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Modification of the content of *n*-3 highly unsaturated fatty acid, chemical composition, and lipid nutritional indices in the meat of grass carp (*Ctenopharyngodon idella*) fed alfalfa (*Medicago sativa*) pellets

Luciano F. Montenegro,[†,‡,‖,1] Adriana M. Descalzo,[†,‖] Sebastián A. Cunzolo,[†,$] and Carolina D. Pérez[†,‖,$]

[†]Centro de Investigaciones de Agroindustria, Instituto de Tecnología de los Alimentos, Instituto Nacional de Tecnología Agropecuaria (INTA), Morón, Buenos Aires, Argentina, [‡]Universidad Nacional de Lomas de Zamora, Lomas de Zamora, Buenos Aires 1836, Argentina, [‖]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina, [$]Universidad de Morón, Morón, Buenos Aires, Argentina

[1]Corresponding author: montenegro.luciano@inta.gob.ar

Abstract

A 120-d feeding trial was conducted to determine the effect of alfalfa (*Medicago sativa*) feeding on growth and chemical composition, fatty acid content, and nutritional and lipid indices of the meat of grass carp (*Ctenopharyngodon idella*). Two experimental diets were used: alfalfa pellet (**AP**) diet and artificial grain diet (**GD**). Final weight, feed conversion rate, and protein efficiency ratio were significantly greater in the GD group ($P < 0.05$). However, no differences in the length and condition factor were observed. The composition of the meat differed between treatments. The protein content was significantly greater in the AP group ($P < 0.05$), while the lipid and cholesterol contents were significantly greater in the GD group ($P < 0.05$). A greater proportion of saturated, *n*-6 polyunsaturated, and *n*-6 highly unsaturated fatty acids was obtained in the GD group. The AP group accumulated a greater concentration of eicosapentaenoic (**EPA**), docosapentaenoic (**DPA**), and docosahexaenoic (**DHA**) acids ($P < 0.05$). The fatty acid composition of the meat determined a significant decrease in the thrombogenicity index and saturation index (S/P) in the AP group ($P < 0.05$). The Elongase index was greater in the GD group ($P < 0.05$). In contrast, the AP group had a greater index of Δ9 Desaturase and Δ5 + Δ6 Desaturase for *n*-3 and *n*-6 fatty acids ($P < 0.05$). These results suggest that alfalfa feeding decreases the growth of *C. idella* but improves the quality of meat by increasing the protein, EPA, and DHA contents. It also reduces cholesterol content and improves nutritional indices.

Key words: fish meat, grass carp, meat nutritional value, nutrition, productive response

Introduction

Aquaculture is currently seeking economically viable and environmentally sustainable alternatives to replace fishmeal in fish diets (Gatlin et al., 2007; FAO, 2016). One alternative is the culture of herbivore species such as grass carp (*Ctenopharyngodon idella*), which is capable of feeding on superior plants and is one of the most widely produced carps (China Fishery Statistical Yearbook, 2013).

In culture conditions, *C. idella* feeds on grain-based diets. However, it may also feed on forage, such as alfalfa (*Medicago sativa*), which is low-cost and environmentally sustainable. Alfalfa is produced worldwide and has great nutritional value,

Abbreviations

AI	atherogenicity
ALA	alpha-linolenic acid
AP	alfalfa pellet
CF	condition factor
DHA	docosahexaenoic acid
DPA	docosapentaenoic acid
EPA	eicosapentaenoic acid
FAMEs	fatty acid methyl esters
FCR	feed conversion rate
GD	grain diet
H	hypercholesterolemic
h	hypocholesterolemic
HUFA	highly unsaturated fatty acids
LA	linoleic acid
MUFA	monounsaturated fatty acids
PER	protein efficiency ratio
PI	peroxidability
PUFA	polyunsaturated fatty acid
TI	thrombogenicity
WG	weight gain

resulting from its protein content and balance between alpha-linolenic (**ALA**; C18:3 *n*-3) and linoleic (**LA**; C18:2 *n*-6) fatty acids (García et al., 2016).

Both fatty acids are important and essential for *C. idella* (Li et al., 2015). These can be elongated and desaturated to fatty acids of 20 or more carbon atoms (highly unsaturated fatty acids: **HUFA**s). LA is elongated to arachidonic acid (**ARA**; C20:4 *n*-6), whereas ALA is elongated to eicosapentaenoic (**EPA**; C20:5 *n*-3), docosapentaenoic (**DPA**; C22:5 *n*-3), and docosahexaenoic (**DHA**; C22:6 *n*-3) acids (Du et al., 2006), the three of which play an important role in the health and growth of fish (Sargent, 2002). Since the enzymes responsible for elongation and desaturation of ALA and LA compete with each other (Brenner, 1999), an adequate ALA/LA ratio could favor the production of EPA, DPA, and DHA (Li et al., 2015). Therefore, in addition to meet the requirements of *C. idella*, these fatty acids could accumulate in greater concentration in the meat.

The content of EPA, DPA, and DHA is one of the attributes for which fish meat is highly recommended and appreciated for human consumption. In this regard, clinical evidence was reported on the benefits that these compounds offer to human health. Some of these benefits are the development of cognitive ability during the first years of life (Richards et al., 2009; Valenzuela et al., 2011), and a decrease in the incidence of neurodegenerative and heart diseases, diabetes risk, and obesity (Simopoulos, 2008; Bazan et al., 2011; Sioen et al., 2017; Saini and Keum, 2018). Therefore, an improvement in the lipid quality of *C. idella* meat could be obtained from alfalfa feeding. Besides, this feeding strategy may have a positive impact on consumer perception. It is known that meat from pasture-fed animals is associated with better quality indices and greater nutritional composition (Nilzén et al., 2001; Descalzo et al., 2005; Forrester-Anderson et al., 2006; Ponte et al., 2008). In recent years, consumers have indicated a preference for fish meat naturally enriched with omega 3, vitamins, and antioxidants (Ramalho Ribeiro et al., 2019; Risius et al., 2019). Therefore, alfalfa feeding may improve the nutritional quality of *C. idella* meat while fulfilling the sustainable criteria of meat production.

The aim of this study was to evaluate the impact of alfalfa (*M. sativa*) feeding on the growth, chemical and fatty acid composition, and metabolic and lipid nutritional indices of *C. idella* meat. We hypothesized that grass carp fed alfalfa pellets (**AP**) can improve the nutritional value of meat for human consumption.

Material and Methods

This research–including all fish handling, sampling, and slaughter procedures–was approved by the Committee of Ethical Use of Animals from Facultad de Ciencias Agrarias, Universidad Nacional de Lomas de Zamora, Lomas de Zamora, Buenos Aires province, Argentina (Protocol number 23060/17), and by the National Institute of Agricultural Technology (INTA).

Experimental diets

Two contrasting diets were used (Table 1): 1) an artificial diet based on grains (**GD**) and 2) a diet based on AP (*M. sativa*). The GD is a specific diet for the species developed by the American Soybean Association (ASA) (Cremer et al., 2003), formulated with 50% soy meal, 20% sunflower meal, 16.8% corn husk, 8% corn gluten, 1% soybean oil, and 4.2% vitamin and mineral mixes. The vitamin mix was constituted by thiamine (50 mg/kg diet), riboflavin (50 mg/kg diet), vitamin A (9 mg/kg diet), vitamin E (400 mg/kg diet), vitamin D3 (6 mg/kg diet), pyridoxine HCl (40 mg/kg diet), cyanocobalamin (0.1 mg/kg diet), biotin (6 mg/kg diet), calcium pantothenate (100 mg/kg diet), folic acid (15 mg/kg diet), niacin (200 mg/kg diet), and inositol (2,000 mg/kg diet). The mineral mix was constituted by $Ca(H_2PO_4)_2$ (9.8 mg/kg diet), calcium lactate (37.9 mg/kg diet), NaCl (2.6 mg/kg diet), K_2SO_4

Table 1. Proximate composition and fatty acid composition of experimental diets

	AP	GD
Composition, g kg^{-1}		
Crude protein	202.8 ± 0.8	175.3 ± 2.0
Crude lipid	22.0 ± 1.3	65.0 ± 1.0
Carbohydrate	123.2 ± 12.1	557.1 ± 11.1
Total fiber	416.3 ± 10.4	47.0 ± 3.0
Moisture	97.5 ± 1.3	111.6 ± 2.0
Ash	138.2 ± 15.7	44.0 ± 1.0
Available energy, kcal kg^{-1}	2,400	3,100
Fatty acid profile, mg		
C10:0	61.9 ± 5.8	–
C12:0	10.1 ± 0.9	–
C14:0	7.1 ± 0.9	8.8 ± 0.1[1]
C15:0	5.1 ± 0.2	–
C16:0	12.1 ± 0.7	726.1 ± 0.6
C17:0	–	8.9 ± 0.8
C18:0	37.2 ± 2.1	331.4 ± 3.3
C20:0	16.1 ± 1.2	2.1 ± 0.2
C22:0	57.6 ± 2.1	28.6 ± 1.6
C24:0	14.5 ± 0.1	–
Total SAT	404.3 ± 11	1,105.2 ± 4.9
C16:1 *n*-7	12.1 ± 0.7	–
C16:1 *n*-9	5.3 ± 0.6	6.4 ± 0.0
C18:1 *n*-7	6.2 ± 0.7	75.1 ± 5.7
C18:1 *n*-9	26.9 ± 1.8	1,415.4 ± 7.4
Total MUFAs	50.5 ± 2.3	1,498.6 ± 1.8
C18:2 *n*-6	111.1 ± 5.5	3,376.6 ± 12.3
C18:3 *n*-6	–	33.3 ± 0.2
C18:3 *n*-3	117.8 ± 2.7	369.6 ± 1.7
Total C_{18} PUFA	228.8 ± 8.2	3,779.6 ± 10.4
C18:3/18:2	1.1 ± 0.0	0.1 ± 0.0

[1]Values are means ± standard deviation (*n* = 4).

(13.1 mg/kg diet), KCl (5.3 mg/kg diet), $FeSO_4$ (0.9 mg/kg diet), ferric citrate (3.1 mg/kg diet), $MgSO_4$ (3.5 mg/kg diet), $ZnSO_4$ (0.4 mg/kg diet), $5H_2O.CuSO_4$ (0.02 mg/kg diet), $CoCl_2$ (0.03 mg/kg diet), and KI (0.02 mg/kg diet). Cellulose was used as a carrier. The animals were fed for a total period of 120 d.

Animals

Approximately, 400 juvenile grass carps (*C. idella*), initial weight 13.42 ± 1.13 g and initial length 10.43 ± 0.55 cm, were obtained from the local fish farm (Oberá, Misiones, Argentina). The assay consisted of two treatments with four replicates each. Replicates were reared in individual tanks with 50 animals. The acclimatization period lasted 10 d. During the feeding period, each tank received individual aeration. Once a week, half the volume of water in the tanks was changed. Water temperature was 23 ± 0.7 °C, dissolved oxygen > 5.0 mg/L, pH 7.8 ± 0.3, NH_4^+-N < 0.5 mg/L, and NO_2–N < 0.05 mg/L. The fish tanks had a physical-biological filtering system to maintain the physicochemical characteristics of the water. The fish were fed to satiation twice a day (9:00 a.m. and 5:00 p.m.) according to the corresponding diets. Food residues were removed from the water after each feeding to determine the feed intake per day.

Sampling procedure

At 120 d of the trial, 12 fish from each treatment (3 fish from each of the four replicates) were slaughtered to determine the chemical and fatty acid composition and the lipid nutritional indices of the meat. Prior to slaughter, the animals were fasted for 24 h. Then, they were desensitized by immersion in water at 0 °C, weighed, and measured. Finally, they were slaughtered with a cervical puncture. To produce the bleeding, a cut was made in the caudal region.

Biometrical parameters

A total of 16 fish from each group (4 fish per tank) were used to determine biometric parameters. The determinations were conducted 24, 48, 72, 96, and 120 d after the beginning of the treatments. The weight gain (**WG**), specific growth rate, feed conversion rate (**FCR**), and other indices were then calculated using the following formulas:

$$\text{Weight gain} = (\text{final weight} - \text{initial weight}) \times 100/\text{initial weight}$$
$$\text{Feed conversion rate} = \text{g feed given/g weight gain}$$
$$\text{Protein efficiency ratio} = \text{fish wet weight gain/protein intake}$$
$$\text{Survival ratio} = (\text{N° final fish/N° initial fish}) \times 100$$
$$\text{Condition factor} = \text{body mass} \times 100/\text{body length}^3$$

Biochemical analysis

Diets

The chemical composition of the diets was performed according to the methods of the Association of Official Analytical Chemists (AOAC, 2000). All determinations were made in triplicate. To determine the moisture content, the samples were placed in a drying oven (60 °C) for 36 h. An aliquot of 2 g was incinerated at 550 °C for 24 h to determine the ash content. Crude protein was analyzed by the Kjeldahl method and estimated as N × 6.25 after sulfuric acid (98% m/m) digestion. An automatic Foss Tecator 2200 Kjeltec model was used. The Soxhlet hot extraction method was used to determine the total lipid content (Soxtec System HT 1043 Extraction unit, Tecator USA).

The fatty acid content was determined from 3 g samples of the dry matter. Lipids were extracted using a chloroform–methanol mixture (2:1), according to the method of Folch et al. (1957). Fatty acid methyl esters (**FAMEs**) were transmethylated with 4% HCl acid in anhydrous methanol, according to the method of Pariza et al. (2001). Methyl nonadecanoate (C19:0) at 1 mg/mL was added as the internal standard.

Analysis of FAME in hexane was performed on gas-liquid chromatography (Varian CP3800, Walnut Creek, CA, USA) fitted with a flame ionization detector. The concentration of fatty acids was expressed in mg/100 g dry matter.

Muscle

The proximate composition analysis of the muscle (three fish per tank) was determined according to AOAC (2000) methods. The moisture was determined by oven drying at 105 °C for 24 h, and the ash content was determined using a muffle furnace at 550 °C for 24 h. Crude protein was analyzed by the Kjeldahl method and estimated as N × 6.25 after sulfuric acid (98% m/m) digestion. An automatic Foss Tecator 2200 Kjeltec model was used. The concentration of lipids was obtained by taking 5 g of muscle using the technique of Folch et al. (1957). The cholesterol level was determined using a cholesterol assay kit (Colestat enzymatic kit, Argentina).

Fatty acid analysis of muscle and lipid nutritional indices

To determine the fatty acid composition, samples of 5 g of muscle (three fish per tank) were taken for lipid extraction using the technique of Folch et al. (1957). FAMEs were transmethylated with 4% HCl acid in anhydrous methanol, according to the method of Pariza et al. (2001). Analysis of FAME in hexane was performed on a gas-liquid chromatography (Varian CP3800, Walnut Creek, CA, USA) fitted with a flame ionization detector. Analytical results were expressed as percentages of total fatty acids. From the lipid content of the meat, the concentration of fatty acids (mg 100 g^{-1} of meat) was calculated.

Fatty acid means were used to calculate atherogenicity (**AI**), thrombogenicity (**TI**), hypocholesterolemic (**h**), hypercholesterolemic (**H**), peroxidability (**PI**), and saturation (S/P) indices using the following formulas, according to Mancini et al. (2017b):

$$\text{AI}: (\text{C14:0} \times 2 + \text{C16:0}) / (\text{MUFA} + \text{PUFA } n\text{-6} + \text{PUFA } n\text{-3})$$
$$\text{TI}: (\text{C14:0} + \text{C16:0} + \text{C18:0}) / (\text{MUFA} \times 0.5 + \text{PUFA } n\text{-6} \times 0.5 + \text{PUFA } n\text{-3} \times 3 + \text{PUFA } n\text{-3}/\text{PUFA } n\text{-6})$$
$$\text{h}: \text{C18:1} + \text{C18:2 } n\text{-6} + \text{C18:3 } n\text{-3} + \text{C18:3 } n\text{-6} + \text{C20:4 } n\text{-6} + \text{C20:5 } n\text{-3} + \text{C22:6 } n\text{-3H}: \text{C14:0} + \text{C16:0}$$
$$\text{PI}: \left(\sum \text{monoenoic acid} \times 0.025\right) + \left(\sum \text{dienoic acid} \times 1\right) + \left(\sum \text{trienoic acid} \times 2\right) + \left(\sum \text{tetraenoic acid} \times 4\right) + \left(\sum \text{pentaenoic acid} \times 6\right) + \left(\sum \text{hexaenoic acid} \times 6\right).$$
$$\text{S/P} = (\text{C14:0} + \text{C16:0} + \text{C18.0}) / (\text{MUFA} + \text{PUFA})$$

Chromatographic conditions

The FAME profile in diets and muscle was determined by split injection (1:100) into a CP-Sil 88 fused silica capillary column (100 m × 0.25 mm i.d., 0.20 μm film thickness, Varian CP7489) using a gradient temperature program. The column oven was held at 45 °C for 4 min, then increased from 45 to 165 °C at 13 °C/min and held for 35 min, and finally, from 165 to 215 °C at 4 °C/min and held for 30 min. The total run time was 90 min. The carrier gas was helium, at a constant flow of 1.0 mL/min. Temperature of injector and detector was 250 °C. Fatty acids were identified by comparing relative retention times with the individual fatty acid standard (PUFA-2 Animal Source; Grain Fatty acid Methyl Ester Mix [Sigma-Aldrich, USA]) and GLC 481B (Nu Chek Prep. Inc. Elysian, MN, USA).

Estimation of metabolic indices in muscle

Some indices were calculated to estimate the desaturase and elongase activity of muscle tissue. The activity of Δ9 desaturase was calculated from the relationship between the percentages of product and precursor (Okada et al., 2005):

$$\Delta 9\ \text{Desaturase}\ (C16) = [C16:1\ c9/\ (C16:1\ c9 + C16:0)] \times 100$$
$$\Delta 9\ \text{Desaturase}\ (C18) = [C18:1c9/\ C18:1c9 + C18:0)] \times 100$$

The elongase and thioesterase indices were calculated according to Zhang et al. (2007) as follows:

$$\text{Elongase} = C18:0/C16:0$$
$$\text{Thioesterase} = C16:0/C18:0$$

The determination of Δ5 and Δ6 desaturase activity index was done by the following equations (Sirri et al., 2010):

$$\Delta 5 + \Delta 6\ \text{Desaturase}\ (n\text{-}6) = [(C20:2\ n\text{-}6 + C20:4\ n\text{-}6 + C22:4\ n\text{-}6) / (C18:2\ n\text{-}6 + C20:2\ n\text{-}6 + C20:4\ n\text{-}6 + C22:4\ n\text{-}6)] \times 100$$
$$\Delta 5 + \Delta 6\ \text{Desaturase}\ (n\text{-}3) = [(C20:5\ n\text{-}3 + C22:5\ n\text{-}3 + C22:6\ n\text{-}3) / (C18.3\ n\text{-}3 + C20:5\ n\text{-}3 + C22.5\ n\text{-}3 + C22:6\ n\text{-}3)] \times 100$$

Statistical analyses

All data were expressed as the means ± standard deviation (SD) for *n* replicates. The effects of each diet were compared by a one-way analysis of variance followed by Tukey's post hoc test ($P < 0.05$). The variation of the biometrical parameters in response to the treatments was studied at different times (24, 48, 72, 96, and 120 d) using repeated measures ANOVA. All analyses were conducted using the XLSTAT Version 2018 (France).

Results

Diet composition

During the trial period, the fish accepted the diets. Voracity, typical of this species, was observed in food consumption. No signs of pathology were visualized in the fish. In addition, the physicochemical parameters of the water were maintained in the optimal ranges.

Table 1 shows the chemical composition and fatty acid concentration of both experimental diets. The AP diet had a significantly ($P < 0.05$) greater protein content than the GD diet and approximately nine times more fiber. In contrast, the GD had three times more total lipids. This indicated that the GD and AP diets had a carbohydrate:lipid (CHO:L) ratio of 8.57 and 5.6, respectively. Despite the lower content of nonstructural carbohydrates, the AP diet provided more structural carbohydrates (fiber) than the GD diet.

Both diets were contrasting in the composition of fatty acids. The greater content of saturated fatty acid (**SFA**) in GD was given mainly by palmitic (C16:0) and stearic (C18:0) acids. In addition, the monounsaturated fatty acids (**MUFA**s) content was 30 times greater in GD. The GD diet also had a greater concentration of LA (C18:2 *n*-6) and ALA (C18:3 *n*-3). Conversely, the AP diet had a greater C18:3/C18:2 ratio.

Biometrical parameters

In both treatments, the fish had similar growth parameters (Table 2), but at 48, 72, and 120 d they had a differential response to diets ($P < 0.05$). Although GD fish were heavier than AP fish, both groups increased their body weight and length (Figure 1)

Table 2. Growth, feed utilization, and biometrical parameters recorded from grass carp (*C. idella*) fed either with AP or with GD diet, at different times of the growth trial[1]

	24 d	48 d		72 d		96 d		120 d			
	AP[2]	GD		AP		GD		AP		GD	
FM, g	15.4 ± 1.2[a]	18.9 ± 1.1[a]		20.6 ± 0.6[b]		25.5 ± 0.6[a]		25.3 ± 0.8[b]		29.5 ± 1.4[a]	
WG, %	15.5 ± 0.5[b]	41.1 ± 0.9[a]		53.7 ± 1.1[b]		90.4 ± 2.6[a]		88.5 ± 2.0[b]		120.4 ± 1.2[a]	
FCR	13.2 ± 0.8[a]	11.2 ± 0.5[b]		14.7 ± 0.3[a]		15.9 ± 0.9[a]		26.2 ± 0.8[a]		25.5 ± 0.6[a]	
PER	0.4 ± 0.0[b]	1.6 ± 0.0[a]		0.7 ± 0.1[b]		1.4 ± 0.1[a]		0.5 ± 0.1[a]		0.6 ± 0.0[a]	
SR, %	100	99		99		99		96		99	
CF	0.9 ± 0.0[b]	1.1 ± 0.2[a]		0.9 ± 0.0[a]		0.9 ± 0.1[a]		0.6 ± 0.0[a]		0.6 ± 0.0[a]	
	AP	GD		AP		GD		AP		GD	
FM, g	29.6 ± 1.7[a]	33.4 ± 0.6[a]		36.6 ± 1.0[b]		41.6 ± 0.5[a]					
WG, %	120.6 ± 2.2[b]	149.0 ± 4.6[a]		173.1 ± 3.1[b]		210.1 ± 5.7[a]					
FCR	35.9 ± 1.4[b]	45.7 ± 0.3[a]		51.9 ± 1.1[b]		56.2 ± 0.5[a]					
PER	0.4 ± 0.1[b]	0.6 ± 0.1[a]		0.5 ± 0.0[b]		1.1 ± 0.1[a]					
SR, %	96	99		96		96					
CF	0.5 ± 0.0[a]	0.4 ± 0.0[a]		0.4 ± 0.0[a]		0.4 ± 0.0[a]					

[1]Values are means ± standard deviation, $n = 16$ per group.
[2]FM, final mass; SR, survival ratio.
[a,b]Adjusted means without a common letter differ statistically from each other (Tukey test, $P < 0.05$).

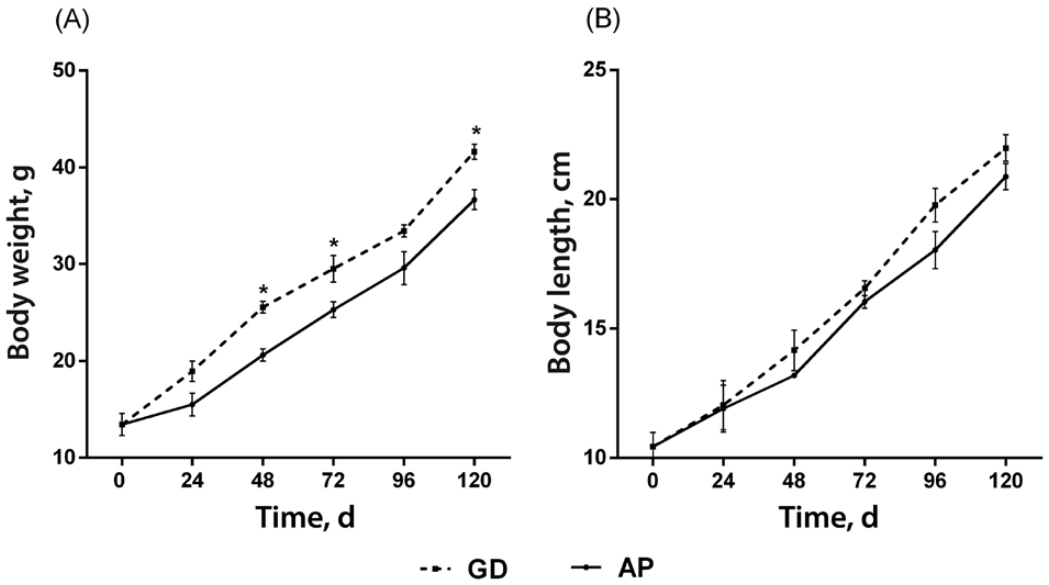

Figure 1. Body weight and body length of *C. idella* juveniles at 24, 48, 72, 96, and 120 d of the growth trial. AP diet treatment; GD treatment. *denotes statistical differences ($P < 0.05$) between treatments for each time of the trial.

over time, and no differences were observed in the condition factor (**CF**).

By contrast, significant differences ($P < 0.05$) were found in the FCR. The AP group had a greater FCR at all times analyzed, except at 72 d. This indicates that the AP group required more feed intake to gain weight probably due to lower efficiency in the use of dietary proteins from AP, as observed in the protein efficiency ratio (**PER**).

Biochemical composition of muscle

The chemical composition of the meat was determined at slaughter time (120 d) (Table 3). Meat from AP and GD had different contents of proteins, lipids, and cholesterol ($P < 0.05$). Crude protein and lipid content in meat reflected the protein and lipid composition of the diets (Table 1). Although the AP group reached lower final weight than the GD group (36.6 vs. 41.6 g), the protein concentration in the meat of alfalfa-fed animals was greater. Furthermore, the lipid and cholesterol contents were lower (approximately twice as low) in the AP than the GD group. Despite differences in the lipid content, the moisture content in meat was similar in both groups.

The diets produced changes in the fatty acid profile of *C. idella* meat in both experimental groups (Table 4). In the GD group, SATs were greater than in AP meat, especially for myristic (C14:0), palmitic (C16:0), and stearic (C18:0) acids, which were the most abundant. This is important because they are known as H and atherogenic. The oleic acid was the most abundant MUFA in both treatments.

The C_{18} polyunsaturated fatty acid (C_{18} **PUFA**) differed between groups ($P < 0.05$). Accordingly, the GD group had a greater LA and a lower ALA proportion than the AP group. This determined a C18:3/C18:2 ratio of 0.08 in the GD group and 1.22 in the AP group. The difference in this relationship was also reflected in the content of HUFAs. Since no HUFAs were detected in the diets, their presence in the meat reflected the metabolic capacity of *C. idella* to elongate and desaturate LA and ALA.

The AP group had a greater proportion of all HUFAs *n*-3: EPA (C20: 5 *n*-3), DPA (C22: 5 *n*-3), and DHA (C22: 6 *n*-3). By contrast, GD had a greater proportion of *n*-6 PUFAs. Differences

Table 3. Chemical composition and cholesterol content determined at the slaughter time on the meat of *C. idella* juveniles fed either with AP or with GD diet[1]

	AP	GD
Crude protein, %	16.03 ± 0.26[a]	14.98 ± 0.34[b]
Crude lipid, %	2.45 ± 0.28[b]	4.35 ± 0.35[a]
Cholesterol, mg	13.72 ± 0.77[b]	22.89 ± 0.94[a]
Moisture, %	80.27 ± 0.71[a]	79.43 ± 0.15[a]
Ash, %	1.25 ± 0.47[a]	1.24 ± 0.32[a]

[1]Values are means ± standard deviation, ($n = 12$ per group).
[a,b]Different superscript letters in columns denote statistical differences ($P < 0.05$).

in the proportions of *n*-3 and *n*-6 fatty acids between groups determined a different *n*-3/*n*-6 ratio. In this regard, although the *n*-3/*n*-6 ratio was low due to the high proportion of total *n*-6 in both groups, the *n*-3/*n*-6 ratio was better in the AP than in the GD group, in terms of the nutritional benefits of the meat (Ruxton et al., 2004, 2005).

The proportions observed in the fatty acids of fish meat produced differences in the concentration of the main fatty acids of interest to human health (Figure 2). The GD group fish doubled the concentration of SFA and MUFA in their meat, compared to the AP group (1,091 vs. 525.9 mg and 1,540.6 mg vs. 738.2 mg/100 g meat, respectively). Furthermore, the GD had three times as much C_{18} PUFA and *n*-6 totals (1,409.4 vs. 538.2 mg and 1,364.1 mg vs. 614.6 mg/100 g meat, respectively). However, the AP group outperformed the GD group in the total *n*-3 content (193.13 vs. 166.3 mg/100 g meat, respectively) and doubled the EPA + DHA content (96.95 vs. 49.67 mg/100 g meat).

Nutritional and metabolic lipid indices of the meat

Differences in fatty acid content in *C. idella* meat, in response to diets, produced differences in metabolic and nutritional lipid indices (Table 5) between the GD and AP groups. The AP group had lower TI, H, and S/P. However, it presented greater PI

Table 4. Fatty acid composition of meat of *C. idella* fed either with AP or with GD diet[1]

Fatty acid, %	AP	GD	Significance
C8.0	0.34 ± 0.06[a]	0.14 ± 0.00[b]	0.0007
C10:0	0.19 ± 0.04[a]	0.05 ± 0.01[b]	0.0005
C12:0	0.12 ± 0.01[a]	0.04 ± 0.00[b]	<0.0001
C14:0	1.36 ± 0.14[b]	1.69 ± 0.05[b]	0.0094
C15:0	0.77 ± 0.04[a]	0.29 ± 0.08[b]	<0.0001
C16:0	13.69 ± 0.26[b]	15.62 ± 0.20[a]	<0.0001
C17:0	0.43 ± 0.03[a]	0.11 ± 0.02[b]	<0.0001
C18:0	2.68 ± 0.33[b]	4.56 ± 0.15[a]	<0.0001
C20:0	0.41 ± 0.09	0.48 ± 0.11	*NS*
C22:0	1.49 ± 0.18	2.10 ± 0.62	*NS*
Total SFA	21.47 ± 0.50[b]	25.09 ± 0.66[a]	0.0001
C14:1 *cis* 9	0.18 ± 0.02[a]	0.11 ± 0.07[b]	0.0359
C16:1 *trans* 9	0.40 ± 0.06[a]	0.15 ± 0.04[b]	0.0019
C16:1 *cis* 7	0.53 ± 0.08	0.46 ± 0.05	*NS*[2]
C16:1 *cis* 9	4.55 ± 0.42[b]	5.71 ± 0.01[a]	0.0120
C17:1 *cis* 10	0.18 ± 0.03[a]	0.12 ± 1.23[b]	0.0033
C18:1 *n*-9	21.29 ± 0.66[b]	25.91 ± 0.31[a]	0.0006
C18:1 *cis* 11 *n*-7	2.70 ± 0.18	2.86 ± 0.01	*NS*
C22:1 *n*-9	0.30 ± 0.09[a]	0.09 ± 1.51[b]	0.029
Total MUFA	30.13 ± 0.86[b]	35.35 ± 0.07[a]	0.0009
C18:2 *n*-6	17.88 ± 0.26[b]	29.66 ± 0.24[a]	<0.0001
C18:3 *n*-6	0.57 ± 0.03[a]	0.24 ± 0.09[b]	0.0025
C18:3 *n*-3	3.52 ± 0.10[a]	2.50 ± 0.37[b]	<0.0001
Total C_{18} PUFA	21.97 ± 0.22[b]	32.40 ± 0.48[a]	<0.0001
C20:4 *n*-6	4.51 ± 0.37	5.09 ± 0.38	*NS*
C20:5 *n*-3	0.38 ± 0.04[a]	0.12 ± 0.03[b]	<0.0001
C22:4 *n*-6	2.14 ± 0.14[b]	2.57 ± 0.02[a]	0.0009
C22:5 *n*-3	0.40 ± 0.06[a]	0.19 ± 0.02[b]	0.0003
C22:6 *n*-3	3.58 ± 0.32[a]	1.02 ± 0.04[b]	<0.0001
Total HUFA	11.01 ± 0.25[a]	8.99 ± 0.33[b]	0.0001
n-3/*n*-6	0.31 ± 0.02[a]	0.10 ± 0.01[b]	<0.0001

[1]Values are means ± standard deviation, (*n* = 12 per group).
[2]*NS*, non significant.
[a,b]Different superscript letters in columns denote statistical differences ($P < 0.05$)..

than the GD group, probably due to its greater content of PUFAs and HUFAs.

No significant differences were observed in the Δ9 Desaturase (C16) index in response to the experimental diets. By contrast, the elongase index was greater (0.09 times) in the GD group. This may be due to the high content of SFA in meat (Table 4). In addition, the AP group had significant differences in the rest of the lipid indices ($P < 0.05$). The thioesterase index was greater in AP than in GD (10.28% vs. 9.22%, respectively). Also, greater Δ5 – Δ6 (*n*-3) and Δ5 – Δ6 (*n*-6) desaturases indices were observed in AP. However, the differences determined for the AP group were greater for *n*-3 fatty acids (19.5%) than for *n*-6 fatty acids (5.96%). Thus, the high proportion of *n*-6 fatty acids in the meat of the GD group was not reflected in a high Δ5 – Δ6 (*n*-6) desaturase index as expected.

Discussion

It is known that the growth and quality of fish meat are affected by a set of factors, the diet being prominent among them (Gutierrez et al., 2014; Gisbert et al., 2016). In this regard, feed sources and their nutrient content are considered the primary contributors (Zhao et al., 2018). In the present trial, the AP group achieved less growth than the GD group. Similar results for forage-based diets were reported in *C. idella* by Camargo et al. (2006), Nekoubin and Sudagar (2012), and Zhao et al. (2018), among others. These works attributed the lower growth to the low protein and carbohydrate contents in forage-based diets. However, many forage crops such as alfalfa (*M. sativa*) and annual ray-grass (*Lolium multiflorum*) can reach great protein contents (Palladino et al., 2009; Garcia et al., 2016), comparable to those of grains. The AP diet had greater protein content than the GD diet (Table 1); therefore, the lower growth observed in the AP group may have been due to the lower carbohydrate and lipid content of the diet and its high fiber content. Previous observations had a negative correlation between increased fiber content in the diet and WG in *C. idella* (Yu et al., 2014; Cheng et al., 2016). Thus, the greater fiber content of the AP diet, compared with the GD diet (413.6 vs. 47 g kg^{-1}), may explain the lower digestibility of the diet, observed from the greater FCR in the AP group. In this regard, Gao et al. (2011) and Guo et al. (2015) reported a decrease in growth in *C. idella* fingerlings with diets whose fiber content was 165 and 173 g kg^{-1}, respectively. The low digestibility of the AP diet may have caused the difference in the PER in the fish of this experimental group.

Biometrical indices other than weight may also be used as indicators of growth. The CF expresses the relationship between length (cm) and weight (g) (Jobling, 1994). In this regard, no differences were observed between both experimental groups for the CF. The CF observed was similar to those reported by Du et al. (2008) when supplementing juveniles of *C. idella* with 1% artificial diet. Therefore, since the fish showed no differences in total length between treatments, the difference observed in total weight may be ascribed to the high lipid deposition in the meat.

As previously mentioned, the diet, in addition to growth, affects the composition of meat (Joo et al., 2013). The processes of muscle growth and meat quality are governed by multiple molecular mechanisms (Salem et al., 2013). These, additionally, depend largely on the nutrient content of the diet (Cheng et al., 2014). In this study, it was observed that the composition of fish meat was related to the composition of the diet. Despite the low relationship reported between the protein content of the diet and the protein content of meat for *C. idella* (Guo et al., 2015), an increase in the protein content of meat was observed in the AP group. This increase may have stemmed from the greater protein content of the AP diet. It might also be attributed to the lower lipid content in the meat of this experimental group, resulting from lower amounts of lipids in the AP diet.

The greater lipid content of the GD diet produced an increase in meat lipids from this group of fish. Numerous studies reported a similar response from *C. idella* to different lipid contents in the diet (Du et al., 2006; Gao et al., 2011; Guo et al., 2015; Jiang et al., 2015; Li et al., 2017; Tian et al., 2019). This increase is linked to the great lipid and carbohydrate content of grain-based diets. However, Tian et al. (2019) found a high accumulation of lipids in *C idella*-fed bean pods (*Vicia faba*) (0.75% lipids), in comparison to that found in a group fed a grain diet.

Given its relationship with the lipid content of meat, cholesterol concentration differed between treatments. The lowest concentration observed in the meat of the AP group can be attributed to the lower lipid content, since the cholesterol concentration per gram of lipids did not differ between treatments (5.43 ± 0.3 mg cholesterol/g lipid). In agreement with these results, Guo et al. (2015) reported that cholesterol concentrations in *C. idella* meat varied according to the content of lipids in the diet, but observed no difference in the concentration expressed in mg cholesterol/g lipid. Regardless of the relative cholesterol content, the fish fed alfalfa had a lower

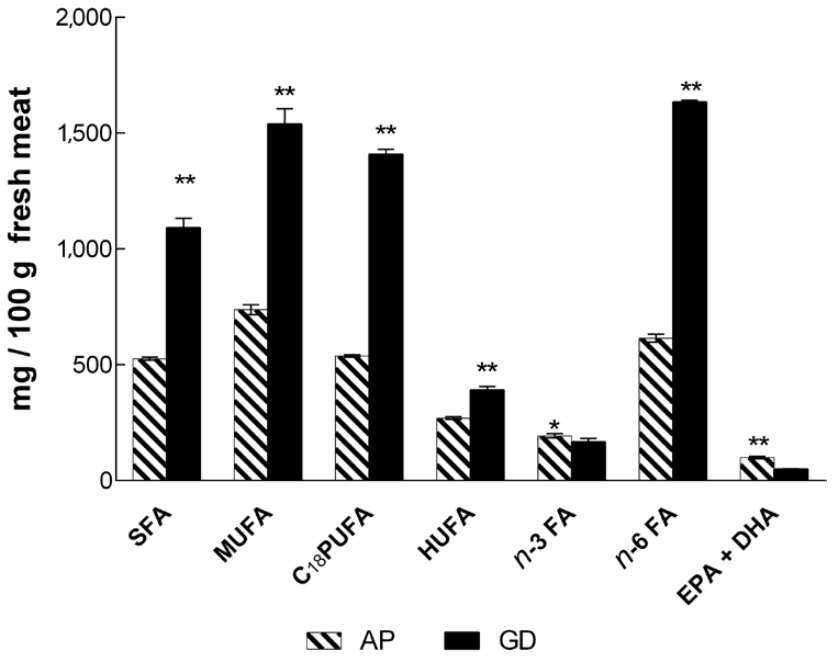

Figure 2. Concentration of fatty acid groups of nutritional interest in juvenile *C. idella* meat, expressed in mg/100 g fresh meat. AP diet treatment; GD treatment; SFA, total saturated fatty acid; MUFA, total monounsaturated fatty acid; C_{18} PUFA, total polyunsaturated fatty acid; HUFA, total highly polyunsaturated fatty acid; *n*-3 FA, total *n*-3 series fatty acid; *n*-6 FA, total *n*-6 series fatty acid. *Significance $P < 0.05$; **Significance $P < 0.01$.

Table 5. Lipid, nutritional, and metabolic indices determined at the slaughter time on the meat of *C. idella* juveniles fed either with AP or with GD diet[1]

	AP	GD	Significance
AI	0.26 ± 0.01	0.25 ± 0.01	NS[2]
TI	0.34 ± 0.01[b]	0.46 ± 0.01[a]	<0.0001
h	49.35 ± 0.67[b]	62.03 ± 1.03[a]	<0.0001
H	15.05 ± 0.31[b]	17.32 ± 0.21[a]	<0.0001
h/H	3.28 ± 0.10[b]	3.58 ± 0.09[a]	0.0051
PI	79.55 ± 0.96[a]	74.63 ± 0.92[b]	0.0003
S/P	1.54 ± 0.04b	1.65 ± 0.05[a]	0.0109
Elongase index	0.20 ± 0.01[b]	0.29 ± 0.02[a]	0.0001
Thioesterase index	10.28 ± 0.65[a]	9.22 ± 0.05[b]	0.0176
Δ 9 Desaturase (C16)	24.92 ± 2.06	26.76 ± 1.93	NS
Δ 9 Desaturase (C18)	88.82 ± 0.85[a]	85.01 ± 1.48[b]	0.0043
Δ 5+ Δ 6 Desaturase (*n*-6)	26.74 ± 0.79[a]	20.78 ± 0.57[b]	<0.0001
Δ 5+ Δ 6 Desaturase (*n*-3)	55.25 ± 0.48[a]	35.75 ± 0.90[b]	<0.0001

[1]Values are means ± standard deviation, (n = 12 per group).
[2]NS, non significant.
[a,b]Different superscript letters in columns denote statistical differences ($P < 0.05$).

total amount of cholesterol. This is of nutritional importance given the recommendations of official health agencies to reduce the consumption of meat with high cholesterol content (Saini and Keum, 2018).

Along with the cholesterol content, fatty acid composition influences the quality of the meat. The meat of the AP group had a lower proportion of SATs, especially myristic (C14:0) and palmitic (C16:0) acids. This is important for consumers because both acids are H (Blank et al., 2002; Russo, 2009; Mancini et al., 2017; Saini and Keum, 2018; Kumar et al., 2019). The difference in the proportion of the acids mentioned reflected their content in the diets. Therefore, the lower concentration of C14:0 in the diet may have resulted in the lower index of the elongases observed in the AP group, as these elongate the C14:0 to C16:0.

As reported by Du et al. (2006, 2008) and Lei et al. (2015), the ability of *C. idella* to elongate and desaturate fatty acids of 18 carbon atoms was also observed in the present work. Differences in the fatty acid profile of the diets produced differences in the profile of the meat. The meat of the AP group had a greater content of ALA (C18: 3 *n*-3) while the meat of the GD group had a greater content of LA (C18: 2 *n*-6); it is known that the growth and quality of fish meat are affected by a set of factors, among which the diet stands out. Besides, based on previous findings, synthesis of ARA (C20:4 *n*-6) was achieved by sequential desaturation and elongation of LA, whereas the synthesis of *n*-3 HUFAs (EPA, DPA, and DHA) was achieved by sequential desaturation and elongation of ALA with desaturase and elongase (Δ5 – Δ6) for the bio-conversion (Luo et al., 2012). The AP diet had lower LA content than the GD diet. This determined a greater ALA/LA ratio in the diet (1.1 vs. 0.1). The obtained relation could have led to the greater activity of Δ5 – Δ6 (*n*-3) desaturase, which favored the biosynthesis of the HUFAs: EPA, DPA, and DHA in the meat of AP. This way, the AP group had a greater endowment of HUFAs (*n*-3), which is beneficial to human health (Saini and Keum, 2018; Hernando et al., 2019). Accordingly, the obtained concentration of EPA + DHA covers half of the daily consumption requirements of EPA and DHA recommended by official health agencies (FAO, 2012; Sioen et al., 2017).

The amount of and differences in the fatty acids in fish meat in response to the diet determined a difference in lipid nutritional indices. TI shows the tendency to clot formation in blood vessels. The decrease in this index, coupled with the decrease in the saturation index, occurred in response to the enrichment of meat with PUFAs and HUFAs *n*-3 from alfalfa (AP) feed. Although there is no report in fish meat, similar results were obtained in the meat of other species, such as rabbit

(Mancini et al., 2017; Dalle Zotte et al., 2018), pork (Mancini et al., 2017), beef (Selani et al., 2016), and broiler (Kumar et al., 2019). This way, it can be inferred from the low TI that the meat of the AP group may contribute to reducing the risk of numerous cardiovascular diseases (König et al., 2005; Nantapo et al., 2015).

We concluded that feeding *C. idella* (grass carp) AP (*M. sativa*) diet is a sustainable alternative in providing meat with attributes of nutritional interest to consumers, although it produces a decrease in the growth rate of fish. Lower lipid and cholesterol contents, coupled with an increase in the number of proteins, EPA, DPA, DHA acids, and a lower TI index, were obtained from the proposed feeding system. Accordingly, not only does this nutritional composition improve the quality of fish meat, but also it fulfills the indications of various official food and human health agencies as well.

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Conflict of interest statement

The authors declare that they have no conflict of interest.

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