species. As reported by Tocher [35] predator fish are not likely to biosynthesize fatty acids *de novo*, normally the large lipid depots these fish accumulate are derived largely if not exclusively from dietary lipids, since they feed on other fish. Those species build up fat reserves mainly from SFA in

drought period, when there is a great diversity of prey and spend those reserves in flood period as food becomes dispersed. Similar results were reported with another piscivorous species, tucunaré, collected in Manaus with seasonal distinction [17, 27].

A significant difference ($p<0.05$) was observed in MUFA content between seasonal periods (Table 2). Higher levels of MUFA were observed in the flood period and a significant difference ($p<0.05$) was noted between the species. *B. filamentosum* and *C. macropomum* showed the highest values during the flood period, while *B. flavicans* showed the highest MUFA content in the drought period. Freshwater fish are capable of desaturating SFA (16:0 and 18:0) to yield their respective MUFA (16:1n-7 and 18:1n-9). However their natural diets are supplied with those fatty acids, so under these conditions, endogenous biosynthesis is likely to be repressed [35].

PUFA content increased in the flood period in comparison to the drought period. A significant difference ($p<0.05$) between seasons was noted for all five species studied. The values varied from 171.23 mg g^{-1} (*P. nigricans*) to 216.17 mg g^{-1} (*C. macropomum*) in the drought period and from 208.37 mg g^{-1} (*B. filamentosum*) to 243.57 mg g^{-1} (*C. macropomum*) in the flood period (Table 2). PUFA are not used as a source or storage of energy, but are used to produce eicosanoids, a class of biochemicals associated with a wide range of physiological process such as egg production, spawning and hatching [34, 35].

C. macropomum showed the highest PUFA contents in both drought and flood periods, which may be associated with diet and fatty acid metabolism. As emphasized before, *C. macropomum* has a great ability to adapt its diet, feeding mainly of animal matter in the drought period and vegetal matter in the flood period [4-6]. Those materials are rich in PUFA (linoleic acid - LA and α-linolenic acid - LNA) and some of them such as algae, have high levels of n-3 long chain polyunsaturated fatty acids (LC-PUFA), such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) [37]. Furthermore, because freshwater fish have delta 6 and delta 5 desaturase, they are able to biosynthesize LC-PUFA from LA and LNA precursors, through the process of elongation and desaturation [35]. These processes could take place in all species studied depending on the requirements of each organism. Thus, the flood period provides conditions for PUFA increase in *L. friderici, C. macropomum* and *P. nigricans* due to availability and diversity of food to these species. In piscivorous species, PUFA also increase in the flood period, once they feed on other fish species, mainly *Curimatidae* and *Characidae* families [12], which present greater PUFA amounts in the same period.

L. friderici showed the highest PUFA variation throughout seasonal periods, presenting higher values in the flood period, when the same species showed minor total lipids (Table 1), suggesting that the reproductive period affected those results [34-36]. According to Inhamuns et al. [27], higher levels of PUFA in the flood period are related to a high energy intake during this period, since SFA and MUFA constitute a source of energy readily available in fish. Meanwhile PUFA are preserved, as they are structural constituents important for metabolic functions of organs and tissues, mainly in reproductive period. Similar results were reported for tucunare (*Cichla sp.*) fillets, in which PUFA content increased during the flood period [27].

The majors PUFA were linoleic acid (LA, 18:2n-6), docosahexaenoic acid (DHA, 22:6n-3), arachidonic acid (AA, 20:4n-6), eicosapentaenoic acid (EPA, 20:5n-3) and α-linolenic acid (LNA, 18:3n-3) (Table 2). A significant difference ($p<0.05$) was observed in the content of these fatty acids between the seasonal periods. The highest content of DHA was noted in *C. macropomum, L. friderici* and *P. nigricans* (Table 2) in the flood period, whereas *B. flavicans* showed the lowest content in the same period. Futhermore, *B. flavicans* and *L. friderici* showed the highest values of AA in both periods (Table 3).

Regarding LNA content, a significant difference ($p<0.05$) was noted between seasonal periods for all the fish species, however no significant difference ($p>0.05$) was observed between four species (*C. macropomum, P. nigricans,*

292 *B. filamentosum* and *B. flavicans*) in the flood period. The species *L. friderici* and *C. macropomum* showed the highest
293 content of LNA in both seasons. These results are in accordance with the diet of those species, feeding of vegetal matter
294 in the flood period, which is rich in precursors of long chain PUFA, as LA and LNA [5, 37]. Silva et al. [5] identified 46
295 vegetal species in the stomach *of C. macropomum* from Rio Negro in the Amazon Basin. Almeida and Franco [29] also
296 observed major contents of LA and LNA in *C. macropomum* fillets collected in Manaus, Amazonas State, during the
297 flood period. LA and LNA contents were higher 11.8% and 5.3%, respectively, than in drought period [29].
298 Eicosapentaenoic acid (EPA) content also varied throughout seasonal periods and *P. nigricans* presented the
299 highest level for all the species. The specie *C. macropomum* showed no significant difference ($p>0.05$) between
300 seasonal periods, whereas other species presented a significant difference ($p<0.05$) in EPA content. Higher contents of
301 n-3 fatty acids, such as LNA, EPA and DHA were observed in *C. macropomum* and *L. friderici* in the flood period. As
302 reported by Tocher [35] the natural diet of many freshwater fish is not rich in 22:6n-3, being rich instead in LA, LNA
303 and to a lesser extent, EPA. Thus, the conversion of LA and EPA to DHA is necessary. As those species have access to
304 diverse foods such as seeds, nuts, fruits and leaves containing precursors of long chain fatty acids [1,2,19], the
305 conversion process may occur through enzymatic delta 5 desaturase and delta 6 desaturase actions, promoting
306 elongation and desaturation [34, 35]. The minor results of n-3 fatty acids in piscivorous species could be associated with
307 deficiencies in delta 6 and delta 5 desaturases expression, causing a lack or low activity, since they obtained those fatty
308 acids in their natural diet [35].
309 According to Bowden et al. [38] fish breeding strategies are a strongly associated with season. Those authors
310 also reported that seasonality affects all life cycles of fish, such as their reproduction, body conditions, food intake and
311 their immune response. In the Amazon Basin seasonality is pronounced by oscillations in river water volume, which
312 changes the food availability. As reported by many researches the changes promoted in the environment during seasonal
313 periods significantly affected the fish species in relation to food intake, reproduction period and migration through
314 Amazon Basin [5-10, 17, 26, 27, 30, 36]. These factors, consequently, influence the total lipid and fatty acid
315 composition, since those substances are used as energy sources and are precursors for production of biochemicals in
316 fish organism, such as eicosanoids and prostaglandins [34, 35].
317 The n-6/n-3 ratio showed a significant difference ($p<0.05$) between the drought and flood periods for all the
318 species studied (Table 2). The n-6 fatty acids presented higher levels than n-3 fatty acids in both periods for all the five
319 species. The n-3 fatty acids, however, increased in the flood period, which reduced the n-6/n-3 ratio during this period.
320 *L. friderici* showed the highest variation in this ratio, which varied from 3.65 in the drought period to 3.05 in the flood
321 period. Results for n-6/n-3 ratio were in accordance with those values reported by Carbonera et al. [39] for Brazilian
322 wild freshwater fish. Other studies have also shown a season-dependent n-6/n-3 ratio as a result of the variation in fatty
323 acids composition [16, 27, 28].
324 Simopoulos [13] reported that a diet with a n-6/n-3 ratio of to 4.0 is associated with a 70% reduction in death
325 caused by coronary diseases. A ratio of 2.5 reduced rectal cell proliferation in patients with colorectal cancer and a
326 ratio between 2.0 – 3.0 suppressed inflammation in patients with rheumatoid arthritis. Results shown in Table 2 suggest
327 that all fish species in both drought and flood periods constitute healthy dietary choices.
328 As opposed to the n-6/n-3 ratio, the PUFA/SFA (P/S) ratio increased in the flood period and showed
329 significant difference ($p<0.05$) from the drought period for all the species studied (Table 2). This behavior is related to
330 n-3 content increasing in the flood period. *C. macropomum* and *L. friderici* showed the highest values of PUFA/SFA
331 ratio, 1.15 and 0.99, respectively. These values are lower than the value reported for sardines (1.47), which have a high
332 content of PUFA [40]. However, the values from the freshwater fish of this study are similar to those of some marine

fish, such as black needle (1.09), white needle (1.11) and mackerel (1.18) [40]. Thus, *C. macropomum* and *L. friderici* be a great source of PUFA and could contribute to human health, mainly in the flood period.

Table 3 exhibits the nutritional profile of the lipid fraction to the five species studied in two seasonal periods. The indices of atherogenicity (IA) and thrombogenicity (IT) indicate the global dietetic quality of lipids and their potential effects on the development of coronary diseases [23]. A decrease was observed in IA and IT from the drought period to the flood period, due to higher contents of PUFA in the flood period, as noted in Table 2. A significant difference ($p< 0.05$) was observed in IA and IT between the two seasonal periods for all the species. Lower values are desirable for both indices due to the better nutritional quality of fat, related to a decrease in cardiovascular disease risk. The results obtained were lower than reported for some freshwater fish, such as *Paulicea luetkeni*, *Pinirampus pirinampu* and similar to *Hemisorubim platyrhynchos* [41].

HH indicates the specific effects of fatty acids on the cholesterol metabolism. Nutricionally, higher HH values are considered more beneficial for human health. HH values obtained in this study were larger in the flood season than the drought season and values varied 1.91 for *B. flavicans* (drought period) to 2.66 for *C. macropomum* (flood period) (Table 3). A significant difference ($p<0.05$) was noted between the species and between seasonal periods, due to high intake of PUFA in the flood period. The results shown in Table 3 are higher than those reported by Ramos Filho et al. [41] for five freshwater fish species from the Pantanal such as *H. platyrhynchos* (1.49) and *P. luetkeni* (1.30), and also higher than some marine species, such as sardine (0.87) and mackerel (1.56) [40].

CONCLUSION

Seasonal variations affected total lipid content, fatty acid composition and the nutritional profile of the five fish species studied. Total lipid content varied according to each fish species behavior and breeding strategies, however higher values of total lipid content were noted in the drought period. Fatty acids composition showed a significant variation between the drought period and the flood period. SFA showed a decrease in flood period, while MUFA and PUFA increased in the same period for all species. In both periods, piscivorous species showed higher amounts of SFA and, omnivorous and herbivorous species showed greater amounts of PUFA. An increase in n-3 fatty acids was noted in the flood period for all five species studied, with a concomitant, decrease in the n-6/n-3 ratio. Consequently, the nutritional profile accessed by quality indices of lipid fraction showed better results in the same period. Indicating that the consumption of the five studied species could promote beneficial effects in human health.

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Table 1. Characteristics of weight (kg), total lipids content (%) and moisture content (%) of the five fish species in two different seasonal periods.

Fish Species	Seasonal period	Moisture content (%)[A]	Total lipids content (%)[A]	Weight (Kg)[B]
C. macropomum	Drought Period	75.90 ^{d} ± 0.33	7.63 ^{c} ± 0.19	2.36 ^{d} ± 0.50
L. friderici		74.47 ^{f} ± 0.21	7.26 ^{d} ± 0.11	1.27 ^{e} ± 0.22
P. nigricans		77.83 ^{a} ± 0.39	4.33 ^{g} ± 0.08	2.88 ^{bcd} ± 0.34
B. filamentosum		73.88 ^{g} ± 0.30	9.48 ^{b} ± 0.10	4.89 ^{a} ± 0.79
B. flavicans		65.92 ^{i} ± 0.45	15.43 ^{a} ± 0.13	3.44 ^{b} ± 0.55
C. macropomum	Flood Period[C]	74.88 ^{c} ± 0.37	7.56 ^{c} ± 0.16	2.55 ^{cd} ± 0.42
L. friderici		76.77 ^{b} ± 0.28	3.97 ^{h} ± 0.09	1.38 ^{e} ± 0.35
P. nigricans		75.89 ^{d} ± 0.42	5.66 ^{f} ± 0.06	3.11 ^{bc} ± 0.53
B. filamentosum		75.44 ^{e} ± 0.33	6.74 ^{e} ± 0.09	4.71 ^{a} ± 0.57
B. flavicans		67.05 ^{h} ± 0.35	6.75 ^{e} ± 0.05	3.19 ^{bc} ± 0.44

[A] Results expressed as mean ± S.D of three replicates. [B] Results expressed as mean ± S.D. [C] Different letters in the same column means significant difference ($p < 0.05$) by Tukey's test throughout the two different periods, drought and flood, and between the five fish species.

457 **Table 2.** Fatty acid composition (mg g^{-1} of total lipids)[A] of the five fish species in two different seasonal periods.

Fatty acid	Drought Period					Flood Period				
	C. macropomum	*P. nigricans*	*B. filamentosum*	*L. friderici*	*B. flavicans*	*C. macropomum*	*P. nigricans*	*B. filamentosum*	*L. friderici*	*B. flavicans*
14:0	12.77 ^{ef} ± 0.23	11.35 ^{g} ± 0.13	13.71 ^{de} ± 0.31	17.60 ^{a} ± 0.74	15.12 ^{c} ± 0.37	11.61 ^{fg} ± 0.05	10.57 ^{g} ± 0.47	13.60 ^{de} ± 0.48	16.37 ^{b} ± 0.58	14.25 ^{cd} ± 0.35
15:0	2.44 ^{ab} ± 0.47	1.79 ^{b} ± 1.03	2.46 ^{ab} ± 0.30	1.74 ^{b} ± 0.22	3.52 ^{a} ± 0.48	1.62 ^{b} ± 0.34	1.51 ^{b} ± 0.41	1.28 ^{b} ± 0.35	1.47 ^{b} ± 0.37	3.49 ^{a} ± 0.43
16:0	149.16 ^{de} ± 0.70	147.96 ^{e} ± 0.74	179.20 ^{a} ± 0.26	149.86 ^{d} ± 0.18	172.34 ^{b} ± 0.58	138.08 ^{g} ± 0.76	141.01 ^{f} ± 0.53	166.92 ^{b} ± 0.72	136.87 ^{g} ± 0.68	158.60 ^{c} ± 0.74
17:00	1.62 ^{de} ± 0.52	1.67 ^{de} ± 0.22	3.27 ^{bc} ± 0.44	4.38 ^{a} ± 0.19	3.50 ^{ab} ± 0.05	1.40 ^{e} ± 0.14	1.59 ^{de} ± 0.12	2.43 ^{cd} ± 0.45	1.61 ^{de} ± 0.39	3.66 ^{ab} ± 0.20
18:0	64.16 ^{c} ± 0.67	93.66 ^{a} ± 0.05	69.59 ^{cd} ± 0.49	58.15 ^{e} ± 0.74	89.37 ^{b} ± 0.79	51.09 ^{f} ± 0.91	81.40 ^{b} ± 0.25	61.86 ^{e} ± 1.50	60.07 ^{de} ± 1.10	64.10 ^{c} ± 1.33
20:0	1.52 ^{c} ± 0.43	2.71 ^{bc} ± 0.33	2.36 ^{bc} ± 0.32	4.11 ^{a} ± 0.63	3.30 ^{ab} ± 0.27	2.54 ^{bc} ± 0.47	2.50 ^{bc} ± 0.18	1.81 ^{c} ± 0.86	2.53 ^{bc} ± 0.45	2.57 ^{bc} ± 0.19
21:00	1.69 ^{cde} ± 0.46	2.85 ^{a} ± 0.28	2.46 ^{ab} ± 0.12	1.78 ^{bcd} ± 0.18	2.14 ^{abcd} ± 0.11	1.64 ^{cde} ± 0.10	2.54 ^{a} ± 0.43	2.36 ^{abc} ± 0.27	1.03 ^{e} ± 0.17	1.52 ^{de} ± 0.02
22:0	2.17 ^{abc} ± 0.24	1.20 ^{c} ± 0.17	2.68 ^{ab} ± 0.43	2.25 ^{abc} ± 0.27	2.20 ^{abc} ± 0.11	1.16 ^{c} ± 0.18	1.63 ^{bc} ± 0.36	1.61 ^{bc} ± 0.01	2.36 ^{abc} ± 0.10	3.03 ^{a} ± 1.09
24:0	3.71 ^{ab} ± 0.44	3.09 ^{bc} ± 0.12	4.13 ^{ab} ± 0.43	1.38 ^{d} ± 0.53	3.79 ^{ab} ± 0.35	2.26 ^{cd} ± 0.34	3.35 ^{abc} ± 0.16	4.33 ^{a} ± 0.52	1.28 ^{d} ± 0.13	2.32 ^{cd} ± 0.51
SFA[B]	241.42 ^{g} ± 2.42	267.49 ^{c} ± 1.69	274.86 ^{c} ± 1.66	241.26 ^{fg} ± 0.68	297.49 ^{a} ± 1.52	211.40 ^{b} ± 1.07	246.10 ^{ef} ± 2.17	256.19 ^{d} ± 2.43	223.59 ^{h} ± 0.78	253.54 ^{de} ± 1.99
14:1n-9	3.22 ^{ab} ± 0.19	1.64 ^{ef} ± 0.35	2.77 ^{abc} ± 0.25	1.52 ^{f} ± 0.27	2.67 ^{bc} ± 0.02	1.80 ^{def} ± 0.19	1.04 ^{cd} ± 0.02	2.29 ^{cd} ± 0.26	3.34 ^{a} ± 0.15	2.18 ^{cde} ± 0.08
15:1n-9	2.38 ^{bc} ± 0.16	2.67 ^{ab} ± 0.24	2.96 ^{a} ± 0.07	2.46 ^{abc} ± 0.24	2.64 ^{ab} ± 0.04	2.05 ^{cd} ± 0.16	1.08 ^{cd} ± 0.07	2.68 ^{ab} ± 0.21	1.80 ^{d} ± 0.00	2.44 ^{abc} ± 0.34
16:1n-9	13.37 ^{a} ± 0.47	5.15 ^{e} ± 0.26	6.22 ^{de} ± 0.23	6.17 ^{de} ± 0.65	7.10 ^{d} ± 0.11	10.90 ^{b} ± 0.69	5.47 ^{de} ± 0.22	6.95 ^{d} ± 0.32	9.15 ^{c} ± 0.01	8.78 ^{c} ± 0.32
17:1n-9	5.23 ^{a} ± 0.12	1.44 ^{e} ± 0.18	5.17 ^{a} ± 0.33	3.64 ^{bc} ± 0.39	3.78 ^{bc} ± 0.06	4.28 ^{b} ± 0.06	1.06 ^{e} ± 0.02	2.56 ^{d} ± 0.40	1.05 ^{e} ± 0.28	3.39 ^{c} ± 0.12
18:1n-9	192.01 ^{e} ± 0.89	204.89 ^{c} ± 0.57	190.66 ^{ef} ± 0.84	189.08 ^{ef} ± 1.24	216.79 ^{g} ± 0.24	209.78 ^{b} ± 0.44	200.41 ^{ab} ± 0.27	216.42 ^{a} ± 2.44	200.36 ^{d} ± 2.52	186.42 ^{f} ± 0.89
18:1n-7	24.79 ^{e} ± 1.01	38.19 ^{b} ± 0.23	25.36 ^{ef} ± 0.16	27.29 ^{de} ± 0.73	22.06 ^{f} ± 0.43	28.79 ^{cd} ± 0.68	34.96 ^{a} ± 0.73	30.11 ^{c} ± 1.70	28.75 ^{cd} ± 0.77	26.70 ^{de} ± 1.44
20:1n-9	11.59 ^{de} ± 0.47	13.89 ^{c} ± 0.93	13.58 ^{c} ± 0.45	14.91 ^{c} ± 0.37	13.31 ^{cd} ± 0.38	11.25 ^{e} ± 0.87	14.48 ^{ab} ± 0.71	16.99 ^{b} ± 0.52	16.81 ^{b} ± 0.09	18.82 ^{a} ± 0.86
24:1n-9	6.74 ^{a} ± 0.25	5.61 ^{c} ± 0.48	3.80 ^{d} ± 0.79	3.67 ^{d} ± 0.29	5.78 ^{c} ± 0.21	6.48 ^{b} ± 0.28	4.13 ^{d} ± 0.58	3.65 ^{d} ± 0.55	3.03 ^{e} ± 0.62	2.82 ^{e} ± 0.55
MUFA	259.34 ^{d} ± 1.27	273.47 ^{b} ± 0.41	248.53 ^{f} ± 1.59	248.75 ^{f} ± 2.32	274.12 ^{b} ± 0.72	275.34 ^{b} ± 0.85	262.62 ^{c} ± 1.18	281.65 ^{a} ± 0.37	264.29 ^{c} ± 1.54	251.57 ^{e} ± 2.28
18:2n-6	129.67 ^{b} ± 1.83	95.34 ^{g} ± 0.56	107.81 ^{e} ± 0.30	108.33 ^{e} ± 0.42	110.26 ^{f} ± 0.37	137.99 ^{a} ± 0.85	122.38 ^{c} ± 0.86	127.20 ^{d} ± 0.75	122.95 ^{c} ± 1.13	130.99 ^{c} ± 0.40
18:3n-6	7.87 ^{b} ± 0.72	7.48 ^{bc} ± 0.44	5.56 ^{de} ± 0.36	3.82 ^{f} ± 0.13	6.72 ^{c} ± 0.16	9.86 ^{a} ± 0.02	10.29 ^{a} ± 0.21	6.54 ^{cd} ± 0.25	4.70 ^{ef} ± 0.32	7.44 ^{bc} ± 0.42
18:3n-3	9.34 ^{de} ± 0.40	7.70 ^{f} ± 0.11	7.60 ^{ef} ± 0.39	9.60 ^{de} ± 0.40	8.58 ^{cd} ± 0.12	12.92 ^{b} ± 0.06	12.11 ^{bc} ± 0.94	10.05 ^{bc} ± 0.47	14.86 ^{a} ± 1.00	10.55 ^{ab} ± 0.60
20:2n-6	12.88 ^{a} ± 0.84	3.33 ^{g} ± 0.25	6.44 ^{ef} ± 0.45	7.32 ^{de} ± 0.23	7.64 ^{cd} ± 0.29	12.44 ^{a} ± 0.25	5.66 ^{f} ± 0.17	7.75 ^{cd} ± 0.32	9.69 ^{b} ± 0.42	8.63 ^{bc} ± 0.14
20:3n-6	9.79 ^{b} ± 0.12	4.28 ^{c} ± 0.17	8.70 ^{b} ± 0.40	8.61 ^{b} ± 0.42	4.33 ^{c} ± 0.29	12.11 ^{a} ± 2.23	4.55 ^{c} ± 0.47	8.92 ^{b} ± 0.11	9.56 ^{b} ± 0.10	4.22 ^{c} ± 0.26
20:3n-3	2.79 ^{g} ± 0.12	22.76 ^{b} ± 0.23	4.34 ^{e} ± 0.50	4.08 ^{e} ± 0.25	2.47 ^{g} ± 0.31	4.95 ^{d} ± 0.14	24.90 ^{a} ± 0.43	5.28 ^{c} ± 0.26	4.43 ^{e} ± 0.63	3.32 ^{f} ± 0.14

Table 2. Fatty acid composition (mg g^{-1} of total lipids)[A] of the five fish species in two different seasonal periods.

20:4n-6	12.72	de	±	0.22	3.52	f	±	0.37	11.50	e	±	0.40	13.87	d	±	0.20	18.77	b	±	0.87	16.08	c	±	0.16	5.14	f	±	0.77	16.16	c	±	1.00	19.47	b	±	0.72	22.10	a	±	0.57
20:5n-3	11.02	cd	±	0.77	11.33	bcd	±	0.22	5.43	d	±	0.36	8.47	e	±	0.43	6.26	g	±	0.15	11.08	bcd	±	0.72	14.52	a	±	0.24	7.29	b	±	0.42	11.82	bc	±	0.02	7.24	f	±	0.27
22:6n-3	20.08	c	±	0.36	15.50	g	±	0.08	15.55	g	±	0.33	16.75	f	±	0.20	15.37	g	±	0.06	26.13	a	±	0.16	23.19	c	±	0.31	19.19	e	±	1.00	23.28	b	±	1.08	18.26	d	±	0.98
PUFA	216.17	ab	±	3.26	171.23	e	±	0.41	172.94	d	±	1.65	180.85	de	±	0.17	172.39	d	±	0.34	243.57	c	±	2.73	222.33	a	±	0.78	208.37	b	±	2.98	220.77	ab	±	2.35	220.75	ab	±	1.55
n-6	172.94	b	±	2.94	113.94	f	±	0.26	140.01	e	±	6.48	141.95	e	±	0.47	147.72	d	±	0.63	188.48	a	±	2.72	148.01	d	±	0.88	166.57	c	±	1.27	166.38	c	±	1.28	173.39	b	±	0.79
n-3	43.23	d	±	0.74	57.28	b	±	0.34	32.92	f	±	1.18	38.90	e	±	0.48	32.68	f	±	0.58	55.09	b	±	0.88	74.32	a	±	0.55	41.81	c	±	1.73	54.39	b	±	1.67	39.37	e	±	0.93
n-6/n-3	4.00	b	±	0.08	1.99	f	±	0.01	4.25	ab	±	0.16	3.65	c	±	0.07	4.52	a	±	0.06	3.42	d	±	0.09	1.98	f	±	0.02	3.98	c	±	0.04	3.05	e	±	0.10	4.40	a	±	0.05
P/S	0.90	b	±	0.02	0.64	f	±	0.01	0.63	f	±	0.01	0.75	e	±	0.00	0.58	g	±	0.00	1.15	a	±	0.02	0.90	bc	±	0.01	0.81	d	±	0.00	0.99	b	±	0.01	0.87	cd	±	0.00

[A] Results expressed as mean ± S.D of three replicates. [B] SFA: total of saturated fatty acid; MUFA: total of monounsaturated fatty acid; PUFA: total of polyunsaturated fatty acid; n-3: total omega-3 fatty acids; n-6: total omega-6 fatty acids, n-6/n-3: ratio of total fatty acids n-6 and n-3, P/S: ratio of polyunsaturated fatty acid and saturated fatty acid. [C] Different letters in the same column means significant difference ($p < 0.05$) by Tukey's test throughout the two different periods, drought and flood, and between the five fish species.

Table 3. Nutritional quality indices of the lipid fraction [A] of five fish species in two different seasonal periods.

Fish Species	Seasonal period	IA	IT	HH
C. macropomum	Drought	0.42 [e] ± 0.04	0.65 [c] ± 0.06	2.24 [c] ± 0.09
P. nigricans		0.43 [de] ± 0.03	0.69 [c] ± 0.08	2.11 [d] ± 0.05
B. filamentosum		0.55 [a] ± 0.02	0.89 [a] ± 0.05	1.69 [h] ± 0.07
L. friderici		0.51 [b] ± 0.02	0.72 [b] ± 0.05	1.98 [f] ± 0.06
B. flavicans		0.51 [b] ± 0.04	0.89 [a] ± 0.02	1.91 [g] ± 0.08
C. macropomum	Flood	0.36 [f] ± 0.03	0.51 [e] ± 0.07	2.66 [a] ± 0.10
P. nigricans		0.38 [f] ± 0.03	0.54 [de] ± 0.04	2.46 [b] ± 0.08
B. filamentosum		0.45 [cd] ± 0.03	0.69 [c] ± 0.04	2.11 [d] ± 0.09
L. friderici		0.42 [e] ± 0.02	0.56 [d] ± 0.03	2.44 [b] ± 0.08
B. flavicans		0.46 [c] ± 0.03	0.72 [b] ± 0.04	2.05 [e] ± 0.05

[A] Results expressed as mean ± S.D. [B] Different letters in the column means significant difference ($p < 0.05$) by Tukey's test throughout the two different periods, drought and flood, and between the five fish species. IA: index of atherogenicity; IT: index of thrombogenicity; HH: hypocholesterolemic/hypercholesterolenic fatty acid ratio.

ARTIGO 2

FATTY ACIDS COMPOSITION OF FRESHWATER FISH FROM CENTRAL AMAZONIA (*Brycon sp.*) USING FOUR DIFFERENTS METHODS OF QUANTIFICATION

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FATTY ACIDS COMPOSITION OF FRESHWATER FISH FROM CENTRAL AMAZONIA (*Brycon sp.*) THROUGH FOUR DIFFERENTS METHODS OF QUANTIFICATION

Abstract

This study aimed to determine the fatty acids composition of two *Brycon* species through four different methods of fatty acid quantification. The methods applied were: area normalization (MAN), internal standard (MIS), alternative theoretical (MAT) and alternative experimental (MAE). A significant difference ($p<0.05$) was observed between the methods applied and the species studied. MAN supplied poor information about fatty acid composition, mainly to diets formulation, which need accurate information. MIS, MAT and MAE supplied information as mass about fatty acid composition of *Brycon cephalus* and *Brycon microlepis,* which showed great contents of n-3 fatty acids. EPA and DHA content totalized, respectively, 104.37 mg $100g^{-1}$ and 117.89 mg $100g^{-1}$ to *B. cephalus* and *B. microlepis*. Principal component analysis (PCA) showed that to each species the variables influenced differently the separation of groups. Thus, to both species MIS showed the most accurate results, whereas MAT and MAE, in general, overestimated the results.

Keywords: quantification, EPA, DHA, fatty acids, PCA

Introduction

Brazil is world-renowned for its great biodiversity and water supply with extensive marine coastline and rivers basins.[1, 2] Thus ranks Brazil first in the world with respect to the number of fish species with 2500 valid species, representing 21% of all known fish species of world. *Characidae* family is the largest and most complex of freshwater subtropical and tropical fish, belong to the order *Characiformes*, encompassing most of the freshwater fish of Brazil with approximately 597 valid species. [2]

Bryconinae subfamily stands out as a group of wide geographic distribution such as South America, Central America and region of the main Brazilian river basins, as the Amazon and Paraná.[3] *Brycon cephalus*, regionally called matrinxã, and *Brycon microlepis*, called piraputanga are an omnivorous characid of the sub-family *Bryconinae*, which are known to undertake periodic upriver migrations associated with reproduction. In general, these species feed of leaves, fruits, seeds and small fishes.[2, 4]

The beneficial effect of fish consumption on human health has been related, among other factors to the content of the health-benefitting long-chain polyunsaturated fatty acids (LC- PUFA) such as eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). [5] Other polyunsaturated fatty acids are also important, as alpha linolenic acid (LNA, 18:3n-3) and linoleic acid (LA, 18:2n-6), since they can be converted to the biologically active LC-PUFA in vertebrates, as fishes. [6] Several beneficial effects of dietary n-3 LC-PUFA have been reported in inflammatory diseases,[7] as rheumatoid arthritis and more recently, on inflammatory bowel disease such as Crohn's disease and ulcerative colitis.[8] LC-PUFA plays an important role in reduction of risk of schizophrenia [9] and depression.[10]

Quantification of omega-3 fatty acids is necessary to know the amount of many important LC-PUFA, as EPA and DHA, which are ingested and the level presented in muscle tissue.[11, 12] This knowledge allows diet be formulated more precisely and also the technological adaptations of several industries processes, seeking to preserve the nutritional value of product and prevent the

oxidation of PUFA. [11, 13] Moreira et al.[12] and Almeida and Franco[13] evaluated the fatty acid profile of *Brycon* species, according to their study shows these fatty acids in percentages from relative areas, which is difficult to understand and translate it to diets.[14]

The method of area normalization expresses the results as percentages from relative areas. According to Visentainer[14] this method has some disadvantages as the errors propagation due to interdependence of results and results difficult to interpret. The absolute quantification method uses an internal standard, the results are expressed as mass. Thus, this method promotes results with greater accuracy, easier to interpret and use in diets formulation and processes design. Results expressed as mass are more reliable and could be used by professionals from different areas.[14]

The alternative methods are based on the study of Exler et al.[15] which necessitates the derivation of a reasonable factor (F) relating the total amount of fatty acids to a given quantity of total lipid. This factor is calculated easily where lipid class composition is given, and the use conversion factors to transform the percentage from relative areas from a methyl esther into mass of correspondent fatty acid. Those conversion factors are based on lipid classes, triglycerides and phospholipids. The alternative methods are very practice and easy to use, some food have those factors tabulated, as beef and fishes.

Therefore, the objective of this study was to determine the fatty acid composition, mainly those from n-3 series, in two fish species from Central Amazonia using and comparing four different methods of fatty acid quantification applying statistical tools.

Experimental

Raw Material

Matrinxã (*B. cephalus*) and Piraputanga (*B. microlepis)* were collected from Teles Pires River, near from Lucas do Rio Verde (13° 1′ 59″ S, 55° 56′ 38″ W), Mato Grosso state, central Amazonia region. Fish samples were divided into three lots, each containing five fish per species of similar sizing. The samples were collected in the period of June to September 2015. Each sample

was weighed individually and kept in polystyrene boxes with ice for transportation. Biometrics data are shown in Table 1. Fishes were beheaded and eviscerated, and the fillets were obtained. Fillets of each species were homogenized, packaged and kept frozen (-30 ºC) until analysis.

Table 1. Biometric data of two *Brycon* species

Species	Weight (kg)[A]	Lenght (cm)
B. cephalus	$1.08^{a} \pm 0.02$	$23.05^{a} \pm 0.05$
B. microlepis	$1.23^{b} \pm 0.06$	$27.34^{b} \pm 0.07$

[A] Results are expressed by mean ± standard deviation, of three triplicates. Different letters in the same column means significant difference ($p<0.05$) by Tukey test.

Proximate Composition

Moisture, ash, and protein contents were determined in accordance with the AOAC.[16] Total lipids were extracted by the Bligh and Dyer[17] method. Analyses were carried out in triplicate.

Separation of Lipid Classes

The procedure described by Johnston et al.[18] was used for the separation of total lipids in lipid classes, using silica gel 60 (70-230 mesh) as adsorbent and the solvents chloroform, acetone and methanol for elution, respectively, of neutral lipids, glycolipids and phospholipids. After solvents evaporation through a rotary vaccum evaporator at 40ºC, the fractions were transferred to amber bottles. The percentage of each lipid classes was calculated based on the weight of the total lipids and are showed in Table 2 (Supplementary information). Those results were used to quantify fatty acids by alternative methods as described by Visentainer and Franco.[19]

Fatty Acid Methyl Esters Preparation

Fatty acid methyl esters were prepared by the method proposed by Hartman and Lago[20] and modified by Maia and Rodriguez-Amaya.[21]

Fatty Acids Identification

Methyl esters were separated by gas chromatography using a Thermo 3300 gas chromatograph fitted with a flame ionization detector (FID) and a fused-silica CP-7420 (SELECT FAME) capillary column (100 m x 0.25 mm i.d. x 0.25 lm of cianopropilpolisiloxane). Operational parameters were as follows: detector temperature, 240ºC; injection port temperature, 230ºC; column temperature, 165ºC for 18 min, programmed to increase at 4ºC min^{-1} up to 235ºC, with final holding time of 14.5 min; carrier gas, hydrogen at 1.2 mL min^{-1}; nitrogen was used as the makeup gas at 30 mL min^{-1}; split injection at 1:80 ratio. For identification, the retention times of the fatty acids were compared to those of standard methyl esters (Sigma, St. Louis, MO, USA). Retention times and peaks area percentages were automatically computed by a Software Chromquest 5.0. Analyses were carried out in triplicate.

Fatty Acids Quantification Methods

Fatty acids quantification was calculated by four different methods: area normalization (MAN), absolute quantification with internal standard (MIS), alternative theoretical method (MAT) and experimental alternative method (MAE).

The area normalization method (MAN) used the percentage of relative area from one fatty acid in relation of sum from all fatty acids eluded, as showed in Equation (1).[19]

$$X\% = \frac{A_x \ x\ 100}{A_t} \quad (1)$$

where X% represents the percentage of relative area of a fatty acid in relation of total area from all fatty acids, A_x is area of fatty acids and A_t represents sum of area from all fatty acids.

The absolute quantification in mg g^{-1} of total lipids (MIS), were made against a tricosanoic acid methyl ester as internal standards from Sigma, as described by Visentainer.[14] The results were converted from mg g^{-1} of total lipids to mg $100g^{-1}$ of sample. Theoretical FID (flame ionization detector) correction factor values were used to calculate fatty acid concentration values in mg g^{-1} of total lipids with Equation (2), according to Visentainer [14]:

$$FA = \frac{A_X W_{IS} CF_X}{A_{IS} W_X CF_{AE}} \quad (2)$$

where FA is mg of fatty acids per g of total lipids, A_X is the peak area (fatty acids), A_{IS} is the peak area of internal standard methyl ester of tricosanoic acid (23:0), W_{IS} is the internal standard weight (mg) added to the sample, W_X is the sample weight (g), CF_X is the theoretical correction factor, and CF_{AE} is the conversion factor necessary to express results as mg of fatty acids rather than as methyl esters.

The alternative methods are based on the study of Exler et al.[15] which employs the derivation of a reasonable factor (F) relating the total amount of fatty acids to a given quantity of total lipid. This factor is calculated easily where lipid class composition is given, and the calculation is based upon the facts that, on the average, 1 g triglyceride (TG) contains 0.956g fatty acid and 1 g phospholipids (PL) contain 0.72 g fatty acid, thus the percentage of total lipid expressed as a decimal, follows as:

$$TG\ x\ 0.956\ +\ PL\ x\ 0.72\ =\ F\ (decimal) \quad (3)$$

The correction factor is used to convert fatty acid methyl esther data to values suitable for food composition tables. It is assumed that, because the average fatty acid mol wt in fish lipids is

relatively high, the methyl esther data can be used as a correspondonding to fatty acid wt percent. Calculation then proceed as follows in Equations (4) and (5):

$$F\ x\ FA\ = \frac{g\ FA}{100 g\ TL} \quad (4)$$

$$F\ x\ FA\ x\ TL\ (decimal)\ = \frac{g\ FA}{100 g\ fish} \quad (5)$$

where FA represents the respective fatty acid and TL is the total lipid content.

Theoric values to fish are tabled by Exler et al.[15] (Supplementary material – S1) and are used to calculate the alternative theoretical method (MAT). To determine experimentally these factors, a lipid class separation was made as described before and results were applied to calculate the factors, and consequently mass of each fatty acid, obtaining fatty acids quantification by alternative experimental method (MAE).

Statistical Analysis

Results of the analysis are presented as mean ± standard deviation (SD). Data were submitted to one-way analysis of variance (ANOVA) and means were compared by Tukey's test at 5% of significance level ($p<0.05$). Total saturated fatty acids (SFA), total monounsaturated fatty acids (MUFA), total polyunsaturated fatty acids (PUFA), Total n-6 fatty acids, total n-3 fatty acids, n-6/n-3 ratio and PUFA/SFA ratio were submitted to Principal Component Analysis – PCA, performed with Statistica software. Data were processed using Statistica software, version 7.0. [22]

Results and Discussion

Proximate composition

Table 2 shows proximal composition of muscle tissue of two *Brycon* species. No significant difference ($P<0.05$) was observed between the species studied only to ash content. Other results, such as protein, ash and moisture are in accordance with other studies with the same species.[12]

Table 2. Proximate composition of two *Brycon* species[A]

Species	*B. cephalus*	*B. microlepis*
Moisture (%)	74.30 [a] ± 0.02	73.89 [b] ± 0.10
Protein (%)	18.55 [b] ± 0.20	19.07 [a] ± 0.15
Total lipids	3.63 [b] ± 0.10	5.34 [a] ± 0.14
Ash (%)	1.23 [a] ± 0.06	1.34 [a] ± 0.09

[A] Results are expressed by mean ± standard deviation, of three triplicates. Different letters in the same line means significant difference ($P<0.05$) by Tukey test.

Regarding total lipids content, Moreira et al.[12] reported minor values of total lipids content in *B. microlepis* (2.49%) collected in Manso River, Mato Grosso State. Almeida et al.[13] reported 4.5% to *B. cephalus* captured in Central Amazon of Brazil. Compared to those researchers, the values found in this study slightly varied, which could be related to different geographical location and season of collection, since lipid deposition in tissue is associated to many factors as reproductive cycle, size, age and season.[23]

Comparing to other fish species, *B. cephalus* and *B. microlepis* showed similar results than reported to *Pimelodus maculatus* (5.55%) and *Piaractus mesopotamicus* (3.30%), respectively.[25]

Fatty acid composition

The fatty acids composition of two *Brycon* species by four different methods of fatty acids quantification is shown in Table 3. A significant difference ($p<0.05$) was noted between the four methods applied.

Regarding the area normalization method (MAN), no significant difference ($p>0.05$) was noted in MUFA, PUFA, n-3 and n-6 fatty acids between the two fish species, *B. cephalus* and *B.*

microlepis. Thus may be due to the characteristics of area normalization method, since that method did not supply the real fatty acids content in samples as reported by Visentainer.[14] According to this author, area normalization method is just a method of fatty acids identification and did not supply fatty acids contents. So, the results obtained with this method cause erroneous appointments, as showed in Table 3. The methods of fatty acid quantification MIS, MAT and MAE shows that n-3 content are significantly different ($p<0.05$) between the species, whereas the MAN shows that there are no significant difference ($p>0.05$) between them.

Comparing the other three methods of fatty acids quantification (MIS, MAT and MAE), a significant difference ($p<0.05$) was noted between them for both species to SFA, MUFA and PUFA. SFA content ranged from 308.46 mg g^{-1} (MIS) to 322.39 mg g^{-1} (MAE) for *B. cephalus*, and from 281.05 mg g^{-1} (MIS) to 306.98 mg g^{-1} (MAT) for *B. microlepis*. Similar results were noted in jurupoca (*H. platyrhynchos*) and mandi-amarelo (*P. maculatus*) as reported by Ramos Filho et al.[25], which evaluated the fatty acids composition of five fish species from the Brazilian Pantanal. PUFA content showed great results to species studied, with higher values observed in *B. cephalus* for all methods of fatty acid quantification (MIS, MAT and MAE).

B. cephalus showed a significant difference ($p<0.05$) to total n-6 fatty acids between the methods MIS, MAT and MAE, whereas *B. microlepis* showed similar results to MAT and MAE. In general, total n-6 fatty acids were higher than n-3 fatty acids in both species and a significant difference ($p<0.05$) was observed between the two species by MIS. Carbonera et al.[26] evaluated fatty acid composition of 21 freshwater fish species and also observed that n-6 fatty acids content were higher than n-3 fatty acids content. Those researchers found values ranging from 6.0 to 24.2% of n-6 fatty acids. *B. cephalus* and *B. microlepis* presented values in accordance with these results.

Linoleic acid (LA, 18:2n-6) was the predominant fatty acid in total n-6 fatty acids, representing approximately 82% to *B. cephalus* and 80% to *B. microlepis* in all methods of fatty acids quantification (MIS, MAT and MAE), respectively. A significant difference ($p<0.05$) was noted between the methods applied and also between the fish species. Arachidonic acid (ARA,

20:4n-6) a LC-PUFA from the n-6 series showed great results, with no significant difference ($p>0.05$) between the methods of fatty acids quantification (MIS, MAT and MAE) studied. Comparing with other species, *B. cephalus* and *B. microlepis* showed higher values of LA than *P. fasciatum* (140 mg g^{-1}) and *P. mesopotamicus* (170 mg g^{-1})[24], and higher values of ARA than *Capoeta damascina* (9.01 mg g^{-1} of total lipids) as reported by Fallah et al. [27]

Total n-3 fatty acids showed a significant difference ($p<0.05$) between the species and methods applied, however with no significant difference ($p>0.05$) between the species to MIS. *B. microlepis* showed no significant difference ($p>0.05$) between MIS, MAT and MAE. Other researchers reported minor values to n-3 fatty acids to *B. cephalus* (3.95%)[13] and *B. microlepis* (4.76%)[12], both evaluated by method of area normalization (MAN). *Brycon* species showed higher values than other freshwater fish species as *P. argenteus* (6.23%) and *P. maculatus* (4.30%).[25]

The LNA, EPA and DHA were the majority fatty acids from n-3 series in *B. cephalus* and *B. microlepis*. LNA is precursor for n-3 LC-PUFA as DHA and EPA through elongation and desaturation process.[6] A significant difference ($p<0.05$) was noted between the methods applied to each fish species. However, both fish species showed similar results to LNA content, around 20.00 mg g^{-1} as noted by method of internal standard (MIS). Lower values of LNA content was observed by Ramos Filho et al.[24] to *P. coruscans* (15.00 mg g^{-1}) and *S. maxillosus* (6.00 mg g^{-1}).

Regarding EPA and DHA content, both species showed great contents, with significant difference ($p<0.05$) between them. To EPA content the MAT and MAE showed no significant difference ($p>0.05$) between them, but they are statically different to MIS. To DHA content those differences were not observed. According to ANVISA[28] food with EPA+DHA content higher than 80 mg are considered source from these fatty acids. Thus, *B. cephalus* and *B. microlepis* attended that recommendation, with 28.72 mg g^{-1} (approximately 104,15mg 100g) and 22.15 mg g^{-1} (around 118,44 mg 100g) respectively. The World Health Organization recommends the consumption of 200 – 500 mg of those fatty acids per day.[29] Thus, a serving of 100g of studied fishes supplied about 50% of daily intake of EPA+DHA.

Comparing the applied methods to fatty acids quantification methods, it was notably that MAN supply poor information about fatty acid composition, mainly to diets formulation. MAT and MAE in most of parameters evaluated could be considered statistically similar. However, MAT needs further investigation, since is an empirical analysis based on tabulated data. MAE depends on the sampling diversity and lipid composition, as the amount of phospholipids and neutral lipids could vary with different forms of lipid class separation in experimental and theoretical quantification, leading to errors in results and consequently to false-true responses. The method of internal standard (MIS), differently from other methods, is the certificate and appropriate method for the fatty acids quantification, since sample and standard runs all analysis stages together, thus any interferences of fatty acids preparation affect both elements.

Table 3. Fatty acid composition of two *Brycon* species through four different methods of fatty acids quantification.

Fatty Acids	*B. cephalus* [A]				*B. microlepis*			
	%	mg g^{-1} TL			%	mg g^{-1} TL		
	MAN[B]	MIS	MAT	MAE	MAN	MIS	MAT	MAE
14:0	0.62±0.03^{d}	5.43±0.25^{c}	5.55±0.26^{c}	5.41±0.26^{c}	0.96±0.01^{d}	8.23±0.01^{b}	8.79±0.03^{a}	8.71±0.03^{a}
14:1n-7	0.02±0.01^{e}	0.13±0.01^{c}	0.14±0.01^{c}	0.13±0.01^{c}	0.09±0.01^{d}	0.75±0.02^{b}	0.81±0.03^{a}	0.80±0.03^{a}
15:0	0.37±0.03^{b}	3.23±0.29^{a}	3.33±0.31^{a}	3.25±0.30^{a}	0.35±0.01^{b}	3.01±0.07^{a}	3.25±0.06^{a}	3.22±0.06^{a}
15:1n-5	0.03±0.01^{b}	0.29±0.06^{a}	0.30±0.06^{a}	0.29±0.06^{a}	0.03±0.01^{b}	0.25±0.01^{a}	0.27±0.01^{a}	0.26±0.01^{a}
16:0	22.69±0.30^{e}	196.34±2.87^{b}	204.18±2.73^{a}	199.07±2.66^{ab}	20.37±0.35^{e}	172.38±2.52^{d}	187.39±3.20^{c}	185.67±3.17^{c}
16:1n-9	0.38±0.02^{d}	3.30±0.14^{c}	3.46±0.15^{bc}	3.37±0.15^{c}	0.43±0.01^{d}	3.64±0.05^{b}	3.99±0.07^{a}	3.95±0.07^{a}
16:1n-7	0.35±0.04^{b}	3.04±0.39^{b}	3.19±0.40^{b}	3.11±0.39^{b}	1.70±0.17^{bc}	14.30±1.48^{a}	15.67±1.58^{a}	15.52±1.56^{a}
16:1n-5	0.09±0.01^{d}	0.78±0.02^{c}	0.82±0.02^{c}	0.80±0.02^{c}	0.11±0.01^{d}	0.93±0.02^{b}	1.02±0.02^{a}	1.01±0.02^{a}
17:0	0.31±0.02^{c}	2.67±0.19^{b}	2.80±0.20^{b}	2.73±0.19^{b}	0.36±0.01^{c}	2.99±0.02^{ab}	3.27±0.03^{a}	3.24±0.03^{a}
17:1n-9	0.30±0.02^{c}	2.58±0.16^{a}	2.72±0.16^{a}	2.66±0.16^{a}	0.13±0.01^{c}	1.08±0.10^{b}	1.19±0.11^{b}	1.18±0.11^{b}
18:0	10.73±0.22^{d}	91.57±1.75^{bc}	96.56±1.98^{a}	94.15±1.93^{ab}	10.51±0.26^{d}	87.75±2.41^{c}	96.73±2.42^{a}	95.84±2.39^{ab}
18:1n-11	0.22±0.01^{f}	1.85±0.02^{c}	1.96±0.02^{a}	1.91±0.02^{b}	0.13±0.01^{g}	1.10±0.01^{e}	1.22±0.01^{d}	1.21±0.01^{d}
18:1n-9	25.84±0.43^{e}	218.95±3.28^{d}	232.55±3.84^{c}	226.73±3.74^{c}	29.36±0.05^{e}	243.33±0.97^{b}	270.15±0.42^{a}	267.66±0.41^{a}
18:1n-7	1.55±0.07^{d}	13.16±0.60^{ab}	13.97±0.62^{a}	13.62±0.60^{ab}	1.38±0.02^{d}	11.41±0.20^{c}	12.67±0.19^{b}	12.56±0.19^{b}
18:2n-6	21.93±0.22^{e}	184.51±1.87^{c}	197.38±1.97^{a}	192.44±1.92^{b}	20.70±0.19^{e}	170.34±1.12^{d}	190.48±1.72^{b}	188.72±1.71^{b}
18:3n-6	0.29±.0.01^{d}	2.43±0.08^{ab}	2.62±0.08^{a}	2.55±0.08^{a}	0.25±0.02^{d}	2.02±0.17^{c}	2.28±0.19^{bc}	2.25±0.19^{bc}
18:3n-3	2.41±0.06^{d}	20.09±0.53^{c}	21.65±0.54^{ab}	21.11±0.53^{b}	2.43±0.03^{d}	19.89±0.33^{c}	22.40±0.31^{a}	22.19±0.31^{a}
20:0	0.23±0.01^{c}	1.91±0.01^{a}	2.04±0.01^{a}	1.99±0.01^{a}	0.14±0.02^{c}	1.14±0.19^{b}	1.27±0.21^{b}	1.26±0.21^{b}
18:4n-3	0.44±0.02^{d}	3.61±0.14^{c}	3.92±0.16^{bc}	3.82±0.15^{bc}	0.50±0.02^{d}	4.06±0.13^{b}	4.61±0.15^{a}	4.56±0.15^{a}

20:1n-9	0.46±0.01^{e}	3.84±0.02^{abc}	4.12±0.02^{a}	4.02±0.02^{ab}	0.40±0.03^{e}	3.29±0.26^{d}	3.70±0.30^{bc}	3.66±0.30^{c}
20:2n-6	0.85±0.02^{e}	7.08±0.13^{b}	7.66±0.15^{a}	7.46±0.14^{a}	0.63±0.01^{e}	5.16±0.02^{d}	5.83±0.03^{c}	5.78±0.03^{c}
21:0	0.05±0.01^{h}	0.42±0.01^{f}	0.45±0.01^{d}	0.44±0.01^{e}	0.10±0.01^{g}	0.81±0.01^{c}	0.91±0.01^{a}	0.90±0.01^{b}
20:3n-6	0.73±0.01^{e}	6.04±0.07^{d}	6.57±0.07^{c}	6.40±0.07^{c}	0.96±0.02^{e}	7.75±0.19^{b}	8.81±0.19^{a}	8.73±0.19^{a}
20:4n-6	1.47±0.07^{b}	12.10±0.53^{a}	13.25±0.59^{a}	12.92±0.57^{a}	1.41±0.21^{b}	11.36±1.73^{a}	12.99±1.95^{a}	12.87±1.93^{a}
20:3n-3	0.91±0.04^{d}	7.56±0.31^{c}	8.22±0.32^{c}	8.01±0.31^{c}	1.16±0.07^{d}	9.39±0.57^{b}	10.68±0.67^{a}	10.58±0.66^{a}
20:5n-3	1.15±0.02^{e}	9.43±0.16^{d}	10.39±0.16^{b}	10.13±0.15^{bc}	1.22±0.04^{e}	9.70±0.33^{cd}	11.18±0.41^{a}	11.08±0.40^{a}
22:0	0.12±0.01^{e}	1.01±0.02^{b}	1.09±0.02^{a}	1.06±0.02^{a}	0.10±0.01^{e}	0.78±0.02^{d}	0.88±0.03^{c}	0.87±0.03^{c}
20:4n-3	0.56±0.02^{c}	4.62±0.14^{a}	5.06±0.14^{a}	4.94±0.14^{a}	0.34±0.04^{c}	2.77±0.31^{b}	3.17±0.34^{b}	3.14±0.34^{b}
20:5n-6	0.35±0.01^{c}	2.83±0.11^{a}	3.12±0.13^{a}	3.04±0.13^{a}	0.20±0.03^{c}	1.61±0.22^{b}	1.86±0.26^{b}	1.84±0.26^{b}
22:4n-6	0.14±0.01^{e}	1.18±0.03^{b}	1.30±0.04^{a}	1.27±0.04^{ab}	0.11±0.01^{e}	0.92±0.06^{d}	1.06±0.08^{c}	1.05±0.08^{c}
22:5n-6	0.82±0.06^{d}	6.66±0.43^{ab}	7.38±0.57^{a}	7.20±0.56^{a}	0.63±0.02^{d}	5.01±0.13^{c}	5.81±0.17^{bc}	5.76±0.16^{bc}
24:0	0.71±0.02^{e}	5.89±0.17^{b}	6.39±0.18^{a}	6.23±0.18^{a}	0.49±0.01^{e}	3.95±0.05^{d}	4.48±0.06^{c}	4.44±0.06^{c}
24:1n-9	0.01±0.01^{d}	0.09±0.01^{a}	0.09±0.01^{a}	0.09±0.01^{a}	0.01±0.01^{e}	0.05±0.01^{c}	0.06±0.01^{b}	0.06±0.01^{b}
22:5n-3	0.47±0.03^{d}	3.82±0.25^{c}	4.24±0.27^{c}	4.13±0.27^{c}	0.72±0.04^{d}	5.69±0.31^{b}	6.60±0.37^{a}	6.53±0.37^{a}
22:6n-3	2.39±0.15^{c}	19.29±1.23^{a}	21.53±1.34^{a}	20.99±1.30^{a}	1.58±0.04^{c}	12.45±0.25^{b}	14.53±0.33^{b}	14.40±0.33^{b}
SFA[C]	35.82±0.09^{f}	308.46±0.97^{c}	322.39±0.80^{a}	314.32±0.78^{b}	33.37±0.07^{g}	281.05±0.05^{e}	306.98±0.66^{c}	304.15±0.65^{d}
MUFA	29.26±0.32^{f}	248.00±2.37^{e}	263.33±2.89^{c}	256.74±2.81^{d}	33.78±0.20^{f}	280.14±2.39^{b}	310.74±1.87^{a}	307.88±1.85^{a}
PUFA	34.92±0.39^{f}	291.26±3.57^{d}	314.28±3.47^{a}	306.42±3.38^{b}	32.86±0.13^{f}	268.13±0.42^{e}	302.28±1.21^{bc}	299.49±1.20^{c}
n-3	8.33±0.21^{d}	68.42±31.84^{b}	75.00±1.89^{a}	73.13±1.85^{a}	6.94±0.12^{d}	63.96±0.95^{c}	63.84±1.13^{c}	63.25±1.12^{c}
n-6	26.59±0.30^{e}	222.84±2.58^{c}	239.28±2.68^{a}	233.29±2.61^{b}	25.92±0.01^{e}	204.17±0.53^{d}	238.44±0.08^{a}	236.24±0.08^{ab}

[A] Results are expressed as mean ± standard deviation of three replicates. Different letters in the same line means significant difference ($P<0.05$) by Tukey test. [B] MAN: method of area normalization; MIS: method of internal standard; MAT: method of alternative theoretical; MAE: method of alternative experimental. [C] SFA: total saturated fatty acids; MUFA: total monounsaturated fatty acids; PUFA: total polyunsaturated fatty acids; n-3: total n-3 fatty acids; n-6: total n-6 fatty acids.

Figure 1A shows the n-6/n-3 ratio and Figure 1B shows PUFA/SFA of two *Brycon* species in four methods of fatty acids quantification. A significant difference ($p<0.05$) was observed to n-6/n-3 ratio between the four methods applied and between the species studied. The MAT and MAE showed no significant difference ($p>0.05$) between them to each species. Results were in accordance with Simopoulos[7] recommendation, which associated a diet with n-6/n-3 ratio minor or equal to 4.0 to a reduction of 70% in death caused by coronary diseases.

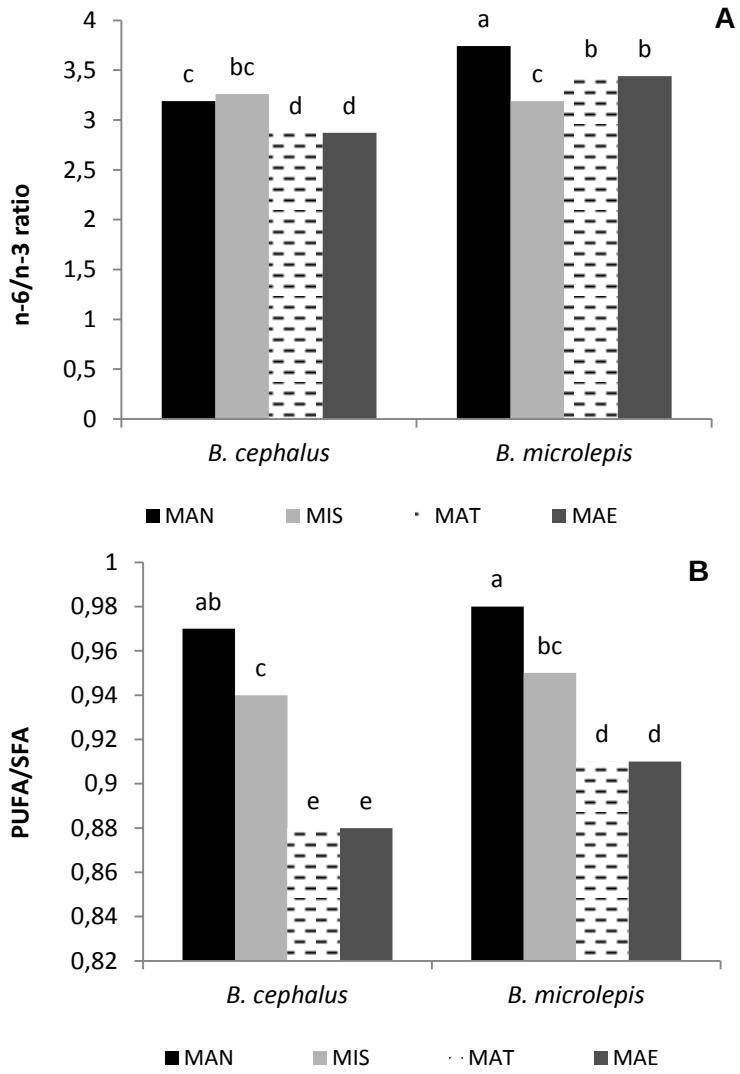

Figure 1. (A) Results of n-6/n-3 ratio of *B. cephalus* and *B. microlepis* in four different methods of fatty acid quantification; (B) Results of PUFA/SFA of *B. cephalus* and *B. microlepis* in four different methods of fatty acid quantification. MAN: method of area normalization; MIS: method of internal standard; MAT: method of alternative theoretical; MAE: method of alternative experimental.

Figure 1B shows the PUFA/SFA ratio, which also showed significant difference ($p<0.05$) between the methods and the fish species. Values varied from 0.88 to 0.97 for *B. cephalus* and 0.91 to 0.98 for *B. microlepis*. These results were higher than those reported by Moreira et al.[12] and Almeida and Franco[13] to same species. However, those values are in accordance to values reported to Brazilian wild freshwater fish.[26]

PCA analysis

PCA was applied to differentiate the sum of fatty acids (SFA, MUFA, PUFA, n-3, n-6) and ratios (n-6/n-3 and PUFA/SFA) between the four methods of fatty acids quantification used in each fish species.

To *B. cephalus*, PCA resulted in two-principal component model that described 91.05% of the total data variance as shown in Figure 2A. PC1 and PC2 accounted for 91.05% and 8.95% of the variance in the data, respectively. To *B. microlepis* a difference was noted in PCA analysis, the PC1 e PC2 accounted for 86.83% and 13.17% of the variance in the data, respectively (Figure 3A).

Regarding loading plot of PC1 x PC2, Figure 2B and Figure 3B, a difference was noted between them. In Figure 2B, PC1 loadings indicated a high positive contributions of SFA (0.9999), MUFA (0.9998) and PUFA (0.9993) and negative contributions of PUFA/SFA (-0.8750); PC2 loadings showed a high negative contributions from n-6/n-3 (-0.5883). In Figure 3B, PC1 loadings also indicated high positive contributions of SFA, MUFA and PUFA, however both PUFA/SFA and n-6/n-3 contributed negatively to PC2. Therefore those variables influenced differently the PC1 and PC2, and consequently the methods applied.

Figure 2C and Figure 3C shows the distributions of treatments on the plan of PC1 x PC2 to *B. cephalus* and *B. microlepis*, respectively. To *B. cephalus* MAN and MIS were isolated from other points, first at the extremely left from PC1 axis, whereas MIS was located

near from zero axis of PC1 and under PC2 axis. MAT and MAE were located on the right of PC1 axis and above PC2 axis. In Figure 3C of *B. microlepis*, MIS and MAN were also isolated from MAT and MAE, which were overlapped.

According to PCA (Figure 2C and Figure 3C), PC1 was significant on separation of MAN and MIS from other methods (MAT and MAE), suggesting a behavior related to the method of fatty acid quantification applied, since difference in positions in score plots were observed between the methods used. Thus, could be associated to the difference in n-6 and n-3 fatty acids contents, and consequently in n-6/n-3 ratio, which influenced those results.

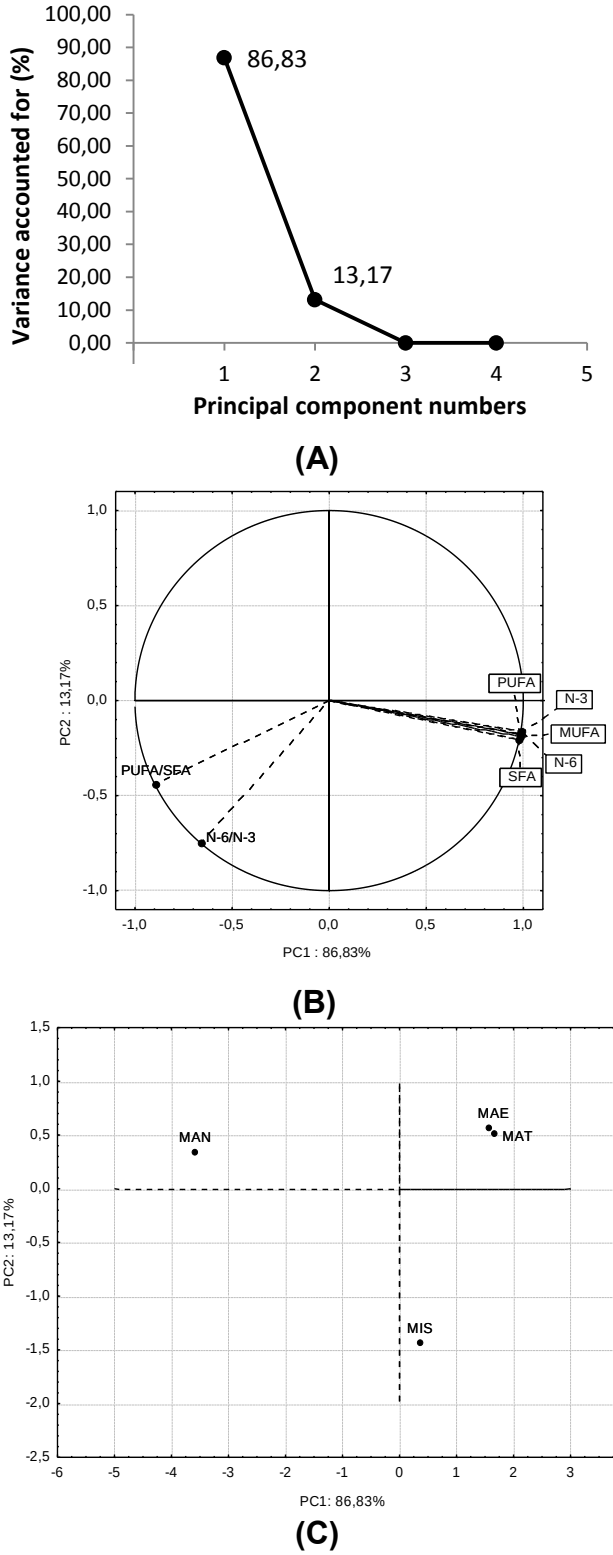

Figure 2. PCA applied to *B. cephalus* fatty acid composition (A) Variance distribution according to PCs. (b) Loading plot of PC1-PC2. (c) Score plots of PC1-PC2. MAN: method of area normalization; MIS: method of internal standard; MAT: method of alternative theoretical; MAE: method of alternative experimental.

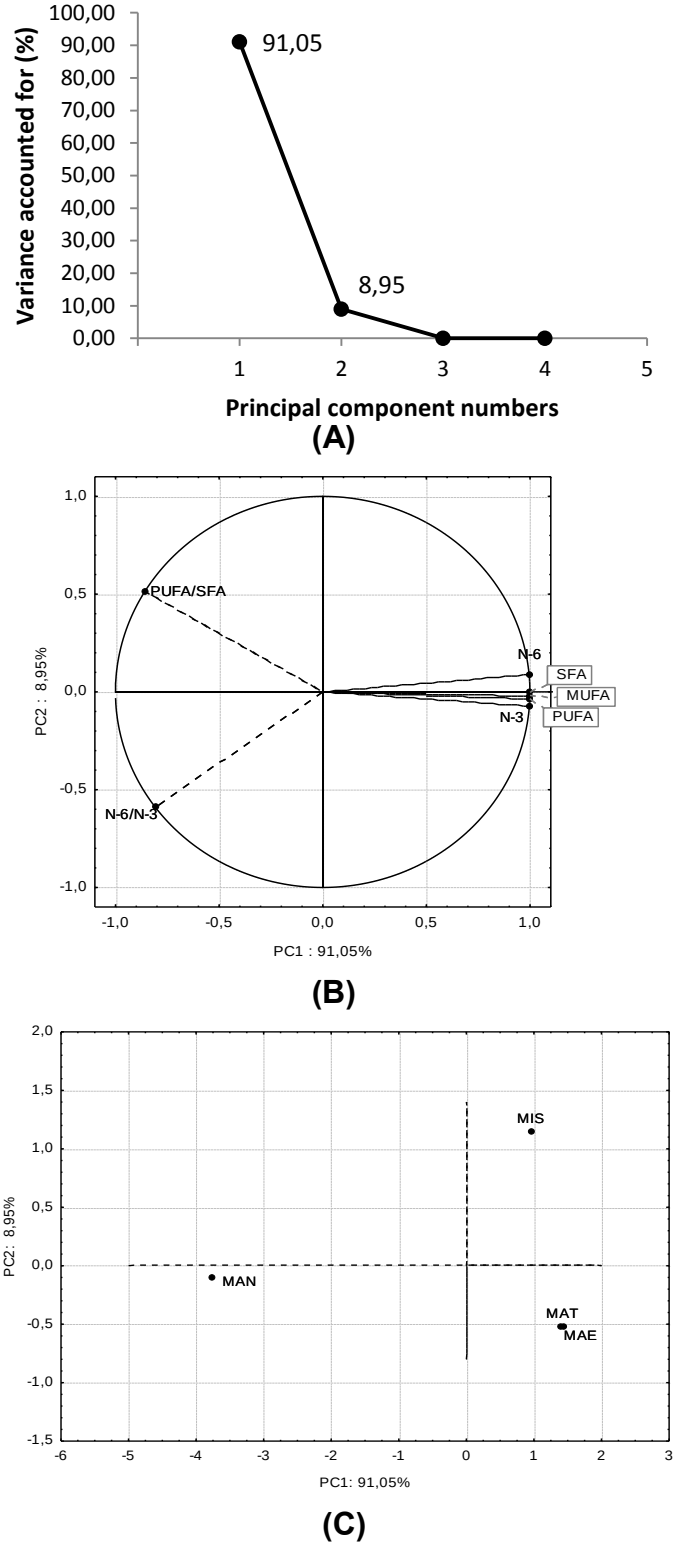

Figure 3. PCA applied to *B. microlepis* fatty acid composition (A) Variance distribution according to PCs. (b) Loading plot of PC1-PC2. (c) Score plots of PC1-PC2. MAN: method of area normalization; MIS: method of internal standard; MAT: method of alternative theoretical; MAE: method of alternative experimental.

Conclusions

The method of fatty acid quantification influenced the results obtained to two Brycon species. Method of internal standard provided the most accurate results to diet formulations, whereas method of area normalization supply only the fatty acids profile and the alternative methods provided results overestimated. *B. cephalus* and *B. microlepis* showed great contents of n-3 fatty acids, mainly the health-benefiting, such as LNA, EPA and DHA. Those species could be considered as higher EPA+DHA content, since a portion of them could supply 50% of daily intake from these fatty acids. PCA showed that variables used influenced differently the separation of groups to each fish species.

Acknowledgements

The authors are grateful to State University of Maringá.

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Supplementary Information

FATTY ACIDS COMPOSITION OF FRESHWATER FISH FROM CENTRAL AMAZONIA (*Brycon sp.*) THROUGH FOUR DIFFERENTS METHODS OF QUANTIFICATION

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Table 1 . Conversion factors based on total lipids content to fish (Visentainer and Franco apud Exler et al., 1975)

% Total lipids	% TG[A]	% FL	F (decimal)
0.65	----	92.3	0.66
0.70	7.1	85.7	0.68
0.80	18.8	75.0	0.72
0.90	27.8	66.7	0.75
1.00	35.0	60.0	0.77
1.25	48.0	48.0	0.80
1.50	56.7	40.0	0.83
1.75	62.9	34.3	0.85
2.00	67.5	30.0	0.86
2.50	74.0	24.0	0.88
3.00	78.3	20.0	0.89
3.50	81.4	17.1	0.90
4.00	83.8	15.0	0.91
4.50	85.6	13.3	0.91
5.00	87.0	12.0	0.92

[A] % Total lipids: g of total lipids in 100 g of sample (%); %TG: percentage of triacylglicerois; %FL: percentage of phospholipids; F: decimal factor

Table 2. Results of lipid class separation to two Brycon species

Lipid Class [A]	*B. cephalus*	*B. microlepis*
% FL	71.07%	84.20%
% NL	27.50%	14.80%

[A] % FL: phospholipids percentage determined by separation lipids method to calculate MAE; %NL: neutral lipids percentage observed in each species through method of lipid class separation

ARTIGO 3

Title

EFFECT OF SOURCES OF ALPHA-LINOLENIC ACID IN DIETS FOR NILE TILAPIA ON FATTY ACID COMPOSITION OF FISH FILET

Authors

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Running Title

Incorporation of LC-PUFA into Nile tilapia fillet

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Key words: chia oil, canola oil, omega-3, *Oreochormis niloticus*, LC-PUFA, PCA.

EFFECT OF SOURCES OF ALPHA-LINOLENIC ACID IN DIETS FOR NILE TILAPIA ON FATTY ACID COMPOSITION OF FISH FILLET

ABSTRACT

This study evaluated the incorporation of n-3 long chain polyunsaturated fatty acids (LC-PUFA) from supplemented feed diets with chia and canola oils as a substitute for soybean oil into the composition of muscle tissue of Nile tilapia. Diets were supplemented with 2.1% (w/w) of each oil and were provided to fish for 15 and 30 days. The supplementation with canola and chia oils increased significantly ($P<0.05$) the contents of eicosapentaenoic acid (EPA), docosapentanenoic acid (DPAn-3) and docosahexaenoic acid (DHA) in Nile tilapia fillet compared to those fed with soybean oil (control diet). At end of 30 days, DHA content increased 97% in Nile tilapia fed with chia oil and 91% in treatment with canola oil. Highest EPA content was noted in treatment with chia oil of 7.33 mg 100 g^{-1}. Precursors of LC-PUFA, linoleic acid (LA) and alpha-linolenic acid (ALA) were observed to increase according to type of treatment and length of feed supplementation. Principal component analysis (PCA) resulted in two-principal component model that described 92.07% of the total data variance. The replacement of soybean oil with canola and chia oils in Nile tilapia diets contributed to increase the concentration of n-3 LC-PUFA in Nile tilapia fillets, improving its nutritional value.

Key word: chia oil, canola oil, omega-3, *Oreochormis niloticus*, LC-PUFA, PCA.

INTRODUCTION

According to FAO an increase in fish farmed was reported in the latest years. Farmed food fish contributed to 42.2 percent of the total 158 million tonnes of fishery production (farmed fish and capture) in 2012 (FAO, 2014). Nile tilapia (*Oreochromis niloticus*) being the second largest group of freshwater fish farming cultivated around the world, due to adaptability of farming, great quality and acceptance (Fülber et al., 2009). Nile tilapia accounted for 43% of the total production of fish farming in Brazil, reaching 169,000 t in 2013 (IBGE, 2013; IBGE, 2014). However, lower contents of long-chain polyunsaturated fatty acids (LC-PUFA) were reported in Nile tilapia farmed when compared to marine fish (Abou et al., 2011).

The LC-PUFA includes the health-benefitting fatty acids (≥C20 and ≥ 3 double bonds) such as arachidonic acid (ARA, 20:4n-6), eicosapentaenoic acid (EPA, 20:5n-3), docosahexaenoic acid (DHA, 22:6n-3) and other important n-3 fatty acids, as docosapentaenoic acid (DPA, 22:5n-3) (Perini et al., 2011). Polyunsaturated fatty acids including alpha-linolenic acid (ALA, 18:3n-3) and linoleic acid (LA, 18:2n-6) can be converted to the biologically active LC-PUFA in vertebrates, as fishes, through process of elongation and desaturation (Tocher, 2003). Several beneficial effects of dietary n-3 LC-PUFA have been reported in the context of inflammatory diseases (Deckelbaum and Calder, 2010; Simopoulos, 2008), such as rheumatoid arthritis and more recently, on inflammatory bowel disease such as Crohn's disease and ulcerative colitis (Cabré, 2012). LC-PUFA plays an important role in reducing the risk of depression and schizophrenia (Morris et al., 2005; Su et al., 2008).

Lipid rich in n-3 LC-PUFA mainly come from marine sources such as fish oil, which is widely used in aquafeeds with supply of these fatty acids (Myers and Worm, 2003). However, fish oil is in increasingly short supply globally due to decrease in marine capture production in some countries (FAO, 2014), rising demand and price hikes. Therefore, many studies

research a replacement for fish oil in the aquaculture industry, especially by vegetable oils. Many oils are commonly included in Nile tilapia diets such as soybean oil, linseed oil, sunflower oil, corn oil and coconut oil (Abou et al., 2011; Myers and Worm, 2003).

Chia (*Salvia hispanica* L) seeds are the vegetable source with the highest known concentration of ALA. Thus, chia oil contains ALA in concentrations of up to 67.8%, which are higher than other vegetable oils as flaxseed and soybean (Ayerza, 2011). Canola oil contain on average 20.0% of LA and 10.0% of ALA, which are precursor of LC-PUFA as ARA, EPA and DHA, respectively (Bocianowsky et al., 2012). These oils have many positive nutritional aspects such as low levels of saturated fatty acids (SFA) and high levels of PUFA. Thus dietary chia and canola oils have the potential of enhancing the n-3 LC-PUFA content in Nile tilapia fillets.

This study aimed to investigate the incorporation of n-3 LC-PUFA from supplemented diets with chia and canola oils as a substitute of soybean oil into the composition of muscle tissue of Nile tilapia.

MATERIAL AND METHODS

Experimental diets

Three types of supplemented diets were formulated as shown in Table 1. A control treatment was formulated with soybean oil (TI), which is commonly used in commercial feeds. Other supplemented diets were prepared with canola oil (TII) and chia oil (TIII). The ingredients were milled, sieved and mixed with water to obtain the pellets (3mm of diameter).

The pellets were dried in an oven with air circulation at 55ºC for 10 h. After pellets were vaccum-packed, protected from light and kept at -18ºC until use for fish feeding.

Materials

All chemicals used in this study were purchased from either Merck (Brazil) or Sigma-Aldrich (St. Louis, MO, USA) unless stated otherwise.

Proximate composition

Moisture, ash, and protein contents were determined in accordance with AOAC methods (AOAC, 1998). Total lipids were extracted by Bligh and Dyer method (1959). Analyses were carried out in triplicate. Proximate composition and lipid composition of experimental diets are also showed in Table 2 and Table 3, respectively.

Breeding and sampling of fish

The experiments were carried out in the Laboratory of Food Chemistry, Department of Chemistry of State University of Maringá. All Ethical Principles, Protocols and Regulations on Experimentation with Laboratory Animals were used according to the standards established internationally and by the approved project by the Institutional Ethics Committee of State University of Maringá (UEM), the Ethics Committee on Animal Use in Experimentation (CEAE)/UEM, Protocol No. 012/2014, Opinion No. 037/2014. Two hundred and forty Nile tilapia (*Oreochromis niloticus*) with initial average weight of 12.00 ± 1.00 g were obtained from Fish Experimental Station UEM/Codapar, located in Floriano District of Maringá-PR. The fish were equally divided into 8 tanks of 40 liters with constant oxygenation and external filtration and circulation of water. Approximately 50% of water volume was replaced by clean water after cleaning each tank. Each treatment was conducted in triplicate. In the first 7 days, all tanks were fed with the diet control containing soybean oil at 2.1% (w/w) for adaptation to the new conditions. On the 8^{th} day, the initial treatment (Control) was established. Then, tanks were divided randomly into three treatments (TI – feed diet containing 2.1% of soybean oil – control; TII – feed diet containing 2.1% of canola oil; TIII – feed diet containing 2.1% of chia oil), where the fish received supplemented diets. Fish were

fed in the morning and late afternoon, during a period of 30 days. Every 15 days after adaptation period (15 and 30 days) a sample (composed of 12 fish) was removed from each tank. Fish were euthanized with a lidocaine overdose (10 g L^{-1}). The samples were disemboweled, washed, filleted, vacuum packed in polyethylene bags and stored at −18°C for further analyses.

Lipid extraction and fatty acid composition

Total lipids were extracted from tilapia fillets by Bligh and Dyer (1959) method. Fatty acid methyl esters were prepared as described by Hartman and Lago (1973) and modified by Maia and Rodriguez-Amaya (1993). Analyses were carried out in triplicate. Methyl esters were separated by gas chromatography in a Thermo model Trace Ultra 3300 equipped with a flame ionization detector and a cyanopropyl capillary column (100 m x 0.25 i.d., 0.25 µm film thickness, CP-7420). The gas flow rates used were 1.2 mL min^{-1} carrier gas (H_2); 30 mL min^{-1} makeup gas (N_2); 35 and 350 mL min^{-1} flame gases (H_2 and synthetic air, respectively). The sample (2 µL) splitting ratio was 1:80. Operating parameters were as follows: detector temperature, 240 ºC; injection port temperature, 230 ºC. Initially, the column temperature was maintained at 165 ºC for 7 min. It was then raised to 185 ºC, at a rate of 4 ºC min^{-1}, and kept at this temperature for 4.67 min. After this period, it was once again raised to 235 ºC at a rate of 6 ºC min^{-1} and maintained for 5 min, totaling 30 min of chromatographic run. Peak areas were determined by the Software Chromquest 5.0. Fatty acids were identified by comparing the retention times with those of standard methyl esters. Fatty acids were quantified against tricosanoic acid methyl ester (Sigma) as an internal standard, as described by Joseph and Ackman (1992). Theoretical FID (flame ionization detector) correction factor values were used to calculate fatty acid concentration values in mg g^{-1} of total lipids as described in Equation 1 (Visentainer, 2012):

$$FA = \frac{A_X W_{IS} CF_X}{A_{IS} W_X CF_{AE}} \quad (1)$$

where FA is mg of fatty acids per g of total lipids, A_X is the peak area (fatty acids), A_{IS} is the peak area of internal standard methyl ester of tricosanoic acid (23:0), W_{IS} is the internal standard weight (mg) added to the sample, W_X is the sample weight (g), CF_X is the theoretical correction factor, and CF_{AE} is the conversion factor necessary to express results as mg of fatty acids rather than as methyl esters. The results were converted from mg g^{-1} to g fatty acid mg $100g^{-1}$ of sample (Visentainer, 2012).

Statistical analysis

Results were submitted to variance analysis (ANOVA) at 5% significance level with Statistica software version 8.0 (Statsoft, Tulsa, OK, USA) and means were compared by Tukey's test. Total n-6 fatty acids, total n-3 fatty acids, ALA, LA, DHA, ARA and the ratio n-6/n-3 to three treatments and periods (IT, 15 and 30 days) were submitted to Principal Component Analysis – PCA, performed with Statistica software.

RESULTS AND DISCUSSION

Feed diets composition

Table 2 and Table 3 show the proximate composition and fatty acids composition of three supplemented diets, respectively. No significant difference ($P>0.05$) was observed in values of crude protein and total lipids, which ensure the isoproteic and isocaloric aspects of diets (Table 2). Total lipids values obtained to both three treatments were in accordance with Jauncey (1998), who recommended values higher than 5% for Nile tilapia feeds. Fatty acids composition showed significant difference ($P<0.05$), mainly in PUFA content, which was higher in treatments TII and TIII. LA was the majority PUFA in all treatments, while LNA presented higher values in TII (33.16 mg g^{-1}) and TIII (57.96 mg g^{-1}) (Table 3). These values were higher than those reported by Schneider et al. (2015b) to feeds diet with borage oil (23.58 mg g^{-1}) and evening primrose oil (25.74 mg g^{-1}) and also to feed diet formulated with sunflower oil (27.40 mg g^{-1}) (Carbonera et al., 2014). The ratio n-6/n-3 was significant

different (P<0.05) between the treatments, TII and TIII were, respectively lower than TI (Table 3), due to higher amounts of n-3 fatty acids in those diets. Similar results were reported in other studies with feed diets supplemented to Nile tilapia (Carbonera et al., 2014; Costa e Silva et al., 2014; Schneider et al. 2015a).

Fatty acid composition

Fatty acids composition of Nile tilapia fillets submitted to three different treatments and times of feed supplementation were shown in Table 4 and Table 5.

SFA content presented a significant difference (P<0.05) according to time and type of treatment (Table 4 and Table 5). The highest content of SFA was noted in TII for 30 days (TII-30), whereas the treatment with chia oil for 15 days (TIII-15) incorporated minor contents of SFA. According to Henderson (1996) the incorporation of fatty acids into tissues is under various metabolic influences such as preferential incorporation, lipogenic activity, fatty acids elongation and desaturation process and may also be affected by environmental factors (Tocher, 2003). In general SFA content increased with length of treatment. However the fatty acid composition of diets affected the extent to which SFA were incorporated in Nile tilapia tissue. Fish fed with diets higher in SFA and 18:0 (TI and TII) retained (or synthetized *de novo*) higher amounts of SFA in those fatty acid composition. These behaviors are consistent to report in other studies to Nile tilapia (Karapanagiotidis et al., 2007) and also to salmonids (Greene, 1990).

MUFA and PUFA contents showed similarity to SFA content behavior, increasing with a longer time of treatment. The major MUFA in all treatments was 18:1n-9. TII that supplied higher amounts of that fatty acid also showed the highest content of MUFA and 18:1n-9 incorporated in the muscle tissue (Table 4 and Table 5). Similar results were also related in Nile tilapia fed with corn and linseed oil (Karapanagiotidis et al., 2007). The replacement of soybean oil with canola oil and chia oil, mainly, increased the PUFA content, promoting greater nutritional quality to human consumption. At the end of 30 days of feed

supplementation TIII-30 and TII-30 showed higher values of PUFA than others treatments (Table 5).

A significant difference ($P<0.05$) was noted in total n-6 fatty acids content between the IT and other treatments. Compared with IT, an increase in n-6 fatty acid was observed in TI, TII and TIII in both times of treatment (15 and 30 days). Generally, total n-6 fatty acids were higher in those treatments that supplied higher amounts of these fatty acids as TI and TII. In other studies with Nile tilapia fed with vegetables oils this behavior was also reported (Karapanagiotidis et al., 2007; Carbonera et al., 2014; Costa e Silva et al., 2014; Schneider et al., 2015a)

LA fatty acid was the predominant fatty acid in total n-6 fatty acids (Table 4 and Table 5). Comparing with IT, an increase of LA was noted in all treatments according to length of treatment, *i.e*, at 30 days of feed supplementation a higher amount of n-6 fatty acids was incorporated to Nile tilapia fillets than at 15 days in all treatments applied. Costa e Silva et al. (2014) reported similar results to LA incorporation in Nile tilapia fed with soybean oil, which incorporated at 30 days (341.4 mg 100 g^{-1}), whereas supplemented diet with chia bran showed 312.2 mg 100 g^{-1}. Turchini et al. (2013) also observed similar behavior to feed supplementation of rainbow trout fed with monola and canola oil.

Total n-6 LC-PUFA incorporated in Nile tilapia fillets increased over treatments and length of treatment (Table 4 and Table 5). After 15 days of feed supplementation, TII-15 incorporated major contents than TI and TIII. Similar behavior was noted after 30 days, when TII-30 also showed the highest n-6 LC-PUFA incorporated. The major n-6 LC-PUFA observed in fillets were ARA (20:4n-6) and DPAn-6 (22:5n-6).

ARA fatty acid is an LC-PUFA constituent of brain and shows some beneficial effects in fetal development (Simopoulos, 2008; Tinoco et al., 2007). ARA content also showed significant difference ($P<0.05$) between IT and all treatments. The differences observed between the treatments (Table 4 and Table 5) could be related to contents of LA in feed diet (Table 3), which is precursor for n-6 LC-PUFA. Fish fed with TIII incorporated (or synthetized

234 through elongation and desaturation process) minor contents of ARA, whereas fish fed with
235 TII (higher amounts of precursor LA) showed the highest content of ARA incorporated.
236 Similar results were reported by Karapanagiotidis et al. (2007) replacing fish oil with corn and
237 linseed oil in Nile tilapia diets. They observed that fish fed LA rich diet retained high levels of
238 all n-6 fatty acids, mainly ARA and 22:5n-6.

239 Regarding n-3 fatty acids content, an increase was observed between IT and other
240 treatments. After 15 days of feed supplementation TI showed minor content of n-3 fatty
241 acids, while TII and TIII presented higher values (Table 4). At end of 30 days of feed
242 supplementation, TII and TIII were significant different ($P<0.05$) with higher amounts of n-3
243 fatty acids incorporated in fillets (Table 5). In comparison to TI-30, TII-30 and TIII-30 showed
244 an increase of 106% and 265% in n-3 fatty acids content, respectively. These results
245 suggested that canola oil and chia oil were greater in transference of n-3 fatty acids to Nile
246 tilapia than soybean oil. Data reported by other researchers also showed that diet with chia
247 bran (Costa e Silva et al., 2014) and chia oil (Scheneider et al., 2015a) were greater in n-3
248 fatty acids transference than diet with soybean oil. At the end of 30 days, those researchers
249 found a content of n-3 fatty acids of 97.4 mg 100 g^{-1} to Nile tilapia fed with chia bran and 43.6
250 mg 100 g^{-1} to diet with soybean oil (Costa e Silva et al., 2014). In our study, TIII-30 showed
251 higher content of n-3 fatty acids than value related to those authors (Table 5).

252 ALA was predominant in total n-3 fatty acids content, increasing according to
253 treatments and time of feed supplementation, with a significant difference ($P<0.05$) between
254 them (Table 4 and Table 5). After 15 days of treatment, TI showed an increase of 90% in
255 ALA content compared with IT, whereas TII and TIII showed an increase of 247% and 423%,
256 respectively. At end of 30 days, these increments on ALA content were higher than at 15
257 days due to greater amount of ALA incorporated (Table 5). The treatment with chia oil for 30
258 days (TIII-30) showed the highest ALA content (103.13 mg $100g^{-1}$). TII-30 presented
259 significant difference ($P<0.05$) from TI, however the amount of ALA was significantly lower
260 than TIII.

Minor contents of ALA were observed in studies with Nile tilapia fed with borage oil and evening primrose oil (Schneider et al. 2015b), and also with chia bran (Costa e Silva et al., 2014). In this last study, a treatment with chia bran for 30 days incorporated 62.2 mg $100g^{-1}$ of ALA in fillets of Nile Tilapia, which is inferior to that obtained with chia oil for 30 days (TIII-30) in this study (Table 5).

The total content of n-3 LC-PUFA in Nile tilapia fillets ranged between the treatments and length of treatments with a significant difference ($P<0.05$) (Table 4 and Table 5). Higher amounts of n-3 LC-PUFA was observed in TIII, in both times of treatments. Those results could be associated with higher amounts of ALA, which is precursor of n-3 LC-PUFA production such as EPA (20:5n-3), DPAn-3 (22:5n-3) and DHA (22:6n-3). Many studies attributed to n-3 LC-PUFA some beneficial effects, as in cancer prevention and coronary diseases (Su et al., 2008; Deckelbaum and Calder, 2010; Perini et al., 2011).

DPAn-3 is a LC-PUFA, intermediary of n-3 fatty acids metabolism by elongation of ALA to DHA (Perini et al., 2011). DPAn-3 showed a significant difference ($P<0.05$) between IT and other treatments, increasing according to time of feed supplementation (Table 4 and Table 5). The TII and TIII showed similar contents of DPAn-3 for both times of feed supplementation, with no significant ($P>0.05$) difference between them. The treatment with soybean oil presented minor amounts of DPAn-3 to Nile tilapia, which could be related to minor contents of the precursor, ALA, in that treatment. Higher amounts of this intermediary, DPAn-3, in TII and TIII may explain the highest content of DHA in these treatments. Schneider et al. (2015b) observed similar results in Nile tilapia fed with borage and evening primrose oils. Higher contents of DPAn-3 were observed in Nile tilapias fed with evening primrose oil, consequently that treatment showed higher amounts of DHA than treatment with borage oil.

EPA and DHA showed great contents in fillets supplemented with canola oil and chia oil (TII and TIII) when compared with control feed. EPA showed the highest values in TIII-15 (5.83 mg 100 g^{-1}) and TIII-30 days (7.33 mg 100 g^{-1}), whereas DHA content was higher in

288 TII-30 days (33.82 mg 100 g^{-1}) and in TIII-30 days (38.12 mg 100 g^{-1}). Costa e Silva et al.
289 (2014) reported lower contents of EPA in Nile tilapia fed with chia bran for 30 days (1.30 mg
290 100 g^{-1}) and similar result to DHA content (28.80 mg 100 g^{-1}). Bonafe et al. (2013) reported a
291 DHA content of 26.12 mg g^{-1} of total lipids (approximately 21.90 mg 100 g^{-1}) in tilapia fed with
292 diet containing 4.2% of tung oil for 30 days. Their result is lower than reported in the present
293 study (Table 5).

294 Ratio n-6/n-3 was observed to decrease according to type of treatment (TI, TII and
295 TIII) as shown in Table 4 and Table 5, with a significant difference ($P<0.05$) between feed
296 control (TI) and other treatments. TII and TIII reduced the n-6/n3 ratio by about 29% and
297 63%, respectively, when compared to TI. Simopoulos (2008) reported that values of n-6/n-3
298 ratio between 2.0 – 3.0 suppressed inflammation in patients with rheumatoid arthritis. The
299 results of TIII were in accordance with that recommendation. Despite the treatment with
300 canola oil showed a significant reduction in n-6/n-3 ratio in comparison to TI in both times (15
301 and 30 days), the results of n-6/n-3 ratio were higher than those recommended by
302 Simopoulos (2008).

303 Other researchers investigated the incorporation of LC-PUFA in Nile tilapia, especially
304 DHA and its precursor, ALA (Carbonera et al., 2014; Schneider et al., 2015a; Santos et al.
305 2014). Santos et al. (2014) investigated changes in fatty acid composition through feed
306 supplementation with perilla seed bran for 45 and 60 days. Their results appointed an
307 incorporation of DHA of 15.50 mg g^{-1} and 16.06 mg g^{-1} of total lipids (approximately 9.9 mg
308 100 g^{-1} and 10.2 mg 100 g^{-1}) for 45 and 60 days, respectively. ALA incorporation ranged from
309 32.42 mg g^{-1} (approximately 20.7 mg 100 g^{-1}) for 45 days to 36.03 mg g^{-1} (approximately 30.2
310 mg 100 g^{-1}) for 60 days of treatment. Compared to that study, the incorporation of DHA and
311 ALA in tilapia through diets incorporated higher amounts of these fatty acids in a shorter time
312 of treatment by using both canola and chia oils. However, supplemented diets with chia oil
313 was observed to be more effective in transference of n-3 fatty acids to Nile tilapia fillets than
314 other treatments, due to higher contents of these fatty acids incorporated and lower n-6/n-3

315 ratio. Results also suggest that the accumulation of ALA and DHA in the fish fillet depends
316 on the length of treatment; so, a longer feeding period would result in a higher incorporation
317 of those fatty acids.
318
319 **Principal component analysis**
320 PCA was applied to differentiate the sum of fatty acids (LA, ARA, ALA, DPAn-3, DHA,
321 n-3, n-6) and ratio n-6/n-3 between the three treatments TI (soybean oil), TII (canola oil), TIII
322 (chia oil) and different periods of experiment IT (initiation of treatment), 15 and 30 days.
323 Principal component analysis resulted in two-principal component model that
324 described 92.07% of the total data variance as shown in Figure 1a. PC1 and PC2 accounted
325 for 69.28% and 22.79% of the variance in the data, respectively. The four remaining
326 generated PCs (i.e. PC3 and PC6) yielded progressively smaller contributions in the variance
327 accounted for and did not explain the data variability significantly (< 8%).
328 PC1 loadings indicated a high negative contributions from DHA (–0.9688), DPA (-
329 0.9404) and n-3 (–0.8933); PC2 loadings showed a high positive contributions from n-6/n-3
330 (0.7267) and ARA (0.6379). Figure 1b presents the graphical loadings for the variables and
331 Figure 1c shows the distributions of treatments on the plan of PC1 x PC2. IT was isolated
332 from other points at the extremely right from PC1 axis, whereas TI-15 and TI-30 (treatment
333 with soybean oil) were located in the right of PC1 axis and above PC2 axis. The treatment
334 with canola oil (TII) to both times (TII-15 and TII-30) was on the opposite quadrant to
335 soybean oil (TI), whereas treatment with chia oil (TIII) was located under PC2 axis and in
336 opposite sides of PC1 axis (Figure 1c).
337 According to PCA (Figure 1c), PC1 was significant on separation of IT from others
338 periods (15 and 30 days) and treatments (TI, TII and TIII), indicating a behavior according to
339 the type of treatment received. The differences of positions in score plots between the
340 treatments suggest the influence of n-3 and n-6 variables, which promotes differences in
341 incorporation of those fatty acids on Nile tilapia fillets.

CONCLUSION

The replacement of soybean oil with canola and chia oils in Nile tilapia diets improved the concentration of n-3 LC-PUFA in Nile tilapia fillets. Both treatments showed greatest increments in EPA, DPAn-3 and DHA contents throughout time of treatment. Treatment with chia oil allowed a considerable incorporation of LC-PUFA into the fish fillet, proving higher effectiveness in fatty acids transference. The precursors of LC-PUFA, LA and ALA also presented higher contents in fillets and increased according to time of feed supplementation for all treatments. PCA showed separation of groups by type of treatment and time of feed supplementation. Therefore, the addition of canola or chia oils as a lipid source modified the fatty acid composition of muscle tissue, contributing to improve its nutritional value.

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Table 1. Composition (g kg^{-1}) of the three supplemented diets

Ingredients (%)	Treatments		
	TI	TII	TIII
Soybean bran	53.65	53.65	53.65
Maize	23.28	23.28	23.28
Wheat bran	8.62	8.62	8.62
Brewers rice	7.66	7.66	7.66
Bicalcium phosphate	2.87	2.87	2.87
Salt	0.48	0.48	0.48
Premix[a]	0.48	0.48	0.48
L-Lysine	0.19	0.19	0.19
DL-Methionine	0.14	0.14	0.14
L-Threonine	0.14	0.14	0.14
L-Tryptophan	0.05	0.05	0.05
Choline chloride	0.10	0.10	0.10
Antifungal	0.10	0.10	0.10
Vitamin C (mono)	0.10	0.10	0.10
Antioxidant (BHT)	0.04	0.04	0.04
Soybean oil	2.10	-	-
Canola oil	-	2.10	-
Chia oil	-	-	2.10

[a] Mineral and vitamin supplement. TI: treatment with soybean oil (control diet); TII: treatment with canola oil; TIII: treatment with chia oil.

Table 2. Proximate composition (g kg^{-1}) of feed diets

	Treatments		
	TI	TII	TIII
Ash	71.4 [a] ± 0.05	73.70 [b] ± 0.04	72.32 [ab] ± 0.05
Crude protein	333.56 [a] ± 0.21	335.59 [a] ± 0.33	336.34 [a] ± 0.27
Moisture	61.11 [a] ± 0.18	61.15 [a] ± 0.19	58.93 [a] ± 0.16
Total lipids	68.81 [a] ± 0.54	66.60 [a] ± 0.32	64.71 [a] ± 0.35

Results expressed as mean ± S.D. of three replicates. Values with different letters in the same row are significantly different ($P < 0.05$) by Tukey's test. TI: treatment with soybean oil; TII: treatment with canola oil; TIII: treatment with chia oil.

Table 3. Fatty acid composition (mg g^{-1} of total lipids) of feed diets

Fatty acids	Treatments		
	TI	TII	TIII
14:0	12.37 [a] ± 0.26	2.33 [b] ± 0.17	1.55 [c] ± 0.39
16:0	137.26 [ab] ± 1.12	135.56 [a] ± 1.97	139.47 [b] ± 0.59
16:1n-7	2.33 [a] ± 0.16	7.21 [b] ± 0.20	5.67 [c] ± 0.34
18:0	32.33 [a] ± 0.32	39.47 [b] ± 0.50	22.29 [c] ± 0.26
18:1n-9	189.32 [a] ± 0.63	180.04 [b] ± 1.28	164.74 [c] ± 2.71
18:1n-7	18.01 [a] ± 1.38	19.14 [a] ± 0.60	14.07 [b] ± 0.66
18:2n-6	364.99 [a] ± 2.49	383.30 [b] ± 2.20	372.08 [c] ± 1.07
18:3n-3	25.44 [a] ± 1.93	33.16 [b] ± 0.62	58.96 [c] ± 1.68
20:0	2.93 [a] ± 0.06	1.68 [b] ± 0.15	0.66 [c] ± 0.07
20:1n-9	2.43 [a] ± 0.16	5.70 [b] ± 0.24	0.81 [c] ± 0.17
24:0	1.74 [a] ± 0.18	0.46 [b] ± 0.36	0.40 [c] ± 0.18
24:1n-9	0.35 [a] ± 0.08	1.38 [b] ± 0.40	0.63 [a] ± 0.13
SFA	186.56 [a] ± 1.24	179.52 [b] ± 2.73	165.04 [c] ± 0.77
MUFA	214.34 [a] ± 0.72	212.09 [b] ± 0.85	185.92 [c] ± 1.97
PUFA	390.43 [a] ± 1.13	416.47 [b] ± 2.31	430.04 [c] ± 0.66
n-6	364.99 [a] ± 2.49	383.30 [b] ± 2.20	372.08 [c] ± 1.07
n-3	25.44 [a] ± 1.93	33.17 [b] ± 0.62	58.96 [c] ± 1.68
n-6/n-3	14.34 [a] ± 1.05	11.55 [b] ± 1.09	6.94 [c] ± 0.91

Results expressed as mean ± S.D. of the three replicates. Values with different letters in the same row are significantly different ($P < 0.05$) by Tukey's test. TI: treatment with soybean oil; TII: treatment with canola oil; TIII: treatment with chia oil. SFA: total saturated fatty acid; MUFA: total monounsaturated fatty acid; PUFA: total polyunsaturated fatty acid; n-6: total omega-6 fatty acids; n-3: total omega-3 fatty acids.

Table 4. Fatty acid composition (mg $100g^{-1}$) of Nile tilapia fillets submitted to different treatments for 15 days of feed supplementation

Fatty acids	IT	TI-15	TII-15	TIII-15
14:0	12.64 ^{a} ± 0.23	12.87 ^{a} ± 0.34	11.47 ^{b} ± 0.17	8.02 ^{c} ± 0.26
14:1n-9	2.60 ^{c} ± 0.14	4.47 ^{a} ± 0.23	3.25 ^{b} ± 0.20	1.66 ^{d} ± 0.01
15:0	1.85 ^{c} ± 0.07	3.69 ^{b} ± 0.12	4.34 ^{a} ± 0.19	1.71 ^{c} ± 0.09
15:1n-9	1.30 ^{ab} ± 0.05	1.86 ^{a} ± 0.36	2.10 ^{a} ± 0.31	0.64 ^{c} ± 0.04
16:0	163.28 ^{a} ± 0.25	157.22 ^{a} ± 0.53	138.15 ^{b} ± 0.35	139.05 ^{b} ± 0.88
16:1n-9	6.15 ^{b} ± 0.21	5.81 ^{b} ± 0.31	8.32 ^{a} ± 0.26	4.13 ^{c} ± 0.14
16:1n-7	29.90 ^{b} ± 0.55	26.49 ^{b} ± 0.64	37.29 ^{a} ± 0.31	19.11 ^{c} ± 0.15
16:1n-5	6.60 ^{a} ± 0.13	5.62 ^{b} ± 0.34	6.93 ^{a} ± 0.32	3.68 ^{c} ± 0.35
17:0	3.40 ^{b} ± 0.10	3.57 ^{b} ± 0.32	4.51 ^{a} ± 0.32	2.77 ^{c} ± 0.24
17:1n-9	5.00 ^{b} ± 0.09	5.80 ^{b} ± 0.08	8.18 ^{a} ± 0.22	4.18 ^{c} ± 0.34
18:0	48.18 ^{c} ± 0.37	57.60 ^{b} ± 0.31	62.46 ^{a} ± 0.50	48.37 ^{c} ± 0.13
18:1n-11	3.00 ^{c} ± 0.01	3.88 ^{b} ± 0.03	4.54 ^{a} ± 0.22	2.21 ^{d} ± 0.11
18:1n-9	172.30 ^{c} ± 1.46	194.39 ^{b} ± 1.19	218.20 ^{a} ± 1.07	169.69 ^{c} ± 1.03
18:1n-7	22.40 ^{c} ± 0.14	26.58 ^{b} ± 0.21	39.07 ^{a} ± 0.35	17.00 ^{d} ± 0.34
18:2n-6	142.90 ^{c} ± 1.08	179.81 ^{b} ± 0.76	203.01 ^{a} ± 0.89	148.94 ^{d} ± 0.55
18:3n-6	6.60 ^{c} ± 0.33	13.62 ^{b} ± 0.10	16.29 ^{a} ± 0.12	7.16 ^{c} ± 0.25
18:3n-3	6.98 ^{d} ± 0.24	13.26 ^{c} ± 0.05	24.29 ^{b} ± 0.39	36.63 ^{a} ± 0.17
20:0	5.50 ^{a} ± 0.19	2.58 ^{c} ± 0.12	4.42 ^{b} ± 0.17	1.91 ^{d} ± 0.27
18:4n-3	0.31 ^{c} ± 0.08	1.29 ^{b} ± 0.55	2.58 ^{b} ± 0.35	3.33 ^{a} ± 0.26
20:1n-9	11.70 ^{b} ± 0.02	10.34 ^{c} ± 0.29	16.60 ^{a} ± 0.27	6.64 ^{d} ± 0.24
20:2n-6	5.56 ^{d} ± 0.08	8.78 ^{b} ± 0.27	11.33 ^{a} ± 0.57	7.35 ^{c} ± 0.32
21:0	5.00 ^{a} ± 0.10	2.82 ^{c} ± 0.52	3.33 ^{b} ± 0.22	1.41 ^{d} ± 0.05
20:3n-6	6.91 ^{d} ± 0.15	11.14 ^{b} ± 0.05	13.28 ^{a} ± 0.47	8.22 ^{c} ± 0.10
20:4n-6	18.08 ^{b} ± 0.09	29.80 ^{c} ± 0.35	36.31 ^{a} ± 0.66	35.40 ^{a} ± 0.43
20:3n-3	1.18 ^{d} ± 0.02	3.94 ^{c} ± 0.03	5.40 ^{b} ± 0.04	7.36 ^{a} ± 0.35
20:5n-6	0.17 ^{c} ± 0.01	1.95 ^{ab} ± 0.15	1.78 ^{b} ± 0.11	2.10 ^{a} ± 0.20
22:0	4.50 ^{a} ± 0.04	1.91 ^{c} ± 0.08	3.26 ^{b} ± 0.22	1.33 ^{c} ± 0.48
20:4n-3	0.28 ^{c} ± 0.07	2.35 ^{b} ± 0.09	2.78 ^{b} ± 0.28	4.92 ^{a} ± 0.05
20:5n-3	0.26 ^{c} ± 0.02	1.98 ^{b} ± 0.08	2.60 ^{b} ± 0.02	5.83 ^{a} ± 0.29
22:4n-6	6.06 ^{d} ± 0.12	12.54 ^{b} ± 0.29	16.60 ^{a} ± 0.36	9.49 ^{c} ± 0.34
22:5n-6	15.09 ^{c} ± 0.18	25.54 ^{b} ± 0.08	30.30 ^{a} ± 0.40	26.93 ^{b} ± 0.33
24:0	5.00 ^{a} ± 0.09	2.15 ^{b} ± 0.14	2.12 ^{b} ± 0.24	1.11 ^{c} ± 0.21
24:1n-9	4.80 ^{a} ± 0.10	2.82 ^{c} ± 0.04	3.66 ^{b} ± 0.14	0.48 ^{d} ± 0.05
22:5n-3	2.82 ^{c} ± 0.20	7.29 ^{b} ± 0.24	10.55 ^{a} ± 0.52	10.62 ^{a} ± 0.23
22:6n-3	8.30 ^{d} ± 0.17	16.96 ^{c} ± 0.19	23.03 ^{b} ± 0.45	30.12 ^{a} ± 0.25

SFA	249.35 [a]	± 0.65	244.40 [b]	± 0.88	234.06 [c]	± 0.97	205.67 [d]	± 0.41
MUFA	265.75 [b]	± 0.95	286.19 [b]	± 1.35	346.04 [a]	± 1.37	228.77 [c]	± 0.78
PUFA	221.50 [c]	± 0.41	330.25 [b]	± 0.69	400.14 [a]	± 0.64	344.38 [b]	± 0.91
n-6	201.36 [d]	± 0.13	283.17 [b]	± 0.45	328.91 [a]	± 0.59	245.57 [c]	± 0.52
n-6 LC-PUFA	44.95 [d]	± 0.07	78.61 [c]	± 0.14	96.33 [a]	± 0.10	81.27 [b]	± 0.62
n-3	20.14 [d]	± 0.11	58.21 [c]	± 0.20	84.51 [b]	± 0.53	107.02 [a]	± 0.84
n-3 LC-PUFA	12.84 [d]	± 0.09	32.52 [c]	± 0.54	44.35 [b]	± 0.31	58.85 [a]	± 0.96
n-6/n-3	10.00 [a]	± 0.33	4.86 [b]	± 0.26	3.89 [c]	± 0.38	2.29 [d]	± 0.47

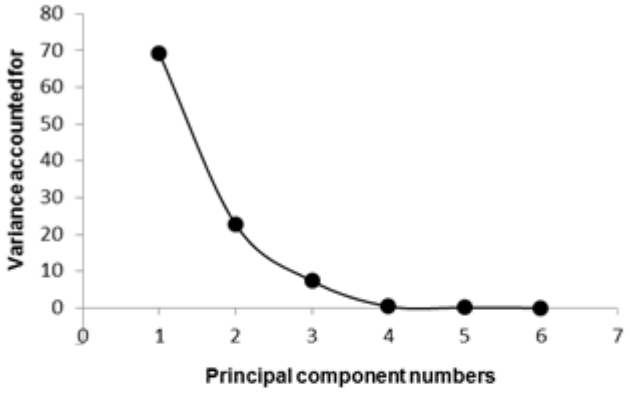
Results expressed as mean ± S,D, of the three replicates, Values with different letters in the same row are significantly different ($P < 0,05$) by Tukey's test, IT: initial treatment; TI-15: treatment with soybean oil for 15 days; TII-15: treatment with canola oil for 15 days; TIII-15: treatment with chia oil for 15 days, SFA: total saturated fatty acid; MUFA: total monounsaturated fatty acid; PUFA: total polyunsaturated fatty acid; n-6: total omega-6 fatty acids; n-6 LC-PUFA: total omega-6 long chain polyunsaturated fatty acids; n-3: total omega-3 fatty acids; n-3 LC-PUFA: total omega-3 long chain polyunsaturated fatty acids.

Table 5. Fatty acid composition (mg $100g^{-1}$) of Nile tilapia fillets submitted to different treatments for 30 days of feed supplementation.

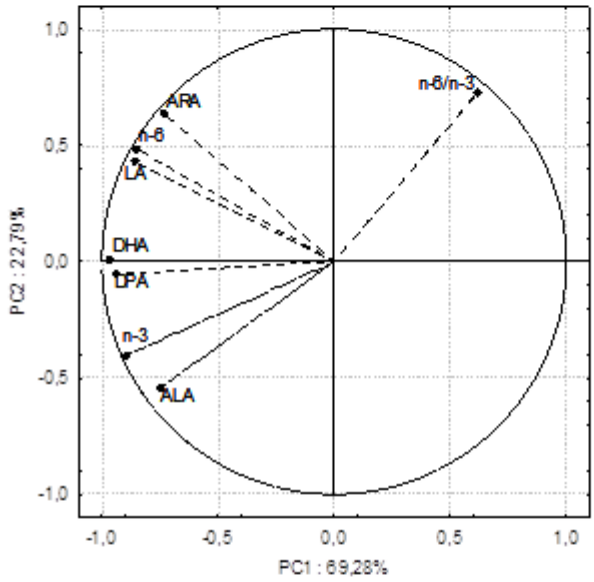
Fatty acids	IT	TI-30	TII-30	TIII-30
14:0	12.64 ^{c} ± 0.23	21.66 ^{a} ± 0.56	20.78 ^{b} ± 0.17	9.55 ^{d} ± 0.24
14:1n-9	2.60 ^{c} ± 0.14	2.65 ^{c} ± 0.17	7.26 ^{a} ± 0.20	3.61 ^{b} ± 0.53
15:0	1.85 ^{d} ± 0.07	2.63 ^{c} ± 0.13	5.37 ^{a} ± 0.19	3.01 ^{b} ± 0.40
15:1n-9	1.30 ^{c} ± 0.05	5.81 ^{a} ± 0.15	3.97 ^{b} ± 0.31	2.09 ^{c} ± 0.41
16:0	163.28 ^{c} ± 0.25	246.04 ^{a} ± 1.01	232.93 ^{b} ± 1.35	241.42 ^{ab} ± 0.31
16:1n-9	6.15 ^{bc} ± 0.21	6.75 ^{b} ± 0.20	13.47 ^{a} ± 0.26	5.41 ^{c} ± 0.04
16:1n-7	29.90 ^{c} ± 0.55	43.53 ^{b} ± 0.82	66.43 ^{a} ± 0.31	28.64 ^{c} ± 0.13
16:1n-5	6.60 ^{b} ± 0.13	5.14 ^{c} ± 0.11	10.34 ^{a} ± 0.32	7.38 ^{b} ± 0.33
17:0	3.40 ^{b} ± 0.10	2.78 ^{c} ± 0.35	3.75 ^{b} ± 0.32	4.42 ^{a} ± 0.49
17:1n-9	5.00 ^{bc} ± 0.09	5.45 ^{b} ± 0.39	11.03 ^{a} ± 0.22	4.80 ^{c} ± 0.28
18:0	48.18 ^{d} ± 0.37	73.68 ^{b} ± 0.46	94.86 ^{a} ± 0.50	58.01 ^{c} ± 0.82
18:1n-11	3.00 ^{b} ± 0.01	3.07 ^{b} ± 0.11	6.92 ^{a} ± 0.22	2.72 ^{b} ± 0.19
18:1n-9	172.30 ^{d} ± 1.46	301.14 ^{c} ± 1.11	367.60 ^{a} ± 1.07	315.90 ^{b} ± 0.82
18:1n-7	22.40 ^{d} ± 0.14	35.48 ^{b} ± 0.13	62.80 ^{a} ± 0.35	32.19 ^{c} ± 0.61
18:2n-6	142.90 ^{c} ± 1.08	245.35 ^{b} ± 1.72	307.92 ^{a} ± 1.89	312.50 ^{a} ± 0.63
18:3n-6	6.60 ^{d} ± 0.33	18.26 ^{b} ± 0.76	25.80 ^{a} ± 0.12	15.59 ^{c} ± 0.34
18:3n-3	6.98 ^{d} ± 0.24	18.54 ^{c} ± 0.73	36.60 ^{b} ± 0.39	103.13 ^{a} ± 0.62
20:00	5.50 ^{b} ± 0.19	3.13 ^{d} ± 0.41	6.30 ^{a} ± 0.17	3.79 ^{c} ± 0.14
18:4n-3	0.31 ^{d} ± 0.08	1.19 ^{c} ± 0.06	3.33 ^{b} ± 0.35	3.76 ^{a} ± 0.28
20:1n-9	11.70 ^{c} ± 0.02	14.44 ^{b} ± 0.69	26.96 ^{a} ± 0.27	9.66 ^{d} ± 0.22
20:2n-6	5.56 ^{d} ± 0.08	12.32 ^{c} ± 0.40	16.89 ^{a} ± 0.57	15.27 ^{b} ± 0.68
21:0	5.00 ^{b} ± 0.10	3.15 ^{c} ± 0.43	6.61 ^{a} ± 0.22	3.08 ^{c} ± 0.55
20:3n-6	6.91 ^{c} ± 0.15	14.53 ^{b} ± 0.30	20.08 ^{a} ± 0.47	14.27 ^{b} ± 0.44
20:4n-6	18.08 ^{d} ± 0.09	34.08 ^{c} ± 0.69	48.92 ^{a} ± 0.66	36.28 ^{b} ± 0.17
20:3n-3	1.18 ^{d} ± 0.02	3.61 ^{c} ± 0.15	7.15 ^{b} ± 0.04	14.42 ^{a} ± 0.18
20:5n-6	0.17 ^{d} ± 0.01	0.65 ^{c} ± 0.09	2.42 ^{b} ± 0.11	4.17 ^{a} ± 0.27
22:0	4.50 ^{a} ± 0.04	1.32 ^{c} ± 0.21	4.55 ^{a} ± 0.22	2.83 ^{b} ± 0.29
20:4n-3	0.28 ^{c} ± 0.07	0.67 ^{b} ± 0.09	3.64 ^{a} ± 0.28	3.54 ^{a} ± 0.16
20:5n-3	0.26 ^{d} ± 0.02	2.66 ^{c} ± 0.44	3.73 ^{b} ± 0.02	7.33 ^{a} ± 0.29
22:4n-6	6.06 ^{d} ± 0.12	13.76 ^{c} ± 0.15	22.77 ^{a} ± 0.36	17.30 ^{b} ± 0.27
22:5n-6	15.09 ^{c} ± 0.18	33.88 ^{b} ± 0.29	45.45 ^{a} ± 0.40	35.53 ^{b} ± 0.66
24:0	5.00 ^{a} ± 0.09	1.55 ^{c} ± 0.27	4.60 ^{ab} ± 0.24	4.30 ^{b} ± 0.05
24:1n-9	4.80 ^{a} ± 0.10	1.55 ^{c} ± 0.10	4.45 ^{b} ± 0.14	1.36 ^{c} ± 0.32
22:5n-3	2.82 ^{c} ± 0.20	5.33 ^{b} ± 0.27	14.12 ^{a} ± 0.52	14.49 ^{a} ± 0.40
22:6n-3	8.30 ^{d} ± 0.17	17.67 ^{c} ± 0.21	33.82 ^{b} ± 0.45	38.12 ^{a} ± 0.38

SFA	249.35	d	± 0.65	355.95	b	± 0.77	379.73	a	± 1.04	330.40	c	± 1.06
MUFA	265.75	d	± 0.95	425.00	b	± 1.03	581.22	a	± 1.11	413.76	c	± 0.88
PUFA	221.50	d	± 0.41	422.50	c	± 0.54	592.64	b	± 0.98	635.72	a	± 0.91
n-6	201.36	d	± 0.13	372.84	c	± 0.42	490.26	a	± 0.37	450.92	b	± 0.45
n-6 LC-PUFA	44.95	d	± 0.07	94.70	c	± 0.33	136.45	a	± 0.25	108.56	b	± 0.19
n-3	20.14	d	± 0.11	49.66	c	± 0.19	102.39	b	± 0.14	184.80	a	± 0.21
n-3 LC-PUFA	12.84	d	± 0.09	29.26	c	± 0.08	58.82	b	± 0.09	74.36	a	± 0.13
n-6/n-3	10.00	a	± 0.33	7.51	b	± 0.11	4.79	c	± 0.17	2.44	d	± 0.09

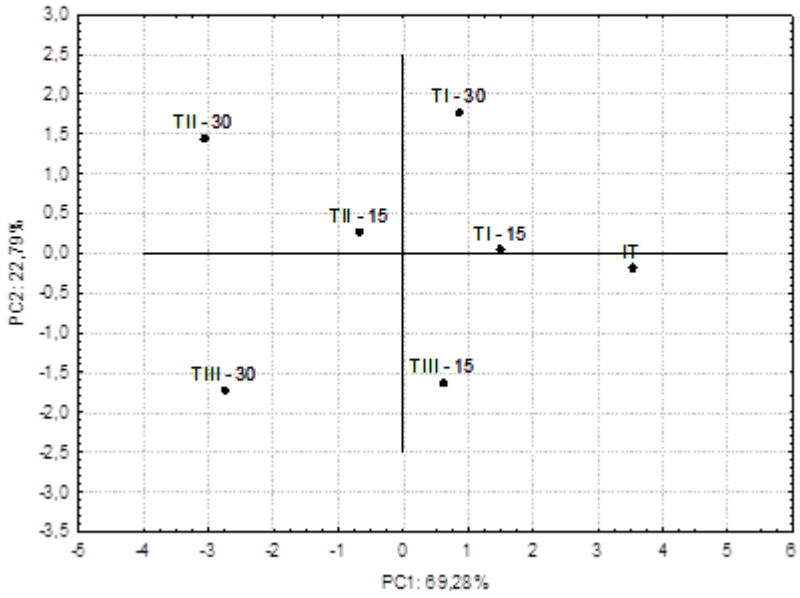
Results expressed as mean ± S.D. of the three replicates. Values with different letters in the same row are significantly different ($P < 0.05$) by Tukey's test. IT: initial treatment; TI-30: treatment with soybean oil for 30 days; TII-30: treatment with canola oil for 30 days; TIII-30: treatment with chia oil for 30 days. SFA: total saturated fatty acid; MUFA: total monounsaturated fatty acid; PUFA: total polyunsaturated fatty acid; n-6: total omega-6 fatty acids n-6 LC-PUFA: total omega-6 long chain polyunsaturated fatty acids; n-3: total omega-3 fatty acids; n-3 LC-PUFA: total omega-3 long chain polyunsaturated fatty acids.


Variance accounted for
Principal component numbers

(a)


n-6/n-3
ARA
n-6
LA
DHA
LPA
n-3
ALA
PC2 : 22,79%
PC1 : 69,28%

(b)


TI - 30
TII - 30
TII - 15
TI - 15
IT
TIII - 30
TIII - 15
PC2: 22,79%
PC1: 69,28%

(c)

Figure Captions

Figure 1 (a) Variance distribution according to PCs. (b) Loading plot of PC1-PC2. (c) Score plots of PC1-PC2. IT: Initial treatment; TI-15: treatment with soybean oil for 15 days; TI-30: treatment with soybean oil for 30 days; TII-15: treatment with canola oil for 15 days; TII-30: treatment with canola oil for 30 days; TIII-15: treatment with chia oil for 15 days; TIII-30: treatment with chia oil for 30 days.