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PAPER

Effect of slaughtering age in different commercial chicken genotypes reared according to the organic system: 2. Fatty acid and oxidative status of meat

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Abstract

The fatty acid profile and the oxidative status of meat of three different commercial chicken genotypes organically reared and slaughtered at two different ages (70 and 81 days) were compared. The genotypes used were Naked Neck (CN1 strain), Kabir (KR4 strain) and Ross 308 (R). All animals were raised in the facilities of a big Italian company, in field conditions. Genotype and slaughtering age affected the main fatty acids and the antioxidant profile of meat. Concerning the content of total saturated fatty acids (SFA), the highest value was observed in R chicks. The CN1 birds showed the lowest SFA values, whereas the KR4 showed intermediate values. Polyunsaturated fatty acids (PUFA) showed a different trend at the two slaughter ages. At 71 days medium-growing chickens had lower values, while at 81 days CN1 birds reached the highest value. The CN1 chickens exhibited lower concentrations of linolenic acid, but higher long chain PUFA derivatives. However, the meat of these chickens showed a lower lipid stability despite a higher antioxidant content probably due to the kinetic behaviour and the resulting high oxidative metabolism. This finding is of importance since health concerns over fatty acid profile are among the main factors contributing to the decline of meat intake. Regarding the slaughtering age, the results of this trial demonstrate that at older age chickens show a better fatty acid profile under a nutritional point of view even if the oxidative status worsens.

Introduction

The organic system requires the use of strains appropriate to free range mainly because of foraging behaviour and immune response. This notwithstanding, commercial poultry farms often use fast-growing genotypes not suitable for the organic system for economic reasons [high body weight (BW), carcass and breast yield].

Previous studies (Dal Bosco *et al.*, 2012; Fanatico *et al.*, 2005) underlined that fast-growing chicks are not appropriate for extensive rearing conditions, as they exhibit muscular-skeletal problems, very low motor activity and foraging behaviour (Castellini *et al.*, 2002a, 2002b; Dal Bosco *et al.*, 2010; Sirri *et al.*, 2010). On the contrary, slow-growing strains generally have a remarkable consumption of fresh forage which implies a reliable intake of antioxidant compounds (tocopherols, tocotrienols and carotenoids; Kerry *et al.*, 2000) and α-linoleic acid (C18:3n-3, ALA) which is partly converted to long-chain derivatives [C20:5n-3, eicosapentaenoic acid (EPA) and C22:6n-3, docosahexaenoic acid (DHA)]. Indeed, fatty acids of the meat derive from dietary uptake, and/or bioconversion; specifically, the bioconversion of long-chain polyunsaturated fatty acids (LCP) of the n-3 series includes endoplasmic D^6-desaturation, chain elongation and D^5-desaturation of ALA to EPA, which is subsequently converted to docosapentaenoic acid (C22:5n-3, DPA). The final metabolite, DHA, is synthesised by chain elongation, D^6-desaturation and peroxisomal b-oxidation of DPA (Poureslami *et al*., 2010).

In a previous study (Dal Bosco *et al.*, 2014a), welfare, carcass traits and meat quality of three commercial genotypes reared under organic system and slaughtered at two different ages were investigated. In the present trial we analyse the fatty acid profile and oxidative status of meat from chickens reared in field condition according to the organic system.

Materials and methods

Animals, housing and feeding

The trial was conducted in the facilities of an European supplier of organic broilers in Central Italy. The used genotypes were Naked Neck (strain CN1), Kabir (strain KR4) and Ross 308 (R); all the birds were furnished by a commercial hatchery (Avicola Berlanda, Carmignano di Brenta, Italy). Kabir and CN1 were of both sexes, while R were only females due to the too high BW reachable by males.

The trial was carried out from April to June 2012 in the facilities of the company in production units of 3000 birds which were also vaccinated against Marek, Newcastle diseases and coccidiosis (Paracox®-8). At 21 days of age all the birds were put in 9 covered shelters (0.10 m^2/bird) with straw litter and access to a grass paddock (4 m^2/bird); feeders and drinkers were available both outdoor and indoor (three replications). Chickens were fed *ad libitum* the same starter (1-21 days) and finisher (22 days to slaughter) diets, containing 100% certified organic ingredients (Table 1). Fatty acids, tocopherol and carotenoid profile of the diets are presented in Table 2. Chemical analyses of diet were done according to AOAC methods (1995).

Blood sampling

Before slaughtering, blood samples were taken from the brachial vein in ten chickens per group and collected in heparinised vacutainers and centrifuged at 1500 g for 10 min at +4°C, to measure the *in vivo* oxidative status. After collection, blood samples were immediately sent to the laboratory of the Department of Agricultural, Food and Environmental Sciences, University of Perugia, Italy where they were centrifuged and frozen at -80°C until analysis.

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Key words: Chickens, Genotype, Fatty acid, Lipid oxidation, Organic system.

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Carcass dissection and sampling

At 70 and 81 days of age, 20 chickens per genotype were slaughtered in the processing plant of the farm, 12 h after feed withdrawal. Chickens were stunned by electrocution (110 V; 350 Hz) before killing. After killing, carcasses were plucked, eviscerated (non-edible viscera: intestines, proventriculus, gall bladder, spleen, oesophagus and full crop) and stored for 24 h at +4°C. Head, neck, legs, edible viscera (heart, liver, gizzard), and fat (perivisceral, perineal and abdominal) were removed in order to obtain the ready-to-cook carcass (Romboli *et al*., 1996). From the carcass, the *Pectoralis major* muscles were excised for successive analysis.

Analytical determinations

Feed and meat fatty acids were quantified as methyl esters (FAME) with a Mega 2 Carlo Erba Gas Chromatograph, model HRGC (Carlo Erba Agents, Milan, Italy), using a D-B wax capillary column (0.25 mm Ø, 30 m long). Fatty acid methyl ester peaks were identified by comparing the retention time with the commercially available FAME standards. The fatty acid compositions were calculated using the peak areas and expressed on percentage basis. The average amount of each fatty acid was used to calculate the sum of the saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids.

Peroxidability index (PI) was calculated according to the equation proposed by Arakawa and Sagai (1986):

$$PI=(\%\ monoenoic\ 0.025)+(\%\ dienoic\ 1)+(\%\ trienoic\ 2)+(\%\ tetraenoic\ 4)+(\%\ pentaenoic\ 6)+(\%\ hexaenoic\ 8)$$

The amount of each fatty acid was used to calculate the indexes of atherogenicity (AI) and thrombogenicity (TI), as proposed by Ulbricht and Southgate (1991), and the hypocholesterolaemic/hypercholesterolaemic ratio (HH), as suggested by Santos-Silva *et al.* (2002):

$$AI=(C12{:}0+4\ C14{:}0+C16{:}0)/[(MUFA+\Sigma(n\text{-}6)+\Sigma(n\text{-}3)];$$
$$TI=(C14{:}0+C16{:}0+C18{:}0)/[(0.5\ MUFA+0.5\ (n\text{-}6)+3\ (n\text{-}3)+(n\text{-}3)/(n\text{-}6)];$$
$$HH=[(C18{:}1n\text{-}9+C18{:}2n\text{-}6+C20{:}4n\text{-}6+C18{:}3n\text{-}3+C20{:}5n\text{-}3+C22{:}5n\text{-}3+C22{:}6n\text{-}3)/(C14{:}0+C16{:}0)]$$

The extent of plasma and muscle lipid oxidation was evaluated by a spectrophotometer set at 532 nm (UV-2550; Shimadzu, Kyoto, Japan) which measured the absorbance of thio-barbituric acid-reactive substances (TBARS), and a 1,1,3,3-tetraethoxypropane calibration curve in sodium acetate buffer (pH=3.5; Dal Bosco *et al*., 2009). Oxidation products were quantified as malondialdehyde index (mg MDA/g muscle). Tocopherol content and retinol of plasma and meat were quantified by high-performance liquid chromatography (HPLC) (Hewavitharana *et al.,* 2004). Briefly, 5 mL of distilled water and 4 mL of ethanol were added to 2 g of sample and then vortexing for 10 sec. After mixing, 4 mL of hexane containing butylated hydroxytoluene (200 mg/L) were added and the mixture was carefully shaken and centrifuged. An aliquot of supernatant (3 mL) was dried under a stream of nitrogen and then redissolved in 300 μL of acetonitrile. 50 μL were injected into the HPLC (PU-1580, equipped with an autosampler sistem AS 950-10; Jasco Int. Co., Tokyo, Japan) on a Ultrasphere ODS column (250 4.6 mm internal diameter, 5 μm particles size; CPS Analitica, Milan, Italy). Tocopherols (α-tocopherol and its isomers β+γ and δ) were identified using a FD detector (FP-1525, excitation and emission wavelength of 295 nm and 328 nm, respectively; Jasco) and quantified using external calibration curves prepared with increasing amounts of pure tocopherols in ethanol.

Statistical analyses

A linear model (StataCorp, 2005; GLM procedure) was used to evaluate the interactive effect of genetic strain and slaughtering age. Differences were assessed by ANOVA test with a Bonferroni multiple *t*-test. Differences with at least a $P<0.05$ value were considered statistically significant.

Results and discussion

The fatty acid and antioxidant profile of the finisher diet is shown in Table 2.

Polyunsaturated fatty acids represented the main class of fatty acids and linoleic acid (LA) was the main n-6 PUFA (47.7%). Oleic acid was the main MUFA (24.9%) and palmitic acid the principal SFA (12.9%). Although α-tocopherol was added in the diet as additive (30 mg/kg), γ-tocopherol was the main tocopherol isoform because it is the isomer mostly represented in corn (Rocheford *et al*., 2002) and soybean (Seguin *et al*., 2009). The same applies to lutein and zeaxanthin, the main carotenoids in finisher diets, mostly due to corn meal.

The fatty acid profile of breast meat is

Table 1. Formulation and chemical composition of the finisher diet.

	Finisher diet
Ingredients, %	
Corn	46.0
Full fat soybean	12.5
Wheat	20.0
Soybean meal°	14.0
Alfalfa meal	2.8
Gluten feed	2.0
Vitamin-mineral premix#	1.0
Dicalcium phosphate	1.0
Sodium bicarbonate	0.5
NaCl	0.2
Chemical composition	
DM, %	90.80
CP, % DM	18.05
EE, % DM	4.98
CF, % DM	4.01
Ash, % DM	5.59
NDF, % DM	10.11
ADF, % DM	5.06
Cellulose, % DM	3.56
ADL, % DM	1.11
Hemicellulose, % DM	5.05
ME,§ MJ kg^{-1}	12.98

DM, dry matter; CP, crude protein; EE, ether extract; CF, crude fibre; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent liquid; ME, metabolisable energy. °From conventional crops. #Amounts per kg: vitamin A, 11,000 U; vitamin D_3, 2000 U; vitamin B_1, 2.5 mg; vitamin B_2, 4 mg; vitamin B_6, 1.25 mg; vitamin B_{12}, 0.01 mg; α-tocopheryl acetate, 30 mg; biotin, 0.06 mg; vitamin K, 2.5 mg; niacin, 15 mg; folic acid, 0.30 mg; pantothenic acid, 10 mg; choline chloride, 600 mg; Mn, 60 mg; Fe, 50 mg; Zn, 15 mg; I, 0.5 mg; Co, 0.5 mg. §Estimated following Carrè and Rozo (1990).

Table 2. Antioxidant and fatty acid profile of the finisher diet.

Antioxidants, mg/100 g	
Lutein	1.03
Zeaxanthin	0.40
α-tocopherol	6.11
δ-tocopherol	2.54
γ-tocopherol	10.25
α-tocotrienol	2.03
γ-tocotrienol	4.10
SFA, % of total fatty acids	
C14:0	1.2
C16:0	12.9
C18:0	3.9
Total	18.0
MUFA, % of total fatty acids	
C16:1	0.6
C18:1	24.9
Total	25.5
PUFA, % of total fatty acids	
C18:2n-6	47.7
C18:3n-3	8.8
Total	56.5

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

shown in Table 3. Both genotypes and slaughtering age affected the main fatty acids of meat. The higher content of SFA was observed in the R chicks, mainly due to the higher amounts of C16:0 and C18:0. The CN1 birds showed the lower SFA values, whereas KR4 was intermediate.

Monounsaturated fatty acid concentration, mainly represented by C18:1n-9, showed the highest levels in CN1 chickens. The low MUFA level, which in chickens depends both on the endogenous synthesis and the gut absorption from the diet, was significantly lower in R birds and at 81 days of age.

Polyunsaturated fatty acids showed a different trend at the two slaughter ages considered: at 70 days of age medium-growing chickens (CN1 and KR4) showed the lowest values, while at 81 days the highest ones. The total n-3 fatty acid and, above all, the LCP derivatives were higher in the medium-growing than in fast-growing strain (R). The lower n-3 amount of these latter birds could be due to different factors: the scarce/null intake of grass and the lower Δ^6-desaturase activity in line with slow-growing lines (Dal Bosco *et al.*, 2012). Indeed, it is widely known that the rate-limiting step in the enzymatic LCP biosynthesis is thought to be Δ^6-desaturase (Yamazaki *et al.*, 1992). On the other hand, medium-growing lines probably eat much more grass than fast-growing ones (Castellini *et al*., 2002b) and the competition for LCP synthesis is more advantageous for n-3 series since grass major PUFA is ALA. Indeed, ALA and LA elongation and desaturation require the same desaturation pathways (Lands, 1992) and higher ALA intake could contribute to the different n-3 profile of medium-growing chickens.

Moreover, the CN1 meat exhibited lower concentrations of ALA, but higher LCP derivatives. As observed in a previous study (Dal Bosco *et al.*, 2012), there is no direct correlation between grass intake and ALA level in the meat. Naked Neck chickens probably ingested more ALA but simultaneously had a higher conversion of ALA into LCP as confirmed by the higher level of EPA, DPA and DHA in the meat. Also, Ponte *et al*. (2008) showed that forage consumption in broiler chickens do not contribute to improve ALA levels in breast meat, while desaturation and elongation of this precursor contribute to improve LCP derivatives and n-6/n-3 ratio.

Older birds showed higher LCP and total PUFA levels (Table 4). These results are in line with Poureslami *et al.* (2010) whose study of the effect of age on fatty acid metabolism revealed that chickens slaughtered at 42 days of age had higher values for PUFA intake, PUFA apparent digestibility and ALA and LCP derivates accumulation when compared with the 7-14 d age period, lied to lower values for b-oxidation. Authors justified this trend with the fact that young birds had a higher metabolism rate compared to the older ones.

Naked Neck chickens showed the best values of total PUFA/total SFA and n-6/n-3 ratio at both considered ages; even peroxidability, atherogenicity and thrombogenicity indexes, as well as hypocholesterolaemic/hypercholesterolaemic fatty acid ratio showed a similar trend among genotypes but without a clear trend with the slaughtering ages.

The tocopherols, tocotrienols, carotenoids and the oxidative status of plasma are presented in Table 5. α-tocopherol was the most represented vitamin E isoform in blood and it is considered as the most active antioxidant. The other two isoforms play a role in reduction of inflammation (Singh *et al.*, 2005). α- and γ-tocotrienol were the only tocotrienols detected in plasma. Tocotrienols, apart for their antioxidant property, are well known for their hypo-

Table 3. Fatty acid profile of *Pectoralis major* muscle at different ages.

	70 days			81 days			Pooled SE
	CN1	KR4	R	CN1	KR4	R	
SFA							
C14:0	0.87^a	1.56^b	1.64^b	0.84^a	1.69^b	1.31^{ab}	0.41
C16:0	29.2	30.1	30.7	28.9	30.0	31.2	3.35
C18:0	9.18^a	10.1^{ab}	11.0^{ab}	10.5^{ab}	11.1^{ab}	12.3^b	2.89
Others	3.32^c	2.06^a	1.99^a	2.65^{bc}	2.19^a	2.41^b	0.48
Total	42.5^a	43.8^a	45.3^{bc}	42.9^a	45.0^b	47.3^c	2.59
MUFA							
C14:1n-6	0.12^b	0.04^a	0.01^a	0.13^b	0.05^a	0.03^a	0.06
C16:1n-7	3.35^e	3.18^d	1.41^a	2.71^b	2.85^c	1.32^a	0.13
C18:1n-9	24.3^{bc}	23.5^b	22.6^a	22.5^a	21.9^a	21.7^a	1.87
Others	0.22^b	0.22^b	0.17^a	0.23^b	0.15^a	0.15^a	0.07
Total	27.9^b	26.8^b	24.1^a	25.5^{ab}	24.9^a	23.2^a	2.06
Polyenoic n-6							
C18:2	20.8^{ab}	20.5^a	20.7^{ab}	22.3^b	21.3^{ab}	21.2^{ab}	1.95
C20:2	0.84^b	0.34^a	0.28^a	1.35^c	1.55^c	0.39^a	0.18
C20:3	0.26^b	0.24^b	0.16^a	0.23^b	0.31^b	0.27^b	0.11
C20:4	4.06^{ab}	5.07^b	7.42^c	3.05^a	3.21^a	5.12^b	1.51
Total	25.9^a	26.1^a	28.5^b	26.9^a	26.3^a	26.9^a	1.29
Polyenoic n-3							
C18:3	0.62^a	0.93^b	0.58^a	0.75^{ab}	0.90^b	1.21^c	0.21
C20:5	0.18^b	0.12^{ab}	0.09^a	0.59^d	0.26^c	0.13^{ab}	0.08
C22:5	0.94^c	0.64^b	0.28^a	1.05^d	0.92^c	0.31^a	0.07
C22:6	0.95^c	0.67^b	0.60^b	1.29^d	0.85^c	0.48^a	0.12
Total	2.72^c	2.41^b	1.57^a	3.76^d	3.00^c	2.16^b	0.36
Total PUFA	28.6	28.5	30.1	30.6	29.3	29.1	2.68

CN1, Naked Neck; KR4, Kabir; R, Ross 308; SE, standard error; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. [a-e]Different letters in the same row denote significant differences ($P<0.05$). Values are expressed as percentage of total fatty acids.

cholesterolemic, anti-cancer and neuroprotective effects that are often not exhibited by tocopherols (Qureshi *et al*., 1996).

Despite the high level of non-α-tocopherol isoforms in diets (about 75% data not shown), in plasma the α-tocopherol represents about 87% of all vitamin E isoform. It should be underlined that oral supplementation of α-tocopherol reduces all the other isoforms because only α-tocopherol is selectively bound to a transfer protein (Oram *et al*., 2001). The presence of such a protein that preferentially selects α-tocopherol seems to explain why all other vitamin E isoforms have a lower biological activity than α-tocopherol. Moreover, tocotrienols belong to a group of phenolic compounds with a lower tissue retention and half-life in respect to α-tocopherol (Qureshi *et al.*, 1996). The plasma of more active chickens (CN1 followed by KR4) had a higher amount of malondialdehyde (Castellini *et al.*, 2006) despite the higher antioxidant content. This fact could be due to the higher oxidative metabolism and free-radical production of this strain which is not fully counterbalanced by the response of organism (Alessio *et al.*, 2000). More kinetic chicks, despite the higher antioxidant intake (Dal Bosco *et al.*, 2010, 2014b), require further antioxidant protection to protect the high LCP level of the body. The antioxidant profile and the oxidative status of *Pectoralis major* are presented in Table 6. The meat followed the same trend of blood plasma: more active chickens had a considerable amount of malondialdehyde (Castellini *et al.*, 2002b; 2006) despite the higher antioxidant content. In agreement with Hewavitharana *et al.* (2004), α-tocopherol was the principal isomer of chicken meat, followed by γ-tocopherol, α-tocotrienol, α- and γ-tocotrienol. β-tocotrienol was coeluted with γ-tocotrienol, while δ-tocotrienol was present only in trace. Ponte *et al.* (2008) did not found any difference in the antioxidant profile of broiler meat when chickens were supplemented with dehydrated forage: the use of fast-growing chickens at very early age (28 days) could explain the difference obtained.

Table 4. Fatty acid indexes of *Pectoralis major* muscle at different ages.

	70 days			81 days			Pooled SE
	CN1	KR4	R	CN1	KR4	R	
P/S	0.67[ab]	0.65[ab]	0.66[ab]	0.71[b]	0.65[ab]	0.62[a]	0.08
n-6/n-3	9.54[b]	10.85[c]	18.19[e]	7.16[a]	8.79[b]	12.48[d]	0.26
Peroxidability index	53.50[a]	54.28[a]	59.15[b]	55.43[ab]	51.00[a]	51.07[a]	3.65
Atherogenicity index	0.58	0.66	0.69	0.57	0.68	0.70	0.12
Thrombogenicity index	1.44[b]	1.11[ab]	1.39[b]	1.07[a]	1.23[ab]	1.42[b]	0.23
HH	1.72	1.62	1.61	1.73	1.55	1.54	0.31

CN1, Naked Neck; KR4, Kabir; R, Ross 308; SE, standard error; [a-e]Different letters in the same row denote significant differences ($P<0.05$). Values are expressed as percentage of total fatty acids.

Table 5. Oxidative status of plasma at different ages.

	70 days			81 days			Pooled SE
	CN1	KR4	R	CN1	KR4	R	
TBAR	24.5[c]	20.3[b]	21.9[bc]	20.4[b]	17.8[a]	18.7[a]	2.04
α-tocopherol	10.1	6.81	6.74	10.8	10.6	9.48	5.80
δ-tocopherol	0.29	0.30	0.30	0.35	0.37	0.32	0.06
γ-tocopherol	0.78	0.62	0.53	0.44	0.50	0.44	0.32
α-tocotrienol	0.25[ab]	0.18[ab]	0.15[a]	0.30[ab]	0.28[ab]	0.27[ab]	0.10
γ-tocotrienol	0.10	0.11	0.09	0.12	0.11	0.10	0.02
Lutein+zeaxantin	31.2[ab]	28.3[a]	29.4[a]	41.0[c]	34.6[b]	34.9[bc]	3.41

CN1, Naked Neck; KR4, Kabir; R, Ross 308; SE, standard error; TBAR, thio-barbituric acid-reactive substances. [a-c]Different letters in the same row denote significant differences ($P<0.05$). Values are expressed as nmol/mL.

Table 6. Oxidative status of *Pectoralis major* muscle at different ages.

	70 days			81 days			Pooled SE
	CN1	KR4	R	CN1	KR4	R	
TBAR, mg/g	0.16[a]	0.15[a]	0.11[a]	0.25[b]	0.16[a]	0.13[a]	0.04
α-tocopherol, ng/g	469.2[ab]	418.5[a]	435.2[a]	589.9[b]	526.8[b]	525.4[b]	45.8
δ-tocopherol, ng/g	22.0[c]	12.9[a]	16.8[b]	34.1[c]	15.4[ab]	15.7[ab]	2.69
γ-tocopherol, ng/g	31.8[a]	25.7[a]	51.0[b]	38.4[b]	56.8[cd]	61.7[d]	5.02
α-tocotrienol, ng/g	23.7[c]	16.5[a]	18.9[a]	34.6[d]	32.4[d]	31.3[d]	2.30
γ-tocotrienol, ng/g	14.4[c]	11.5[ab]	10.0[a]	14.7[c]	12.6[b]	12.5[b]	1.25
Lutein+zeaxantin, ng/g	31.2[ab]	28.3[a]	29.1[a]	41.0[c]	34.6[b]	34.9[b]	3.41

CN1, Naked Neck; KR4, Kabir; R, Ross 308; SE, standard error; TBAR, thio-barbituric acid-reactive substances. [a-d]Different letters in the same row denote significant differences ($P<0.05$).

More consideration to antioxidant stability of organic meat should be devoted by improving the pasture allowance or by adding antioxidants to the diets.

Conclusions

In conclusion, the results of this study indicate that both genotypes and age affect the fatty acid content of chicken breast. In organic farming, chicken genotype plays a fundamental role in meat nutritional value (fatty acid, antioxidant, oxidative stability) owing to its peculiar foraging behaviour, metabolism and kinetic activity. This finding assumes considerable importance as health concerns over fat intake are among the main factors contributing to the decline of meat intake. Regarding the slaughtering age, the results of this trial demonstrate that older chickens show a better fatty acid profile from a nutritional point of view even if the oxidative status gets worse. These results open new research perspectives on management and nutrition techniques in order to maintain a good oxidative status and ultimately provide an optimal fatty acid profile to the consumer.

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