http://dx.doi.org/10.1590/1415-52732014000500004

Hematology and plasma biochemistry in rats fed with diets enriched with fatty fishes from Amazon region

Hematologia e bioquímica plasmática em ratos alimentados com dietas enriquecidas com peixes gordurosos da bacia Amazônica

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A B S T R A C T

Objective

Rats fed diets enriched with fatty fish from the Amazon region had Hematology and plasma biochemistry analyzed.

Methods

Forty Wistar rats were divided into four groups: control group fed a standard diet; *mapará* group fed a diet enriched with *Hypophthalmus edentatus*; *matrinxã* group fed a diet enriched with *Brycon* spp.; and *tambaqui* group fed a diet enriched with *Colossoma macropomum*. After thirty days the rats had an red blood count and plasma biochemistry.

Results

Hematocrit and hemoglobin levels were higher in rats fed *tambaqui* and *matrinxã* than in those fed the standard diet of *mapará*. However, *mapará* increased cholesterol, especially low-density lipoprotein cholesterol and high-density lipoprotein cholesterol. All fish-enriched diets reduced triacylglycerols.

Conclusion

Diets enriched with fatty fish from the Amazon region reduce triacylglycerol and increase high-density lipoprotein cholesterol, especially the diet enriched with *tambaqui*. *Tambaqui* and *matrinxã* affected hematocrit and hemoglobin levels, but not *mapará*. Further research is needed to determine the benefits of diets enriched with fatty fish from the Amazon region.

Indexing terms: Cholesterol. Diet. Parameters. Triacylglycerols.

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Support: Fundação de Amparo à Pesquisa do Estado do Amazonas (Proccess nº 01212009 and 020/2013).

http://dx.doi.org/10.1590/1415-52732014000500004

RESUMO

Objetivo

A hematologia e bioquímica plasmática foram avaliadas em ratos submetidos a dietas enriquecidas com peixes gordurosos da região amazônica.

Métodos

Ratos machos da linhagem Wistar foram divididos em quatro grupos: grupo-controle (dieta-padrão); grupo mapará (dietas enriquecidas com Hypophthtalmus edentatus); grupo matrinxã (dietas enriquecidas com Brycon spp.); grupo tambaqui (dietas enriquecidas com Colossoma macropomum). Os parâmetros hematológicos e as variáveis bioquímicas plasmáticas foram analisadas nos animais após 30 dias de experimentação.

Resultados

Animais alimentados com dietas enriquecidas com tambaqui e matrinxã apresentaram valores de hematócrito e concentração de hemoglobina mais elevados que aqueles alimentados com dieta padrão. Não foram observadas alterações nas variáveis hematológicas em ratos alimentados com dietas enriquecidas com mapará. Porém, os ratos desse grupo apresentaram elevados teores de colesterol total plasmáticos, principalmente de colesterol da lipoproteína de baixa densidade e colesterol da lipoproteína de alta densidade. Todos os tratamentos com dietas enriquecidas reduziram os níveis de triacilgliceróis plasmáticos.

Conclusão

Dietas enriquecidas com carne de peixes amazônicos gordurosos reduzem os teores de triacilgliceróis plasmáticos e aumentam os níveis de colesterol da lipoproteína de alta densidade, especialmente nos ratos do grupo tambaqui. Com exceção do grupo mapará, ratos alimentados com dietas enriquecidas com outras dietas enriquecidas com peixes apresentaram alterações hematológicas. Porém, fazem-se necessário mais estudos para se estabelecerem os benefícios das dietas enriquecidas com peixes gordurosos da Bacia Amazônica.

Termos de Indexação: *Colesterol. Dieta. Parâmetros. Triglicerídios.*

INTRODUCTION

The number of studies on the health benefits of seafood has increased in the last twenty years, especially because of the importance of Polyunsaturated Fatty Acids (PUFA) in reducing cardiovascular disease risk[1,2]. Omega-3 fatty acids have been shown to prevent cardiovascular diseases by reducing the number of cardiac arrhythmias, lowering triglycerides, lowering blood pressure, and reducing platelet aggregation[3]. However, it is not clear whether all omega-3 fatty acids reduce cardiovascular risk similarly.

Seafood is an important source of omega-3 fatty acids. Although omega-3 fatty acids are also essential for these organisms, they obtain both omega-3 and omega-6 fatty acids. Omega-3: omega-6 ratio is higher in saltwater fish than in freshwater fish because of their different diets[4]. For example, Greenlandic Inuit consume roughly 400 g of fish *per capita per* day[5] and have low cardiovascular disease mortality rate[6] like the Japanese, who consume roughly 100 g of fish *per capita per* day[7].

In the last years, fish intake in Brazil increased by approximately 24%[8]. Yet, according to the Ministry of Fishing and Aquaculture[8], Brazilians consume only 30.0 g of seafood *per capita per* day. This amount is similar to the minimum amount of 33.0 g *per capita per* day recommended by the World Health Organization (WHO) and lower than the global consumption of 46.5 g *per capita per* day[9]. According to the *Instituto Brasileiro de Geografia e Estatística* (IBGE, Brazilian Institute of Geography and Statistics)[10], fish intake in the Amazon region (104.0 g *per capita per* day) is higher than the Brazilian average. However, intake varies greatly by location: in the high *Solimões* River area, *per capita* intake reaches 800.0 g/day[11], while in *Manaus'* metropolitan area, the mean *per capita* intake is 92.0 g/day[12]. This clearly shows that fish is one of the most important and intensely harvested resources in the Amazon region.

The approximate number of freshwater fish species in the Amazon region is 2,500, representing 30% of the total number of freshwater fish on the planet[13]. Freshwater fish have high levels of C-16 and C-18 and low levels of C-20 and C-22 PUFA (among them Eicosapentaenoic [EPA] and Docosahexaenoic [DHA]) when compared with saltwater fish[14], but according to many studies, the levels of EPA and DHA are still very high in freshwater fish. Inhamuns & Franco[15] found that the catfish *mapará* (*Hypophthalmus* spp.) has high levels of EPA and DHA in their muscles during the Amazon basin flood stage, when more food is available. *Mapará* muscle contains more PUFA than *tambaqui* (*Colossoma macropomum*) and *matrinxã* (*Brycon* spp.) muscles. Proportionally, *tambaqui* has more Saturated Fatty Acids (SFA) than Monounsaturated Fatty Acids (MUFA) and PUFA. Although *mapará* is classified as a fatty fish, most of its fatty acids are PUFA and MUFA instead of SFA[16]. Hence, in terms of nutrition, *mapará* is an excellent source of MUFA and PUFA, reducing cardiovascular risk. Additionally, these fatty acids may also reduce triacylglycerols and total cholesterol and increase High Density Lipoprotein-cholesterol (HDL-c).

However, there are reports that fatty foods affect blood variables[17]. For example, high-fat diets change blood cell-related characteristics, increase total cholesterol and Low Density Lipoprotein-cholesterol (LDL-c), and decrease HDL-c. On the other hand, low levels of fatty acids increase blood viscosity and the risk of atherosclerosis[18]. Blood viscosity increases when hematocrit, number of circulating erythrocytes, or cell volume increases. Thus, diets enriched with fish containing highlevels of SFA, such as *tambaqui*, may have negative effects.

The study objective was to analyze the effects of fatty fish from the Amazon region on the blood cell count and lipids of male Wistar rats.

METHODS

Forty male Wistar rats (*Rattusnorvegicus*) aged about thirty days and weighing 240 g ± 0.60 were obtained from the *Universidade Federal do Amazonas* (UFAM) central vivarium. All animals were fed the standard diet for three days. They were then divided into four groups of ten animals each, kept in individual cages maintained under a 12/12-hour light-dark cycle and controlled temperature, andgiven free access to water and the study diet. This study was approved by the Animal Research Ethics Committee of the UFAM under Protocol CEUA- 014/12.

Diet preparation

The diets were prepared as recommended by Souza *et al*.[19]. In summary, the fish muscle was deboned mechanically (Baader 694 Bone Separator) and immediately frozen to -30°C. The bulk of the experimental diets wasthe commercial chow Nuvilab CR-1 (Nuvilab® *Nutrientes* Ltda, *Curitiba*, PR). The diets based on Amazon fish consisted of commercial chow (72.5%), casein (12.5%), and mechanically deboned and minced muscle (15.0%) of the following species: *mapará* (*Hypophthtalmus edentatus*), *matrinxã* (*Brycon* spp.), and *tambaqui* (*Colossomama cropomum*). All components were ground and mixed. The final composition of each experimental diet was 22.0% proteins, 10.5% lipids, 40.0% carbohydrates, and 16.0% fibers.

Red blood count

At the end of the experiment, the animals were anesthetized with Ketamine® (0.15 mL/ 100 g of body weight) and the painkiller Rompun® (0.015 mL/ 100 g of body weight) tocollect blood by cardiac puncture. Red blood cells were counted as recommended by Kampen & Zijlstra[20]. Hemoglobin level was estimated by the cyanmethemoglobin method as ([Hb g/dL] = Absorbance (540 nm) x 0.146 (correction factor)

 http://dx.doi.org/10.1590/1415-52732014000500004

x 200 (blood sample dilution). Hematocrit (Ht%) was determined by collecting blood in micro-hematocrit heparinized tubes and centrifuged at 1200 rpm for five minutes. The reading was done in a standard card. Circulating erythrocytes ($x10^6$/mm^3) were counted after diluting the blood in saline (1:200 v:v). The erythrocytes were counted directly under 400x magnification using the chamber Neubauer. The corpuscular constants were estimated as follows: Mean Corpuscular Volume (MCV = (Ht/RBC) x 10) in μm^3; Mean Corpuscular Hemoglobin (MCH = ([Hb]/RBC) x 10) in picograms; and Mean Corpuscular Hemoglobin Concentration (MCHC = ([Hb]/Ht) x 100) in %.

Lipoprotein panel

The plasma was separated by centrifugation at 7,500 rpm for two minutes to determine glucose, total protein, triacylglycerol, total cholesterol, and HDL-c levels. Glucose was determined by the colorimetric assay kit Glucox 500 Doles®. This assay uses the enzyme glucose oxidase and a spectrophotometer to measure absorbance at 510 nm. Total protein was determined by the Bradford protein assay, which uses the dye Coomassie Brilliant Blue G-250 and measures absorbance at 595 nm, proportionally reflecting protein concentration. Triacylglycerols were determined by the colorimetric assay kit Triglycerides 120 Doles®. This method uses the enzyme glycerol-3-phosphate oxidase and measures absorbance at 510 nm. Total cholesterol was determined by the colorimetric assay kit Colesterol 250 Doles®, which uses the enzyme cholesterol oxidase and measures absorbance at 510 nm. HDL-c was determined by the colorimetric assay kit Colesterol-HDL from Renylab®, which uses phosphotungstic acid and magnesium chloride, and measures absorbance at 510 nm. Very Low Density Lipoprotein-cholesterol (VLDL-c) and LDL-c were given by Friedwald's equation: VLDL-c = (triacylglycerols/5) and LDL-c = total cholesterol - (HDL-c + VLDL-c).

Statistical analyses

Blood variables are expressed as mean ± standard deviation. The Kolmogorov-Smirnov test assessed whether each variable had a normal distribution. No data group was transformed. The treatments (diets enriched with *mapará, matrinxã*, and *tambaqui*) were compared with the control group (standard diet) by one-factor Analysis of Variance (Anova) followed by Dunnett's *post hoc* test. The significance level was set at 5% for all tests ($p<0.05$). The data was treated by the software SigmaPlot (Systat Software, Inc).

RESULTS

After the 30-day experimental period, rats fed *tambaqui*- and *matrinxã*-enriched diets had higher Hematocrit (Ht) and Hemoglobin Levels (Hb) than those fed the standard diet (Figure 1) ($p<0.05$). Moreover, the total number of circulating erythrocytes increased significantly in animals fed the *tambaqui*-enriched diet ($p<0.05$). *Matrinxã* significantly increased erythrocyte volume and decreased ($p<0.05$) mean corpuscular hemoglobin concentration (Figure 2). *Mapará* caused no RBC-related changes.

Figure 3 shows the glucose, total protein, and triacylglycerol data. Rats fed the *tambaqui*-enriched diet had significantly lower blood glucose than those fed the standard diet ($p<0.05$). Blood glucose was not affected by the other diets. None of the experimental groups experienced changes in total protein levels, but all experimental groups experienced a reduction in triacylglycerol and VLDL-c levels ($p<0.05$). However, total cholesterol increased in rats fed the *mapará*-enriched diet. Their HDL-c and LDL-c were both high (Figure 4). The only experimental diet that increased HDL-c was the *tambaqui*-enriched diet, but it did not affect LDL-c or total cholesterol.

DISCUSSION

High low density lipoprotein-cholesterol and low HDL-c increase blood viscosity, and this

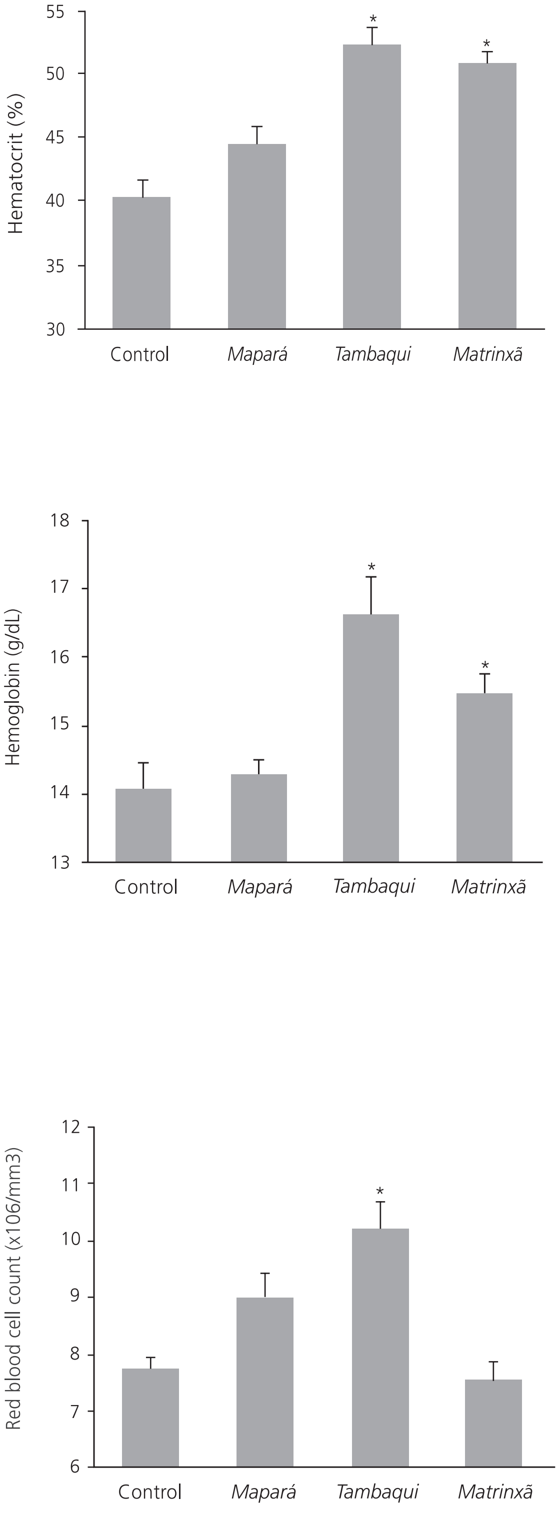

Figure 1. Hematocrit, hemoglobin level, and number of circulating erythrocytes of Wistar rats fed diets enriched with Amazon region fatty fish.

Note: *Indicates a significant difference from the control group fed the standard diet ($p<0.05$).

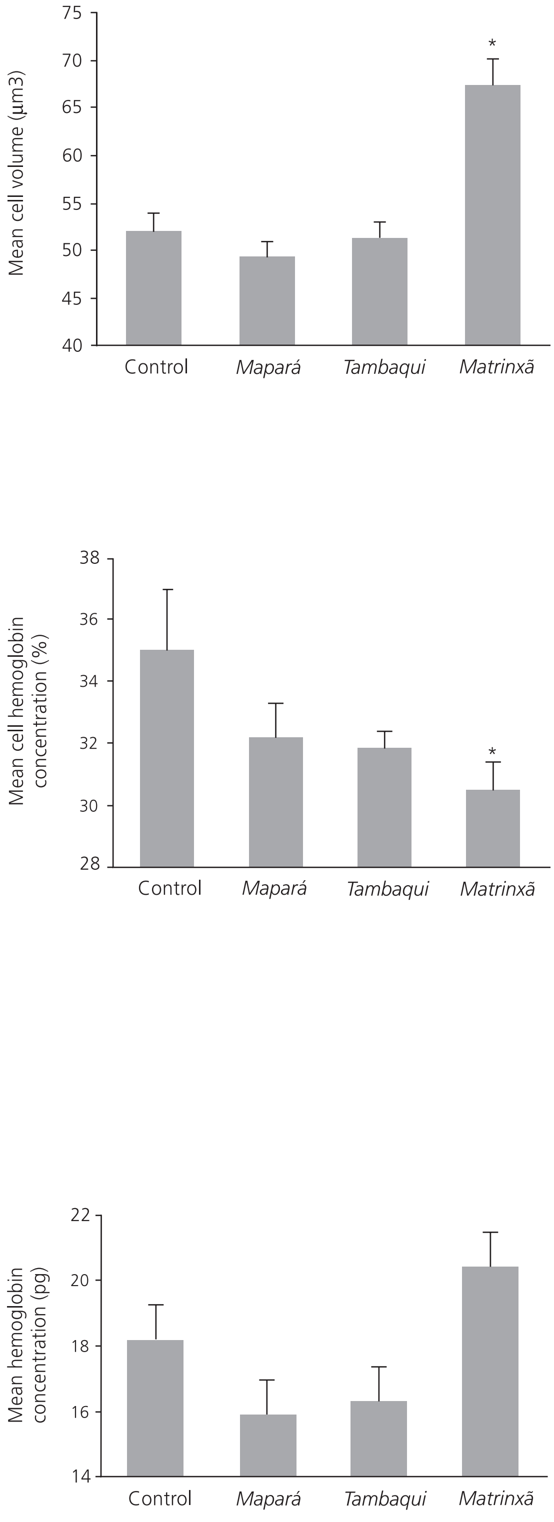

Figure 2. Red blood cell indices, cell volume, mean corpuscular hemoglobin concentration, and Mean Corpuscular Hemoglobin (MCH) in Wistar rats fed diets enriched with Amazon region fatty fish.

Note: *Indicates a significant difference from the control group fed the standard diet ($p<0.05$).

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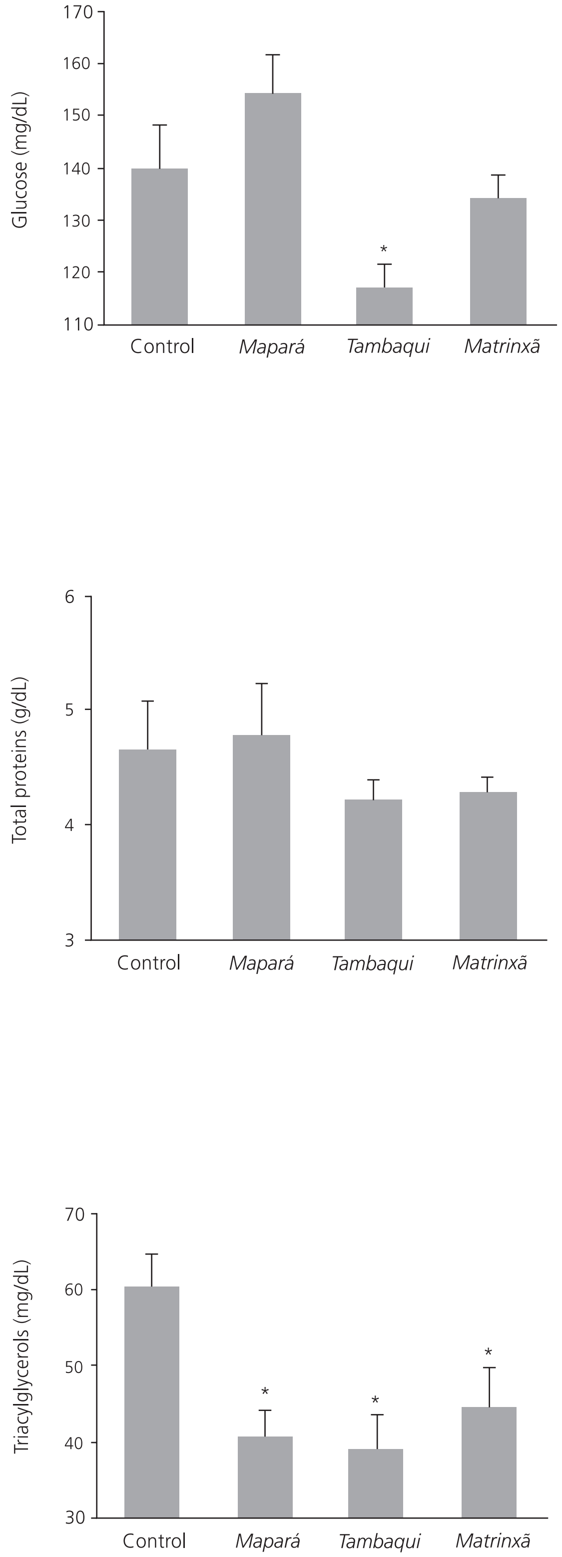

Figure 3. Glucose, triacylglycerols, and total proteins of rats fed diets enriched with Amazon region fatty fish.

Note: *Indicates a significant difference from the control group fed the standard diet (p<0.05).

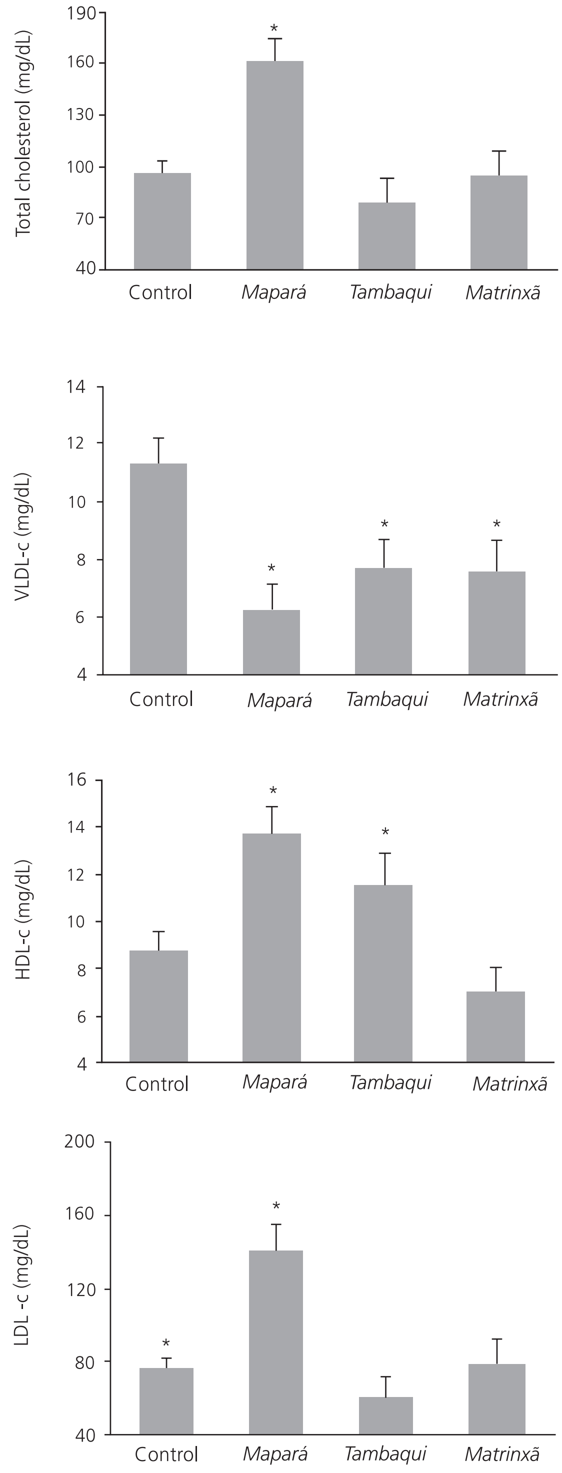

Figure 4. Variations of the total cholesterol, HDL-c, LDL-c, and VLDL-c levels of Wistar rats fed diets enriched with Amazon region fatty fish.

Note: *Indicates a significant difference from the control group fed the standard diet (p<0.05).

HDL: High Density Lipoprotein-cholesterol; LDL: Low Density Lipoprotein-cholesterol; VLDL: Very Low Density Lipoprotein-cholesterol.

abnormal rheological property increases the risk of atherogenesis[21,22]. The increase in the number and volume of circulating red blood cells affect Ht and blood viscosity[23]. Rats fed the *tambaqui*- and *matrinxã*-enriched diets experienced changes in Ht and hemoglobin levels but the associated physiological mechanisms are distinct: in the *matrinxã* group, high Ht occurred because of an increase in the number of circulating erythrocytes, while in the *tambaqui* group, high Ht was due to erythrocyte swelling, that is, the cells increased in volume. An increase in cell volume causes dilution of erythrocyte hemoglobin (low MCHC). Erythrocyte volume may increase due to changes in osmoregulation, which include fewer plasma solutes[24] and failure in the erythrocyte membrane ion transport system[25]. The mechanism by which high-lipid diets affect blood variables has not yet been clarified. Therefore, the secondary effects of these diets should be investigated routinely by RBC because many studies have found that high triacylglycerol levels increase the volume of circulating erythrocytes[26].

Interestingly, diets enriched with minced Amazon fish reduced triacylglycerol and VLDL-c levels significantly, possibly due to the high levels of EPA and DHA in these fish[27]. Additionally, fish oil has been shown to reduce the total cholesterol, LDL-c, and triacylglycerol levels of women aged 51 to 71 years[24]. Suprijana *et al*.[28] too found that fish oil reduced rats' triacylglycerol, total cholesterol, and cholesterol fractions, corroborated by Kim *et al.*[29]. Dyslipidemic patients treated with fish oil rich in omega-6 fatty acids experienced a reduction in total cholesterol, LDL-c, VLDL-c[30] and triacylglycerols, and an increase in HDL-c[31]. Another nutritional benefit at least in murine models is that Amazon fish-enriched diets increase HDL-c, especially if the fish is *tambaqui* or *mapará*. However, the minced *mapará*-enriched diet increased LDL-c. MUFA levels in *tambaqui* and *mapará* are 28.2% and 35.3%, respectively, and PUFA levels are 10.1% and 23.9%, respectively[16]. Although SFA in *tambaqui* is high (68.7% of the total fat content), it did not affect rats' LDL-c. Studies using labeled VLDL-c apoB-100 found that this fraction is the precursor of LDL-c[32]. This lipid metabolism pathway suggests that higher LDL-c synthesis lowered the VLDL-c of rats fed minced *mapará*.

In summary, the study data suggest that in murine models: (1) diets enriched with Amazon fish reduce triacylglycerol levels; (2) this result is corroborated by low VLDL levels; (3) *mapará* is considered a fatty fish, so diets enriched with minced *mapará* increase total cholesterol, especially LDL-c and HDL-c; (4) *tambaqui* was the only fish that increased HDL-c; (5) while *matrinxã*- and *tambaqui*-enriched diets affect RBC counts, the minced *mapará*-enriched diet increased total cholesterol, LDL-c (85%), and HDL-c (56%) compared with the standard diet; (6) minced *tambaqui*- and *matrinxã*-enriched diets change blood variables. However, more studies are needed to understand the physiological effects of diets enriched with fatty fish from the Amazon region.

A C K N O W L E D G M E N T S

The authors thank *Fundação de Amparo à Pesquisa do Estado do Amazonas* for sponsoring Francisca das Chagas do Amaral Souza (process 062.01725.2014/FAPEAM). We thank *Instituto Nacional de Pesquisas da Amazônia* for their help and for sponsoring the study.

C O N T R I B U T O R S

FCA SOUZA has contributed in planned and carried out all experiments. Processed the samples and wrote and reviewed the final version of the manuscript. WP DUNCAN has contributed in conducted the complete blood counts and lipoprotein panels. Performed the statistical analyses and graphed the results. Reviewed the final version of the manuscript. RP CARVALHO has contributed in planned and coordinated the experiments. Coordinated the laboratory tests and spreadsheet development. Wrote and reviewed all versions of the manuscript.

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Received on: 12/12/2013
Final version on: 7/15/2014
Approved on: 8/5/2014