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Impact of chia meal and hydroxytyrosol on the nutritional quality of broiler chicken meat

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Aditional keywords

Broiler.
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SUMMARY

In poultry production, the composition of the diet directly influences the meat quality of birds. The present research deals with the effect of the addition in the diet of two by-products, chia meal and/or hydroxytyrosol, on lipid content, fatty acids profile, lipids indices and enzymes activity and lipid oxidation in the breast. Ninety-six broiler chicks were randomly divided into 16 groups of 6 animals, which were distributed in 4 blocks. In each block experimental treatments were randomly allocated 1) C: control; 2) CM: 10% chia meal; 3) CM+HT: 10% chia meal + hydroxytyrosol and 4) HT: hydroxytyrosol. The experimental period lasted from day 22 to day 46, at which the slaughter was carried out and breast samples were obtained. The dietary chia meal increased total PUFA, total n-3 PUFA, α- linolenic, n-3 PUFA-LC (EPA, DPA, DHA), PUFA/SFA, UI and reduced the n-6/n-3, LA/ALA, thrombogenicity index and TFA, although worsened lipid peroxidation. However, the addition of hydroxytyrosol and its combination with chia meal increased α-linolenic levels and decreases the lipid oxidation value of the breast, exhibiting its action as an antioxidant. Therefore, the results outline the potential effect of chia meal combined with hydroxytyrosol, in the modification of the composition of fatty acids and their oxidative stability in chicken breast, thereby resulting in meat with higher nutritional quality for human health.

Impacto de la harina de chía e hidroxitirosol sobre la calidad nutricional de la carne de pollo

Palabras clave

Pollos parrilleros.
Antioxidante.
Omega n-3.
Perfiles ácidos grasos.
Índices de salud.

RESUMEN

En la producción avícola la composición de la dieta presenta una influencia directa sobre la calidad de la carne de las aves. El objetivo del presente trabajo fue evaluar el efecto de la adición de dos subproductos, harina de chía y/o hidroxitirosol en la dieta, sobre el contenido de lípidos, el perfil deácidos grasos, los índices lipídicos y de actividad enzimática y la oxidación de lípidos en la pechuga. Noventa y seis pollos parrilleros fueron divididos en 16 grupos de 6 animales, los cuales se distribuyeron en 4 bloques. En cada bloque se asignaron uno de los 4 tratamientos experimentales: 1) C: control; 2) HC: 10% harina de chía; 3) HC+HT: 10% harina de chía + hidroxitirosol y 4) H T: hidroxitirosol. El período experimental se extendió desde el día 22 hasta los 46 días, momento en el que se realizó la faena y se obtuvieron muestras de pechuga. El agregado de harina de chía aumentó los niveles de AGPI, AGPI n-3, α-linolénico, AGPI-CL (EPA, DPA, DHA), la relación AGPI/AGS, el índice UI y redujo n-6/n-3, AL/ALA, el índice de trombogenicidad y AGT, aunque empeoró la peroxidación lipídica. Sin embargo, la adición de hidroxitirosol y su combinación con harina de chía aumentó los niveles de α-linolénico y disminuyó el valor de oxidación de los lípidos en la pechuga, exhibiendo su acción como antioxidante. Por lo tanto, los resultados delinean el efecto potencial del uso combinado de harina de chía con hidroxitirosol, en la modificación de la composición de los ácidos grasos y la estabilidad oxidativa de cortes de pechuga, dando como resultado la obtención de carnes con mayor calidad nutricional para la salud de la población.

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INTRODUCTION

Currently, there is a growing interest in investigating possible alternatives in the diet of broilers in the early stages of breeding that result in obtaining products of higher nutritional value and hygienically safe quality for public health. The ultimate goal is to obtain a functional food with direct benefits for the consumer.

In this sense, it is highlighted the use of different sources that provide high levels of omega n-3 polyunsaturated fatty acids (n-3 PUFA) in order to modify the lipid profile of the meat (Ayerza *et al*. 2002, Gallinger 2015, Anjum *et al*. 2013). Enriched poultry meat with n-3 PUFA allows obtaining a nutraceutical or functional product with direct benefits on consumers health (Connor 2000). De Lorgeril & Salen (2004), Ayerza *et al*. (2002), Volpato & Hull (2018), Manson *et al*. (2019) showed that an increased intake of n-3 PUFA through the human diet would contribute to the prevention of the occurrence of coronary heart disease, depression, cancer and other pathologies.

A source of interest in Argentine with a high content of omega n-3 PUFA is chia (*Salvia hispánica*, L.), as whole seed or meal. It is a natural source of plant origin with a high content of fatty acids α-linolenic and linoleic. Chia flour is a low-cost agribusiness by-product obtained after extracting oil from seeds.

In recent years the addition of chia in the diet of broiler has become substantial to enhance n-3 PUFA content in meat (Ayerza *et al*. 2002, Azcona et al. 2008). Ayerza & Coates (2000, 2001), observed high n-3 PUFA content, reduced saturated fatty acids (SFA) and better n -6/n-3 ratio in eggs of laying hen feed chia seed diet. It is also known that by adding these seeds or meals to the diet, it is possible to modify the profile of fatty acid in broiler meat (Mohd Ali et al. 2012). Ayerza *et al*. (2002) in white and dark meat in broilers fed with chia seed at 10 % and 20 %, observed higher n-3 PUFA and reduced SFA. It is also accounted that rising the degree of unsaturation strongly increases susceptibility to lipid peroxidation affecting permeability, membrane fluidity, protein membrane and metabolic function (Yu *et al*. 1998).

Oxidation is a process that leads to the formation of free radicals traduced in adverse health effects, due to their potential for altering lipids and proteins generating oxidative stress (Rodríguez Perón *et al*. 2001). Oxidative processes affect meat quality deterioration, being the oxidation of lipids the main factor of quality loss (Campo *et al*. 2006). Antioxidants are substances that protect the cell from the action of oxidants or free radicals, counteracting their harmful effect.

The addition of a source of n-3 PUFA and an antioxidant combined in the diet could exert a complementary activity. The antioxidant would preserve the PUFA provided by the n -3 from oxidation processes allowing them to perform the function they are intended, eg. as members of the cell membrane. This effect would be enhanced when the diet promotes n -3 enriched meat, increasing the content of these PUFA in the human's diet (Mendonça *et al*. 2020).

Antioxidants have been classified according to their solubility into fat-soluble (lipophilic) or water- soluble (hydrophilic). The first group can be mentioned vitamin E, tocopherols, and carotenoids while in the second, vitamin C, glutathione, selenium, and polyphenols. A novel antioxidant polyphenol obtained from the olive oil production process is hydroxytyrosol, a non -flavonoid subtype - mono- phenolic acid. Its antioxidant activity is one of the highest among polyphenols exerting a chelating action of metal ions and a free radical sequestering effect (Visioli & Galli 199.; Tripoli *et al*. 2005, Rietjens *et al*. 2007). There is currently little information regarding the use of hydroxytyrosol obtained from the olive industry as an antioxidant in broilers diet. Based on the current information and the significance of using chia as a source of omega n-3, the purpose of the present study has been undertaken to investigate the effect of the addition of the novel antioxidant (hydroxytyrosol) through the chia/antioxidant combination, as an enhancer of chia (a by-product of the oil industry) functions.

MATERIAL AND METHODS

The experience was carried out in the Experimental Broiler Unit of the Department of Agronomy of the Universidad Nacional del Sur (UNS), facility located in the city of Bahía Blanca, (Lat. 38°47' Lo 62°37'), Argentina.

Animals, diets and experimental procedure

Ninety-six, 1-d-old, Cobb-500 broiler chicks were randomly divided into 16 groups of 6 animals (3 male and 3 female), housed in experimental pens (1 x 1 m) which were distributed in 4 blocks. The floor of the pens was slatted and raised 30 cm. In each block experimental treatments were randomly allocated 1) C: control (without chia meal (CM), without hydroxytyrosol (HT)); 2) CM: diet with 10% chia meal; 3) CM+HT: diet with 10% chia meal + hydroxytyrosol and 4) HT: diet with hydroxytyrosol.

Feed for all treatments was prepared weekly and stored under ambient conditions until fed. The CM (Desus S.A., Buenos Aires, Argentina) presented 59.7% of linolenic acid / total fat (18%) and 4% of soluble fiber / total dietary fiber (40%). The antioxidant hydroxytyrosol (Hytolive, Genosa, Madrid, Spain) was added to provide 7 mg/kg BW/day. This additive was weighed and added to the diet daily before being offered to the animals. The dose was adjusted weekly according to the weight gain of the animals. Chicks from each treatment were fed two diets during the experimental period. The first diet, fed from day 0 to day 21, was identical for all treatments and was a ty pical starter diet (**Table I**). The second diet was consumed from day 22 to day 46 and was the experimental diets, formulated to be isocaloric and isonitrogenous (**Table II**). Analysis of crude protein, crude fiber and lipid content of feed were carried out following the Kjeldahl procedure (AOAC 1990), the detergent system (Goering & Van Soest, 1970) and the Soxhlet method (AOAC 1990), respectively. All animals had ad libitum access to feed and freshwater throughout the study, and ventilation was controlled. During the first 48 h of the trial, lighting was provided 23 h a day; after that continuous lighting was used. The experimental initial temperature was 31°C for the first week and was reduced by 3 °C each consecutive week until the temperature reached 23 °C. Handling of animals and experimental protocols in the present study met the code of practice for the care and handling of farm animals recommended by the Servicio Nacional de Sanidad y

Table I. Composition of starter diet (0-21 days). (Composición del alimento iniciador (0-21 días)).

Items %	
Ground corn	62,00
Soybean meal	30,00
Meat meal	5,75
Ground shell	0,75
Lysine	0,20
Salt	0,25
Vitamin-mineral premix[1]	0,50
Dl-methionine	0,15
Chemical composition	
Crude protein (%)*	19,66
ME ($Kcal.Kg^{-1}$)	3035
Lipid (%)*	4,1
Crude fiber (%)*	2,85
Ca (%)**	1,05
Total P (%)**	0,69
Methionine+Cystine (%)**	0,82
Lysine**	1,26

[1] Vitamin A: 8.000.000 UI; vitamin D3: 1.500.000 UI; vitamin E: 30.000 UI; vitamin B2: 3.800 mg; vitamin B6: 1.800 mg; vitamin B1: 1.200 mg; vitamin K3: 1.500 mg; nicotinic acid: 26.000 mg; pantotenic acid: 9.000 mg; folic acid: 600 mg; Biotin: 40 mg; Cholin: 180 g; vitamin B12: 10.000 µg; Copper 8.500 mg; Iron: 50.000 mg; Iodine: 1000 mg; Manganese: 70.000 mg; Selenium: 250 mg; Cobalt: 200 mg; Zinc: 60.000 mg; Antioxidant: 125 mg: Excipient C.S.P.: 1000 g. ME: metabolizable energy. *Analysed values. **Calculated values.

Calidad Agroalimentaria (SENASA 2015), following international animal ethics guidelines.

Meat samples

At forty-six-days-old, thirty-two chicks (2 male/block/treatment) were randomly selected and slaughtered at the Agriculture and Livestock abattoir of the UNS, after a fasting time of 12 hours (overnight), by cutting the jugular vein until total bleeding. After slaughtering, the carcasses were cooled and kept at 4° C for 24 hours postmortem. After that, the *Pectoralis* muscle (breast) were withdrawn and samples were stored at −80° C until they were analyzed.

Lipid and fatty acid content

The intramuscular lipids of breast were extracted according to the method described by Folch et al. (1957). The determination of fatty acids was carry up by gas chromatography. Samples were injected into a gas split/splitless chromatograph Shimadzu GC 14B (Shimadzu Corporation, Japan) equipped with a capillary column (100m, 0.25mm ID, 0.20 µm df; Restek Rt 2650, USA). Helium 5.0 was used as carrier gas and Nitrogen 5.0 was used as Make up. The thermal conditions were injector/detector temperature (FID) 240°C/290°C. The initial oven temperature was 140° C with a ramp of 4° C/minute up to 240° C and 20 minutes at 240° C. The data was retrieved and processed by Software LabSolutions GCSoluction Release 2.31 (Shimadzu), and individual fatty acid was quantified as a percentage of total fatty acids methylated esters (FAMEs) identified through the SupelcoTM 37 Component FAME MIX standard. The total SFA, monounsaturated fatty acids (MUFA), PUFA, total n-3 PUFA and total n-6 PUFA were calculated by the sum of the individual quantity of the fatty acids.

Lipids indices and enzyme activity indices

The atherogenicity (IA) and thrombogenicity (IT) indices were calculated according to Ulbricht & Southgate (1991) using the following equations: IA = [(4 x C14:0) + C16:0] / [ΣMUFA+ Σ(n-6) + Σ(n-3)], and IT = (C14:0 + C16:0 + C18:0)/[(0.5 × ΣMUFA) + (0.5 × Σ(n -6)) + (3×Σ(n-3)) + (Σ(n-3))/Σ(n-6)]. In terms of health prevention, these two calculates lipids indices estimate the potential of the diet to form plaques or clots in blood vessels. Hypocholesterolemic/Hypercholesterolemic ratio (h/H) was calculated according to Fernández et al. (2007), as follows: 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). This last index would indicate the potential effect of fatty acids composition on cholesterol. Unsaturation index (UI) indicates PUFA diet quality including dose with low degree of unsaturation and was calculated according to Logue *et al*. (2000) using the formula: UI = 1 × (% monoenoics) + 2 × (% dienoics) + 3 × (% trienoics) + 4 × (% tetraenoics) +5 × (% pentaenoics) + 6 × (% h exaenoics). Trans fatty acid index (TFA) was estimated as the sum of trans-MUFA and trans-PUFA, according to the European Food Safety Authority (EFSA, 2010). Conjugated fatty acid (CLA; C18:2 c9, t11) was excluded from TFA because presents a different function than those of TFAs.

The activity of desaturases enzymes was estimated according to Del Puerto *et al*. (2017). The Δ9 desaturase was estimated by the ratio C16:1/C16:0 and by the ratio C18:1/C18:0. The total delta-9 desaturase index (for both C16:1 and C18:1) was estimated by the sum of the previous two indexes. The activity of both Δ5-desaturase and Δ6-desaturase were calculated using the following equation (Dal Bosco et al. 2012): Δ5 desaturase + Δ6 desaturase = [C20:2n -6 + C20:4n-6 + EPA + C22:5n-3 + DHA/C18:2n-6 (LA) + ALA + C20:2n-6 + C20:4n-6 + EPA + C22:5n-3 + DHA] × 100. These two desaturases are indices of desaturation and elongation in the n-6 and n-3 PUFA series of fatty acids from their precursors C18:2n6 and C18:3n3. The elongase and the thioesterase enzymes were calculated as the ratio of C18:0/C16:0 and C16:0/C14:0, respectively (Del Puerto *et al*. 2017).

Lipid oxidation

A sample of 25 g from Pectoral muscle was homogenized in 64 ml of HCl 0.00475 M, following the Tarladgis procedure (1960) based in TBARS (thiobarbituric acid reactive substances) method. The malonyl dialdehyde (MDA) concentration was determined from a standard calibration curve with 1,1,3,3-tetraethoxypropane (Fluka, Honeywell International Inc.), the results were expressed in µg of MDA/g of fresh meat. Color development reading was performed at 530 nm in a Lambda 20 UV - Visible spectrophotometer (Perkin Elmer Corp. - Norwalk, CT, USA).

Table II. Composition of experimental diets (22-46 days) (Composición de las dietas experimentales (22-46 días)).

Items%	Experimental diets			
	C	CM	CM+HT	HT
Ground corn	69,00	66,00	66,00	69,00
Soybean meal	23,50	16,66	16,66	23,50
Meat meal	6,20	6,20	6,20	6,20
Chía meal	-	10,00	10,00	-
Ground Shell	0,30	0,30	0,30	0,30
Lysine	0,13	-	-	0,13
Salt	0,25	0,25	0,25	0,25
Vitamin-mineral premix[1]	0,50	0,50	0,50	0,50
Dl-methionine	0,12	0,09	0,09	0,12
Hidroxitirosol	-	-	yes	yes
Chemical composition				
Crude protein (%)	18,48	18,18	18,18	18,48
ME ($Kcal.Kg^{-1}$)	3141,14	3.127,87	3.127,87	3141,14
Lipid (%)	4,33	5,86	5,86	4,33
Crude fiber (%)	2,59	4,73	4,73	2,59
Ca (%)	0,91	0,99	0,99	0,91
Total P (%)	0,70	0,73	0,73	0,70
Methionine+Cystine (%)	0,74	0,80	0,80	0,74
Lysine	1,04	1,17	1,17	1,04

C: control; CM: 10% chia meal; CM + HT: 10% chia meal and hydroxytyrosol and HT: hydroxytyrosol. 1 Vitamin A: 8.000.000 UI; vitamin D3: 1.500.000 UI; vitamin E: 30.000 UI; vitamin B2: 3.800 mg; vitamin B6: 1.800 mg; vitamin B1: 1.200 mg; vitamin K3: 1.500 mg; nicotinic acid: 26.000 mg; pantotenic acid: 9.000 mg; folic acid: 600 mg; Biotin: 40 mg; Cholin: 180 g; vitamin B12: 10.000 µg; Copper 8.500 mg; Iron: 50.000 mg; Iodine: 1000 mg; Manganese: 70.000 mg; Selenium: 250 mg; Cobalt: 200 mg; Zinc: 60.000 mg; Antioxidant: 125 mg: Excipient C.S.P.: 1000 g. ME: metabolizable energy.

Statistical Analyses

Data are expressed as the mean ± standard error of mean of 8 repetitions (n=8 birds). Lipid and fatty acids content, lipid indices, enzyme activity index and lipid oxidation data were analyzed as a randomized complete block ANOVA. Statistical analyses were computed using Infostat software (Di Renzo *et al*. 2008). The comparison between mean values were made using the Tukey test ($p<0.05$).

RESULTS AND DISCUSSION

In the present investigation, the lipid content of *Pectoralis* muscle of omega n -3 and/or hydroxytirosol enriched diets yielded no differences ($p>0,05$) with the control diet (**Table III**). The effect of diet on intramuscular lipid content is limited and the absence of results could be attributed to the fact that the fatty acids are structural components of cell membranes that limit its levels of deposition with the purpose to maintain the fluidity and permeability of different components (Cortinas *et al*. 2004). Our findings agree with Aziza *et al*. (2010) and Rahimi *et al*. (2011) independently of the source of omega n-3 supplied. Also, Crespo & Esteve-Garcia (2001), showed that the level of polyunsaturation in the dietary fat does not influence the intramuscular lipid content of the breast. Even, Taşdelen & Ceylan (2017) and Cortinas *et al*. (2014) who used different sources of PUFA combined with vitamin E showed no effect. The same results were obtained by Branciari *et al*. (2017) in meat of chickens fed with semisolid olive cake.

In **Table III**, fatty acid content of *Pectoralis* from birds receiving diets with chia meal and/or hydroxytyrosol or control diet is shown. The comparison between the fatty acids compositions of the different treatment showed that the type of diet supplied did not modify ($p>0,05$) the content of SFA, represented principally by C16:0 and, on a smaller scale, by C18:0 (**Table III**). Previous works (Ayerza *et al*. 2002, Azcona *et al*. 2008) reported that the SFA content in chicken breasts decreased by adding chia seed or meal into diets, attributing it to the decrease in palmitic acid. Probably the chia seed or meal incorporated into the poultry diet in substitution of soya meal and corn was higher and these could explain the differences with our work. Other rich ingredients as Camelina sativa and canola- flaxseed meals, studied by Aziza *et al*. (2010) and Rahimi *et al*. (2011) did not affect the SFA values in breast muscle. Furthermore, according to Rahimi *et al*. (2011), the ability of broiler chickens to alter the SFA content of the breast muscle is limited. Regarding antioxidant effect on SFA, the information available is discussed. Ajuyah *et al*. (1993) observed lower values of SFA in breast when adding flaxseed with tocopherol and/or canthaxanthin to broiler diet, while Tomažin *et al*. (2013) and Gallinger (2015) found no differences in the level of SFA when adding flaxseed or flax oil combined with vitamin E and selenium as antioxidants. In other study, Papadomichelakis *et al*. (2019) observed

Table III: Fatty acids composition (as % of total fatty acids) of *Pectoralis muscles* of broiler fed chia meal and/ or hydroxytyrosol in the diet (Composición de ácidos grasos (% total de ácidos grasos) en la pechuga de pollos parrilleros alimentados con dietas adicionadas con harina de chía y/o hidroxitirosol).

Fatty acid	C	CM	CM+HT	HT	SEM	*p*-Value
Lipids %	1,91	1,87	1,92	2,27	0,11	NS
14:0	0,27	0,27	0,25	0,34	0,02	NS
14:1	0,07	0,06	0,06	0,09	0,01	NS
16:0	25,91	26,17	26,28	26,38	0,82	NS
16:1 n-7	4,27	4,34	4,49	4,41	0,47	NS
17:0	0,19	0,33	0,16	0,26	0,09	NS
17:1	0,10	0,11	0,10	0,12	0,02	NS
18:0	4,46	5,04	4,60	5,57	0,43	NS
18:1 n-9	48,20 [a]	39,77 [b]	40,95 [bc]	44,98 [ac]	1,53	0,013
18:2 n-6	11,44 [a]	14,05 [b]	14,24 [b]	12,29 [a]	0,55	0,014
18:2 c9, t11	0,03 [a]	0,09 [b]	0,10 [b]	0,04 [a]	0,01	0,008
20:0	0,08	0,07	0,09	0,09	0,02	NS
20:1	0,19 [a]	0,14 [b]	0,13 [b]	0,21 [a]	0,01	0,008
18:3 n6	0,07	0,05	0,06	0,09	0,01	NS
18:3 n-3	0,21 [a]	3,61 [b]	4,54 [c]	0,30 [a]	0,29	0,0001
20:2 n6	0,04 [ab]	0,02 [c]	0,03 [ac]	0,05 [b]	0,01	0,008
22:0	0,08	0,12	0,11	0,11	0,02	ns
20:3 n6	0,29	0,23	0,21	0,34	0,05	NS
20:4 n-6 (AA)	1,08	1,07	0,82	1,32	0,17	NS
20:5 n-3 (EPA)	0,06 [a]	0,37 [b]	0,25 [b]	0,06 [a]	0,05	0,028
22:5 n3 (DPA)	0,13 [a]	0,53 [b]	0,39 [b]	0,13 [a]	0,07	0,012
22:6 n3 (DHA)	0,06 [a]	0,23 [b]	0,14 [ab]	0,09 [a]	0,05	0,049
SFA	30,98	32,01	31,52	32,62	1,08	NS
MUFA	53,37 [a]	44,93 [b]	46,22 [bc]	50,43 [ac]	1,70	0,023
PUFA	13,45 [a]	20,23 [b]	20,78 [b]	14,76 [a]	0,61	0,0001

SEM: standard error of the mean. a-c Means in the same row with no common superscript differ significantly (p<0.05). NS: not significant (P> 0.05). C: control; CM: 10% chia meal; CM + HT: 10% chia meal and hydroxytyrosol and HT: hydroxytyrosol. SFA: saturated fatty acids, MUFA: monounsaturated fatty acids and PUFA: polyunsaturated fatty acids.

reduced SFA with dry olive pulp addition to broiler diet. The different results within these studies could be related to types of antioxidants,their chemical composition, and the dietary level of inclusion. In our case, little information is available on the possible effect of hydroxytyrosol olive by-product on SFA of *Pectoralis* chicken muscle. Thereby, the dose of 7 mg/kg BW/day is novel with no main effect on SFA.

MUFA, were represented mainly by C18:1 and, on a smaller scale, by C16:1 (**Table III**). In the present study, the broiler fed CM and CM+HT diet presented lower content (p<0,05) of MUFA than those receiving the control diet, being intermediate diet HT. This finding concurs with the results of Ajuyah *et al*. (1991), Ayerza *et al*. (2002) and Gallinger (2015). The decrease in C18:1 content found in diets containing chia meal could be related to the inhibition effect of PUFA against Δ9 - desaturase activity, preventing the formation of MUFA from their precursors (Ayerza *et al*. 2002). The Δ9 desaturase is primarily in charge of C18:1 biosynthesis from C18:0 and also C16:1 from C16:0 (Brenner 1989). The addition of HT to the diet did not modify the MUFA values. These last findings agree with Tomazin *et al*. (2013) and Ajuyah *et al*. (1993) who showed no differences in MUFA values in breast muscle when adding flaxseed with vitamin E or vitamin E + canthaxanthin, respectively to broiler chickens diet.

Regardless the diet, PUFAs were represented mainly by fatty acids C18:2n6 (linoleic acid -LA) and C18:3n-3 (*α*-linolenic acid-ALA). Nevertheless, in the present study, diets containing chia meal (CM and CM+HT) showed the highest levels (P<0,05) of these two essential PUFA in breast muscle (**Table V**). Compared to the control, the CM diet increased the linoleic acid content by approximately 22,8%, and the *α*-linolenic acid content by 1619%. These results are in accord with Ayerza *et al*. (2002); Azcona *et al*. (2008); Salazar-Vega *et al*. (2009); Mendonça *et al*. (2020) and could be explained by the fact that α-linolenic acid is the main fatty acid in chia meal and seeds. Even more, when hydroxytyrosol was added combined with chia meal (CM+HT), C18:3n -3 showed a higher level (+25,76%; P<0,05) in comparison to the CM diet (**Table III**). These last finding agree with Ajuyah *et al*. (1993), who used flax seeds with the antioxidant tocopherol and/or canthaxanthin and are in contrast with Cherian et al. (1996) and Leskovec *et al*. (2018), who found no differences when adding vitamin E to diets enriched with omega n-3. In the present experiment, the differences in C18:3n3 level could be explained by the antioxidant capacity of hydroxytyrosol to slow the lipid peroxidation of these fatty acids in the diet.

It is known that PUFAs long-chain (n-3 PUFA-LC), eicosapentaenoic (EPA), docosapentaenoic (DPA) and docosahexaenoic (DHA) are synthesized from the precursor alpha-linolenic acid, whereas arachidonic acid (AA, C20:4 n-6) is produced from linoleic acid through the action of Δ5 and Δ6-desaturases. In the present experiment, AA remained unchanged (P>0.05), and all the other n-6 PUFA fatty acids showed bit variation. The increase in the different n-6 PUFA due to chia meal was not as prominent as the increase in n-3 PUFA and could be due to the competition with α-linolenic fatty acid for Δ5-Δ6 enzymes involved in desaturation and elongation (Jing *et al*. 2013). These results agree with Ajuyah *et al*. (1993) and Azcona *et al*. (2008) who fed other omega n-3 enriched diets.

Regarding n-3 PUFA-LC, increased (P<0.05) content of EPA, DHA, DPA in CM and EPA and DPA in the CM+HT diet was observed in contrast with the control and HT diet (**Table III**). The results of the present experiment would support the hypothesis that chick can convert, though at a low rate, α-linolenic acid to longer-chain n-3 fatty acids (López-Ferrer *et al*. 2001; Cachaldora *et al*. 2005). ALA conversion to these fatty acids depends on multiple factors such as LA and ALA competition for enzymes of the same metabolic pathway, Δ6-desaturases preference for ALA, gene expression of elongases and desaturases, dietary LA:ALA ratio, the dose of ALA, LA, n-3 PUFA or n-6 PUFA, negative feedback from product mediated PUFA synthesis, hormonal factors such as insulin which stimulates the expression of Δ6D, among others (Reyna *et al.* 2018). Our findings are in accord with Mendonça *et al*. (2020) who showed a higher EPA and DHA level in the breast and thigh muscle of broilers fed chia oil or seed. Also, López-Ferrer *et al*. (2001) and Long *et al*. (2020) found more EPA, DPA, and DHA levels in the muscle when differents sources of n -3 PUFA were added to the broiler diet.

However, the addition of hydroxytyrosol combined with chia meal (diet CM+HT) increased ALA content but did not promote EPA and DPA levels concerning the CM diet. These results are in accord with the findings of Botsoglou *et al*. (2013), who obtained no egg lipid profile changes from hens fed fish oil with olive leaves and α-tocopherol as antioxidants. In agreement, Leskovec *et al*. (2018) did not observe differences when using different antioxidants combined with flax oil in broiler diet. Meanwhile, Ajuyah *et al*. (1993) found higher EPA, DPA and DHA values when combining flax seeds with or without tocopherol and/or canthaxanthin in birds diet. In the present study, one of the possible reasons would seem to indicate that hydroxytyrosol only managed to protect omega n -3 present in the diet, therefore, it increased the content of C18:3 in the breast, however, it failed to protect the n-3 PUFA-LC (EPA, DHA, DPA) and as they were synthesized, they were oxidized. Another possibility would suggest that the dose of 7 mg/kg BW/day of hydroxytyrosol and the feeding period length could have been insufficient to express antioxidant activity on n-3 PUFA-LC.

Regarding the DHA level, no differences were observed in CM+HT diet concerning the CM, HT and C diet, displaying intermediate values. Studies by Smink *et al*. (2012) showed that high ALA intakes could lower DHA concentration in pigs liver. The reduction in the synthesis of this fatty acid could be explained by the higher affinity of Δ6-desaturase for ALA than for C24:5n-3 (tetracosapentaenoic acid), which is an intermediate in the synthesis of DHA from DPA. Consequently, the desaturation of C24:5n-3 would be inhibited, conditioning the synthesis of DHA (Smink *et al*. 2012). Based on this background, it could be suggested that the antioxidant hydroxytyrosol promoted an increase in ALA supply with the diet and consequently a higher level in the breast, restraining DHA synthesis. Also, Ounnas *et al.* (2014) determined that the conversion from EPA to DHA is probably lower than from ALA to EPA because they involve additional elongation, desaturation and β-oxidation reactions, subject to hormonal regulation. In addition, Reyna *et al*. (2016) observed that high-dose of chia oil as a source of ALA in rat diet inhibited the gene expression of Δ5 and Δ6 -desaturase. It should also be noted that ALA is elongated and desaturated in a tissue-dependent manner (Barceló-Coblijin & Murphy, 2009).

However, the effect of hydroxytyrosol olive byproduct on the fatty acid composition of breast muscle in broiler n-3 enriched diet has scarcely been studied, while more research is needed on this controversial subject to understand the specific function of antioxidant compounds on C18:3 and its conversion to PUFA acids. The underlying mechanism involved in PUFAs convertion need further investigation.

As for the n-3 PUFA-LC level in HT diet, the dietary addition of hydroxytyrosol did not modify (P> 0.05) the content ALA, EPA, DPA concerning C diet (**Table III**). A similar result was obtained by Tomažin *et al*. (2013) and Paiva-Martins *et al*. (2009) who used vitamin E in chickens and olive leaves in pigs, respectively. Also, Botsoglou *et al*. (2012) showed that olive leaves or α-tocopherol did not affect the fatty acid profile in eggs. Contrarily, Del Puerto *et al*. (2017) observed an increase in α-linolenic acid, EPA, DPA, and DHA fatty acids level in *Gastrocnemius* muscle after supplementation with selenium. The different responses among authors could be based on the different types, chemical composition, half-life and the dose of antioxidants used.

Gutiérrez (2015) and Reboredo *et al*. (2010) indicates that the presence of PUFA (5-6 double bonds) strongly increases susceptibility to lipid peroxidation. Specifically, in poultry meat, lipid oxidation is one of the primary causes of limiting its quality and leads to a decrease in nutritional value s of meat products (Arshad *et al*. 2013). Natural antioxidants play a key role in enhancing the stability and quality of meat and are safer than synthetic ones (Arshad *et al*. 2013). Breasts corresponding to the CM diet presented the highest value of MDA (P<0.05), indicating less oxidative stability concerning the other treatments (**Table VI**). This find could be a direct consequence of the increase in n -3 PUFA content provided by chia meal and are consistent with the results of Anjum *et al*. (2013) and Zanini *et al*. (2006) and in contrast with the findings of Long *et al*. (2020). Moreover, the addition of hydroxytyrosol and its combination with chia meal lowered (P<0.05) MDA values for breast muscle in contrast with CM diet, showing no differences with the C diet (**Table VI**). These results suggest that the dietary incorporation of hydroxytyrosol could be an effective way to increase the stability of n-3 PUFA enriched broilers meat through oxidative damage. Previous studies have shown that some of the major polyphenols of olive oil mill wastewater, including hydroxytyrosol, effectively reduce lipid peroxidation (Gerasopoulos *et al*. 2015, Cardinali *et al*. 2012, Rubio-Senent *et al*. 2014). A similar observation was previously reported by Taşdelen & Ceylan (2017); Leskovak *et al*. (2018) who use vitamin E supplementation with fish or rapeseed oil-enriched diet, respectively. In the present experiment, MDA levels recorded in the diets were below the limit acceptability value set for oxidized meat (<2µg / g) by Campos *et al*. (2006).

In the present study, nutritional lipid indices were calculated to associate with human nutrition and health (**Table IV**). Enriched CM diets (CM and CM+HT) increased (P<0.05) PUFA/SFA ratio in the breast, which is interesting because the consumption of a diet high in SFA is associated with higher plasma cholesterol level, especially low-density lipoprotein content, which is related to coronary heart disease (Ulbricht & Southgate, 1991). Western diets are characterized by high consumption of foods rich in fat, especially SFA, as well as n-6 PUFA leading to ratios very high for n-6/n-3 (15-20/1) and lower for PUFA/SFA. Nutritional studies suggest an n-6/n-3 ratio no greater than 4:1, for the prevention of obesity, cancer, depression, anti-inflammatory and cardiovascular diseases, being the ideal 1:1 (Simopoulos 2008, Simopoulos 2016, Long *et al*. 2020). The results of the present study are relevant because they denote that the use of chia meal in the diet of broilers promoted a better n-6/n-3 ratio due to the increase of n-3 PUFA deposition in breast muscle.

PUFA/SFA is the most common index used to evaluate the effect of fatty acid quality in meat, however, MUFA is missing in the formula; this is why IA, IT indices and h/H ratio were calculated. Considering MUFA, oleic acid (C18:1 n-9 cis) is the most common fatty acid which was associated with lower serum cholesterol and total triglycerides levels reducing heart failure (Natali *et al*. 2007, Lopez-Huertas 2010). In the current study, no difference (P>0.05) was observed in the IA index and h/H ratio between treatments (**Table IV**). However, a reduced (P<0.05) IT was observed in omega n-3 enriched diet, which is suggestive because this index estimates the potential to form clots in the blood vessels and the lower level is associated with a reduced risk of cardiovascular diseases and directly with healthier food (Ulbricht & Southgate 1991). All these indices include unsaturated fatty acids and are used to evaluate cardiovascular health, although to determine the effect of the proportion of fatty acids with different degrees of unsaturation in the total fatty acid composition, the UI index was included. In this experiment, considering that the higher degree of unsaturation of the membrane fatty acids maintains permeability and fluidity, a higher (P <0.05) UI index was recorded in CM and CM+HT diets, suggesting a high degree of total unsaturation and better nutritional quality of breast in these diets (**Table IV**). There is no data on the levels of UI in broiler chicken breast and the current information is based in meat pig, finding values between 111 and 124 (Realini *et al*. 2013).

Table IV. Lipid oxidation (MDA, µg/g meat), PUFA/SFA ratio, n-3, n-6 and their ratios and calculated lipid indices in *Pectoralis* muscles of broiler fed chia meal (CM), or CM + hydroxytyrosol (HT) or HT, from 21 to 46 days of age (Oxidación lipídica (MDA, µg/g carne), relación AGPI/AGS, n-3, n-6 y sus relaciones e índices lipídicos en la pechuga de pollos parrilleros alimentados con dietas adicionadas con harina de chía (HC) o HC + hidroxitirosol (HT) o HT, desde los 21 hasta los 46 días de edad).

	CM	CM+HT	HT	SEM	*p*-Value
MDA (µg/g)	1,55 ^{b}	1,07 ^{a}	0,61^{c}	0,10	0,003
PUFA/SFA	0,63 ^{b}	0,66^{b}	0,45^{a}	0,02	0,0001
PUFAn-3	4,67 ^{b}	5,29^{b}	0,58^{a}	0,26	0,0001
PUFAn-6	15,47	15,39	14,14	0,64	NS
n-6/n-3	3,36 ^{b}	2,95 ^{b}	25,52^{a}	2,92	0,0001
LA/ALA	3,89 ^{b}	3,14 ^{b}	40,96 ^{a}	3,28	0,0001
IA	0,42	0,41	0,43	0,02	NS
IT	0,71 ^{b}	0,66 ^{b}	0,95^{a}	0,05	0,0018
h/H	2,46	2,51	2,41	0,12	NS
UI	94,76 ^{b}	96,34 ^{b}	83,65 ^{a}	1,25	0,0001
TFA	0,34 ^{b}	0,33 ^{b}	0,53 ^{a}	0,05	0,009

SEM: standard error of the mean.$^{a-c}$ Means in the same row with no common superscript differ significantly (P<0.05). NS: not significant. C: control; CM: 10% chia meal; CM+HT: 10% chia meal and hydroxytyrosol and HT: hydroxytyrosol. PUFA/SFA: polyunsaturated fatty acids/ saturated fatty acids. LA/ALA: linoleic acid/α-linolenic acid. IA: index of atherogenicity, IT: index of thrombogenicity, h/H: hypocholesterolemic/ hypercholesterolemic ratio, UI: unsaturation indice, TFA: trans fatty acids.

Table V. Enzyme activity index of lipid metabolism estimated on the basis of fatty acid composition of *Pectoralis* muscles of broiler fed chia meal and/or hydroxytyrosol in the diet. (Indices de actividad enzimática del metabolismo lipídico estimado sobre la base de la composición de ácidos grasos en la pechuga de pollos parrilleros alimentados con dietas adicionadas con harina de chía y/o hidroxitirosol).

	C	CM	CM + HT	HT	SEM	*p*-Value
Δ-9 desaturase						
16:1/16:0	0,16	0,17	0,17	0,17	0,02	NS
18:1/18:0	10,90	8,24	9,18	8,45	0,91	NS
16:1 + 18:1/16:0 + 18:0	1,74	1,43	1,48	1,55	0,10	NS
Δ-5 + Δ-6 desaturases	10,40	10,80	7,91	11,50	1,66	NS
Elongase 18:0/16:0	0,17	0,19	0,17	0,21	0,01	NS
Thioesterase 16:0/14:0	99,71	106,40	107,04	79,78	10,12	NS

SEM: standard error of the mean. NS: not significant (P> 0.05). C: control; CM: 10% chía meal; CM + HT: 10% chia meal and hydroxytyrosol and HT: hydroxytyrosol.

It could consider that the UI only targets the degree of unsaturation of fatty acids and does not discern between n-6 and n-3 fatty acids, a condition that was also repeated in the previous indices. These fatty acids have different physiological effects on the human body (Chen *et al*. 2020). In our experiment, n- 6 PUFA yielded no difference (P>0.05) between treatments (**Table IV**). However, n -3 PUFA increase (P<0,05) in breast muscle of CM and CM+HT diets in contrast with the C and HT diet (**Table IV**). In recent years, human meat enriched with these fatty acids has gained importance due to increasingly widespread knowledge of its effect as hypocholesterolemic, anti-inflammatory properties and its ability to prevent cardiovascular and autoimmune diseases and cancer (Simopoulous 1991, Manson et al. 2019). LA/ALA ratio was calculated considering that the principal fatty acids within the n-6 and n- 3 series are LA and ALA. These fatty acids are considered essential and must be supplied through the diet (Glick et al. 2013). In the present study, a lower LA/ALA index (P <0.05) was observed in the CM and CM+HT diets due to the high content of ALA in CM, which would indicate a better balance between these two fatty acids and healthier food. There is no information associated with the treated ratio in broiler chickens. This index has been used in sheep (Majdoub -Mathlouthi *et al*. 2015) and cattle (Sharma *et al*. 2018) to determine the quality of meat and milk, respectively.

In the current study, TFA was included considering that the consumption of these fatty acids may injure human health (Micha & Mozaffarian, 2009). The World Health Organization (WHO) recommended less than 1% of the total energy of TFA intake. Due to the negative effect of TFA on vital human function, they could be taken as an indicator of food security (Chen 2020). In our finding, lower (P<0,05) TFA values were reported for CM and CM+HT diets providing the benefit of the addition of CM on meat quality. TFA definition does not consider CLA fatty acid, even though it has one trans double bond, because information shows a beneficial health effect as anti-cancer, anti-obesity, anti-hypertensive and anti-atherosclerosis (Knekt *et al*. 1996, Koba *et al*. 2014, den Hartigh 2019). When the main effect of diet was considered, CLA showed a higher (P<0,05) level when CM was added to feed in comparison to the control and HT diet (**Table IV**), displaying a better meat fatty acid profile.

In the current study, the indices and ratios in the breast muscle met the recommended guidelines (Ayerza *et al*. 2002, Winiarska *et al*. 2020, Long *et al*. 2020, Chen *et al*. 2020), which could suggest that the meat of birds offered chia meal diets present higher nutritional quality for human health. In experimental studies, the fatty acid composition of meat should be evaluated to determine their nutritional and medicinal value, especially in n-3 PUFA enriched diets.

By last, the indices related to the activity of desaturase, elongase, and thioesterase enzymes were calculated in order to consider the possible effect of those enzymes in elongating and desaturating fatty acids presented in the experimental diets. However, it is important to clarify that all the indices are indirect measures, and cannot be assumed to reflect enzyme activities directly (Hodson & Fielding 2013). In the current study, for all the considered enzymes indices, no main effects (P>0,05) have been observed, independently of the diet supplied (**Table V**). More research is needed to understand the mechanisms behind the enzyme's activity action on PUFA synthesis.

CONCLUSION

The results obtained in this study suggest that the source of dietary fatty acids clinch the fat ty acid profile in meat. The supply of a broiler diet containing chia meal enriches breast meat with n-3 PUFA, with a slight increase in the generation of free radicals, and improves lipids indices and ratios. The dietary addition of hydroxytyrosol combined with chia meal seems to be an effective way to increase the stability of n-3 PUFA through oxidative damage and α-linolenic level, providing improvements in the meat nutritional quality, with consequent health benefits for its consumers.

The effects of a dietary by-product of olive industry, hydroxytyrosol, on meat fatty acid profile in broiler diets appear as being limited. In our current study, diets containing 7 mg/kg BW/day of hydroxytyrosol was novel. Further research is needed to determine the possible effect of higher doses of this antioxidant on the susceptibility of n-3 PUFA to lipid peroxidation and fatty acid profile.

In conclusion, chia incorporated in poultry diet improve the nutritional value of meat, the *Pectoralis* in this study, by an increase in n-3 PUFA content. When combining chia meal with hydroxytyrosol in poultry diet, this antioxidant has a protector effect on the lipid oxidation of the polyunsaturated fatty acids of the enriched meat. Hydroxytyrosol is also a good alternative to other antioxidants.

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