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# Lipid profile of yacarés overo meat fed with diets enriched with flax seeds

## Perfil lipídico de la carne de yacarés overo alimentados con dietas enriquecidas con semillas de lino

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### ABSTRACT

Diet influences fatty acid composition in meat of monogastric animals; increasing essential fatty acids in meats would improve its nutritional quality for human consumption. The objective of this research is to estimate meat lipid profile of commercial purpose raised caimans, and evaluate the effect addition of flax seed to the diet, on the meat n-3 fatty acids concentration. Caimans were randomly assigned to three treatments: Control diet (CD), control diet plus 10% whole flax seed (B), control diet plus 10% mashed flax seed (C). Diet composition affected fatty acid profiles in the meat, and differences were more evident between CD and C groups. Caiman meat presents a healthy fatty acid profile for human consumption and an ideal ratio n-6 / n-3. Addition of mashed flaxseeds to diet improves caiman meat qualities, by reducing the concentration of saturated fatty acids, and increasing concentration of oleic, and alpha-linolenic fatty acids.

**Key words**: *Caiman latirostris*; fatty acids; α-linolenic acid; crocodile farming.

### RESUMEN

La dieta influye en la composición de los ácidos grasos de la carne de los animales monogástricos; el incremento del contenido de ácidos grasos en las carnes para consumo humano, mejoraría su calidad nutricional. El objetivo de esta investigación es estimar el perfil lipídico de la carne de caimanes cultivados con fines comerciales, y evaluar el efecto de la adición de semillas de lino a la dieta, sobre la concentración de ácidos grasos n-3 de la carne. Los caimanes fueron asignados aleatoriamente a tres tratamientos: Dieta control (DC), dieta control con 10% de semillas de lino enteras (B), dieta control con 10% de semillas de lino molidas (C). La composición de la dieta afectó los perfiles de ácidos grasos en la carne, y las diferencias fueron más evidentes entre los grupos DC y C. La carne de caimán presenta un perfil de ácidos grasos saludable para el consumo humano y una proporción ideal entre n-6 / n-3. La adición de semillas de lino molidas mejora la calidad de la carne de caimán, reduciendo la concentración de ácidos grasos saturados, e incrementando la concentración de ácidos grasos oleicos y α-linolénicos.

**Palabras clave**: *Caiman latirostris*; ácidos grasos; ácido α-linolénico; cría de cocodrilos.

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## INTRODUCTION

In the province of Santa Fe (Argentina), Proyecto Yacaré (Province goverment/MUPCN) has been working since 1990 in the conservation and sustainable use of the broad-snouted caiman (*Caiman latirostris*) through ranching. Today the program is producing caiman leather for industries and meat for human consumption (Larriera and Imhof 2006; Larriera *et al.,* 2008).

In recent years, the number of publications concerning experimental diets and their influence on fatty acid composition has increased considerably. Most of them show that fatty acid deposition in the different tissues is affected by the lipid profile of food (Al-Souti *et al.,* 2012; Fernandes *et al.,* 2012). The general trend is that the most represented fatty acid in the diet, will be the more abundant in the tissues of the feed animals (Caldironi and Manes 2006; Depetris *et al.,* 2003; Maroof Bahurmiz and Wing-Keong 2007; Realini *et al.,* 2004).

In order to improve nutritional quality for humans, reducing chances to develop arteriosclerotic illness, some recent researchs have been focusing on increasing essential fatty acids in many species that are regularly consumed by humans (Justi *et al.,* 2003; Moloney *et al.,* 2012; Visentainer *et al.,* 2005). Basal fatty acid composition in crocodilians has been studied previously (Huchzermeyer 2003; Lance *et al.,* 2001; Vicente-Neto *et al.,* 2010), indicating that crocodiles tend to have levels of fatty acids that are ideal for human consumption. Because caimans are monogastric animals, there is some evidence that diet influences its meat composition. This has not been tested previously and also should depend on the species under study.

A healthy diet for humans should include low concentrations of saturated fatty acids and be rich on mono- and poli-unsaturated ones (Calañas-Continente and Bellido 2006; De Caterina *et al.,* 2006; Harris 2006). Food modification for human consumption is a valid strategy to obtain a diet that reaches such conditions, in order to improve population health (De Lorgeril *et al.,* 1999). Considering that non-communicable diseases represent the major impact to the public health of developed countries, and it is growing fast in undeveloped ones (Tavella and Peterson 2000), and assuming it would be possible to prevent those illnesses with some changes in food. The goals of the present work are to know lipid profile of comercialy-raised caimans and to evaluate if the addition of flaxseed into the diet improves n-3 fatty acids concentration in the meat. It is also evaluated if there are differences in the ability to incorporate fatty acids from flaxseeds, in the case they are mashed or whole, since crocodilians are carnivorous.

## MATERIALS AND METHODS

### Sampling

All procedures with animals followed the ethical research standards, established by Proyecto Yacaré/SENASA. The animals were sacrificed at slaughterhouses participating in the meat production programs (approved by SENASA – establishment slaughter N°4081).

We use twenty-seven individuals about two years old of C. latirostris, 95cm in total length and four kg body mass in average, from three different nests (nine individuals each), provided by Proyecto Yacaré program of sustainable use. Three caimans from each nest were randomly assigned to three nutritional treatments.

Control Diet (CD) which consisted in regular food, was based in crushed chicken heads and a balanced supplement. Diet B had CD + 10% whole flaxseed, and finally, diet C had CD + 10% mashed flaxseed. For this last diet, flaxseeds were mashed just before feeding, in order to prevent oxidation. Animals received the food *ad libitum*, six times a week on a daily basis, from Monday through Saturday.

The experiment began on January 11$^{th}$ 2005, and finished on February 15$^{th}$ 2005, when the animals were sacrificed. Animals were fed the last time on February 14$^{th}$. During the experiment five individuals escaped from the enclosures; due to this, a final number of 22 animals was analyzed: nine belonging to the control group (27 samples), seven to the whole flaxseed fed group (21 samples), and six to the mashed flaxseed fed group (18 samples). From each carcass two meat samples from arms (*M. Tricipitis branchii*), legs (*M. Quadriceps femoris*) and tail (*M. Ilio-ischio-caudalis*) were taken for the chemical analysis 24h post-mortem. Macroscopic fat was removed previously to analyze the fatty acid composition of meat. Samples were covered with polypropylene film and aluminum foil, frozen and stored at -18°C until their analysis. Each meat sample was crushed, thus producing a homogeneous mass before chemical analysis.

### Chemical analysis

The chemical analysis consisted of the determination of fatty acids composition using a gas-liquid chromatography technique. All solvents and reagents were of analytical grade and 99% purity standards of fatty acid methyl esters were purchased from NuCheck Prep, Inc (Minnesota, USA). Total lipids were extracted with chloroform: methanol (2:1 v/v) using Folch's technique (Folch *et al.,* 1957), then, a partition was made with the 20% v/v water of the resulting extract, which was completely dried in a $N_2$ current.

In a second step, cholesterol and other non saponifiable compounds were separated by saponification by 10% KOH/methanol for 45 minutes. The remnant of the saponification process was acidified with concentrated HCl and free fatty acids were extracted three times with petroleum ether and, after vaporizing until dry. This free fatty acid were transformed into methyl esters by using 10% BF3/methanol at 80°C for 45 minutes. A nitrogen atmosphere was kept during the entire procedure. Methyl esters were extracted with hexane and analyzed using a Hewlett-Packard 6890 gas chromatography instrument. The fatty acid composition was obtained with a 50m capillary column (0.25mm inside diameter, CPSil 88, Chrompack, The Netherlands). Retention times of each of the fatty acids were compared to those of commercial standards. Chromatographic conditions were as follows: injection temperature - 250 °C, flame ionization detector (FID) temperature - 250 °C, initial temperature - 185°C, initial time - 3 min, final temperature - 230 °C, rate - 3°C/min, with nitrogen as carrying gas, pressure of 19 psi and a split ratio of 70/1. Gas-liquid chromatography (GLC) peak areas for methyl esters were not corrected for losses of procedure and response to the detector of flame ionization, and were considered as directly proportional to the percentages in weight.

### Statistical analyses

Data were analyzed using Kruskal Wallis. The grouping variable was food treatment and the response variable was fatty acid concentration, expressed as g% of total fatty acid present in the sample. On the data base, each value was the average of two measurements. Statistical analyses were done using InfoStat (2017) for Windows.

## RESULTS AND DISCUSSION

All the animals increased their body masses during the experiment. However, individuals from the three treatments had similar body masses at the end (4.4 ± 0.8kg control, 3.9 ± 0.5kg whole flaxseeds, and 3.4 ± 0.9kg mashed flaxseeds; P>0.05). Fatty acids composition of food affected fatty acid profiles of caiman meat (Table 1), being more evident differences between control and mashed flaxseeds group. Diets with masched flaxseeds reduced SFA contents, and increased the unsaturated fatty acids contents (P<0.05); differences were found in saturated fatty acids (SFA), 16:1 n-7 (palmitoleic acid), 18:1 n-9 (oleic acid), and 18:3 n-3 (alpha linolenic acid).

Previous studies made in caiman latirostris fed with beef (Secretary of agriculture, livestock, fishing and food, 2007), showed 41.4% of SFA in meat. Results of the present work show lower concentrations of SFA, considering regular food (35.71+-1.39%), and reduced SFA contents with the adition of mashed flaxseed diet (30.70+-1.35%). Yacare caiman meat presented similar values of SFA than those of our control treatment (35.1% Vicente-Neto *et al.,* 2010), but higher than meat from animals fed by a diet including mashed flaxseed. Caiman meat showed lower concentrations of SFA than beef and pork, but higher than chicken or freshwater fish pirá-pitá (*Brycon orbignyanus*, Table 2). Addition of mashed flaxseeds improved SFA quality in caimans, making it equal to chicken meat. Low concentration of SFA is an advantage for caiman meat consumers, since SFA have a negative impact in human health (Mensink and Katan 1992).

Concentrations of 18:1 trans fatty acid in beef, pork and chicken determined in other studies were higher than values found in *C. latirostris* in this work. On the other hand, in *B. orbygnyanus*,

Table 1. Fatty acids profile in Caiman latirostris meat fed with diets enriched with flax seed

| Fatty acids | (Control diet) (n=27) | B diet (n=21) | C diet (n=18) | Differences |
|---|---|---|---|---|
| SFA | 35.71 ± 1.39 | 32.21±0.45 | 30.70±1.35 | P=0.0429 |
| 18:1 trans | 0.75 ± 0.05 | 0.91±0.07 | 0.92±0.05 | P=0.0290 |
| 16:1 n-7 | 1.71 ± 0.12 | 2.21±0.27 | 2.38±0.24 | P=0.0322 |
| 18:1 n-9 | 23.68 ± 0.89 | 26.31±0.92 | 27.75±1.08 | P=0.0124 |
| Total n-6 | 29.96 ± 1.09 | 31.69±0.97 | 32.15±0.81 | None |
| 18:2 n-6 | 23.21 ±0.73 | 25.26±0.53 | 26.19±0.94 | None |
| 20:4 n-6 | 6.75±0.53 | 6.42±0.71 | 5.96±0.60 | None |
| Total n-3 | 6.62±0.36 | 5.97±0.38 | 6.19±0.38 | None |
| 18:3 n-3 | 1.45±0.14 | 1.56±0.06 | 2.17±0.10 | P=0.0001 |
| 20:5 n-3 + 22:5 n-3 + 22:6 n-3 | 5.17±0.39 | 4.46±0.41 | 4.02±0.42 | None |
| n-6/n-3 | 4.69±0.16 | 6.10±0.76 | 5.69±0.60 | None |

SFA= Saturated Fatty Acids. Results are expressed as mean ± S.E. Differences are significant when P values are less 0.05 for diet treatment.

this fatty acid was not detected (Table 2). Excluding, pirá-pitá meat, concentrations of 18:1 trans fatty acid found in caimans were lower than half of the value found in pork, and almost ten times lower than those found in beef.

Similarly, contents of 18:1 n-9 cis fatty acid (oleic acid) in caiman meat were lower than in other meats used for comparison (Table 2), but were similar to *C. yacare* (Vicente-Neto *et al.,* 2010). Addition of flaxseed increased oleic acid concentration from 23.7 ± 0.89 to 27.75% ± 1.08. This could influence health of consumers, since consumption of this acid reduces cholesterol in blood, thus reducing ischemic origin coronary illness frequency (Dilzer and Park 2012; Erener *et al.,* 2007; Molendi-Coste *et al.,* 2011).

Concentrations of n-6 fatty acids (linoleic 18:2 n-6 + arachidonic 20:4 n-6) in caiman meat were higher than all values appearing for animal meats used for human consumption (Table 2), including *C. yacare* (24% Vicente-Neto *et al.,* 2010). As shown in trans fatty acids, variations in diet did not change n-6 fatty acids concentration. Comparatively, in caimans fed with the control diet, concentrations of linoleic acid were higher than in the other meats (Table 2). This is a beneficial aspect for consumers, since it is an essential fatty acid and there is lot of evidence of its blood cholesterol reducing effects (Phillipson *et al.,* 1985). Diets enriched with flaxseeds produced a significant increment in the concentration of this fatty acids, improving its beneficial effects (Mapiye *et al.,* 2013; Morel *et al.,* 2013), and results showed that even higher levels were obtained with mashed flaxseeds diet, suggesting that the crushing of the seeds facilitate its absorption and assimilation.

Fatty acids of the n-3 family (represented by alpha linolenic acid (18:3 n-3) + eicosapentaenoic acid 20:5 n-3 (EPA) + docosapentaenoic acid 22:5 n-3 (DPA) + docosahexaenoic acid 22:6 n-3 (DHA)) in the intramuscular caiman meat, were two to almost ten times higher than in the other species (Table 2). In this study, caimans fed with the control diet showed a concentration of alpha linolenic acid (18:3 n-3) higher than in the other meat products (Table 2). Animals fed on the diet with mashed flaxseed presented a higher concentration of alpha-linolenic acid than caimans fed on the other diets (Table 1). The increase of this fatty acid in caiman meat can be explained by the fact that flaxseeds have high

Table 2. Comparison between fatty acid profiles found in *Caiman latirostris* meat (control diet) and other meat of local human consumption.

| Fatty acids | Caiman meat (Control diet) | Beef fat (*) | Pork fat(*) | Chicken fat(*) | Pira-pitá meat(**) |
|---|---|---|---|---|---|
| SFA | 35.71±1.39 | 49.2±1.97 | 36.1±1.05 | 29.1±0.79 | 33.73±0.24 |
| 18:1 trans | 0.75± 0.05 | 6.87±0.39 | 1.62±0.17 | 2.18±0.68 | ND |
| 16:1 n-7 | 1.71±0.12 | 3.18±0.39 | 2.81±0.17 | 6.02±0.46 | 3.5±0.09 |
| 18:1 n-9 | 23.68±0.89 | 32.08±1.66 | 40.73±0.55 | 36.37±0.96 | 39.74±0.28 |
| Total n-6 | 29.96±1.09 | 2.9±0.49 | 14±0.45 | 21.5±1.36 | 15.46±0.12 |
| 18:2 n-6 | 23.21±0.73 | 2.4±0.34 | 13±0.4 | 20.4±1.44 | 13.77±0.46 |
| 20:4 n-6 | 6.75±0.53 | 0.40±0.13 | 0.35±0.08 | 0.49±0.12 | 1.06±0.03 |
| Total n-3 | 6.62±0.36 | 1.2±0.23 | 0.71±0.08 | 1.4±0.23 | 2.42±0.01 |
| 18:3 n-3 | 1.45±0.14 | 0.81±0.12 | 0.56±0.02 | 1.23±0.22 | 1.02±0.02 |
| 20:5 n-3 + 22:5 n-3 + 22:6 n-3 | 5.17±0.39 | 0.39±0.09 | 0.09±0.01 | 0.12±0.02 | 1.28±0.07 |
| n-6/n-3 | 4.69±0.16 | 2.36 (2.86/1.21) | 19.70 (13.99/0.71) | 15.79 (21.48/1.36) | 6.38±0.07 |

SFA= Saturated Fatty Acids. Results are expressed as mean ± S.E. Fatty acid composition of each lipid class is expressed as percentage of the total fatty acid present. ND: No detected.
(*) Baylin *et al.* 2007. (**) Moreira *et al.* 2001

contents of alpha-linolenic acid (about 50 – 60% Ayerza and Coates 2011; Taha *et al.,* 2012).

In humans, consumption of high levels of alpha-linolenic acid, such as in "Mediterranean diet", produces reduction in 50 to 70% of cardiovascular risk, and maintains protective efects up to four years after suffering the first heart attack (De Lorgeril *et al.,* 1999; Calañas-Continente and Bellido 2006; Urpi-Sarda *et al.,* 2012). Alpha-linolenic acid also serves as the precursor of other long chain acids of n-3 series such as DHA and EPA (Simopoulos 1991; Orton *et al.,* 2008; Molendi-Coste *et al.,* 2011), which are also beneficial for human health.

Long chain n-3 fatty acids (20:5 n-3 (EPA) + 22:5 n-3 (DPA) +22:6 n-3 (DHA)) concentrations in caiman meat, were approximately four times higher than the concentrations found in other meats (Table 2). The origin of these fatty acids is probably neural tissues contained in the chicken mashed heads, basis in the diet of this animal (Surai and Sparks 2000). EPA and DHA were associated with benefits such as prevention of cardiovascular illness (Calañas-Continente and Bellido 2006; Jensen *et al.,* 2007), some types of cancer (Trombetta *et al.,* 2007), and in development of visual and nervous tissue in children (Jensen *et al.,* 2007). Apparently DHA is important for mental development and general health (Shirai *et al.,* 2004; Orton *et al.,* 2008).

Caimans did not increase total n-3 fatty acids when mashed flaxseeds were added to the diet, in comparison to whole grain feed fed animals. This could be the result of a reduced ability to synthesize polyunsaturated fatty acids using alpha-linolenic acid as a precursor. Many researchers have mentioned that the n-6 / n-3 ratio should be around five (Moreira *et al.,* 2001; Coronado Herrera *et al.,* 2006). This was approximately the value found in caiman meat (4.69 ± 0.16). Freshwater fish (pirá-pitá) contains similar values of 6.4 ± 0.07 (15.5 / 2.4; Moreira *et al.,* 2001), but beef [2.4 (2.9 / 1.2)], chicken [15.8 (21.5 / 1.4)] and pork [19.7 (14 / 0.7)] have extreme values of this ratio (Baylin *et al.,* 2007). Wild caiman (*C. yacare*) meat had values of 6.4, but those from captivity had a higher ratio (10.9; Vicente-Neto *et al.,* 2010), indicating that caiman, in general, tend to produce a balanced relation between n-6 / n-3 fatty acids, but some diets in captivity could modify that ratio (Al-Souti *et al.,* 2012).

Caiman meat (as other crocodilians) is a product that is increasing in acceptance in the world food market. Currently there is a proper supply of meat from many management plans from Argentina, Bolivia, Brazil and USA in the Americas. Added to its palatability, it exhibits other nutritional benefits when compared to other meat products found in the market (soft water fish, beef, chicken, and pork). Considering all the results of this research, the improvement of benefits for human health in caimans meat, obtained with the addition of mashed flaxseed in diet should be noted. There is also a commercial exploitation of wild crocodilian in some countries such as Australia and United States of America. That meat is in some way attractive for the market because it is game meat, but also should present a higher proportion of poly-unsaturated fatty acids (PUFA, about 6%) compared to captive animals (Vicente-Neto *et al.,* 2010).

Caiman meat presents a healthy fatty acid profile for human consumption. It is characterized by low saturated fatty acid content and high levels of unsaturated fatty acids, including essential fatty acids such as linoleic (18:2 n-6) alpha-linolenic (18:3 n-3), and respective derivatives: ARA-arachidonic acid of n-6 family, EPA and DHA of the n-3 family. Caiman meat also presents an ideal relationship between n-6 / n-3. Fatty acid profile observed in caiman, is as good quality meat, compared to other products of regular human consumption (pirá-pitá, fish, chicken, beef, or pork).

Addition of mashed flaxseeds during a short time, like one month to the regular diet, improves the qualities of caiman meat, by reducing concentration of saturated fatty acids, and increasing concentration of oleic, and alpha-linolenic acids.

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