

Impact of chia meal and hydroxytyrosol on the 10 nutritional quality of broiler chicken meat

Fernández, H.T.¹; Echevarría, G.¹; Saadoun, A.^{2,3} and Cabrera, M.C.^{2,3}

¹ Departamento de Agronomía. Universidad Nacional del Sur. Bahía Blanca. Argentina.

² Facultad de Ciencias. Universidad de la República. Montevideo. Uruguay.

³ Facultad de Agronomía. Universidad de la República. Montevideo. Uruguay.

ADDITIONAL KEYWORDS

Broiler.
Antioxidant.
Omega n-3.
Fatty acid profile.
Health indices.

SUMMARY

In poultry production, the composition of the diet directly influences the meat quality of birds. The 34 present research deals with the effect of the addition in the diet of two by-products, chia meal and/or 35 hydroxytyrosol, on lipid content, fatty acids profile, lipids indices and enzymes activity and lipid 36 oxidation in the breast. Ninety-six broiler chicks were randomly divided into 16 groups of 6 animals, 37 which were distributed in 4 blocks. In each block experimental treatments were randomly allocated 1) 38 C: control; 2) CM: 10% chia meal; 3) CM+HT: 10% chia meal + hydroxytyrosol and 4) HT: 39 hydroxytyrosol. The experimental period lasted from day 22 to day 46, at which the slaughter was 40 carried out and breast samples were obtained. The dietary chia meal increased total PUFA, total n-3 41 PUFA, α -linolenic, n-3 PUFA-LC (EPA, DPA, DHA), PUFA/SFA, UI and reduced the n-6/n-3, 42 LA/ALA, thrombogenicity index and TFA, although worsened lipid peroxidation. However, the 43 addition of hydroxytyrosol and its combination with chia meal increased α -linolenic levels and 44 decreases the lipid oxidation value of the breast, exhibiting its action as an antioxidant. Therefore, the 45 results outline the potential effect of chia meal combined with hydroxytyrosol, in the modification of 46 the composition of fatty acids and their oxidative stability in chicken breast, thereby resulting in meat 47 with higher nutritional quality for human health.

Impacto de la harina 11 de chía e hidroxitirosol sobre la calidad nutricional de la carne de pollo

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 de ácidos 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) HT: 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 TAG, 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 delimitan 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.

PALABRAS CLAVE

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

INFORMATION

Cronología del artículo.
Recibido/Received: 11.04.2019
Aceptado/Accepted: 10.10.2022
On-line: 15.10.2022
Correspondencia a los autores/Contact e-mail:
hfernand@criba.edu.ar

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 Argentina 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 po-

lyphenols 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 linoleic 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 typical 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 UEA 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 Calidad

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; pantothenic 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.

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 GCSolution Release 2.31 (Shimadzu), and individual fatty acid was quanti fied

as a percentage of total fatty acids methylated esters (FAMES) identified through the Supelco™ 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: $IA = [(4 \times C14:0) + C16:0] / [\Sigma MUFA + \Sigma(n-6) + \Sigma(n-3)]$, and $IT = (C14:0 + C16:0 + C18:0) / [(0.5 \times \Sigma MUFA) + (0.5 \times \Sigma(n-6)) + (3 \times \Sigma(n-3)) + (\Sigma(n-3)) / \Sigma(n-6)]$. 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: $UI = 1 \times (\% \text{ monoenoics}) + 2 \times (\% \text{ dienoics}) + 3 \times (\% \text{ trienoics}) + 4 \times (\% \text{ tetraenoics}) + 5 \times (\% \text{ pentaenoics}) + 6 \times (\% \text{ hexaenoics})$. 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): $\Delta 5 \text{ desaturase} + \Delta 6 \text{ desaturase} = [C20:2n-6 + C20:4n-6 + EPA + C22:5n-3 + DHA / C18:2n-6 (LA) + ALA + C20:2n-6 + C20:4n-6 + EPA + C22:5n-3 + DHA] \times 100$. 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; pantothenic 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 \pm 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 hydroxytyrosol 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

Branciaro *et al.* (2017) in meat of chickens fed with semi-solid 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 chia y/o hidroxitiroso).

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 $\Delta 9$ - desaturase activity, preventing the formation of MUFA from their precursors (Ayerza *et al.* 2002). The $\Delta 9$ 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 findings 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 diffe-

rences 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 $\Delta 5$ and $\Delta 6$ -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 $\Delta 5$ - $\Delta 6$ 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, $\Delta 6$ -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 $\Delta 6D$, 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 different 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 $\Delta 6$ -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 $\Delta 5$ and $\Delta 6$ -desaturase. It should also be noted that ALA is elongated and desaturated in a tissue-dependent manner (Barceló-Coblijn & Murphy, 2009).

However, the effect of hydroxytyrosol olive by-product 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 conversion 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 Tomazin *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 values 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 ($< 2 \mu\text{g} / \text{g}$) 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

Table IV. Lipid oxidation (MDA, $\mu\text{g}/\text{g}$ 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, $\mu\text{g}/\text{g}$ 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 + hidroxitiroso (HT) o HT, desde los 21 hasta los 46 días de edad).

	CM	CM+HT	HT	SEM	p-Value
MDA ($\mu\text{g}/\text{g}$)	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. (Índices 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% chia meal; CM + HT: 10% chia meal and hydroxytyrosol and HT: hydroxytyrosol.

the current information is based in meat pig, finding values between 111 and 124 (Realini *et al.* 2013).

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 (Simopoulos 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 fatty 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.

ACKNOWLEDGEMENTS

The authors thank DESUS S.A (Argentina) y GENOSA I+D (Spain) to donate of chia meal and Hydroxytyrosol, respectively.

BIBLIOGRAPHY

- Ajuyah, AO, Lee, KH, Hardin, RT & Sim, J 1991, 'Changes in the yield and in the fatty acid composition of whole carcass and selected meat portions of broiler chickens fed full-fat oil seeds', *Poultry Science*, vol. 70, pp. 2304-2314.
- Ajuyah, AO, Hardin, RT & Sim, JS 1993, 'Effect of Dietary Full-Fat Flax Seed With and Without Antioxidant on the Fatty Acid Composition of Major Lipid Classes of Chicken Meats', *Poultry Science*, vol. 72, pp. 125-136.
- Anjum, FM, Haider, MF, Khan, M, Sohaib, M & Arshad, MS 2013, 'Impact of extruded flaxseed meal supplemented diet on growth performance, oxidative stability and quality of broiler meat and meat products', *Lipids in Health and Disease*, vol. 12, pp. 13-24.
- AOAC 1990, Official Methods of Analysis of AOAC International, 15th (ed) AOAC International, Washington, DC, USA, Official Method.
- Arshad, MS, Anjum, FM, Khan, MI, Shahid, M, Akhtar, S & Sohaib, M 2013, 'Wheat germ oil enrichment in broiler feed with α -lipoic acid to enhance the antioxidant potential and lipid stability of meat', *Lipids in Health and Disease*, vol. 4, no. 2, pp. 164-178.
- Ayerza, R & Coates, W 2000, 'Dietary levels of chia: influence on yolk cholesterol, lipid content and fatty acid composition for two strains of hens', *Poultry Science*, vol. 79, no. 5, pp. 724-739.
- Ayerza, R & Coates, W 2001, 'Omega-3 enriched eggs: the influence of dietary linolenic fatty acid source combination on egg production and composition'. *Canadian Journal of Animal Science*, vol. 81, pp. 355-362.
- Ayerza R, Coates, W & Lauria, M 2002, 'Chia as an n-3 fatty acid source for broilers: influence on fatty acid composition, cholesterol and fat content of white and dark meat, on growth performance and on meat flavor', *Poultry Science*, vol. 81, pp. 826-837.
- Azcona, JO, Schang MJ, Garcia PT, Gallinger, C, Ayerza, RJ & Coates, W 2008, 'Omega-3 enriched broiler meat: The influence of dietary-linolenic-3 fatty acid sources on growth, performance and meat fatty acid composition'. *Canadian Journal of Animal Science*, vol. 88, pp. 257-269.
- Aziza, AE, Quezada, N & Cherian, G 2010, 'Feeding Camelina sativa meal to meat-type chickens: Effect on production performance and tissue fatty acid composition'. *Journal of Applied Poultry Research*, vol. 19, pp. 157-168.
- Barceló-Coblijn, G & Murphy, EJ 2009, 'Alpha-linolenic acid and its conversion to longer chain n-3 fatty acids: Benefits for human health and a role in maintaining tissue n-3 fatty acid levels', *Progress in Lipid Research*, vol. 48, no. 6, pp. 355-374.

- Botsoglou, E, Govaris, A, Fletouris, D & Botsoglou, N 2012, 'Effect of supplementation of the laying hen diet with olive leaves (*Olea europaea* L.) on lipid oxidation and fatty acid profile of α -linolenic acid enriched eggs during storage', *British Poultry Science*, vol. 53, no. 4, pp. 508–519.
- Botsoglou, EN, Govaris, AK, Ambrosiadis, IA, & Fletouris, DJ 2013, 'Olive leaves (*Olea europaea* L.) versus α -tocopheryl acetate as dietary supplements for enhancing the oxidative stability of eggs enriched with very-long-chain n-3 fatty acids', *Journal of the Science of Food and Agriculture*, vol. 93, no. 8, pp. 2053–2060.
- Branciarri, R, Galarini, R, Giusepponi, D, Trabalza, M, Forte, C, Roila, R, Miraglia, D, Servili, M, Acuti, G & Valiani, A 2017, 'Oxidative Status and Presence of Bioactive Compounds in Meat from Chickens Fed Polyphenols Extracted from Olive Oil Industry Waste', *Sustainability*, vol. 9, pp. 1566–1579.
- Brenner, RR 1989, 'Factors influencing fatty acid chain elongation and desaturation', in AJ Vergoesen & M Crawford (eds.), *The Role of Fats in Human Nutrition*, Academic Press, New York, USA.
- Cachaldora, P, Blas, JD, García-Rebollar, P, Álvarez, C & Méndez, J 2005, Short communication. 'Effects of type and level of supplementation with dietary n-3 fatty acids on yolk fat composition and n-3 fatty acid retention in hen eggs', *Spanish Journal of Agricultural Research*, vol. 3, no. 2, pp. 209–212.
- Campo, M, Nute, G, Huegues, S, Enser, H, Wood, J & Richardson, R 2006, 'Flavor perception of oxidation in beef', *Meat Science*, vol. 72, pp. 303–311.
- Cardinali, A, Pati, S, Minervini, F, D'Antuono, I, Linsalata, V & Lattanzio, V 2012, 'Verbascoside, isoverbascoside, and their derivatives recovered from olive mill wastewater as possible food antioxidants', *Journal of Agricultural and Food Chemistry*, vol. 60, no. 7, pp. 1822–1829.
- Chen, J & Liu, H, 2020, 'Nutritional Indices for Assessing Fatty Acids: A Mini-Review', *International Journal of Molecular Sciences*, vol. 21, no. 16, pp. 5695–5719.
- Cherian, G, Wolfe, FW & Sim, JS 1996, 'Dietary oils with added tocopherols: effects on egg or tissue tocopherols, fatty acids, and oxidative stability', *Poultry Science*, vol. 75, no. 3, pp. 423–431.
- Connor, WE 2000, 'Importance of n-3 fatty acids in health and disease'. *The American Journal of Clinical Nutrition*, vol. 71, pp. 171S–175S.
- Cortinas, L, Villaverde, C, Galobart, J, Baucells, MD, Codony, R & Barroeta, AC 2004, 'Fatty acid content in chicken thigh and breast as affected by dietary polyunsaturation level', *Poultry Science*, vol. 83, no. 7, pp. 1155–1164.
- Crespo, N & Esteve-García, E 2001, 'Dietary fatty acid profile modifies abdominal fat deposition in broiler chickens', *Poultry Science*, vol. 80, pp. 71–78.
- Dal Bosco, A, Mugnai, C, Ruggeri, S, Mattioli, S & Castellini, C 2012, 'Fatty acid composition of meat and estimated indices of lipid metabolism in different poultry genotypes reared under organic system', *Poultry Science*, vol. 91, no. 8, pp. 2039–2045.
- Del Puerto, M, Cabrera, C & Saadoun, A 2017, 'A Note on Fatty Acids Profile of Meat from Broiler Chickens Supplemented with Inorganic or Organic Selenium'. *International Journal of Food Science*, vol. 2017, articleID7613069, 8 pages. <https://doi.org/10.1155/2017/7613069>.
- Den Hartigh, LJ 2019, 'Conjugated Linoleic Acid Effects on Cancer, Obesity, and Atherosclerosis: A Review of Pre-Clinical and Human Trials with Current Perspectives', *Nutrients*, vol. 11, no. 2, pp. 370–398.
- De Lorgeril, M & Salen, P 2004, 'Alpha-linolenic acid and coronary heart disease', *Nutrition, metabolism, and cardiovascular diseases*, vol. 14, no. 3, pp. 162–169.
- Di Renzo, JA, Casanoves, F, Balzarini, MG, Gonzalez, L, Tablada, M & Robledo, CW 2008, InfoStat, Versión 2008. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina.
- European Food Safety Authority (EFSA) 2010, Scientific opinion on dietary reference values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol, *EFSA Journal*, vol. 8, no. 3, pp. 1461–1568.
- Fernández, M, Ordóñez, JA, Cambero, I, Santos, C, Pin, C & de la Hoz L 2007, 'Fatty acid compositions of selected varieties of Spanish dry ham related to their nutritional implications', *Food Chemistry*, vol. 101, no. 1, pp. 107–112.
- Folch, J, Lees, M & Stanley, GHS 1957, 'A simple method for isolation and purification of total lipides from animal tissues', *Journal of Biological Chemistry*, vol. 226, no. 1, pp. 497–509.
- Gallinger, C 2015, 'Estabilidad oxidativa y calidad sensorial de carne de pollo enriquecida con ácidos grasos n-3 proveniente de fuentes de origen vegetal y animal, protegida con vitamina E y selenio orgánico', Tesis doctoral, Universitat Politècnica de València, España, vista 1 de abril, 2021, <http://hdl.handle.net/10251/59250>.
- Gerasopoulos, K, Stagos, D, Kokkas, S, Petrotos, K, Kantas, D, Goulas, P & Kouretas, D 2015, 'Feed supplemented with byproducts from olive oil mill wastewater processing increases antioxidant capacity in broiler chickens', *Food and Chemical Toxicology*, vol. 82, pp. 42–49.
- Glick, NR & Fischer, MH 2013, 'The Role of Essential Fatty Acids in Human Health', *Journal of Evidence-Based Complementary & Alternative Medicine*, Vol. 18, no. 4, pp. 268–289.
- Goering, HK & Van Soest, PJ 1970, 'Forage fiber analyses: Apparatus, Reagents, Procedures and Some Applications, in USDA-Agricultural Research Service (eds.), *Agricultural Handbook*, vol. 379, Washington, DC.
- Gutiérrez, AM 2015, 'Composición de ácidos grasos y peroxidación lipídica de mitocondrias y microsomas aislados de hígado, corazón y cerebro de aves de diferentes especies: relación con el tamaño corporal', Tesis doctoral, Universidad Nacional de La Plata, Argentina, vista 14 de junio, 2021. <http://sedici.unlp.edu.ar/handle/10915/45722>.
- Hodson, L & Fielding, BA 2013, 'Stearoyl-CoA desaturase: rogue or innocent bystander?', *Progress in Lipid Research*, vol. 52, no. 1, pp. 15–42.
- Jing, M, Gakhar, N, Gibson, RA & House, J D 2013, 'Dietary and ontogenic regulation of fatty acid desaturase and elongase expression in broiler chickens', *Prostaglandins, Leukotrienes, and Essential Fatty Acids*, vol. 89, pp. 107–113.
- Knekt, P, Jarvinen, R, Seppanen, R, Pukkala, E & Aromaa, A 1996, 'Intake of dairy products and the risk of breast cancer', *British Journal of Cancer*, vol. 73, pp. 687–691.
- Koba, K & Yanagita, T 2014, 'Health benefits of conjugated linoleic acid (CLA)', *Obesity Research & Clinical Practice*, vol. 8, no. 6, pp. e525–e532.
- Leskovec, J, Levart, A, Nemeč Svete, A, Peric, L, Đukic Stojic, M, Žikic, D, Salobir, J & Rezar, V 2018, 'Effects of supplementation with α -tocopherol, ascorbic acid, selenium, or their combination in linseed oil-enriched diets on the oxidative status in broilers', *Poultry Science*, vol. 97, no. 5, pp. 1641–1650.
- Logue, JA, De Vries, AL, Fodor, E & Cossins, AR 2000, 'Lipid compositional correlates of temperature-adaptive interspecific differences in membrane physical structure', *The Journal of Experimental Biology*, vol. 203, pp. 2105–2115.
- Long, S, Liu, S, Wu, D, Mahfuz, S & Piao, X 2020, 'Effects of Dietary Fatty Acids from Different Sources on Growth Performance, Meat Quality, Muscle Fatty Acid Deposition, and Antioxidant Capacity in Broilers', *Animals*, vol. 10, no. 3, pp. 508–521.
- López-Ferrer, S, Baucells, M. D, Barroeta, AC & Grashorn, MA 2001, 'N-3 enrichment of chicken meat. 1. Use of very long-chain fatty acids in chicken diets and their influence on meat quality: fish oil', *Poultry Science*, vol. 80, no. 6, pp. 741–752.
- Lopez-Huertas, E 2010, 'Health effects of oleic acid and long chain omega-3 fatty acids (EPA and DHA) enriched milks. A review of intervention studies', *Pharmacological Research*, vol. 61, no. 3, pp. 200–207.
- Majdoub-Mathlouthi, L, Saïd, B & Kraïem, K 2015, 'Carcass traits and meat fatty acid composition of Barbarine lambs reared on rangelands or indoors on hay and concentrate', *Animal*, vol. 9, no. 12, pp. 2065–2071.
- Manson, JE, Cook, NR, Lee, IM, Christen, W, Bassuk, SS, Mora, S. & Buring, JE 2019, 'Marine n-3 fatty acids and prevention of cardio-

- vascular disease and cancer', *New England Journal of Medicine*, vol. 380, no. 1, pp. 23-32.
- Mendonça, N, Sobrane Filho, ST, Oliveira, DH, Lima, E, Rosa, P, Faria, PB, Naves, LP & Rodrigues, PB 2020, 'Dietary chia (Salvia hispánica L.) improves the nutritional quality of broiler meat', *Asian Australasian Journal of Animal Sciences*, vol. 33, no. 8, pp. 1310-1322.
- Micha, R & Mozaffarian, D 2009, 'Trans fatty acids: effects on metabolic syndrome, heart disease and diabetes. Nature reviews', *Endocrinology*, vol. 5, no. 6, pp. 335-344.
- Mohd Ali, N, Yeap, SK, Ho, WY, Beh, B., Tan, SW & Tan, SG 2012, 'The promising future of chia, Salvia hispanica L', *Journal of Biomedicine and Biotechnology*, vol 2012, article ID171956, 9 pages, <http://doi.org/10.1155/2012/171956>.
- Natali, F, Siculella, L, Salvati, S & Gnoni, GV 2007, 'Oleic acid is a potent inhibitor of fatty acid and cholesterol synthesis in C6 glioma cells', *Journal of Lipid Research*, vol. 48, no. 9, pp. 1966-1975.
- Unnas, F, Privé, F, Salen, P, Hazane-Puch, F, Laporte, F, Fontaine, E, Rio, DD, Calani, L, Melegari, C, Bianchi, MA, Demeilliers, C & de Lorgeril, M 2014, 'Wheat aleurone polyphenols increase plasma eicosapentaenoic acid in rats', *Food Nutrition Research*, vol. 58, pp. 24604-24621.
- Paiva-Martins, F, Barbosa, S, Pinheiro, V, Mourão, JL & Outor-Monteiro, D 2009, 'The effect of olive leaves supplementation on the feed digestibility, growth performances of pigs and quality of pork meat', *Meat science*, vol. 82, no. 4, pp. 438-443.
- Papadomichelakis, G, Pappas, AC, Tsiplakou, E, Symeon, GK, Sotirakoglou, K, Mpekkelis, V & Zervas, G 2019, 'Effects of dietary dried olive pulp inclusion on growth performance and meat quality of broiler chickens', *Livestock Science*, vol. 221, pp. 115-122.
- Rahimi, S, Kamran Azad, S & Karimi Torshizi, M 2011, 'Omega-3 Enrichment of Broiler Meat by Using Two Oil Seeds'. *Journal of Agricultural Science and Technology*, vol. 13, pp. 353-365.
- Realini, CE, Pérez-Juan, M, Gou, P, Díaz, I, Sárraga, C, Gatellier, P, García-Regueiro, JA 2013, 'Characterization of Longissimus thoracis, Semitendinosus and Masseter muscles and relationships with technological quality in pigs. 2. Composition of muscles', *Meat Science*, vol. 94, pp. 417-423.
- Reboredo, GR, Gutiérrez, AM, Piergiacomi, VA, Zeinsteger, PA & Palacios, A 2010, 'Resistencia a la peroxidación lipídica en organelas de ovario e hígado de gallinas ponedoras' Brown Nick jóvenes', *Revista Veterinaria*, vol. 21, no. 2, pp. 86-92.
- Reyna, S, Gonzáles, C, Valenzuela, R & Villanueva, ME 2016, 'Effect of purple corn extract on gene expression of srebp-1, A5d y A6d in mammary gland of nursing rats supplemented with chia oil (Salvia Hispánica L.)', *Revista Chilena de Nutrición*, vol. 43, no. 3, pp. 296-302.
- Reyna, S, Valenzuela, R & Villanueva, ME 2018, 'Acción de flavonoides sobre la conversión de ácidos grasos poliinsaturados de cadena larga a partir de ácidos grasos esenciales', *Revista Chilena de Nutrición*, vol. 45, no. 2, pp. 153-162.
- Rietjens, SJ, Bast, A & Haenen, GR 2007, 'New insights into controversies on the antioxidant potential of the olive oil antioxidant hydroxytyrosol'. *Journal of Agricultural and Food Chemistry*, vol. 55, pp. 7609-7614.
- Rodríguez Perón, J M, Menéndez López, JR & Trujillo López, Y 2001, 'Radicales libres en la biomedicina y estrés oxidativo'. *Revista Cubana de Medicina Militar*, vol. 30, pp. 15-20.
- Rubio-Senent, F, de Roos, B, Duthie, G, Fernández-Bolaños, J & Rodríguez-Gutiérrez, G 2014, 'Inhibitory and synergistic effects of natural olive phenols on human platelet aggregation and lipid peroxidation of microsomes from vitamin E-deficient rats', *European Journal of Nutrition*, vol. 54, no. 8, pp. 1287-1295.
- Salazar-Vega, M, Rosado-Rubio, J, Chel-Guerrero, L, Betancur-Ancona, D & Castellanos-Ruelas, A 2009, 'Composición en ácido graso alfa linoléico (W3) en huevo y carne de aves empleando chíca (Salvia hispánica L.) en el alimento'. *Interciencia*, vol. 34, no. 3, pp. 209-213.
- SENASA 2015, Manual de bienestar animal. Un enfoque práctico para el buen manejo de especies domésticas durante su tenencia, producción, concentración, transporte y faena, Servicio Nacional de Sanidad y Calidad Agroalimentaria (SENASA), Buenos Aires, Argentina, viewed 9 September 2021, <https://www.argentina.gob.ar/senasa/bienestar-animal/manuales>.
- Sharma, R, Ahlawat, S, Aggarwal, R, Dua, A, Sharma, V & Tania, MS 2018, 'Comparative milk metabolite profiling for exploring superiority of indigenous Indian cow milk over exotic and crossbred counterparts', *Journal of Food Science and Technology*, vol. 55, pp. 4232-4243.
- Simopoulos, AP 1991, 'Omega-3 fatty acids in health and disease and in growth and development', *American Journal of Clinical Nutrition*, vol. 54, pp. 438-463.
- Simopoulos, AP 2008, 'The Importance of the Omega-6/Omega-3 Fatty Acid Ratio in Cardiovascular Disease and Other Chronic Diseases', *Experimental Biology and Medicine*, vol. 233, no. 6, pp. 674-688.
- Simopoulos, AP 2016, 'An Increase in the Omega-6/Omega-3 Fatty Acid Ratio Increases the Risk for Obesity', *Nutrients*, vol. 8, no. 3, pp. 128-235.
- Smink, W, Gerrits, WJJ, Gloaguen, M, Ruiter, A & van Baal, J 2012, 'Linoleic and α -linolenic acid as precursor and inhibitor for the synthesis of long-chain polyunsaturated fatty acids in liver and brain of growing pigs', *Animal*, vol. 6, no. 2, pp. 262-270.
- Tarladgis, BG, Watts, BM & Younathan, MT 1960, 'A distillation method for the quantitative determination of malonaldehyde in rancid foods', *Journal of the American Oil Chemists' Society*, vol. 37, pp. 44-48.
- Taşdelen, EÖ & Ceylan, N 2017, 'Effects of Dietary Inclusion of Oil Sources With or Without Vitamin E on Body Composition and Meat Oxidation Level in Broilers', *Brazilian Journal of Poultry Science*, vol. 19, pp. 103-116.
- Tomazini, U, Korošec, T, Voljc, M, Rezar, V, Levart, A & Salobir, J 2013, 'The potency of alpha- and gamma-tocopherol, and their combination, in reducing dietary induced oxidative stress in vivo and improving meat lipid stability in broilers', *Archiv fur Geflugelkunde*, vol. 77, no. 4, pp. 266-274.
- Tripoli, E, Giammanco, M, Tabacchi, G, Di Majo, D, Giammanco, S & La Guardia, M 2005, 'The phenolic compounds of olive oil: structure, biological activity and beneficial effects on human health', *Nutrition Research Review*, vol. 18, pp. 98-112.
- Ulbricht, TLV & Southgate, DAT 1991, 'Coronary heart disease: seven dietary factors', *Lancet*, vol. 338, pp. 985-992.
- Visioli, F & Galli, C 1998, 'The effect of minor constituents of olive oil on cardiovascular disease: new findings', *Nutrition Reviews*, vol. 56, pp. 142-147.
- Volpato, M & Hull, MA 2018, 'Omega-3 polyunsaturated fatty acids as adjuvant therapy of colorectal cancer', *Cancer Metastasis Reviews*, vol. 37, no. 2-3, pp. 545-555.
- Winiarska-Mieczan, A, Kwiecien, M, Kwiatkowska, K, Baranowska-Wójcik, E, Szwajgier, D & Zaricka, E 2020, 'Fatty acid profile, antioxidative status and dietary value of the breast muscle of broiler chickens receiving glycine-Zn chelates', *Animal Production Science*, vol. 60, no. 8, pp. 1095-1102.
- Yu, B, Tsai, CC, Hsu, JC & Chiou, PW 1998, 'Effect of different sources of dietary fibre on growth performance, intestinal morphology and caecal carbohydrases of domestic geese', *British Poultry Science*, vol. 39, no. 4, pp. 560-567.
- Zanini, SF, Colnago, GL, Bastos, MR, Pessotti, BMS, Casagrande, FP & Lima, VR 2006, 'Oxidative stability and total lipids on thigh and breast meat of broilers fed diets with two fat sources and supplemented with conjugated linoleic acid', *LWT-Food Science Technology*, vol. 39, pp. 717-723.