Effect of flaxseed and flaxseed oil supplemented in caiman diet on meat fatty acids

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Abstract
Increasing polyunsaturated or long-chain fatty acids in meat for human consumption improves both nutritional quality and consumer perception. The increase could occur through the addition of rich sources of omega-3 fatty acids (such as flaxseed or flaxseed oil) to the animal diet. The aim of this study was to evaluate the effects of dietary supplement with two presentations of flax (crushed seeds or oil) on the change of FA content in two cuts of caiman meat (tail and neck). We measured fatty profile in two different caiman meat cuts (neck and tail) from 30 animals (total length $96.7 \pm 4.9$ cm, snout-vent length $47.8 \pm 3$ cm, weight $4.2 \pm 0.6$ kg), raised in individual enclosures, fed three a week for 50 days with crushed chicken head and a dry food formulated for these reptiles in a 70/30 ratio (C, $n=10$), control diet with 10% crushed flaxseed (FS, $n=10$), and control diet with 10% flaxseed oil (FO, $n=10$), while the remaining days animals were fed the control diet. Meats from animals fed both enrichment diet (FS and FO) showed an increase of C18:3n-3 and ΣUFA with respect to control diet. Although both enriched diets raised the levels of C18:3n-3, the neck showed higher values than the tail. We observed that the neck is more susceptible than the tail to be improved by FO, which could suggest that it is more beneficial to consume neck meat. In order to be implemented in caiman farms, flaxseed oil is more expensive than seed, but more effective, easier to manage, and is practical for application on caiman farms.

Keywords Caiman latirostris · n-3 fatty acids · Oils · Seeds

Introduction
Around the world, consumers are increasingly interested in healthy eating habits, with concern for their physical health (Tobin, 2013; Shan et al., 2017). This change in attitude has put pressure on the meat industry to offer healthier products and/or to change the lipid composition of meats by modifying animal diets during their rearing (De Smet and Vossen, 2016; Gómez et al., 2016; Jankowski et al., 2018; Kumar et al., 2019). Supplementing animal diets with sources rich in the n-3 family fatty acids (FA) can increase those FA for the benefit of human consumption. The most common FA to be increased are monounsaturated fatty acids (MUFA) and some polyunsaturated fatty acids (PUFA), particularly n-3, for example, α-linolenic acid (ALA, C18:3 n-3), eicosapentaenoic acid (EPA, C20:5 n-3), and docosahexaenoic acid

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(DHA, C22:6 n-3). They are FAs whose benefits come from prevention of chronic non-communicable diseases (NCD), such as cardiovascular diseases (Siegel and Ermilov, 2012; del Gobbo et al., 2016; Mir et al., 2018). The importance of n-6 and n-3 PUFAs has been known since the 1950s, both for adequate neural development and modulation of eicosanoid synthesis. A recommended level of n-6/n-3 ratio of approximately 4:1 is associated with a decreased risk of atherosclerosis, thrombosis, stroke, certain cancers, and immune disorders. In addition, increasing dietary n-3 PUFA can lower plasma triglycerides (Patterson et al., 2012; Wu et al., 2014; Li et al., 2019; Uljević et al., 2019). Consequently, health professionals recommend increased consumption of n-3 polyunsaturated fats because of their positive health effects (Marventano et al., 2015). A natural food source of PUFA such as C18:3 n-3 are flaxseeds and flaxseed oil (the latter used less frequently), applied as a supplement to animal diets to enrich their content in meat (Gómez et al., 2016).

Caiman farming and ranching operations produce skins and meat. It is of great interest of producers to promote an alternative source of FA such as caiman meat (Caiman latirostris or Broad-snouted caiman). Crocodilian meat is traded widely but is particularly favored in the Far East, especially China; the top species in trade are Crocodylus niloticus and Crocodylus siamensis, peaked at nearly 1,000 t traded in good years (Caldwell, 2017). In Argentina and in the world, Broad-snouted caiman meat has special characteristics that makes it unique related to other species that are traditionally marketed, which makes it a product with high potential (Vicente Neto et al., 2010; Simoncini et al., 2020; Nepote et al., 2021). Due to the limited production (in kilograms), compared to raising chickens or cows, caiman meat should be marketed as gourmet or delicatessen meat. Around the world, some preparations based on crocodile meat include crocodile herbal soup, croc bistek, croc mechado, croc ribs, dried chili crocodile meat, and crocodile filet (Coralfarms, 2021). In Argentina, caiman meat is usually eaten in pieces and fried, pickled, used as a filling in empanadas, or even roasted.

Compared to the meat of other animals, crocodilian meat is generally high in protein, low in fat (less than 1% in caimans and 2% in crocodiles) and rich in PUFA (Osthoff et al., 2010; Vicente-Neto et al., 2010; Hoffmann and Cawthorn, 2013; Canto et al., 2015; Simoncini et al., 2020), and can be enriched with PUFA (Piña et al., 2016). Considering these qualities if we could improve the lipid profile of caiman meat, a very healthy product could be obtained. Also, at the same time, with the consumption of n-3 fats the animal welfare may be improved, since cell membrane enrichment with n-3 PUFA may decrease the inflammatory response, improve growth rate, erythropoiesis, leukopoiesis, and increase specific immunity as evidenced in other oviparous species (Korver and Klasing, 1997; Radwan et al., 2012; Abdulwahid and Mudheher, 2017). Regard that the increase of long-chain PUFAs in caiman meats for human consumption would improve their nutritional quality, the present work compares two sources of n-3 PUFA, flaxseed oil or crushed flaxseed, as the dietary supplement for farmed caimans. In the case of flaxseed oil, the FA are available and are absorbed more quickly and in greater percentage than when offered as crushed seeds, whose lignins and cuticles of the seeds could hinder this process of digestion and absorption (Goyal et al., 2014). In addition, we must consider that dietary supplement of oil (despite a higher cost) makes application to the diet easier compared, to flaxseed as the seed must be crushed prior to addition to the diet, in order to facilitate the availability of the lipids.

Tail and neck meat cuts provide the highest meat yield in this species, the most representative of the meat and the one preferred by consumers (Vicente Neto et al., 2010; Hoffman and Cawthorn, 2012; Kluczkovki et al., 2015). Currently caiman meat in Argentina is marketed complete (without head and eviscerated). A positive outcome of our research would lead to a proposal to offer the market cuts with an added value of lipids that are favorable to health such as the increase of PUFAs. The aim of this study was to evaluate the effects of dietary supplement with two presentations of flax (crushed seeds or oil) on the change of FA content in two cuts of caiman meat (tail and neck).

Materials and methods

All the animals were treated following the Reference ethical framework for biomedical research: ethical principles for research with laboratory, farm and wild animals (CONICET 2005) and all the experiments were approved by the Animal Ethics Committee of the School of Biochemistry (Universidad Nacional del Litoral).

Animal used in this work are part of the harvest of a sustainable management program that uses wild caiman populations, approved by Provincia de Santa Fe (Law 11,820), registered in Dirección de Fauna Silvestre de la Nación (following resolutions No 283/00 y 03/04), fulfills CITES normative, and follows recommendations of the Crocodile Specialist Group (CSG/SSC/IUCN). These caiman populations are not endangered.

Animal hatching and breeding

Caiman latirostris individuals used for this study were provided by Proyecto Yacaré (Larrriera et al., 2008). Eggs collected from wild sources were incubated and hatched in an artificial incubator and kept under controlled captive conditions in a heated room (30 ºC), in a cement pool (4.5 m wide × 0.8 m high × 0.7 m deep). Inside the pool, each...
Individuals had access to water and dry areas; pools were cleaned and the water was renewed every other day. The animals were fed ad libitum 6 days a week, with a base diet composed of crushed chicken heads and dry feed formulated for reptiles (Avigan®; Table 1) (70/30; FA profile of diet in Table 2; Larriera et al., 2008; Simoncini et al., 2020).

**Animal testing**

The specimens were raised on average for 2 years to reach the weight and length suitable for being marketed (X ± SD; total length 96.7 ± 4.9 cm, snout-vent length 47.8 ± 3 cm, weight 4.2 ± 0.6 kg). Thirty individuals were randomly selected from the six pools (five individuals per each pool) and moved to individual plastic containers with a capacity of 150 L. Animals were under conditions suitable for raising caimans, having one-part dry and the other with water, at an average temperature of 31 °C. Each caiman was randomly assigned to an experimental treatment. Their weight was recorded with a digital scale (0.1 kg precision) and total length and snout-vent length were measured with a tape measure (0.5 cm precision). After a 7-day habituation, during which the condition of the animals and their normal feeding activity were checked the dietary experiment was initiated.

For 50 days, caimans were fed according to the assigned treatment: 1. control diet (C, n = 10): crushed chicken head and a dry food formulated for these reptiles in a 70/30 ratio, 2. flax seed diet (FS, n = 10): control diet with 10% of mass in crushed flax seed, and 3. flax oil diet (FO, n = 10): control diet with 10% flaxseed oil (Table 2). We used 30 individual plastic containers: 10 replicates per treatment.

The supplemented diets were given three a week during the entire experimental period, while the remaining days animals were fed the control diet. While previous results obtained from caiman meat enhancement with dietary enrichments were supplemented 6 days a week (Piña et al., 2016), prior data (Valli, 2020) evidenced modification in caiman energy and tissue samples were obtained (meat cuts from tail and neck).

**Physico-chemical characterization of diet and meat**

Diets were freshly prepared, gassed with nitrogen, and stored at −10 °C for subsequent FA profile analysis. The animals were slaughtered following the protocol of the Proyecto Yacaré (Yacarés Santafesinos/MUPCN, approved slaughterhouse N° 4081) and according to the sanitary conditions established by SENASA (National Service of Agrifood Health and Quality) for meat production for human consumption. Two samples of 5 g were taken from the tail (ilio-ischio-caudalis muscle) and neck (occipito-cervicalis-medialis muscle) of each animal and preserved at −18 °C until further processing.

The FA composition of the tissues and experimental diets was determined by gas chromatography with a Shimadzu (GC 2014) chromatograph equipped with an automatic injector (AOC-20i auto injector Shimadzu) and a flame ionization detector (SFID1). Analyses were carried out with a capillary column CP Sil 88 (100 m length, 0.25 mm i.d., 0.25 mm film thickness) (Varian, Walnut Creek, CA, USA, Part N° CP7489). The column temperature was held a 75 °C for 2 min after injection, then 5 °C/min to 170 °C, held for 40 min, 5 °C/min to 220 °C and held 40 min. Nitrogen was the carrier gas with an inlet pressure set at 100 kPa and a split ratio of 1:20. The injector and detector temperatures were maintained at 220 and 250 °C, respectively. Injection volume was 0.5 mL and the column flow was 0.8 mL/min. Total fat in tissues, serum, and diets were extracted using the method described by Bligh and Dyer (1959). Briefly, samples were homogenized in trichloromethane:methanol 1:2 (vol:vol). After drying under nitrogen, the samples were dissolved in 1 mL of hexane for methylation. The fatty acids methyl esters (FAME) were formed by transesterification with

| Centesimal composition of dry feed formulated for reptiles | g/1000 g |
|----------------------------------------------------------|----------|
| Proteins                                                 | 47%      |
| Fat                                                      | 13%      |
| Fiber                                                    | 2%       |
| Moisture                                                 | 12%      |
| Phosphorus                                               | 1.5%     |
| Calcium                                                  | 3%       |
| Energy value                                             | 4500 kcal/kg |
Table 2 Fatty acid composition determined in flaxseed oil, flaxseed, and the three diets provided to individuals of *C. latirostris* in captivity

| Fatty acids | Oil | Seed | Diets treatment |
|-------------|-----|------|-----------------|
|             | C   | FO   | FS              |
| Total lipids | 99% | 34%  | 26%            |
| Fatty acids |     |      | 46%            |
|             |     |      | 31.5%          |
| SFA         |     |      |                |
| 14:0        | 0.05 ± 0.01 | 0.05 ± 0.2 | 0.49 ± 0.01 | 0.41 ± 0.00 | 0.38 ± 0.02 |
| 15:0        | ND  | ND   | 0.06 ± 0.01    | 0.05 ± 0.01 | 0.07 ± 0.00 |
| 16:0        | 5.82 ± 0.07 | 5.48 ± 0.05 | 20.63 ± 0.16 | 17.50 ± 0.23 | 16.41 ± 0.60 |
| 17:0        | 0.04 ± 0.01 | 0.04 ± 0.01 | 0.33 ± 0.01 | 0.22 ± 0.01 | 0.28 ± 0.02 |
| 18:0        | 4.45 ± 0.03 | 4.64 ± 0.08 | 6.24 ± 0.07 | 5.87 ± 0.03 | 5.88 ± 0.03 |
| MUFA        |     |      |                |
| 14:1 – cis 9 | ND  | ND   | 0.09 ± 0.01    | 0.10 ± 0.00 | 0.07 ± 0.06 |
| 16:1 – cis 9 | 0.07 ± 0.01 | 0.08 ± 0.01 | 4.41 ± 0.02 | 3.73 ± 0.09 | 3.02 ± 0.11 |
| 18:1 – cis 9 | 18.56 ± 0.22 | 18.11 ± 0.04 | 33.13 ± 0.40 | 30.97 ± 0.12 | 29.18 ± 0.01 |
| 18:1 – cis 11| ND  | ND   | 1.70 ± 0.03    | 1.53 ± 0.07 | 1.44 ± 0.01 |
| 18:1 – trans-8| ND | ND   | 0.03 ± 0.00    | 0.03 ± 0.00 | 0.04 ± 0.00 |
| 18:1 – trans-9| ND | ND   | 0.47 ± 0.56    | 0.06 ± 0.00 | 0.06 ± 0.00 |
| 18:1 trans-10| ND | ND   | 0.06 ± 0.00    | 0.08 ± 0.01 | 0.07 ± 0.01 |
| 18:1 trans-11| 0.77 ± 0.02 | 0.74 ± 0.02 | 0.06 ± 0.00 | 0.05 ± 0.00 | 0.06 ± 0.00 |
| 20:1 – cis 11| 0.27 ± 0.08 | 0.22 ± 0.03 | 0.30 ± 0.02 | 0.31 ± 0.01 | 0.32 ± 0.00 |
| PUFA        |     |      |                |
| 18:2 n-6    | 14.26 ± 0.13 | 11.75 ± 0.58 | 27.18 ± 0.15 | 25.83 ± 0.30 | 25.43 ± 0.03 |
| 18:3 n-6    | 0.27 ± 0.00 | 0.28 ± 0.01 | 0.24 ± 0.01 | 0.24 ± 0.01 | 0.23 ± 0.01 |
| 18:3 n-3    | 54.96 ± 0.06 | 58.69 ± 0.18 | 2.49 ± 0.09 | 11.66 ± 0.41 | 15.75 ± 0.53 |
| 20:2        | ND  | ND   | 0.22 ± 0.03    | 0.15 ± 0.02 | 0.15 ± 0.00 |
| 20:3 + 22:0 | ND  | ND   | 0.22 ± 0.01    | 0.20 ± 0.01 | 0.20 ± 0.00 |
| 20:4 n-6    | 0.12 ± 0.02 | ND   | 0.78 ± 0.00    | 0.53 ± 0.05 | 0.49 ± 0.01 |
| 20:5 n-3    | ND  | ND   | 0.03 ± 0.01    | 0.02 ± 0.00 | 0.02 ± 0.00 |
| 22:4 n-6    | ND  | ND   | 0.18 ± 0.01    | 0.11 ± 0.03 | 0.12 ± 0.01 |
| 22:5 n-6    | ND  | ND   | 0.04 ± 0.00    | 0.02 ± 0.01 | 0.03 ± 0.00 |
| 22:5 n-3    | ND  | ND   | 0.11 ± 0.01    | 0.06 ± 0.01 | 0.09 ± 0.01 |
| 22:6 n-3    | ND  | ND   | 0.11 ± 0.02    | 0.09 ± 0.00 | 0.07 ± 0.01 |
| ∑SFAa       | 10.35 ± 0.04 | 10.20 ± 0.11 | 27.94 ± 0.27 | 24.17 ± 0.33 | 23.13 ± 0.69 |
| ∑UFAb       | 89.27 ± 0.25 | 89.85 ± 0.79 | 71.92 ± 1.32 | 75.76 ± 1.12 | 76.81 ± 0.77 |
| ∑MUFAc      | 19.66 ± 0.11 | 19.14 ± 0.04 | 40.30 ± 10.02 | 36.86 ± 0.30 | 34.24 ± 0.16 |
| ∑PUFAd      | 69.61 ± 0.14 | 70.71 ± 0.75 | 31.63 ± 0.30 | 38.91 ± 0.82 | 42.57 ± 0.61 |
| ∑n-3e       | 54.96 ± 0.06 | 58.69 ± 0.18 | 2.74 ± 0.11 | 11.83 ± 0.41 | 15.93 ± 0.55 |
| ∑n-6f       | 14.65 ± 0.11 | 70.71 ± 0.75 | 28.88 ± 0.19 | 27.07 ± 0.41 | 26.64 ± 0.06 |
| n-6/n-3     | 0.27 ± 0.01 | 0.21 ± 0.01 | 10.53 ± 1.82 | 2.29 ± 1.01 | 1.67 ± 0.11 |

Values are expressed as mean (% of total FAME). Control diet (C): crushed chicken head and dry feed formulated for reptiles (70%/30%), flax oil diet (FO): control diet+10% flax oil, flax seed diet (FS): control diet+10% mass crushed flaxseeds saturated fatty acid (SFA), unsaturated fatty acid (UFA), polyunsaturated fatty acid (PUFA).

*a*Saturated = C14:0 + C15:0 + C16:0 + C17:0 + C18:0

*b*Unsaturated = 14:1 cis-9 + 16:1 cis-9 + 18:1 cis-9 + 18:1 cis-11 + 18:1 trans-9 + 18:1 trans-10 + 18:1 trans-11 + 20:1 cis-11 + C18:2 n-6 + C18:3 n-6 + 18:3 n-3 + 20:2 + 20:3 + 20:4 n-6 + 20:5 n-3 + 22:4 n-6 + 22:5 n-6 + 22:5 n-3 + 22:6 n-3

*c*Monounsaturated = 14:1 cis-9 + 16:1 cis-9 + 18:1 cis-9 + 18:1 cis-11 + trans-8 + trans-9 + trans-10 + 18:1 trans-11 + 20:1 cis-11 + C18:2 n-6 + C18:3 n-6 + 18:3 n-3 + 20:2 + 20:3 + 20:4 n-6 + 20:5 n-3 + 22:4 n-6 + 22:5 n-6 + 22:5 n-3 + 22:6 n-3

*d*Polyunsaturated = C18:2 n-6 + C18:3 n-6 + 18:3 n-3 + 20:2 + 20:3 + 20:4 n-6 + 20:5 n-3 + 22:4 n-6 + 22:5 n-6 + 22:5 n-3 + 22:6 n-3

*e*∑n-3 PUFA = 18:3 n-3 + 20:5 n-3 + 22:5 n-3 + 22:6 n-3

*f*∑n-6 PUFA = C18:2 n-6 + C18:3 n-6 + 20:4 n-6 + 22:4 n-6 + 22:5 n-6

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methanolic potassium hydroxide solution as an interim stage before saponification (ISO 5509:2000, Point 5 IUPAC method 2.301). FAME were identified by comparison of their retention times relative to those of commercial standards using GC Solution Postrun software (version 2.30 00 SU6). Standards GLC-463 reference standard containing 52 FAME mixture (purity > 99%) and trans-mix GLC 481 (purity > 99%) were purchased from Nu-Chek (Nu-Chek Prep, Inc., Elysian, MN, USA). Linoleic acid methyl esters, cis/trans mix (Catalog #47,791) was obtained from Supelco (Bellefonte, PA, USA). Conjugated linoleic acid, cis/trans mix (Catalog #05,507) was purchased from Sigma Chemical Co. Others FAME standards were provided by the International CYTED Net (208RT0343). All solvents and reagents used for the FA quantification were of chromatography grade, and all the other chemicals used were at least American Chemical Society (ACS) degree. Values of FA content were expressed as percentage of total FAME.

We expressed grouped as ΣSFA (saturated fatty acids), ΣUFA (unsaturated fatty acids), ΣPUFA (polyunsaturated fatty acids), Σn-3 FA (C18:3 + C20:5 + C22:5 + C22:6), Σn-6 FA (C18:2 + C18:3 6c9c12c + C20:3 + C20:4 + C22:4 + C22:5 + C20:2), and the ratio of ΣSFA/ΣPUFA. Also, we determine the ratio of C20:4/C18:2 n-6 (ARA/LA), C22:6/C18:3 n-3 (DHA/ALA), and n-6/n-3 ratio.

We estimated the index of D9- and D6-desaturase activities and elongase according to Malau-Aduli et al. (1997), Kazala et al. (1999), and Pitchford et al. (2002), as follows: Index of C16 desaturase activity = 100 [(C16:1 cis-9)/(C16:1 cis-9 + C16:0)]; Index of C18 desaturase activity = 100 [(C18:1 cis-9)/(C18:1 cis-9 + C18:0)]; and Index of C16–C18 elongase activity = 100 [(C18:0 + C18:1 cis-9)/(C16:0 + C16:1 cis-9 + C18:0 + C18:1 cis-9)]. The atherogenic index, considered as a health indicator related to the risk of cardiovascular disease, was computed according to Ulbricht and Southgate (1991) as [4(C14:0) + C16:0] / (ΣMUFA + ΣPUFA).

### Statistical analysis

To compare the proportion of FA in two cuts of meat from animals fed different diets, we analyzed the data with general linear models (GLM). In all cases, the assumptions of normality and homogeneity of variances were tested (analyzed graphically and with Shapiro–Wilks’s test). In all GLMs, we analyzed each fatty acid according to the type of cut (two levels: tail, neck) and the diet (three levels: C, FS, and FO) and we evaluated the interactions between the cut and diet variables. If the interaction was not significant (p > 0.05), it was dropped from the model, and the data re-analyzed with a model including only the main effects of cut and diet. In cases where we detect differences (p < 0.05), we make a posteriori comparisons using the DGC test. Data were analyzed using the InfoStat software, version 2018 (Di Rienzo et al., 2018).

### Results

Throughout the experiment, the diets were well accepted. We observed that individuals consumed the same amount of the supplemented diet as the control and animals showed a healthy status without any pathological manifestation associated with the treatments such as lack of appetite, changes in behavior, abnormality in the body, or death. In the dietary treatments enriched with the addition of FS and FO (Table 2), we observed a reduction in SFA, and an increase in UFA, PUFA, more specifically in C18:3 n-3, and Σn-3, with a decrease in Σn-6 and Σn-6/n-3.

The meat cuts (tail and neck) evaluated showed different characteristics. Tail meat presented a higher proportion of C14:0, C16:0, C18:3 n-6 FA, ΣMUFA, index of C18 desaturase activity, and atherogenic index with respect to neck and higher proportion in neck of C18:0, C18:1 trans-10, C18:1 cis-11, C22:4, and ΣPUFA with respect to tail. Furthermore, meats from animals fed both enrichment diets (FS and FO diets) showed an increase of C18:3 n-3 and ΣUFA with respect to control. In addition, meats from animals fed the FO diet presented an increased the ratios of C18:1 trans-10, C22:5 n-3, C22:6n-3 (DHA), Σn-3, and reduced the n-6/n-3 ratio in both cuts compared to animals fed with other diets (Table 3). More importantly are that dietary treatments influenced those meat cuts in different way. Although both enriched diets raised the levels of C18:3 n-3, the neck showed higher values than the tail. Regarding FS, it was less effective on the tail, since we observed that the meat of animals fed with C and the meat of the tail of animals fed with FS showed less C22:4, higher MUFA, index of C18 desaturase activity, and atherogenic index, with respect to the other cuts and treatments.

### Discussion

In this study, we observed that with the addition of flaxseed oil and crushed flaxseeds in the caiman food, we achieved an increase in UFA and PUFA (more specifically C18:3 n-3 and Σn-3 FA) and a decrease in Σn-6/n-3 FA with respect to caiman food of control diet. These changes are mainly due to the C18:3 n-3 increase produced by the addition of flaxseed oils and seeds, which was about 5 times higher than the control diet. It should be noted that although the supplementation was 10% in both diets, the FA profiles obtained
Table 3  Differences in fatty acid composition determined in meat of *C. latirostris* (g/100 g) fed different diets (C, FO, and FS), between cuts (tail and neck) and interactions between the cut and diet variables; F value of parameter and P value (differences with *p* value *<* 0.05 highlighted in bold). DGC test showed the differences among groups of diets, cuts, or interactions

|            | Tail | Neck |        | FO   |        |        |        |        |        |
|------------|------|------|--------|------|--------|--------|--------|--------|--------|
|            | C    | FS   |        | C    | FS    |        |        |        |        |
| Total lipids | 0.91% | 0.99% | 1.2%  | 0.81% | 0.84%  | 0.89%  |        |        |        |
| Fatty acids | C 14:0 | 0.16 ± 0.01 | 0.21 ± 0.07 | 0.20 ± 0.14 | 0.13 ± 0.06 | 0.13 ± 0.03 | 0.17 ± 0.03 | 4.92 | 0.0330 |
|            | C 16:0 | 18.83 ± 1.55 | 19.01 ± 1.59 | 17.29 ± 1.00 | 17.39 ± 0.98 | 17.54 ± 0.58 | 17.58 ± 0.62 | 4.73 | 0.0362 |
|            | C 16:1cis 9 | 1.54 ± 0.32 | 1.95 ± 0.52 | 1.46 ± 0.56 | 1.13 ± 0.15 | 1.41 ± 0.34 | 1.64 ± 0.31 | 3.01 | 0.0913 |
|            | C 18:0 | 9.71 ± 1.17 | 8.92 ± 0.86 | 10.07 ± 1.22 | 10.86 ± 0.77 | 10.45 ± 0.75 | 10.23 ± 0.91 | 9.04 | 0.0048 |
|            | C 18:1cis 9 | 0.02 ± 0.01 | 0.02 ± 0.01 | 0.02 ± 0.02 | 0.02 ± 0.01 | 0.03 ± 0.01 | 0.03 ± 0.01 | 0.29 | 0.5918 |
|            | C 18:1trans 10 | 0.01 ± 0.00 | 0.02 ± 0.01 | 0.03 ± 0.01 | 0.02 ± 0.01 | 0.03 ± 0.01 | 0.04 ± 0.01 | 12.87 | 0.0010 |
|            | C 18:1trans 11 | 0.01 ± 0.01 | 0.05 ± 0.03 | 0.06 ± 0.04 | 0.05 ± 0.03 | 0.06 ± 0.02 | 0.06 ± 0.04 | 1.72 | 0.1980 |
|            | C 18:1 9-cis 9 | 19.91 ± 5.35 | 20.44 ± 2.35 | 17.86 ± 3.88 | 15.89 ± 0.44 | 17.10 ± 2.96 | 18.49 ± 2.05 | 3.86 | 0.0571 |
|            | C 18:1 11-cis 11 | 4.00 ± 1.22 | 4.35 ± 0.30 | 4.49 ± 1.07 | 5.22 ± 0.75 | 5.10 ± 0.55 | 4.62 ± 0.34 | 6.95 | 0.0123 |
|            | C 18:2 n-6 | 29.01 ± 1.20 | 29.45 ± 1.65 | 28.95 ± 0.96 | 30.42 ± 1.16 | 29.85 ± 1.15 | 29.84 ± 1.39 | 2.40 | 0.1298 |
|            | C 18:3 n-6 | 0.15 ± 0.05 | 0.17 ± 0.05 | 0.18 ± 0.06 | 0.13 ± 0.01 | 0.14 ± 0.02 | 0.15 ± 0.03 | 5.76 | 0.0217 |
|            | C 18:3 n-3 | 0.89 ± 0.15 | 1.08 ± 0.12 | 0.94 ± 0.17 | 0.83 ± 0.06 | 0.96 ± 0.14 | 1.09 ± 0.16 | 0.03 | 0.8543 |
|            | C 20:3 n-6 | 0.30 ± 0.08 | 0.31 ± 0.08 | 0.34 ± 0.05 | 0.32 ± 0.08 | 0.32 ± 0.02 | 0.29 ± 0.04 | 0.05 | 0.8332 |
|            | C 20:4 n-6 | 8.52 ± 2.25 | 7.44 ± 1.83 | 8.97 ± 2.50 | 9.64 ± 0.83 | 9.01 ± 1.90 | 8.17 ± 1.66 | 0.86 | 0.3610 |
|            | C 20:5 n-3 | 0.12 ± 0.04 | 0.11 ± 0.04 | 0.11 ± 0.04 | 0.13 ± 0.04 | 0.14 ± 0.03 | 0.11 ± 0.02 | 2.59 | 0.1164 |
|            | C 22:4 n-6 | 1.08 ± 0.41 | 1.23 ± 0.17 | 1.66 ± 0.31 | 1.55 ± 0.23 | 1.55 ± 0.21 | 1.40 ± 0.22 | 4.49 | 0.0416 |
|            | C 22:5 n-6 | 0.40 ± 0.23 | 0.35 ± 0.09 | 0.51 ± 0.15 | 0.40 ± 0.06 | 0.44 ± 0.09 | 0.44 ± 0.10 | 0.24 | 0.6280 |
|            | C 22:5 n-3 | 1.34 ± 0.59 | 1.27 ± 0.19 | 1.80 ± 0.40 | 1.49 ± 0.17 | 1.49 ± 0.29 | 1.53 ± 0.34 | 0.06 | 0.8094 |
|            | C 22:6 n-3 | 2.01 ± 0.65 | 1.75 ± 0.42 | 2.86 ± 0.90 | 2.14 ± 0.13 | 2.38 ± 0.70 | 2.92 ± 0.78 | 2.24 | 0.1434 |
| ΣSFAa       | 28.86 ± 1.01 | 28.32 ± 0.92 | 27.71 ± 0.98 | 28.53 ± 0.66 | 28.25 ± 0.36 | 28.10 ± 0.80 | 0.03 | 0.8553 |
| ΣMUFAc      | 69.47 ± 1.36 | 71.34 ± 0.78 | 71.80 ± 0.93 | 71.00 ± 0.73 | 71.35 ± 0.36 | 71.55 ± 0.78 | 2.51 | 0.1223 |
| ΣPUFAd      | 33.73 ± 8.98 | 27.50 ± 2.16 | 24.66 ± 3.93 | 22.90 ± 1.16 | 24.32 ± 2.35 | 25.46 ± 2.12 | 12.04 | 0.0014 |
| Σn-3e       | 44.64 ± 4.17 | 43.83 ± 2.16 | 47.15 ± 3.52 | 48.10 ± 1.25 | 47.03 ± 2.56 | 46.09 ± 1.59 | 3.44 | 0.0420 |
| Σn-6f       | 4.47 ± 1.20 | 4.24 ± 0.55 | 5.74 ± 1.13 | 4.90 ± 0.45 | 5.02 ± 0.86 | 5.41 ± 1.13 | 0.97 | 0.3313 |
| n-6/n-3     | 40.15 ± 3.06 | 39.56 ± 2.17 | 41.37 ± 2.66 | 41.72 ± 1.65 | 41.19 ± 1.93 | 40.66 ± 0.91 | 3.73 | 0.0612 |
| C20:4       | 0.30 ± 0.09 | 0.25 ± 0.06 | 0.31 ± 0.09 | 0.32 ± 0.03 | 0.30 ± 0.07 | 0.28 ± 0.06 | 0.47 | 0.4095 |
| C18:2       |                  |                  |                  |                  |                  |                  | 0.58 | 0.5674 |
### Table 3 (continued)

|                     | Tail | Neck             | $F_{out}$ | $P_{out}$ | $F_{dist}$ | $P_{dist}$ | DGC | $F_{ln}$ | $P_{int}$ | DGC |
|---------------------|------|------------------|----------|----------|-----------|-----------|-----|----------|-----------|-----|
| DHA/C18:3           |      |                  |          |          | 1.99      | 0.1666    |     | 1.24     | 0.3023    |     |
| Index of C16 desaturase activity | 7.60±1.69 | 9.20±1.69 | 7.66±2.51 | 6.08±0.45 | 7.40±1.51 | 8.47±1.24 | 1.82 | 0.1861   | 1.89      | 0.1661 |     |
| Index of C18 desaturase activity | 66.49±5.68 | 69.53±2.43 | 63.35±7.11 | 59.43±2.26 | 61.66±5.73 | 64.26±4.19 | 7.85 | 0.0083   | 7.66      | 0.6153 |     |
| Index of C16 to C18 elongase activity | 58.83±6.45 | 58.28±4.42 | 59.74±2.62 | 59.12±1.93 | 59.18±1.68 | 59.90±1.67 | 0.24 | 0.6277   | 0.49      | 0.6153 |     |
| Atherogenic index | 1.03±0.07 | 1.06±0.07 | 0.94±0.08 | 0.92±0.06 | 0.94±0.06 | 0.96±0.05 | 10.66 | 0.0025   | 2.68      | 0.0827 | 5.28 |

Values are expressed as mean (% of total FAME) [control diet (C): crushed chicken head and dry feed formulated for reptiles (70%/30%), flax oil diet (FO): control diet + 10% flax seeds oil, flax seed diet (FS): control diet + 10% mass crushed flaxseeds] of *C. latirostris*, saturated fatty acid (SFA), unsaturated fatty acid (UFA), polyunsaturated fatty acid (PUFA), arachidonic acid (AA), linoleic acid (LA), docosahexaenoic acid (DHA), linolenic acid (ALA)

$a$ ΣSaturated = C14:0 + C15:0 + C16:0 + C17:0 + C18:0

$b$ ΣUnsaturated = 14:1 cis-9 + 16:1 cis-9 + 18:1 cis-9 + 18:1 cis-11 + 18:1 trans-9 + 18:1 trans-10 + 18:1 trans-11 + C18:2 n-6 + C18:3 n-6 + 18:3 n-3 + 20:3 + 20:4 n-6 + 20:5 n-3 + 22:4 n-6 + 22:5 n-6 + 22:6 n-3

$c$ ΣMonounsaturated = 14:1 cis-9 + 16:1 cis-9 + 18:1 cis-9 + 18:1 cis-11 + 18:1 trans-9 + 18:1 trans-10 + 18:1 trans-11

$d$ ΣPolyunsaturated = C18:2 n-6 + C18:3 n-6 + 18:3 n-3 + 20:3 + 20:4 n-6 + 20:5 n-3 + 22:4 n-6 + 22:5 n-6 + 22:6 n-3

$e$ Σn-3 PUFA = 18:3 n-3 + 20:5 n-3 + 22:5 n-3 + 22:6 n-3

$f$ Σn-6 PUFA = C18:2 n-6 + C18:3 n-6 + 20:4 n-6 + 22:4 n-6 + 22:5 n-6

$g$ Index of C16 desaturase activity = 100 [(C16:1 cis-9)/(C16:1 cis-9 + C16:0)]

$h$ Index of C18 desaturase activity = 100 [(C18:1 cis-9)/(C18:1 cis-9 + C18:0)]

$i$ Index of C16–C18 elongase activity = 100 [(C18:0 + C18:1 cis-9)/(C16:0 + C16:1 cis-9 + C18:0 + C18:1 cis-9)]

$j$ Atherogenic index = [4(C14:0 + C16:0)/(ΣMUFA + ΣPUFA)]
from the FS feed show an incorporation of approximately 21% of C18:3 n-3 available in the seed; however, FO showed 27% of C18:3 n-3 available in the flaxseed oil. Also, the total lipids content on diets reflected similar relation.

There is limited information about the apparent metabolizable energy of flaxseed oil and flaxseed; in avian oviparous it was shown that extracted flaxseed oil would have a better C18:3 n-3 deposition rate to egg yolk compared to a crushed flaxseed ingredient when supplemented in a ration of laying hens (Ehr et al., 2017), showing that oil has a higher availability of C18:3 n-3 than seeds. Even in further processed ingredients such as crushed flaxseed, the flaxseed still contains the components of the seed’s cellular matrix that may trap some of the lipid fraction as seen with other seed types (Cassady et al., 2009), which may be a factor in reducing digestibility or availability for transfer omega-3 fatty from flaxseed. In our work, the values of C18:3 n-3 and total lipids obtained in the diets seem to indicate that it is due to the amount of supplementation used, and not because of a higher availability in the flaxseed oil with respect to the flaxseed.

Regarding the characteristics of caiman meat, we observed differences in the FA profile between cuts; mainly, we could highlight that neck cut presented higher values of PUFA and certain MUFA than the tail. Moreover, atherogenic index was lower on neck cut than tail and we observed that the neck is more susceptible than the tail to be improved by FO, which could suggest that it is more beneficial to consume neck meat.

However, in Argentina the entire caiman carcass is sold, so with the few differences found we could not recommend any cut that stands out for its characteristics. Comparison with another caiman such as Caiman crocodilus, Huang et al. (2018) highlighted the higher proportion of PUFAs in anterior body position (foreleg and anterior ventral meat) compared to other cuts. Also, for this same species, Vicente Neto et al. (2010) observed other differences between cuts; tail meat is more tender than that of the back in this species, attributing these differences to the functions, i.e., adaptations of the body to different activities. In our study, probably differences between cuts could be attributed to the same cause; however, we only evaluated the FA profile of the two meat cuts.

When analyzing the total lipid content, the recommended in meat is less than 50% SFA and more than 70% UFA (MUFA and PUFA; Jiménez-Colmenero et al., 2001). Thus, the meats obtained in this work present similar and improved values with respect to the recommended ones (between 27.5 and 28.8% of SFA and 70.3 and 71.8% of UFA). Replacing animal lipids with FA of the n-3 family, in particular C18:3 n-3 and C22:6, is a strategy used to improve the nutritional profile of meats or meat products (Moghadasian, 2008) such as those obtained from pigs, cattle beef, and sheep (Berthelot et al., 2012; Kronberg et al., 2012; Mapiye et al., 2013; Morel et al., 2013; Scollan et al., 2014). We obtained in caiman meats when these animals fed diets enriched with n-3 family FA a 1.2% C18:3 n-3. As mentioned above, similar studies had been conducted on other species. In those species, the effect of fatty acid exchange was much more pronounced than observed for the caiman. This low C18:3 n-3 level could be due to several factors. Mainly, most of dietary supplementation studies conducted were conducted on mammals, avian, or fish. The caiman is a reptile and has very little intra-muscular fat (1%, Simoncini et al., 2020) and with a different metabolism rate (more slow), which may also affect the turnover of nutrients.

Second, the feeding frequency of the enriched diet only was three times a week, lower than in other studies. For this reason, this work seeks to compare different types of supplementation sources in order to suggest better and more economical practices to producers. Surely, if we increase the frequency of supplementation and period of time of experimentation, the changes evidenced in the meat would be greater.

The supplementation FO increased in meat of animals the ratios of C22:5 n-3, C22:6n-3, Σn-3, and reduced the n-6/n-3 ratio compared to animals fed with other diets. Results reported here show that FO is more efficient ability to enrichment to caiman meats as compared to FS. For this reason, enriching the caiman diet with 10% flaxseed oil for a short period of time, before the animals are slaughtered, is a viable and good strategy to improve the final product as it is the caiman meat. This allows us to obtain a non-traditional product improved in its nutritional value and provide a feasible alternative to be considered as a component of the human diet and an important tool in local or regional economic development in many countries.

Dietary intake of foods rich in n-3 FA family has been reported to be effective in protecting against cardiovascular disease, breast and colon cancer, rheumatoid arthritis, and inflammatory bowel disease (Pelser et al., 2007; Patterson et al., 2012; Wu et al., 2014; Li et al., 2019; Uljević et al., 2019). According with that and the European Commission Regulation (EU) No 432/2012, several health claims can be made on foods containing them. Food containing DHA presents healthy properties such as contributing to the maintenance of normal brain function and normal vision, as well as fatty acids such as EPA and DHA contributing to normal heart function (European Commission).

Bolger et al. (2017) mention that Western European countries consume food with a ratio n-6/n-3 between 15 and 16:1, the recommended ratio being between 2.5 and 5:1, with an optimum below 4:1 (Simopoulos, 2002). Although the ratio obtained from the enriched meat is not below optimal values, the diet with flaxseed oil addition managed to improve the ratio n-6/n-3 in the meat,
with respect to the control diet. It should be noted that these changes were obtained by offering caimans (only for 50 days, three times a week) a supplement that does not have a high cost compared to others used as marine and algal oils, as they are good sources of fatty acids EPA and DHA. However, we must consider that the incorporation of the latter is limited due to unpleasant flavors associated with oxidation and a product with a fishy taste or odor (Josquin et al., 2012).

Regarding its implementation of enrichments diets in farms, our work shows that flaxseed oil is a well-tolerated and more efficient ability to transfer the omega-3 FA to meat compared to crushed flaxseed. Although the cost of flaxseed oil is 20 times higher than that of flax seeds, the use of the oil would require less frequency or time of supplementation to obtain an enriched meat, besides being easy and simple to incorporate into the diet, as it does not require previous treatments such as seed crushing. In addition, working with flaxseed oil would require less storage space, in relation to the comparative flaxseeds, although stocking of both (seeds and oil) require fresh and dark places and cannot be stored for a long time. Moreover, we must recognize that both flax seeds and oil are functional ingredients that are easily accessible, can be obtained at any time of the year, and are not expensive.

Our studies continue in the development of other qualities of healthy meat and the benefits generated to caimans, since diet enrichment could improve the health status of individuals (immune system, growth, among others) and the quality of the production system. It is considered that in the future new studies related to the enrichment of the diet of these animals with flaxseed will be carried out since they have shown that in addition to having numerous benefits they have antioxidant effects due to their lignans content (Prasad et al., 1998; Pattanaik and Prasad, 1998; Prasad, 1999) and also due to the presence of phytoestrogens (Babu et al., 2000).

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Author contribution The author’s contributions were as follows: M.S.S. and C.I.P. conceived the study; M.A.G., M.C.L., M.S.S., and C.I.P. designed the study; P.M.L.L. and M.S.S. performed the experiment and collected the samples; J.L., M.A.G., and P.M.L.L. carried out the laboratory analyses; and A.E.F., P.M.L.L., and M.S.S. analyzed the data. All authors interpreted results, drafted the manuscript, and they critically revised the manuscript.

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Data availability All data in this research are availability. Compositions of diets shown in Table 1 and Table 2; fatty acid composition of meat is included in Table 3.

Declarations

Ethics approval All the animals were treated following the Reference ethical framework for biomedical research: ethical principles for research with laboratory, farm and wild animals (CONICET 2005) and all the experiments were approved by the Animal Ethics Committee of the School of Biochemistry (Universidad Nacional del Litoral).

Conflict of interest The authors declare no competing interests.

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