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The incorporation of Chia seeds (*Salvia hispanica* L.) in the chicken diet promotes the enrichment of meat with n-3 fatty acids, particularly EPA and DHA

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A B S T R A C T

Chia seeds, an ancestral food produced in South America, have been utilized to supplement the diet of chickens with the objective of increasing the meat content of n-3 fatty acids. Chia seeds are notably rich in α-linolenic acid, an essential fatty acid comprising 62% of the total fatty acids in chia. The expectation was that feeding chickens with such a level of α-linolenic acid would promote its conversion to EPA, DPA, and primarily DHA, considering the favorable impact of these fatty acids on consumer health. To achieve this goal, 96 male Ross chickens were provided ad libitum diets supplemented with 0%, 2.5%, 5%, and 10% of chia seeds. At 49 days of age, the animals were slaughtered, and the *Gastrocnemius, Iliotibialis lateralis*, and *Pectoralis major* muscles were analyzed. The results indicated that chia seeds did not have a negative impact on the productive parameters. Regarding meat color, the inclusion of chia in the feed appeared to lower the redness of meat, especially in *Pectoralis major*, without apparent effects on the pHu neither for the drip loss of the meat. Chia seeds led to an increased deposition of C18:3n3, EPA, DPA, and DHA into the muscles. The DHA levels detected in the muscles in our study could be considered relatively high when compared to the findings of other investigations using chia seeds in chickens, especially given the extent of chia seed incorporation in the feed. On the other hand, meat indices such as total n-3 fatty acids, n-6/n-3 ratio, AI (atherogenicity), TI (thrombogenicity), and h/H (hypocholesterolemic effect) are favorable for consumers' health when chia seeds are included in the feed of chickens, except for AI and h/H when chia is included at 10% in the feed.

1. Introduction

Worldwide poultry meat production in 2022 was reported at approximately 124 Mtons (FAOStat) and projections indicate that global poultry meat consumption in 2031 is expected to reach approximately 154 Mtons (OECD/FAO 2022). This projection is based on worldwide prospective studies, which also suggest that poultry and pig meat will remain the most consumed meat products in 2031, while the consumption of bovine and ovine meat is expected to continue to decline during the same period (OECD/FAO 2022, ABC, 2024).

The widespread preference for chicken meat worldwide can be attributed to several factors. It is relatively inexpensive in most countries, easily produced locally, and has fewer religious and cultural restrictions when compared to pork and ruminant meats (Pinto da Rosa et al., 2021).

Nutritionally, chicken meat boasts high-quality proteins, essential vitamins, and minerals. It also boasts a low content of saturated fatty acids (SAT) and a high content of polyunsaturated fatty acids (PUFA). These nutritional benefits, often highlighted in the media, contribute to consumers perceiving chicken meat as a safe and healthy diet choice (Katiyo et al., 2020; Barbut & Leishman, 2022).

Taking advantage of these features, chicken meat has the potential to serve as a vehicle for delivering valuable nutrients to consumers. In this context, the focus could be on the n-3 family fatty acids, which are among the most promising targets. The consumption of n-3 polyunsaturated fatty acids (PUFA), particularly docosahexaenoic acid

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(DHA, C22:6n3) and eicosapentaenoic acid (EPA, C20:5n3), is associated with numerous health benefits (Endo & Arita, 2016). These fatty acids have been linked to a reduced risk of cardiovascular diseases in humans (Calder, 2016; Calder, 2018; Harris & Zotor, 2019). Moreover, research has indicated that a diet enriched with n-3 fatty acids may have the potential to mitigate inflammation processes (Marion-Letellier et al., 2015), lower the risk of certain cancers (Nasir et al., 2020), and possibly even contribute to the prevention of some neurodegenerative diseases (Shramko et al., 2020).

The enrichment of chicken meat with n-3 fatty acids have been the subject of investigations reported in the scientific literature. The primary ingredients used to achieve this enrichment through diet included mainly flaxseed, fish meal, fish oil, marine algae, canola and chia seeds (Ayerza et al., 2002; Azcona et al., 2008; Figuerola et al., 2008; González-Esquerra & Leeson, 2001; Remize et al., 2021). . However, some of those ingredients present limitations due to their high costs and commercial availability, or the presence of anti-nutritional factors like trypsin inhibitors, cyanogenic glycosides, phytates, oxalates, and tannins (Dzuvor et al., 2018). When it comes to chia seeds, this product is readily available in South America at affordable prices. Furthermore, chia seeds appear to contain less anti-nutritional factors (Motyka et al., 2023). Another noteworthy feature is that chickens are non-ruminant animals capable of efficiently transferring lipid nutrients, such as fatty acids, from their food to their tissues with minimal alteration (Bień et al., 2022).

Chia seeds (*Salvia hispanica* L.) are native to Mexico and Guatemala. This plant has historically been cultivated alongside amaranthus, quinoa, and maize, and it was a staple food in the diets of the Mayas and Aztec populations for centuries (Muñoz et al., 2013; Jamshidi et al., 2019). Today, chia is cultivated as a valuable seed crop and is regarded as a functional food for human nutrition. Chia is a rich source of proteins, contains minerals, vitamins and antioxidants (Kulczyński et al., 2019). However, its popularity in human nutrition is related to the high content in α-linolenic acid, around 60–65% of total fatty acids, an essential fatty acid precursor of EPA and DHA (Motyka et al., 2023). The presence of the α-linolenic acid in such level in chia seeds could be taken advantage to promote its incorporation in the chicken's meat, but above all to boost the synthesis of its products, such as EPA and particularly DHA. This could make that the meat of those chickens could be considered as a fortified food in these fatty acids, taking into account their health benefits for human as described above in the text.

Hence, the primary objective of this study was to augment the n-3 fatty acid content in chicken meat through the incorporation of chia seeds into the animals' diet. To accomplish this goal, we examined three distinct levels of chia seed supplementation to identify the optimal amount that would facilitate a more efficient transfer of α-linolenic acid from the feed to the meat. This, in turn, would promote the increasing of EPA and DHA in the chicken meat.

2. Materials and methods

2.1. Animals, management and diets

Three hundred Ross male broiler chickens (*Gallus domesticus*) at 1 day of age were obtained from a commercial hatchery and raised until 21 days on a floor pen with wood shavings, in a climate controlled room. The first 3 days maintaining the temperature at 35°C and the following 21 days reducing it until 25°C; with a photoperiod of 23 h light: 1 h darkness. They were fed ad libitum with a corn-soya diet (21.7% crude protein and 2998 kcal/kg of metabolizable energy). Diets were formulated based on Tablas brasileñas para aves y cerdos (Tablas brasileñas para aves y cerdos 2017). Tap water was given ad libitum. At 21 days, 96 birds were selected on a homogenous live weight basis and assigned randomly into four groups of 24 birds each. The birds were located in experimental pens on floor (90 cm x 90 cm) with wood shavings as litter. Each pen located three birds, fed ad libitum with one of the experimental diets, until slaughtering. The experimental unit was each pen locating 3 birds for each treatment.

Four experimental diets were formulated with increasing levels of chia seeds, a control diet (Control) based in corn, soybean meal, meat meal and vegetal oil, and three other diets containing chia 2.5% (2.5), 5% (5) and 10% (10). Ingredients and composition of diets, including fatty acids, were presented in Table 1. The nutritional composition, including fatty acids, of chia seeds used in the experiment was presented in Table 2.

At 49 days of age, birds were sacrificed in a commercial slaughterhouse after a fasting time of 16 h. Afterwards, carcasses were chilled at +4°C for 24 h and the *Gastrocnemius, Iliotibialis lateralis* and *Pectoralis major* muscles were withdrawn and stored at −20°C until analysis.

2.2. Productive parameters

The animal's weight (BW) was determined individually at the beginning and at the end of the experiment. The total feed intake during the experiment and the feed conversion ratio (FCR) were also

Table 1
Ingredients and chemical composition (as feed basis) of the experimental diets containing increasing levels of chia seeds offered to chickens from 21 to 49 days of age.

	Diets			
Ingredients (g/kg)	C	T1	T2	T3
Yellow corn grain, ground	549.9	537.6	523.8	493.8
Soybean meal, 48%, crude protein	353.4	353.4	353.4	353.4
Chia seed	0.0	25.0	50.0	100.0
Meat and bone meal, 40/45% crude protein	38.0	30.0	20.0	10.0
Monocalcium phosphate, feed grade	8.0	10.0	12.0	14.5
Calcium carbonate, feed grade	11.0	11.0	14.0	16.0
NaCl	3.0	3.0	3.0	3.0
Sunflower oil, high oleic acid	28.0	21.2	15.0	0.5
L-Lysine monoclorhidrate	2.8	2.8	2.8	2.8
DL-Methionine	1.0	1.0	1.0	1.0
Anticoccidial [1]	0.1	0.1	0.1	0.1
Premix [2]	4.0	4.0	4.0	4.0
Choline chloride	0.3	0.3	0.3	0.3
Ascorbic acid, feed grade	0.2	0.2	0.2	0.2
Analyzed composition				
Gross Enery (Mcal/kg)	4.82	4.67	4.66	4.81
Crude Protein (%)	24.0	23.5	25.2	25.0
Lipids (%)	6.17	6.36	6.23	6.90
ADL (%)	0.75	1.00	1.81	1.46
ADF (%)	3.29	4.34	4.39	5.45
NDF with amylase (%)	13.6	11.6	11.4	12.9
Ashes (%)	6.72	6.49	7.45	7.09
Dry matter (%)	88.6	88.6	88.5	88.5
Calculated composition				
Calcium (%)	1.10	1.10	1.10	1.10
Available phosphorus (%)	0.40	0.40	0.40	0.40
Analyzed fatty acid composition (g/100 g fatty acids)				
C14:0	0.31	0.23	0.20	0.10
C14:1	0.02	0.01	0.05	0.03
C16:0	9.19	9.11	8.99	8.99
C16:1	0.29	0.25	0.29	0.33
C18:0	4.17	3.67	3.55	2.85
C18:1	56.4	46.7	36.8	22.1
C18:2n6	25.8	28.1	28.5	29.8
C18:3n3	1.36	10.0	18.7	33.3
Unidentified fatty acids	2.48	1.96	2.92	2.41

[1] Monensin sodium.
[2] The premix (provided the following per kg of premix: 3.000,00 KIU vitamin A; 625,00 KIU vitamin D3; 15,63 mg 25 (OH)$D_{3;}$ 20.000,00 mg vitamin E; 800 mg vitamin K3; 800 mg vitamin B1; 2.150,00 mg vitamin B2; 1.075,00 mg vitamin B6; 4,25 mg de vitamin B12;16.250,00 mg niacin; 5.000,00 mg pantothenic acid; 550,00 mg folic acid; 55,00 mg biotin; 100.000,00 mg choline chloride; 4.000,00 mg Cu; 5.000,00 mg Fe; 30.000,00 mg Mn; 62,50 mg Co; 312,50 mg Y; 27.500,00 mg Zn; 75,00 mg Se (Rovimix ® ROSS, DSM, Uruguay).

C= Control without Chia. T1, T2 and T3 are diets with Chia included at 2.5%, 5% and 10%, respectively.

Table 2
Chemical composition of Chia seeds (as feed; *Salvia hispánica* L.).

Items	Chemical composition of Chia seeds
Dry matter (%)	94.4
Ashes (%)	4.46
NDF with amylase corrected by ashes (%)	37.8
ADF corrected by ashes (%)	20.9
Acid Detergent Lignin (%)	10.0
Ether Extrait (%)	38.4
Crude Protein (%)	20.9
Gross energy (Mcal/kg)	6.32
Fatty acid composition (%)	
C14:0	0.03 ± 0.00
C16:0	7.01 ± 0.01
C16:1	0.11 ± 0.01
C18:0	2.56 ± 0.01
C18:1n-9	6.62 ± 0.02
C18:2n-6	20.5 ± 0.01
C18:3n-3	62.4 ± 0.12
Undefined fatty acids	0.73 ± 0.10

Data are mean ±SD of three samples.
Samples of chia were ground before to perform the analysis.

determined. FCR was calculated using the equation: FCR = Feed intake (g) / BW (g).

2.3. Analytical determinations

2.3.1. Color, pH and drip loss

The pH, color and drip loss were measured 24 horas *post mortem* in the three muscles. For pH measure, a Luton pH-201 penetration pH meter was used. For color, the CIELab method was used (L*; lightness, a*; redness and b*; yellowness) utilizing a Minolta Lab (CR-10) colorimeter with a D65 standard light CIE, 1976 . For the determination of drip loss, 5 g of samples of each muscle were taken. They were weighted and suspended into polyethylene closed bags inside the refrigerator (+4 °C). After 24 h, the samples were weighed and the drip loss percentage was determined.

2.3.2. Fatty acid profile

The intramuscular lipids were extracted according to Folch et al. (Folch et al., 1957). A sample of 2 g of *Gastrocnemius, Iliotibialis lateralis* and *Pectoralis major* muscles (free of visible fat) was homogenized at 12, 000 rpm with an IKA T25 homogenizer, during 1 min, with 50 ml of chloroform: methanol (2:1). Subsequently, the homogenate was filtered on fritted funnel (Graduation M), moved to a separating funnel, mixed by inversion for 1 min, and decanted overnight. The lower phase (chloroform containing lipids) was recuperated in a glass balloon, evaporated at 45°C with a light vacuum in a Rotavapor (IKA basic). Afterward, the balloon was dried in an oven at 35–40 °C for 30 min and cooled at ambient temperature overnight in a vacuum desiccator. To determine the percentage of lipids of each sample the balloon was weight at 0.0001 g. The methylation of fatty acids followed the procedure described by Ichihara et al. (Ichihara et al., 1996), using methanolic KOH. The determination of fatty acids by gas chromatography followed the procedure according to Eder (Eder, 1995), using fused-silica capillary column CPSIL-88 of 100 m installed in a split/splitless chromatograph Clarus 500, and the samples (1 μl in hexane) were injected using an autosampler (Perkin Elmer Instruments, USA).

2.3.3. Lipids enzymes activity indices

The enzyme activities of desaturase, elongase, and thioesterase were determined by measuring the conversion of specific substrates to their corresponding products for each respective enzyme. The activity of stearoyl-CoA desaturase (delta-9-desaturase) was estimated by calculating the ratios 16:1n-7 to 16:0 and 18:1n9 to 18:0. The delta-5 desaturase and delta-6 desaturase activities were calculated to estimate the conversion of corresponding substrate/products ratio into long-chain n-6 and n-3 fatty acids The ratio 18:0 to 16:0 was also calculated to estimate the elongase activity, and the thioesterase was estimated from the ratio of C16:0 to C14:0 (Boschetti et al., 2016; del Puerto et al., 2017). These indices are used as surrogates of the measure of the true enzyme activities (Dal Bosco et al., 2012; Vessby et al., 2002).

2.4. Calculus of nutritional and lipids health indices

Selected indices were calculated to establish the nutritional characteristics of meat chickens fed diet supplemented with chia seeds. Those indices were PUFA/SAT ratio, total n-6 fatty acids, total n-3 fatty acids, n6-n3 ratio and the n-3 indices. The n-3 indices were based on Dal Bosco et al. (Dal Bosco et al., 2022). Furthermore, usual lipids health indices were also calculated as follow:

- *Indices of Atherogenicity (AI)*.These indices compute the relation between the proatherogenic and the antiatherogenicfatty acids. It was calculated as described by Ulbricht and Southgate (Ulbricht & Southgate, 1991); (4 x C14:0 + C16:0) / [∑ MUFA + ∑(n-6) + ∑(n-3)].
- *Indices of Thrombogenicity (TI)*.These indices estimates the relation between the prothrombogenic and the antithrombogenic fatty acids. It was calculated as described by Ulbricht and Southgate (Ulbricht & Southgate, 1991).; (C14:0 + C16:0 + C18:0) / [0,5 x ∑MUFA + 0,5 x ∑(n-6) + 3 x ∑(n-3) + ∑(n-3) / ∑(n-6)].
- *Hypocholesterolemic/Hypercholesterolemic ratio (h/H)*.These indices estimate the relation between unsaturated fatty acids and the saturated fatty acids 14:0 and 16:0. The h/H ratio was estimated as described by Fernández et al. (Fernández et al., 2007); h/*H* = [(C14:1 + C16:1 + C18:1 + C20:1 + C22:1 + C18:2 + C18:3 + C20:3 + C20:4 + C20:5 + C22:4 + C22:5 + C22:6) / (C14:0 + C16:0)].

2.5. Contribution of n-3 intake to consumer

The contribution of intake of n-3 fatty acids from 100 g of chicken meat has been estimated based on the total n-3 fatty acids present in the three muscles. The calculation was performed by applying conversion factors to the total fat, in order to determine the values for total fatty acids. This method was carried out in accordance with the guidelines as outlined in Greenfield and Southgate (Greenfield & Southgate, 2003).

2.6. Statistical analysis

Data are presented as mean ± SEM. Animal response parameters were analyzed by one-way ANOVA. The pH, color, drip loss, fatty acids composition and lipids indices were analyzed by ANOVA with a GLM procedure using diet and muscle type as fixed effects. Also, was used a Tukey-Kramer post hoc test ($P < 0.05$). The Software NCSS 2019 NCSS, LLC, Kaysville, Utah, USA, has been used.

3. Results and discussion

3.1. Productive responses

The composition of chia used in our experiment, with regards to oleic acid, linoleic acid, and α-linolenic acid (Table 1), aligns with the levels reported for various sources of these seeds by Motika et al. (Motyka et al., 2023)

The inclusion of chia seeds in our experiment, at three different levels in the chickens' diets, did not have an impact on the final body weight or the body weight gain throughout the experimental period (Table 3). Nevertheless, the incorporation of chia seeds at levels of 5% and 10% resulted in a significantly higher feed intake, consequently leading to a higher feed conversion ratio, in comparison to the control group and the animals fed diets with 2.5% of chia seeds (Table 3). In the work by Ayerza et al. (Ayerza et al., 2002), the body weight has been

Table 3
Performance parameters of chickens fed diets with Chia seeds included at 0% (C), 2.5% (T1), 5% (T2) and 10% (T3).

	Diets				Significance
	C	T1	T2	T3	
Final body weight (g)	4138 ± 85	4090 ± 79	4005 ± 60	4096 ± 64	NS
Feed intake (g/bird)	4997 ± 55	5079 ± 65	5210 ± 33	5556 ± 68	***
Body weight gain (g/bird)	3112 ± 78	3092 ± 67	3019 ± 51	3063 ± 54	NS
Feed conversion ratio (g feed intake / g body weight)	1.63 ± 0,04	1.66 ± 0,04	1.74 ± 0,03	1.82 ± 0,03	***

The data represents the mean ± SD (Standard Error) of 10 birds for each diet. NS: Not significant.
*** P < 0.001. Feed intake, body weight gain, and feed conversion ratio have been calculated throughout the experimental period.

reduced when chia was incorporated at 10% and 20%. At the same time, the feed conversion ratio was higher for those both incorporation levels of chia. These effects on the feed intake and the feed conversion ratio, after the incorporation of chia seeds in feed, have not been observed by Azcona et al. (Azcona et al., 2008). However, in our experiment, while the feed conversion ratio was higher than that of the control group when chia was incorporated at 5% and 10% in the feed, the absolute values fall within the typical range from both economic and productivity perspective for the chicken meat industry. In fact, the absolute values of feed conversion ratio were even lower than those reported in similar trials by Ayerza et al. (Ayerza et al., 2002) and Azcona et al. (Azcona et al., 2008), where chia was incorporated into the feed at 10% and 15%, respectively.

3.2. Color, pH and drip loss

Regarding the meat quality parameters such as color, pH and drip loss, it could be observed that there is not a diet effect for L* (Table 4). The values observed in the three muscles ranged from 50.7 to 52.2 for GN, 47.8 to 48.5 for ITL and 52.0 to 53.9 for PM. At the same time, L* was lower in ITL compared to GN and the PM (Table 4). There is limited report that compares L* between the three muscles evaluated in our work. However, when L* for GN was compared to PM within the same experiment, this last generally showed a higher value (Cruz et al., 2018; Weng et al., 2022; Cai et al., 2023). Overall, when considering L* levels observed in the present study, which ranged from 47.8 to 53.9, they align with the ranges reported in various investigations using commercial lines of chickens (Weng et al., 2022; Cai et al., 2023; Petracci et al., 2004; Bianchi et al., 2007; Petracci et al., 2013).

In the case of a*, there is a clear declining of the redness for all treatments and particularly for T2 and T3 in comparison to C and T1. The inclusion of chia in feed seems to have a lowering effect on the redness of chicken meat, particularly in PM, less rich in myoglobin, in comparison to GN and ITL muscles. That effect of chia on a* could be explained by the presence of polyphenol compounds in chia. The polyphenols, depending of the dose, could act having a capacity to accelerate the oxidation of myoglobin and hemoglobin causing their decline in chicken meat (Wu et al., 2022). For b*, the effect of chia seems to have a similar tendency to reduce the values of that parameter (Table 4). Color is a critical factor influencing consumers' decisions when purchasing chicken meat, with preferences often shaped by cultural norms and varying according to the country and market. Hence, the reduction in a* and b* values resulting from the inclusion of chia in the feed could be seen as an argument against its use in enriching meat with valuable fatty acids, through dietary means. Nevertheless, the feed can be adjusted by incorporating natural and approved pigments to achieve a balanced meat color (Barbut & Leishman, 2022).

Table 4
Color parameters L*, a* and b* of muscles *Gastrocnemius*. (GN) *Ileotibialis lateralis* (ITL) and *Pectoralis major* (PM) in chickens fed diet with Chia seeds included at 0% (C). 2.5% (T1). 5% (T2) and 10% (T3).

Muscles		Diets				Significance
		C	T1	T2	T3	
GN	L*	52.2 ± 0.52	52.1 ± 0.66	50.7 ± 0.41	51.2 ± 0.42	NS
	a*	0.50 ± 0.13	0.50 ± 0.30	−0.19 ± 0.13	−0.03 ± 0.17	*
	b*	6.36 ± 0.45	4.97 ± 0.60	6.02 ± 0.51	6.05 ± 0.43	*
ITL	L*	48.0 ± 0.56	48.1 ± 0.55	48.5 ± 0.46	47.8 ± 0.50	NS
	a*	1.34 ± 0.25	1.43 ± 0.27	0.97 ± 0.16	0.55 ± 0.21	*
	b*	6.92 ± 0.41	6.81 ± 0.37	7.03 ± 0.39	5.64 ± 0.37	*
PM	L*	53.9 ± 0.40	52.8 ± 0.57	52.3 ± 0.36	52.0 ± 0.48	NS
	a*	−0.16 + 0.16	−0.46 ± 0.22	−0.88 ± 0.16	−0.48 ± 0.26	*
	b*	7.84 ± 0.31	6.63 ± 0.35	6.10 ± 0.29	5.58 ± 0.26	*
Main effects		Diet		Muscle		
	L*	NS		***, ITL < GN, PM		
	a*	***, T2, T3 < C, T1		**, PM < GN, ITL		
	b*	***, T1, T2, T3 < C		**, GN < ITL, PM		

Data are means ±SD of 24 birds each diet.
* = $P < 0.05$.
** = P <0.01.
*** = P<0.001. NS= Not significant.

For ultimate pH (pHu), the results showed a muscle effect but not a diet effect (Table 5). Indeed, the PM muscle showed a lower pHu value in comparison to GN and ITL, (Bianchi et al., 2007; Glamoclija et al., 2015). As observed in the current experiment, the PM generally exhibits a lower pHu in comparison to the GN (Cruz et al., 2018; Cai et al., 2023). However, the inclusion of chia seeds in the feed does not appear to have affected the pHu of the meat (Table 5).

Regarding the drip loss, the results did not show any diet or muscle effect (Table 6).

As noted for pHu, the inclusion of chia seeds to supplement the feed does not appear to have affected the drip loss of the three muscles. To our knowledge there are not scientific reports that evaluated the color, pHu and drip loss in chicken's meat when chia seed was included in the feed.

Table 5
Levels of pHu of muscles *Gastrocnemius*, (GN) *Ileotibialis lateralis* (ITL*)* and *Pectoralis major* (PM) in chickens fed diet with chia seeds included at 0% (C), 2.5% (T1), 5% (T2) and 10% (T3).

Muscles	Diets				Significance
	C	T1	T2	T3	
GN	6.43 ± 0.04	6.43 ± 0.04	6.43 ± 0.04	6.41 ± 0.04	NS
ITL	6.40 ± 0.05	6.41 ± 0,04	6.34 ± 0.03	6.36 ± 0.04	NS
PM	5.98 ± 0.03	5.97 ± 0.04	6.00 ± 0.03	5.98 ± 0.01	NS
Main effects:	Diet: NS		Muscle:***, PM < GN, ITL		

The data represents the mean ± SD of 24 birds for each diet.
*** = P<0.001. NS= Not significant.

Table 6
Drip loss (%) of muscles *Gastrocnemius*, (GN) *Iliotibialis lateralis* (ITL) and *Pectoralis major* (PM) in chickens fed diets with Chia seeds included at 0% (C), 2.5% (T1), 5% (T2) and 10% (T3).

Muscles	Diets				Significance
	C	T1	T2	T3	
GN	4,60 ± 0.71	5.29 ± 0.99	4.68 ± 0.60	4.39 ± 0.73	NS
ITL	5.32 ± 0.90	5.37 ± 1.37	4.86 ± 0.57	4.47 ± 0.77	NS
PM	6.73 ± 0.80	8.65 ± 0.91	6.97 ± 0.68	7.06 ± 0.72	NS
Main effects	Diet: NS		Muscle: NS		

The data represents the mean ± SD of 10 birds for each diet. NS= Not significant.

3.3. Lipids content and fatty acid profile

The inclusion of chia seeds in the diet of animals does not affect the lipid content of meat, but the GN and the PM muscles presented lower lipid content in comparison to the ITL muscle (Table 7). However, it is important to note that the overall lipid content in all three muscles falls within a range that has been previously reported by other researchers. (del Puerto et al., 2017; Dal Bosco et al., 2012; Mourot & Hermier, 2001; Castromán et al., 2013; Gallinger et al., 2016; Giampietro-Ganeco et al., 2020).

Regarding the fatty acids composition of meat, the sum of SAT (∑SAT) presents a lower value in animals fed diets C and T1, compared to the diets T2 and T3. The SAT values are comparable to those documented by Mendonça et al. (Mendonça et al., 2020), using chia seeds included in the feed at 16.4%, but higher to other earlier reports. First by Ayerza et al. (Ayerza et al., 2002), using feed supplemented with chia seeds at 10% and 20%, and secondly by Azcona et al. (Azcona et al., 2008) with chia seeds included at 15% in the feed. The SAT in our study were represented mainly by C14:0, C16:0, C18:0 and C22:0. Only the levels of C16:0 and C22:0 showed a diet effect (Table 7). In the case of C16:0, C and T1 presented a lower level of this fatty acid in comparison to T2 and T3. The levels of C16:0 observed in our work were of the same order as reported by Mendonça et al. (Mendonça et al., 2020) and close as or slightly higher than those reported by Ayerza et al. (Ayerza et al., 2002). However, Azcona et al. (Azcona et al., 2008) reported lower values. For C22:0, C and T1 showed a lower content than T3 but not to T2. For other part, only C22:0 showed a muscle effect having ITL with a lower content in that fatty acid compared to PM, but not to GN (Table 7). For comparison, only Mendonça et al. (Mendonça et al., 2020) reported the level of C22:0 in meat. The same was of 0.096%, that is, approximatively one third of the value observed in our work (Table 7).

In the case of MUFA, the animals fed diets supplemented with chia at 5% and 10% showed a lower level of MUFA in their meat. It was an inverse result than the observed for SAT. That is, more% of chia in the feed results in a lower presence of MUFA in meat. For comparison, in the work of Ayerza et al. (Ayerza et al., 2002) the level of MUFA in animals supplemented with chia seeds (10% and 20%) was of the same order than those observed in our study (Table 7). To the contrary, in the reports of Azcona et al. (Azcona et al., 2008) and Mendonça et al. (Mendonça et al., 2020), the levels of MUFA in meat of chickens supplemented with chia presented a lower level of MUFA in comparison of our own results (Table 7). Note that Azcona et al. (Azcona et al., 2008) and Mendonça et al. (Mendonça et al., 2020) supplemented their animals with chia seeds at 15% and 16.4%, respectively. For other part, there was a muscle effect where GN showed a lower content in MUFA in comparison to ITL (Table 7). In our study, MUFA were principally represented by C14:1, C16:1 and C18:1. However, only the last two fatty acids showed a relevant amount in meat regardless to the diets and the muscles. In the case of C16:1, C and T1 showed a lower level in meat than T3. For other part, PM contained less C16:1 than GN and ITL. For C18:1, meat of animals fed T3 contained less of that fatty acids than C, T1 and T2. Also, ITL and PM showed less C18:1 than GN (Table 7). For comparison, Ayerza et al. (Ayerza et al., 2002) found that after feeding chickens with chia seeds, the C16:1 level detected in meat were ranged 6.91–7.41% and 7.27–7.44%, when the level of supplementation was 10% and 20%, respectively. Those levels were surprisingly high when compared to the results of other reports after supplementation with chia seeds. It is important to highlight that in the same study, the control group also displayed elevated levels of C16:1, ranging from 8.92% to 9.48%. Therefore, highest level of this fatty acid in meat of animals supplemented with chia seeds probably cannot be linked with a specific effect of that kind of seeds. The levels of C18:1 in meat in the same work were ranged between 34.13–34.96% and 33.07–34.62%, for animals supplemented with 10% and 20%, respectively. In the investigation by Azcona et al. (Azcona et al., 2008), the chickens were supplemented with 15% of chia seeds. In that experiment, the level of C16:1 and C18:1 was of 2.20% and 32.2%, respectively. These values were within a similar range, or slightly lower, when compared to our own findings. In a more recent investigation by Mendonça et al. (Mendonça et al., 2020), the animals were fed a diet supplemented with 16.4% of chia seeds. In that experiment, the amount of C16:1 and C18:1 in meat was of 4.16% and 31.9%, respectively. These values were within a similar range, or slightly higher when compared to our results (Table 7).

In the case of PUFA detected in meat, the groups supplemented with chia seeds (T1, T2, and T3) exhibit higher levels of PUFA compared to the control group (C). At the same time T3 showed the higher levels of PUFA in meat than T1 and T2, while T1 and T2 present similar level. For other part, there is not any muscle effect for PUFA (Table 7).

The two essential fatty acids, C18:2n6 and C18:3n3 showed a higher level in T1, T2 and T3 in comparison to C. However, that increasing was more consistent quantitatively for C18:3n3 than for C18:2n6. It seems that the transfer of C18:3n3, from diet to muscles, has been accomplished. Indeed, in comparison to C, the groups T1, T2 and T3 presented a consistent increase in the incorporation of C18:3n3 into the muscles. That increasing of C18:3n3 deposition in muscles increased, independently to the muscle, that is approximatively of 50% between C and T1, 68% between T1 and T2, and 75% between T2 and T3. That increasing of the deposition of C18:3n3 in tissues has been also reported by Ayerza et al. (Ayerza et al., 2002) and Azcona et al. (Azcona et al., 2008) using chia seed included in the diet of chickens. Furthermore, that increasing showed a high magnitude compared to the control, that is, between 4 and 9 times the values detected in the control group, depending of the level of chia seeds incorporated in the diet. It must be important to note that the level of incorporation of chia seeds in the diet was of 10% and 20% in the work of Ayerza et al. (Ayerza et al., 2002) and 15% in the work of Azcona et al. (Azcona et al., 2008). In our work, the chia seeds were incorporated at 2.5%, 5% and 10%, and the deposition of C18:3n3 was 2 to 4 times higher compared to the levels observed in the control group. This is true for the three studied muscles. In the experiment by Mendonça et al. (Mendonça et al., 2020), the control group was missed, thus the magnitude of deposition of C18:3n3 in the muscles cannot be determined in comparison to a control group, without chia seeds included in the feed.

For other part, in our experiment, the increasing deposition of C18:3n3 into the muscles was accompanied by a similar increasing of the level of its products such as EPA, DPA, and DHA (Table 7). Indeed, the level of EPA increased by 1.5 to 4 times when the incorporated level of chia seed increased from 0 to 10% in comparison to the control. A similar pattern of increasing was observed for DPA and DHA. For comparison, Ayerza et al. (Ayerza et al., 2002) did not report EPA, DPA nor DHA in the meat obtained in their experiment. Azcona et al. (Azcona et al., 2008) reported a level of EPA, DPA and DHA of 0.60–0.92%, 1.23–1.51% and 0.72–1.02%, respectively. Those levels represent 4–8 times, 2 times and below one time for EPA, DPA and DHA, respectively,

Table 7
Fatty acids composition of muscles *Gastrocnemius* (GN), *Iliotibialis lateralis* (ITL) and *Pectoralis major* (PM) of chickens fed diets with Chia seeds included at 0% (C), 2.5% (T1), 5% (T2) and 10% (T3).

	Muscles												Significance	
	GN				ITL				PM				Maineffects	
	C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3	Diet	Muscle
Total lipids (g/100 g raw meat)														
	2.37 ±0.41	2.62 ±0.39	2.47 ±0.25	2.35 ±0.23	3.60 ±0.57	3.57 ±0.24	3.69 ±0.50	2.39 ±0.21	2.15 ±0.19	2.20 ±0.20	2.32 ±0.14	1.96 ±0..18	NS	**, PM, GN<ITL
Fatty acids (g/100 g fatty acids)														
Saturated fattyacids (SAT)														
∑SAT	31.7 ± 0.94	29.1 ± 0.77	31.4 ± 0.70	33.3 ± 0.60	29.2 ± 0.82	27.8 ± 0.55	30.8 ± 0.54	33.2 ± 0.66	28.9 ± 0.58	30.2 ± 0.93	30.8 ± 0.57	34.4 ± 0.79	***,C,T1, T2<T3, T1 < T2	NS
C14:0	0.74 ±0.89	0.54 ±0.15	0.57 ±0.18	0.61 ±0.13	0.56 ±0.19	0.54 ±0.16	0.56 ±0.12	0.57 ±0.14	0.52 ±0.12	0.55 ±0.19	0.54 ±0.13	0.62 ±0.12	NS	NS
C15:0i	0.03 ±0.03	0.02 ±0.01	0.01 ±0.01	0.01 ±0.01	0.02 ±0.01	0.02 ±0.01	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.02 ±0.01	0.01 ± 0.01	0.01 ±0.003	**, C, T1<T3,T2	NS
C15:0ai	0.03 ±0.05	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.004	0.01 ± 0.004	0.01 ± 0.004	0.01 ±0.004	0.01 ±0.004	0.01 ±0.01	0.01 ±0.004	NS	NS
C15:0	0.14 ±0.13	0.12 ± 0.02	0.11 ± 0.02	0.13 ± 0.02	0.12 ± 0.03	0.12 ± 0.03	0.12 ± 0.02	0.13 ± 0.02	0.11 ± 0.02	0.11 ± 0.03	0.11 ± 0.03	0.13 ± 0.03	NS	NS
C16:0	20.3 ± 1.88	19.3 ± 2.35	21.2 ± 2.53	22.5 ± 1.64	19.4 ± 2.15	18.9 ± 1.97	21.1 ± 1.60	22.6 ± 1.63	19.3 ± 1.46	20.0 ± 3.15	21.1 ± 1.82	23.5 ± 1.92	**, C,T1< T2,T3 T2<T3	NS
C18:0	10.3 ± 2.16	8.80 ± 1.61	9.19 ± 1.62	9.69 ± 1.48	8.85 ± 3.13	7.97 ± 2.41	8.70 ± 2.38	9.53 ± 1.57	8.72 ± 1.41	9.16 ± 1.86	8.68 ± 0.96	9.80 ± 1.58	NS	NS
C22:0	0.26 ±0.09	0.25 ± 0.08	0.29 ± 0.10	0.30 ± 0.09	0.18 ± 0.073	0.21 ± 0.09	0.28 ± 0.14	0.34 ± 0.13	0.28 ± 0.08	0.31 ± 0.13	0.32 ± 0.09	0.37 ± 0.11	*, C,T1<T3	*, ITL< PM
Monounsaturated fatty acids (MUFA)														
∑MUFA	43.0 ± 1.24	43.2 ± 1.01	40.0 ± 0.85	34.9 ± 1.11	47.4 ± 1.02	46.3 ± 0.79	41.6 ± 1.28	35.2 ± 1.17	46.2 ± 0.91	42.5 ± 0.91	41.4 ± 0.72	34.2 ± 1.15	***,T3<C, T1,T2 T2 < C,T1	**, GN < ITL
C14:1	0.07 ±0.05	0.07 ± 0.02	0.07 ± 0.04	0.09 ± 0.03	0.08 ± 0.03	0.08 ± 0.03	0.08 ± 0.03	0.08 ± 0.03	0.05 ± 0.02	0.05 ± 0.02	0.06 ± 0.02	0.08 ± 0.03	NS	***, PM< ITL,GN
C16:1	2.67 ± 0.89	2.89 ± 0.73	3.25 ± 0.83	3.69 ± 0.90	3.10 ± 0.65	3.20 ± 0.64	3.44 ± 0.95	3.50 ± 0.95	2.18 ± 0.42	2.15 ± 0.57	2.60 ± 0.64	2.99 ±1.19	**, C, T1<T3	**, PM<ITL, GN
C18:1	40.2 ± 3.82	40.3 ± 3.22	36.7 ± 2.61	31.1 ± 3.41	44.2 ± 3.55	43.0 ± 2.32	38.1 ± 3.91	31.7 ± 3.39	44.0 ± 3.23	40.3 ± 3.19	38.7 ± 2.28	31.2 ± 3.60	*, T3<C,T1, T2 T2< T1,C	*,ITL, PM<GN
Polyunsaturated fatty acids (PUFA)														
∑PUFA	23.8 ± 0.89	26.3 ± 0.68	27.3 ± 0.66	30.5 ± 0.79	22.3 ± 0.60	24.8 ± 0.53	26.5 ± 0.87	30.3 ± 0.73	23.3 ± 0.47	25.9 ± 0.82	26.5 ± 0.61	29.9 ± 0.62	***,C < T1, T2,T3. T1, T2 < T3	NS
C18:2n6	16.2 ± 1.82	18.2 ± 1.28	18.5 ± 1.13	19.8 ± 1.62	17.2 ± 1.12	18.6 ± 1.30	18.7 ± 1.37	19.8 ± 1.43	16.5 ± 0.90	17.4 ± 1.80	18.0 ± 1.31	18.9 ± 1.52	*, C < T1, T2,T3 T1,T2<T3	*, PM<ITL, GN
C18:3n3	0.96 ±0.21	1.88 ±0.37	2.64 ±0.87	3.87 ± 1.20	1.04 ±0.13	2.07 ±0.31	2.85 ±0.93	3.78 ±1.06	0.98 ±0.10	1.88 ±0.53	2.88 ±0.90	3.80 ± 0.82	*, C < T1< T2 < T3	NS
C20:2	0.05 ±0.01	0.05 ± 0.03	0.06 ± 0.03	0.05 ± 0.03	0.05 ± 0.01	0.06 ± 0.02	0.06 ± 0.04	0.06 ± 0.03	0.05 ± 0.01	0.05 ± 0.02	0.05 ± 0.02	0.06 ±0.03	NS	NS
C20:3n6	0.20 ±0.09	0.14 ± 0.06	0.15 ± 0.05	0.14 ± 0.07	0.15 ± 0.10	0.10 ± 0.06	0.12 ± 0.05	0.14 ± 0.08	0.14 ± 0.09	0.10 ± 0.05	0.10 ± 0.03	0.11 ± 0.04	*, T1,T2<C	*, PM< GN
C20:3n3	0.54 ±0.19	0.48 ± 0.14	0.54 ± 0.17	0.60 ± 0.20	0.40 ± 0.23	0.36 ± 0.13	0.47 ± 0.22	0.66 ± 0.27	0.58 ± 0.25	0.57 ± 0.27	0.58 ± 0.16	0.75 ± 0.21	*, C,T1, T2<T3	*, ITL< PM
C20:4n6	3.98 ±1.74	3.55 ± 1.40	3.25 ± 1.22	3.38 ± 1.07	2.36 ± 1.27	2.25 ± 0.94	2.52 ± 1.29	3.27 ± 1.22	3.45 ± 1.42	3.70 ± 1.95	2.85 ± 0.78	3.45 ± 1.06	NS	*, ITL< PM
EPA	0.13 ± 0.05	0.19 ±0.06	0.26 ±0.08	0.38 ± 0.11	0.11 ±0.06	0.14 ±0.05	0.22 ±0.07	0.38± 0.12	0.12 ± 0.07	0.20 ± 0.07	0.27 ± 0.09	0.46 ± 0.15	*, C < T1< T2 < T3	*, ITL< PM
C22:4n6	1.22 ±0.54	0.90 ± 0.35	0.79 ± 0.37	0.74 ± 0.25	0.69 ± 0.39	0.56 ± 0.26	0.60 ± 0.35	0.71 ± 0.29	1.08 ± 0.47	0.98 ± 0.59	0.67 ± 0.22	0.74 ± 0.22	*, T2,T3<C	*, ITL<PM, GN
DPA	0.30 ±0.13	0.53 ± 0.20	0.67 ±0.22	0.94 ±0.37	0.17 ±0.10	0.36 ± 0.17	0.55 ± 0.29	0.92 ±0.32	0.26 ±0.10	0.54 ± 0.28	0.62 ± 0.23	0.96 ±0.33	*, C < T1< T2< T3	***, ITL< GN
DHA	0.23 ± 0.10	0.45 ± 0.23	0.49 ± 0.19	0.62 ± 0.22	0.16 ± 0.10	0.28 ± 0.14	0.43 ± 0.26	0.63 ± 0.26	0.22 ± 0.08	0.45 ±0.24	0.49 ±0.18	0.64 ±0.26	*, C < T1< T2< T3	NS
Undefined	1.55 ±0.64	1.38 ± 0.53	1.31 ± 0.30	1.25 ± 0.30	1.13 ± 0.40	1.18 ± 0.35	1.21 ± 0.43	1.30 ± 0.31	1.49 ± 0.36	1.46 ± 0.36	1.33 ± 0.42	1.45 ± 0.91	–	–

Data are means ± SD of 14 birds by diet.
* $= P < 0.05$,.
** $= P < 0.01$,.
*** $= P < 0.001$. NS: No significant.

in comparison to the control. In our experiment, the DHA reached between 2 and 4 times the levels observed in the control (Table 7). Interestingly, in our experiment the levels of DHA showed very close levels in the three studied muscles. The same observation has been made by Azcona et al. (Azcona et al., 2008) working on the muscles *Pectoralis major* and *Gastrocnemius*. In the work of Mendonça et al. (Mendonça et al., 2020), the reported level of EPA was of 0.62% and the DHA of 0.45%. The level of DPA has not been reported. As previously mentioned before, the absence of a control group makes difficult to determine the extent of the increase in these fatty acids, after the addition of chia seeds to the feed.

Overall, the DHA levels detected in the muscles examined in our study could be regarded as relatively high when compared to the findings of other cited reports, especially when considering the extent of chia seed incorporation in the feed. Nonetheless, it is worth considering why the DHA levels are not higher as it could be expected, in the three muscles studied here, particularly when considering the deposition levels of C18:3n3. This observation holds true for the other studies referenced in this text that utilized chia seeds as a supplement in chicken feed. Furthermore, the use of other source of n-3, such as flaxseeds containing high level of C18:3n3, reach the same result, that is the level of DHA is not as high as it could be expected (Azcona et al., 2008; Elkin & Harvatine, 2023). However, to the contrary to the observation by Elkin and Harvatine (Elkin & Harvatine, 2023) that the conversion of DHA is inversely correlated to the amount of C18: 3n3 present in feed, in the present experiment, the level of DHA has increased proportionally to the level of C18:3n3 included in the feed through the chia seeds (Table 7). Those different results could be explained by the fact that two different kinds of seeds, chia seeds versus flaxseeds, could have different molecular structures and nutrient bioaccessibility that probably affect the intestinal absorption of C18:3n3.

Various hypotheses have been proposed to explain the low presence of those valuable n-3 fatty acids, such as EPA, DPA and particularly DHA, in muscles of chickens fed high level of C18:3n3. Ayerza et al. (Ayerza et al., 2002), using chia seeds, explained the absence of EPA and DHA in the meat of chickens used in their experiments, by the low activities of the desaturase and elongase in chicken's tissues, mainly the liver. Hence, this enzymatic activity appears to be insufficient to guarantee an adequate conversion of C18:3n3 into EPA and DHA prior to its inclusion in muscle tissues. Nevertheless, this explanation is not substantiated by the findings of Gregory et al. (Gregory et al., 2013), which confirmed that chickens possess highly active fatty acid elongase enzymes, even surpassing the activity observed in rat tissues. Another explanation has been proposed by Elkin and Harvatine (Elkin & Harvatine, 2023) that relates to a possible limitation of the ability of liver to incorporate dietary DHA on VLDL (very low-density lipoproteins) before its secretion in the blood stream to reach tissues to be incorporated in the cell membranes. This explanation has the merit to be supported by two observations, 1) the liver is the main site of lipid metabolism in chickens (Saadoun & Leclercq, 1983), and 2) the capacity of hepatic lipid metabolism in liver of chickens is limited. Indeed, if the *de novo* lipogenesis and the intake of preformed lipids from the feed, including their desaturation and elongation processes, surpass the liver's capacity to produce VLDL, the transportation of lipids from the liver to the tissues is delayed and diminished. In some case, this situation causes hepatic steatosis that is the accumulation of lipids in the liver (Hermier, 1997). Moreover, research indicates that chickens fed with a diet consisting of 9% total lipids have inhibition of their hepatic lipid metabolism; while a diet containing 2% total lipids stimulates it (Saadoun & Leclercq, 1987). To the best of our knowledge, most research that focused to increase the n-3 fatty acid levels in the chicken's muscles, typically formulate diets with a lipid content ranging from 5% to 11% of the total composition. In our own experiment, the level of total lipids was established at 6%. Of course, it is very difficult to formulate a regime for chickens with 10% or more of chia seeds, or flaxseeds, and at the same time restricting the level of total lipids in the diet. Thus, it could be interesting to investigate, in future research, the influence of the percentage of total lipids in the feed on the hepatic lipid metabolism, having the objective to increase the level of DHA in the chicken's muscles.

3.4. Enzyme activity indices

Regarding the estimation of the activities of enzymes of lipid metabolism in the three muscles studied here, it could be observed that at higher levels of chia seeds of 10% in the feed, the activities of enzymes delta 9-C18 desaturase were lower than in C, T1 and T2. This effect has not been observed for delta 9-C16 desaturase. The sum of both delta 9 (C16+C18) showed a similar results than for delta 9-C18 desaturase (Table 8). The comparison between muscles showed that the activities in PM were lower than in GN and ITL for delta 9-C16 desaturase. For delta 9-C18 desaturase the ITL has lower activity than GN. Mendonça et al. (Mendonça et al., 2020) using chia seeds included at 16.4 in feed, found as well differences in the activities of delta 9-C16 and delta 9-C18 desaturases between the breast and the thigh. As noted before, the absence of a control group restricts the possibility to make further comparisons in that investigation.

In the case of delta 5 desaturase activity, estimated by the ratio C20:4n6/C20:3n6, C showed a lower activity compared to T1 and T3. The comparison between muscles showed that GN and ITL have higher activity compared to the PM (Table 8).

The delta 6 desaturase activity exhibited a relatively limited diet effect in almost all the calculated ratio considered, except for the ratio C22:6n3/C20:5n3, where T3 present a lower activity compared to T1, and for the ratio C20:5n3/C18:3n3 where T1 present a lower activity than C. Overall, it could be observed that the activity of delta 6 desaturase remains relatively stable regardless of the quantity of chia seeds added to the diet of the chickens (Table 8). However, there is a muscle effect for almost all the calculated ratio except for the delta 6 calculated with the ratio C22:6n3/C20:5n3. It seems that there is a different activity for delta 5 and delta 6 desaturase depending of the considered muscle (Table 8). The absence of published reports on the study of these two enzymes, in experiments involving the addition of chia seeds to the chicken feed, makes it difficult to compare our findings with existing research.

The calculated elongase activities based on the ratio C18:0/C16:0 showed no diet or muscle effect (Table 8). Mendonça et al. (Mendonça et al., 2020) found a similar lack of differences, between breast and thigh for the same elongase, in their investigation with chia seeds added in the feed of the chickens.

In the case of thioesterase calculated through the C16:0/C14:0 ratios, it does not reveal any discernible diet or muscle-related effects (Table 8).

3.5. Nutritional and lipids health indices

Concerning the nutritional value of meat from the different muscles obtained in chickens fed chia seeds, various indices have been estimated and the results showed that the ratio PUFA/SAT presents a diet effect but not a muscle effect. Indeed, C displayed lower values compared to all chia seeds fed groups (Table 9). However, it can be noted that all the values for these indices are below 1, which is in conformity with the advised ratio regarding the health of consumers (Saadoun & Cabrera, 2008). Mendonça et al. (Mendonça et al., 2020) found a ratio near or slightly higher than 1 for breast and thigh muscles, respectively. Other indices such as the sum of n-6 and n-3 and their ratio, useful to estimate the richness of meat in those two classes of fatty acids important for the health of consumers, showed opposed results. Indeed, while the sum of n-6 fatty acids presents a lower content for C, T1 and T2 compared to T3, the content of n-3 fatty acids present an expected increase as the level of chia seeds increases in the feed. Conversely, for these both indices, there is not any muscle effect (Table 9). For the n-6/n-3 ratio, it can be observed an inverse pattern than the observed for the sum of n-3 fatty acids, that is, a lower ratio as the level of chia seeds increases in the feed.

Table 8
Enzymes activities of lipid metabolism in muscles *Gastrocnemius* (GN), *Iliotibialis lateralis* (ITL) and *Pectoralis major* (PM) of chickens fed diets supplemented with Chia seeds included at 0% (C), 2.5% (T1), 5% (T2) and 10% (T3).

	Muscles												Significance	
	GN				ITL				PM				Main effects	
	C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3	Diet	Muscles
Delta 9/C16 16:1/16:0	0.16 ±0.01	0.17 ±0.01	0.16 ±0.01	0.16 ±0.01	0.13 ±0.01	0.15 ±0.01	0.15 ±0.01	0.16 ±0.01	0.11 ±0.005	0.11 ±0.005	0.12 ±0.01	0.13 ±0.01	NS	***PM<GN, ITL
Delta9/C18 18:1/18:0	5.55 ±0.51	5.86 ±0.46	4.71 ±0.38	3.46 ±0.24	4.19 ±0.38	4.79 ±0.36	4.17 ±0.31	3.32 ±0.22	5.20 ±0.28	4.61 ±0.31	4.54 ±0.20	3.30 ±0.21	***T3<C, T1,T2	***ITL<GN
Sum delta 9 C16:0 + C18:0	5.71 ±0.51	6.03 ±0.47	4.87 ±0.39	3.61 ±0.25	4.32 ±0.39	4.94a ±036	4.33 ±0.32	3.49 ±0.23	5.31 ±0.29	4.72 ±0.31	4.66 ±0.20	3.42 ±0.22	***T3<C, T1,T2	***ITL<GN
Delta 5 C20:4n6/ C20:3n6	17.4 ± 1.07	25.1 ± 1.61	21.6 ± 1.92	26.9 ± 4.33	20.2 ± 1.58	28.0 ± 3.17	22.4 ± 1.18	28.2 ± 3.17	27.4 ± 2.64	37.3 ± 3.74	30.0 ± 3.23	38.5 ± 6.39	***C<T1, T3	***GN, ITL<PM
Delta 6 C22:6n3/ C20:5n3	1.38 ±0.17	1.97 ±0.20	1.85 ±0.15	1.64 ±0.09	1.89 ±0.21	2.39 ±0.27	1.86 ±0.13	1.66 ±0.08	1.97 ±0.18	2.25 ±0.28	2.08 ±0.43	1.40 ±0.10	**T3<T1	NS
Delta 5+Delta 6 C20:4n6/ C18:2n6	0.14 ±0.02	0.12 ±0.01	0.13 ±0.02	0.17 ±0.02	0.25 ±0.03	0.19 ±0.02	0.18 ±0.02	0.17 ±0.02	0.21 ±0.03	0.22 ±0.04	0.16 ±0.01	0.18 ±0.02	NS	***GN<ITL, PM
Delta 5+Delta 6 C20:5n3/ C18:3n3	0.11 ±0.02	0.07 ±0.01	0.08 ±0.01	0.11 ±0.02	0.14 ±0.02	0.11 ±0.01	0.11 ±0.01	0.11 ±0.01	0.13 ±0.02	0.12 ±0.02	0.10 ±0.01	0.13 ±0.02	*T1<C	*GN<ITL
Delta 5+Delta 6 C22:6n3/ C18:3n3	0.16 ±0.03	0.14 ±0.02	0.17 ±0.03	0.19 ±0.03	0.27 ±0.04	0.26 ±0.04	0.21 ±0.03	0.17 ±0.02	0.23 ±0.03	0.27 ±0.06	0.18 ±0.02	0.18 ±0.03	NS	*ITL<GN
Elongases C18:0/ C16:0	0.47 ±0.05	0.43 ±0.04	0.42 ±0.04	0.42 ±0.02	0.51 ±0.03	0.46 ±0.05	0.44 ±0.03	0.43 ±0.019	0.45 ±0.02	0.47 ±0.03	0.41 ±0.02	0.42 ±0.018	NS	NS
Thioesterase C16:0/ C14:0	36.5 ± 1.85	36.1 ± 1.46	38.4 ± 1.47	41.2 ± 2.28	37.4 ± 2.49	37.0 ± 1.64	39.1 ± 2.04	38.4 ± 1.74	38.4 ± 1.69	38.6 ± 2.10	40.5 ± 2.06	38.8 ± 1.50	NS	NS

Data are mean ± SD of 14 birds by diet.
[*] = P<0.05,.
[**] = P<0.01,.
[***] = P<0.001. NS: No significant.

In our work, the values were ranging between 3.56 and 11.2 (Table 9). No muscle effect has been detected for that ratio. Ayerza et al. (Ayerza et al., 2002) obtained also a ratio, slightly higher than 2, when the chia seeds have been added at 10% as well as at 20%, in both breast and thigh. Azcona et al. (Azcona et al., 2008) found even a lower ratio, below of 2, in their experiment with chickens feed chia seeds added at 15% in the feed. In the work of Mendonça et al. (Mendonça et al., 2020), the reported ratio n-6/n-3 fatty acids were 2.70 and 3.30 for breast and thigh, respectively. Taken together, our results and those of Ayerza et al. (Ayerza et al., 2002) and Azcona et al. (Azcona et al., 2008), it seems that the inclusion of chia seeds in chicken feed positively lowers the ratio n-6/n-3 fatty acids, in comparison to the control. The advised ratio in favor of the health of consumers has been established to be low between 2 and 6 depending of the source of information (Simopoulos, 2002; Wijendran & Hayes, 2004; Simopoulos, 2010; Liput et al., 2021). However, nowadays that ratio becomes open to question about its usefulness regarding the human health, as pointed out by Harris (Harris, 2018). Another nutritional index proposed by Dal Bosco et al. (Dal Bosco et al., 2022) take into account the level of n-3 fatty acids to highlight the importance of a dietary product, regarding its composition in n-3 fatty acids. As expected, the indices were high, thus convenient for the health of consumers, as the level of chia seeds increases in the feed. No muscle effect has been observed either (Table 9).

Regarding the indices related to cardiovascular diseases, such as AI, TI and h/H, it can be see that the AI indices ranged 0.30–0.41. The preferred values must be as low as possible. Although the values are low for the three groups, T3 present the higher values compared to C, T1 and T2. No muscle effect has been observed (Table 9). In the investigation of Mendonça et al. (Mendonça et al., 2020), the AI indices were of the same order than those reported in our work. For TI indices, as for AI, the preferred values must be as low as possible. The values ranged from 0.63 to 0.81, indicating that the inclusion of chia seeds in the chicken feed reduced the TI in T1, T2, and T3 in comparison to the control group C. No muscle effect has been reported (Table 9). In the case of h/H, the values ranged 2.68–3.70 showing that T3 present the lower values in comparison to C. T1 and T2. For these indices, the preferred values in regard to health effect have to be as high as possible. No muscle effect has been observed for h/H either (Table 9). In the study conducted by Mendonça et al. (Mendonça et al., 2020), it is noteworthy that they reported lower h/H values compared to those observed in our research. Specifically, they found h/H indices of 0.52 for the breast and 0.54 for the thigh.

3.6. Contribution of n-3 intake to consumer

There are studies that have used chia seeds added at high levels, such as 15% and 20%, in chicken feed. Nevertheless, that increase in the level of chia seeds in the feed did not result in a substantial higher content of EPA and DHA in the chicken's meat, when compared to the control group (Ayerza et al., 2002; Azcona et al., 2008). In Fig. 1, the estimation of content of n-3 fatty acids in 100 gs of chicken meat obtained in our experiment is depicted. The figure underscores the potential intake of these health-valuable n-3 fatty acids to consumers, through the intake of chicken meat, thanks to the inclusion of chia seeds in the diet of the animals. While there are indeed other vegetable sources of n-3 fatty acids that can be utilized for fortifying chicken meat with C18:3n3, EPA,

Table 9
Nutritional and lipids health indices of muscles *Gastrocnemius* (GN), *Iliotibialis lateralis* (ITL) and *Pectoralis major* (PM) of chickens fed diets supplemented with Chia seeds included at 0% (C), 2.5% (T1), 5% (T2) and 10% (T3).

	Muscles												Significance	
	GN				ITL				PM				Main effects	
	C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3	Diet	Múscles
PUFA/SAT	0.76 ± 0.03	0.91 ± 0.03	0.88 ± 0.03	0.92 ± 0.03	0.77 ± 0.03	0.90 ± 0.03	0.86 ± 0.03	0.92 ± 0.02	0.81±0.02	0.87 ±0.05	0.87 ±0.03	0.87 ±0.02	***, C < T1,T2,T3	NS
∑n-6	21.6 ± 0.84	22.8 ± 0.57	22.7 ± 0.55	24.1 ± 0.46	20.4 ± 0.50	21.5 ± 0.47	21.9 ± 0.74	23.9 ± 0.46	21.1 ± 0.38	22.2 ± 0.65	21.6 ± 0.44	23.2 ± 0.41	***, C,T1,T2<T3	NS
∑n-3	2.15 ± 0.08	3.52 ± 0.16	4.60 ± 0.27	6.42 ± 0.39	1.88 ± 0.11	3.21 ± 0.13	4.50 ± 0.34	6.36 ± 0.33	2.17 ± 0.11	3.63 ± 0.20	4.84 ± 0.31	6.60 ± 0.26	***, C < T1< T2 < T3	NS
n-6/n-3	10.1 ± 0.39	6.59 ± 0.24	5.11 ± 0.25	3.85 ± 0.15	11.2 ± 0.48	6.80 ± 0.23	5.05 ± 0.26	3.81 ± 0.11	9.94 ± 0.30	6.29 ± 0.26	4.66 ± 0.26	3.56 ± 0.10	***, T1< T2< T3 <C	**, PM < ITL
n-3 indices	17.8 ± 1.05	30.3 ± 1.20	42.5 ± 2.95	60.1 ± 2.90	20.3 ± 0.75	33.2 ± 1.50	43.4 ± 2.54	60.7 ± 3.65	20.4 ± 1.00	34.2 ± 1.86	45.7 ± 2.97	62.4 ± 2.47	***, C < T1< T2 < T3	NS
AI	0.35 ± 0.03	0.31 ± 0.01	0.35 ± 0.02	0.38 ± 0.01	0.31 ± 0.01	0.30 ± 0.01	0.34 ± 0.01	0.38 ± 0.01	0.31 ± 0.01	0.33 ± 0.02	0.34 ± 0.01	0.41 ± 0.01	***, C,T1,T2< T3 T1<T2	NS
TI	0.81 ± 0.03	0.66 ± 0.03	0.69 ± 0.03	0.67 ± 0.02	0.73 ± 0.03	0.63 ± 0.02	0.67 ± 0.01	0.67 ± 0.02	0.71±0.02	0.69 ±0.03	0.66 ±0.02	0.70 ±0.02	***, T1,T2,T3 < C.	NS
h/H	3.22ab ±0.13	3.55a ±0.14	3.15ab ±0.13	2.85b ±0.09	3.53bc ±0.12	3.70ab ±0.13	3.16c ±0.08	2.85 cd ±0.08	3.54a ±0.10	3.4 ± 0.17	3.16 ± 0.10	2.68 ± 0.08	***, T3 <C.T1.T2. T2 < C, T1	NS

Data are mean ± SD of 14 birds by diet. SAT= saturated fatty acids, PUFA= polyunsaturated fatty acids, AI= atherogenic index, TI= thrombogenic index, h/*H*= hypocholelesterolemic index.
** = P<0.01,.
*** = P<0.001, NS= No significant.

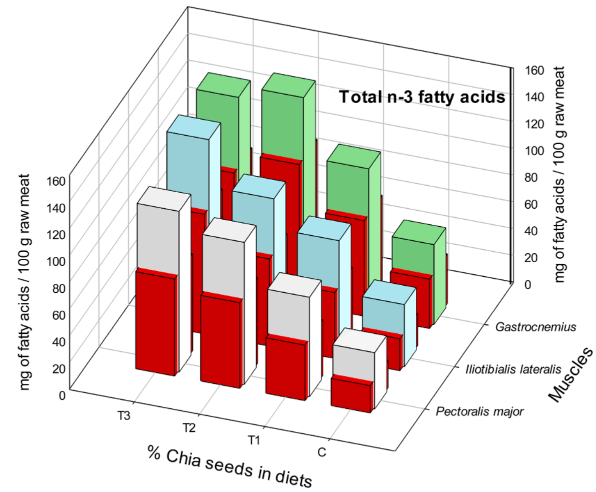

Fig. 1. Estimating the contribution of n-3 fatty acids intake from 100 gs of chicken meat fed diets supplemented with Chia seeds. at 0% (C), 2.5% (T1), 5% (T2) and 10% (T3). Total n-3 fatty acids are the sum of α-linolenic acid, C20:3n3, EPA, DPA and DHA. In red, the part of the α-linolenic acid in that contribution. The error bars have been omitted to enhance clarity.

and DHA, namely flaxseeds, stearidonic acid-enriched oil, and others (Elkin & Harvatine, 2023; El-Zenary et al., 2023; Rizzo et al., 2023), it's important to note that chia seeds remain an economical and readily available source of n-3 fatty acids in South America. Indeed, chia seeds can serve as a valuable local choice for fortifying chicken meat with these important fatty acids, such as DHA, contributing to the overall health benefits for consumers.

4. Conclusion

The use of chia seeds in the chicken feed is a good and relatively cheap way to increase the n-3 fatty acids, and particularly DHA, in their meat. Chia seeds can be easily managed and integrated into chicken feed, and this can be done by both small local farmers and larger chicken meat industry. Furthermore, no noticeable negative effects have been detected in the productive or meat quality parameters of the animals fed regime with chia seeds even at 10% in feed. In the present experiment, the levels of C18:3n3, EPA, DPA and especially DHA, showed a significant increase in their levels in meat in comparison to the control without chia.

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Ethical statement

The experiment has been assessed and approved in accordance with good animal welfare practices by the Honorary Committee for Animal Experimentation (CHEA, protocol N° 702-Uruguay).

CRediT authorship contribution statement

Ayrton da Silva: Methodology, Writing – original draft. **María Cristina Cabrera:** Conceptualization, Data curation, Funding acquisition, Investigation, Project administration, Supervision, Writing – review & editing. **Roberto Olivero:** Supervision. **Marta del Puerto:** Methodology, Supervision. **Alejandra Terevinto:** Supervision. **Ali Saadoun:** Conceptualization, Data curation, Formal analysis, Investigation, Project administration, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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