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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 of meat in monogastric animals; increasing essential fatty acids of meats would improve its nutritional quality for human consumption. The objective of the present research was to estimate the lipid profile of commercially-raised caimans, and to evaluate if addition of flaxseed into diet improves n-3 fatty acids concentration in meat. Caimans were randomly assigned to three treatments: regular food; addition of 10% whole flaxseed; and addition of 10% mashed flaxseed. Diet composition affected fatty acid profiles in the meat, and differences were more evident between control and mashed flaxseeds. 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; incrementar los ácidos grasos en las carnes para consumo humano, mejoraría su calidad nutricional. En este estudio, se conoció el perfil lipídico de la carne de caiman comercializada, y se evaluó si la adición de semillas de lino a la dieta mejoraría la concentración de ácidos grasos n-3 en la carne. Los caimanes fueron asignados aleatoriamente a tres tratamientos: dieta regular; dieta regular con adición del 10% de semillas de lino enteras; y dieta regular con adición del 10% de semillas molidas. La composición de la dieta afecta los perfiles de ácidos grasos en la carne, y las diferencias fueron más evidentes entre la dieta control y la dieta con adición de semillas molidas. 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 (Gob. de la Provincia/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 researches 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 a healthy fatty acids composition for human consumption. Because caimans are monogastric animals there is some evidence that diet does influence its meat composition in the animals, but this has not been tested previously in any crocodilian, and also that 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 that it would be able to prevent those illnesses with some changes in food, the goals of the present work were to know the lipid profile of commercially-raised caimans, and to evaluate if the addition of flaxseed into the diet improves n-3 fatty acids concentration in the meat. It was also evaluated if there were differences in the ability to incorporate fatty acids from flaxseeds; in the case they were 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).

Only 27 individuals of *C. latirostris*, were used in the experiment due to the logistic difficulties to maintain and control feeding of a larger group of animals; furthermore, considering that these animals were provided by a program of sustainable use, we were prompted to use the minimal possible number. Caimans used were approximately two years of age, and they were from three different nests (nine individuals each). Under the raising conditions of Proyecto Yacaré, those animals were in average about 95cm in total length and a body mass of four kg. caimans were randomly assigned to three nutritional treatments, in such a way that each treatment received three animals from each nest.

The Control Diet (CD) consisted of the regular food provided by the project, which was based on 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 three meals *ad libitum*, six times a week on a daily basis, from Monday through Saturday.

The experiment began on January 11th 2005, and finished on February 15th 2005, when the animals were sacrificed. Animals were fed for the last time on February 14th. During the experiment five individuals escaped from the enclosures; due to this, a final number of 22 animals were analyzed: nine belonging to the control group, seven to the whole flaxseed fed group, and six to the mashed flaxseed fed group. 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 used were of analytical grade. Standards of fatty acid methyl esters of 99% purity were purchased from NuCheck Prep, Inc (Minnesota, USA). Total lipids were extracted with chloroform: methanol (2:1 v/v) using the Folch's technique (Folch *et al.,* 1957), and 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, they were transformed into methyl esters by using 10% BF3/methanol at 80°C for 45 minutes. A nitrogen atmosphere was kept as long as possible during the entire procedure. Methyl esters were extracted with hexane and were analyzed by 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). The retention times of each of the fatty acids were compared to those of commercial standards. The chromatographic conditions were as follows: injection temperature - 250°C, (FID) detector temperature - 250°C, initial temperature - 185°C, initial time - 3 min, final temperature - 230°C, rate - 3°C/min, with nitrogen as the carrying gas, with a pressure of 19 psi and a split ratio of 70/1. The GLC peak areas for methyl esters were not corrected for losses of procedure and response to the detector of flame ionization, and they 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 Info Stat for Windows

RESULTS AND DISCUSSION

All the animals increased their body mass during the experiment. However, the caiman from the three treatments had similar body masses at the end of the experiment (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 the fatty acid profiles in caiman meat (Table 1). Differences were more evident between control and mashed flaxseeds; 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). Mashed flaxseeds in the diet reduced SFA contents, and increased the unsaturated fatty acids contents ($P<0.05$).

Previous studies conducted in *Caiman latirostris*, based on beef feeding to animals in captivity (Secretaría de Agricultura, Ganadería, Pesca y Alimentos 2007), showed 41.4% of SFA in meat. Results of the present work showed lower concentrations of saturated fatty acids, considering the regular food (35.71 ± 1.39%), and the addition of mashed flaxseed reduced SFA contents to 30.70 ± 1.35%. *Caiman yacare* 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

Table 1. Fatty acid profi les of lipids in*Caiman latirostris* intramuscular fat from tail, hind limb, and forelimb (mean ± S.E.) of the nine control animals, seven control + whole fl axseeds, and six control + mashed fl axseeds. Differences are signifi cant when P values are less 0.05 for Diet treatment.

Fatty acids	(Control) (n=27)	Control + Flaxseeds (n=21)	Control + Mashed Flaxseeds (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 quality in caimans, making it equal to chicken meat. The 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*, this fatty acid was not detected (Table 2). Excluding, pirá-pitá meat, the concentration of 18:1 trans fatty acid found in caimans was lower than half of the value found in pork, and almost ten times lower than those found in beef.

Similarly, the content of 18:1 n-9 cis fatty acid (oleic acid) in caiman meat was lower than in other meat used for comparison (Table 2), but was similar to *C. yacare* (Vicente-Neto *et al.,* 2010). Addition of fl axseed increased oleic acid concentration from 23.7 ± 0.89 to 27.75% ± 1.08, which could infl uence health of consumers, since consumption of this acid produces a reduction on cholesterol in blood, thus reducing coronary illness frequency of ischemic origin (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, the concentration of linoleic acid concentration is higher than in the other meats (Table 2). This is a benefi cial aspect for consumers, since it is an essential fatty acid and there is lot of evidence of blood cholesterol reducing effects (Phillipson *et al.,* 1985). Diets enriched with fl axseeds produced a signifi cant increment in the concentration of this fatty acids, improving its benefi cial effects (Mapiye*et al.,* 2013; Morel *et al.,* 2013). Once again, results showed that higher levels were obtained with the mashed fl axseeds diet, suggesting that the crushing of the fl axseeds facilitate the absorption and assimilation of these fatty acids.

Table 2. Comparison between fatty acid profi les 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. Fatty acid composition of each lipid class is expressed as percentage of the total fatty acid present. ND: None detected.
(*) Baylin *et al.* 2007. (**) Moreira *et al.* 2001

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

For humans, consumption of a diet with high levels of alpha-linolenic acid, as the “Mediterranean diet”, produces a reduction of the cardiovascular risk of 50 to 70%, reducing the relative risk of heart attack, and maintaining its protective effects up to four years after suffering the fi rst heart attack (De Lorgeril *et al.,* 1999; Calañas-Continente and Bellido 2006; Urpi-Sarda *et al.,* 2012). Beyond this important benefi t of alpha-linolenic acid to human health, it 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 benefi cial 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 the neural tissues contained in the chicken mashed heads that forming the basis of the diet of this animal (Surai and Sparks 2000). EPA and DHA were associated with benefi ts such as prevention of cardiovascular illness (Calañas-Continente and Bellido 2006; Jensen *et al.,* 2007), and some types of cancer (Trombetta *et al.,* 2007). Moreover, they are involved in development of visual and nervous tissue in children (Jensen *et al.,* 2007), and apparently DHA is important for mental development and health (Shirai *et al.,* 2004; Orton *et al.,* 2008).

Caiman did not increase the total n-3 fatty acids when mashed fl axseeds were added to the diet, relative to caiman fed whole, intact grain. 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 fi ve (Moreira*et al.,* 2001; Coronado Herrera *et al.,* 2006). This was approximately the value found in caiman meat (4.69 ± 0.16). Freshwater fi sh (pirá-pitá) have been found to contain a similar value 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 benefi ts when compared to other meat products found in the market (soft water fi sh, beef, chicken, and pork). Considering all the results of this research, the improvement of the benefi ts for human health of the caiman meat obtained with the addition of mashed fl axseed in the animal´s meals 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 PUFA (about 6%) compared to captive animals (Vicente-Neto *et al.,* 2010).

Caiman meat presents a healthy fatty acid profi le 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) and alpha-linolenic (18:3 n-3), and the respective derivatives: ARA-arachidonic acid of the n-6 family, EPA and DH belonging to the n-3 family. Caiman meat also presents an ideal relationship between n-6 / n-3. The fatty acid profi le, presented the caiman meat as good quality meat, compared to other meat products of regular human consumption (pirá-pitá, fi sh, chicken, beef, or pork).

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

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