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Effect of dietary supplementation of Spirulina (*Arthrospira platensis*) and Thyme (*Thymus vulgaris*) on rabbit meat appearance, oxidative stability and fatty acid profile during retail display

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a b s t r a c t

The objective of this study was to evaluate the effect of Spirulina and Thyme supplementation on rabbit meat during retail display. At weaning 294 rabbits were allocated to 7 different treatments (42 rabbits/treatment). Rabbits of the control group (C) received a diet without any supplementation throughout the experiment (5–11 weeks of age). The other groups were fed diets containing 5% Spirulina (S), 3% Thyme (T) or both sup- plements (ST) for the whole trial (5–11 weeks; treatments S, T and ST), or for a part of the growing period (8–11 weeks; treatments C–S, C–T and C–ST). Colour parameters, pH, water holding capacity and drip loss were determined on fresh and stored *Longissimus dorsi* muscle of 5 rabbits/treatment. Spirulina- and Thyme-supplemented diets had a significant effect on redness and yellowness of *Longissimus dorsi*. Drip loss was significantly reduced in C–T and T groups that also showed the highest content of α-tocopherol and $n-3$ fatty acids content and the lower lipid oxidation.

Keywords: Spirulina Thyme Rabbit meat Physical traits Lipid oxidation

1. Introduction

Different studies show that the main quality aspects of consumer's choice for the purchase of meat are taste, tenderness, juiciness, fresh- ness, leanness, healthiness and nutritious (Grunert, 1997). Appearance, in particular colour and loss of exudates, determines how consumers perceive quality and influences purchasing behaviour (Resurreccion, 2003). In the specific case of rabbit meat, which is very rich in un- saturated fatty acids (Dalle Zotte, 2002), a certain degree of lipid oxida- tion, mainly during processing and storage (Castellini, Dal Bosco, & Bernardini, 1998; Cavani & Petracci, 2004; Dalle Zotte, 2002), is expected with a detrimental effect on its physical characteristics, like colour and water holding capacity. To counteract this process, together with meat appropriate modified atmosphere packaging, there has been an increasing interest in the use of antioxidants in rabbit feed formulas (Corino, Pastorelli, Pantaleo, Oriani, & Salvatori, 1999; Dal Bosco, Castellini, Bianchi, & Mugnai, 2004). Synthetic antioxidants were widely used in the meat industry, but consumer concerns over safety and toxicity pushed the food industry to find natural sources (Coronado, Trout, Dunshea, & Shaha, 2002). In rabbit, many studies were carried out to evaluate the effect of dif- ferent antioxidants derived from olive oil (Lopez-Bote, Rey, Sanz, Gray, & Buckley, 1997), oats (Lopez-Bote, Sanz, Rey, Castaño, & Thos, 1998), soy-isoflavones (Yousef, Kamel, Esmail, & Baghdadi, 2004), oregano- essential oils (Botsoglou, Florou-Paneri, Christaki, Giannenas, & Spais, 2004), grape polyphenols (Sgorlon, Stradaioli, Stefanon, Altimer, & Della Loggia, 2005), grape pomace (Eid, 2008), olive pomace (Dal Bosco et al., 2012), red quebracho tannins (Dalle Zotte & Cossu, 2009), chestnut hydrolysable tannins (Dalle Zotte et al., 2012), alfalfa polysac- charides (Liu, Dong, Tong, Xu, & Zhang, 2011), algae (Peiretti & Meineri, 2011) and green tea (Eid, Zeweil, Ahmed, Basyony, & Farok, 2011).

Spirulina (*Atrhrospira platensis*) is a rich source of phycocyanin, an antioxidant biliprotein pigment, and carotenoids (Belay, Kato, & Ota, 1996; Cheong et al., 2010). Thyme (*Thymus vulgaris*) essential oil contains more than 60 ingredients, which are known to have anti- oxidant properties and antimicrobial activity (Rota, Herrera, Martínez, Sotomayor, & Jordán, 2008).

On the basis of these considerations, in this trial the main rabbit meat traits for assessing the consumer choices (colour, drip loss during a simulated retail display) at time of purchase was investigated. This study is a part of an extensive research aimed to evaluate the effect of the dietary supplementation (between 5 and 11 or 8 and 11 weeks of age) of Spirulina (*Arthrospira platensis*, 5%) powder and/or Thyme (*Thymus vulgaris*, 3%) dried leaves on live performance, health status, and carcass and meat quality of growing rabbits, where this part fo- cused on the oxidative status and fatty acid profile of rabbit meat during a simulated retail display. The dietary levels of the two used antioxi- dants were selected on the basis of the few previous experiments on this issue (Nieto, Díaz, Bañón, & Garrido, 2010; Peiretti & Meineri, 2008, 2009, 2011).

Materials and Methods

1.1. Animals and experimental design

The experiment was conducted at the experimental rabbit farm of Kaposvár University (Hungary) using maternal line rabbits (n = 294). Rabbits received a control diet (C) from the age of 3 weeks. After weaning (5 weeks of age), they were randomly sorted to 7 groups (42 rabbits/group) and housed in wire net cages (0.61 × 0.32 m, 16 rabbits/m^2) until 11 weeks of age, when they were slaughtered. Rabbits of the control group received a diet throughout the experiment without any supplementation (C diet). In the other groups the diets were completed by 5% Spirulina (S), 3% Thyme (T) or by both (ST) for the whole (5–11 weeks of age; groups: S, T, ST), or for a part of the growing period (8–11 weeks of age; groups: C–S, C–T, C–ST) (Table 1). The latter was conceived to evaluate a short-period of natural additives supple- mentation to reduce the feeding costs. Water and feed were available *ad libitum* for every group and the diets did not contain medication. The applied temperature and lighting schedule in the rabbitry were 15–18 °C and 16 L:8D, respectively.

1.2. Collection and analytical determinations

At 11 weeks of age 5 rabbits per group, with a live weight close to the average of the group (2562 g ± 10%) (Gerencsér et al., 2012) were selected and, after 12 hours feed withdrawal, slaughtered; ani- mals did not undergo transport. Following electro-stunning, rabbits were killed by cutting the carotid arteries and jugular veins. After 24 h carcass refrigeration at +4 °C, the two sides of *Longissimus dorsi* (LD) muscle were removed and carefully freed from connective and adipose tissues. The same day, samples were transported refrigerated to the Department of Applied Biology (Perugia, Italy) to be analyzed. The day after, on the left LD muscle side, pH, colour parameters, water holding capacity (WHC), antioxidants content, oxidative processes (TBARs) and fatty acid (FA) profile were determined as described below.

The right side of LD muscle was weighed and left whole for the determination of drip loss.

All the samples were successively placed on plastic foams, over- wrapped with PVC film (600 cm^2) and displayed at +4 °C under continuous cool white fluorescent illumination (2300 lux). All the analyses were conducted at day 1 and again at days 3, 6 and 9, where- as the FA profile determination was repeated only at the end of the storage period (day 9).

The pH was measured with a Knick digital pHmeter (Broadly Corp., Santa Ana, CA, USA) after grinding 1 g of muscle into 10 mL of distilled water for 30 s (

Korkeala, Mäki-Petais, Alanko, & Sorvettula, 1986).

The colour parameters (L*, a*, b*) were evaluated using a tristimulus analyser (Minolta Chroma Meter CR-200; Azuchi-Machi Higashi-Ku, Osaka 541, Japan) with the CIElab (1976). The L*a*b* color system con- sists of a luminance or lightness component (L*) and two chromatic components: the a* which goes from green (-a*) to red (+a*) and the b* which ranges from blue (-b*) to yellow (+b*). The colorimeter was calibrated using a standard pink plate. It has an 8 mm diameter measuring area and uses diffuse illumination and 0° viewing angle (spectral component included) for accurate measurement of a wide variety of subjects.

The WHC was estimated by centrifuging 1 g of muscle for 4 min at 1500 ×*g* and determining the residual water by drying the sample at 70 °C overnight (Cyril, Castellini, & Dal Bosco, 1996). Meat tocopherol (α-tocopherol and its isoform β+γ and δ) and retinol contents were quantified by HPLC according to Hewavitharana, Lanari, and Becu (2004). In particular, 500 μL of distilled water and 1 mL of ethanol were added to 500 μg of sample and then vortexed for 10 s. Successively, 0.2 mL hexane and butylhydroxytoluene (0.01%) were added and the mixture was carefully shaken and centrifuged. An aliquot of supernatant (0.8 mL) was taken and injected into the HPLC (CM 4000, Milton Roy, Riviera Beach, FL, USA), using a silica column (Beckman, Fullerton, CA, USA). Fluorescence detection was performed with a spectrofluorimeter (excitation and emission wavelengths of 292 nm and 330 nm, respectively). The extent of muscle lipid peroxidation was evaluated by a spec- trophotometer (set at 532 nm, Hitachi U-2000, Theodor - Heuss Anlage 12, Mannheim, F.R. Germany), which measured the absor- bance of thio-barbituric acid-reactive substances (TBARs), and a tetraethoxypropane calibration curve in sodium acetate buffer (pH = 3.5, Dal Bosco et al., 2009). Oxidation products were quantified as malondialdehyde (MDA) equivalents (mg MDA/kg muscle). The FA profile of meat was determined by gas-chromatography (Fisons mega 2, equipped with a flame ionization detector; Fisons In-

struments S.p.A., Rodano, Milano, Italy) after lipid extraction (Folch, Lees, & Stanley, 1957) and consecutive hot derivatization with a

methanolic solution of sulfuric acid (3%). Separation of the resulting fatty acid methyl esters (FAME) was carried out on an Agilent (J&W) capillary column (30 m × 0.25 mm ID) coated with a DB-Wax sta- tionary phase (film thickness of 0.25 mm). The individual FAME were identified by reference to the retention time of authentic FAME standards. The FA composition of the samples was expressed as a per- centage of total FA and calculated with Chrom-Card software.

1.3. Statistical analysis

Meat characteristics were evaluated with a linear model for the analysis of repeated measures estimating the interactive effect of time (1.9 days) × treatment (StataCorp, 2005 - GLM procedure). The statistical significance of differences was assessed by a multiple t-test.

1. Results and discussion

In Table 2, the physical characteristics of the LD muscle are presented. Independently to the dietary treatment, storage at simulated retail display conditions significantly increased pH (P b 0.0001; data

not shown). During storage, pH variations depend on two opposite events: the hydrolysis of proteins, with NH_3 release, and the hydrolysis of lipids with release of FA (Cabanes, Ouhayoun, & Gilbert, 1996). We observed the maximum pH value in all groups after six days of storage and successively a reversal trend (average pH values = 5.83, 5.88, 5.93 and 5.90, for day 1, 3, 6 and 9, respectively), probably for the above mentioned release of free FA. Concerning WHC it should be noted that the refrigeration for long periods should reduce it due to membrane breakage; however we observed an opposite situation with an improvement of WHC during storage, probably due to the higher pH and to the lower water content of the meat. The LD meat colour was affected by the storage time with an increase of L*, a* and b* values in accordance with the findings of Cabanes-Roiron, Ouhayoun, and Gilbert (1994). Concerning the effect of Spirulina and Thyme, meat colour differences were mainly observed on a* (redness) and b* (yellowness), even if it did not always reach the statistical significance. In particular, C–T and T treatments showed the lower values for a* at day 1 of dis- play, as well as at the end of storage. The a* value lowering observed only in rabbits fed T diet, could be explained by a less intense oxidation of myoglobin with consequent lower levels of metmyoglobin. This could also explain the slight higher WHC of C–T and T groups compared to the average value of C, C–S, C–ST, S and ST groups (58.7 and 59.7 *vs* 57.7%). As expected, TBARs values increased during storage in all groups(Table 3). At day 1, the highest value was recorded in the S group(even if not significant) and the lowest in the T one. The rate of oxida- tive processes during storage was not similar for all treatments. Infact, at the end of trial, the highest MDA content was observed in C and the lowest in T treatment, followed by the C–T one (*P* b 0.001). This is quite surprising because of the demonstrated *in vitro* antioxi- dant activity of Spirulina (Wang, Pan, Sheng, Xu, & Hu, 2007) and the reason of the lack of the same positive effect in muscle tissue is unclear. Eid et al. (2011) reported that feeding rabbits with diets containing 0.5% of green tea (very rich in catechins), significantly decreased TBARs of the thigh and loin rabbit meat stored for two months, but did not affect Total Reactive Antioxidant Potential values of the rabbit serum. These results would confirm the hypothesis of different mechanisms of action exerted by the different antioxidants in various vegetal essences (scavenger *in vivo*, chain-breaking in membrane, etc.). Concerning Thyme, it has a strong antioxidant activity related to the high content of thymol and carvacrol;

indeed biphenyl compounds, dimerization products of thymol and carvacrol and a flavonoid (eriodicytol), have also been isolated as efficient antioxidants inhibiting superoxide anion production in the xanthine/ xanthine oxidase system and mitochondrial and microsomal lipid peroxidation (Kahkonen et al., 1999).

Lee, Umano, Shibamoto, and Lee (2005) identified twelve aroma constituents of Thyme and evaluated their antioxidant activities. Eugenol, thymol, carvacrol, and 4-allylphenol showed strong antioxi- dant activities that were comparable or higher to those of the stan- dard antioxidants, α-tocopherol and butylated hydroxy toluene. These claims are also supported by a comparison with the results of our previous study, which was carried out to assess the effect of the combined action of dietary vitamin E and ascorbic acid on the oxidative status of rabbit meat, using the same storage condition protocol of the present trial (Castellini, Dal Bosco, & Bernardini, 2000). Surprisingly, T feeding group showed a meat TBARs value similar to that of meat whose rabbits fed a diet supplemented with 200 IU of α−tocopheryl acetate and 1000 mg/L of ascorbic acid. Concerning the amount of some antioxidants in the LD muscle during the trial, time had a significant effect in all groups (P b 0.0001; data not shown), showing a decreasing trend in antioxidants amount during the display period. In agreement with the above mentioned results, the groups supplemented with Thyme only (C–T and T) showed the highest content of α-tocopherol, at the beginning and at the end of the storage period (P b 0.001). This situation can be explained considering that Thyme is, as already said, a good source of powerful antioxidants such as phenols, but also such as ascorbic acid and tocopherols (Barros, Heleno, Carvalho, & Ferreira, 2010; Lee et al., 2005; Youdim & Deans, 2000).

The FA profile of the LD meat (Table 4) differed on the basis of the inclusion level of T and S supplements, that was characterized by amounts of total $n-3$ and n-6 FA of 3.13 and 11.35, and 31.7, and 21.08 %, respectively (data not shown). Thus, Thyme presence (C–T and T-T groups) determined a significant increase in meat of $n-3$ FA both at the beginning and at the end of the trial (P b 0.001), whereas, at day 1, S group showed the highest amount of n-6 FA and thus PUFA levels (P b 0.01). Specifically, the meat of S and ST animals presented the highest amount of C18:2n-6, and S, C-T and ST the highest values of C18:3n-6 (P b 0.001). The total n-6 FA increase in LD meat of rabbits fed S diets was also evidenced by Peiretti and Meineri (2011) who investigated the effects of four inclusion levels (0, 5, 10, or 15 %) of Spirulina on meat quality of growing rabbits.

Further confirmation on the positive action of Thyme have been obtained from the analysis of the percentage increase of the TBA-Rs and leakages of $n-3$ FA during the period of display (Table 5). C–T and T treatments induced significantly (P b 0.05) lower increases of lipid oxidation and at the same time the lower losses of $n-3$ FA acid. Only the ST treatment caused a non-linear trend between increase of TBARs and loss of $n-3$ FA, with a high development of oxidative process not accompanied by significant reductions of poly- unsaturated fatty acids

In agreement with the above mentioned statements, Thyme- supplemented diets (C–T and T) showed a significant reduction of the drip loss during display and such improvement was probably due to the positive effect of Thyme antioxidants on the integrity of muscle fibres, thus implementing their capability to retain water. With regards to poultry meat, Asghar et al. (1989) suggested that antioxidants preserve the functionality of membranes and thus im- prove their role as semi permeable barriers against exudative loss. According to Cheah, Cheah, and Krausgrill (1995) the beneficial effect of dietary antioxidants on drip loss is due to their ability to stabilize membranes, presumably achieved by inhibiting the phospholipase A_2 activity and by lowering Ca^{++} release, determining in turn, a re- duction in the rate of post-mortem glycolysis with a subsequently higher pH. An analogous positive action on drip loss was reported by Monahan, Buckley, Gray, and Morrissey (1990) and Mitsumoto, Arnold, Schaefer, and Cassens (1995) in pork and beef meat, respec- tively. Contrary to our expectations, supplementing the diet with Spirulina had no substantial effect on the membrane integrity of rabbit muscle.

Different hypothesis could be advanced to explain such trend. Firstly, the bioactive compounds of Spirulina have a demonstrated scavenger effect in the reduction of free radicals *in vivo*, but the effect in the reduction of oxidation processes in meat membrane phospho- lipids is not demonstrated. Another possibility is related to the even- tual pro-oxidant effect of these compounds at certain levels. Some Authors investigated this issue, and concluded that the phycocyanin, which is abundant in Spirulina, can become pro-oxidant at certain conditions and concentrations (Macari et al., 2011).

In conclusion, Thyme improved colour parameters and reduced exudative losses during a simulated retail display, also considering a shorter supplementation period (C–T group). This situation could determine a better impact on consumers at the time of purchase, and the shorter supplementation period would be a good compro- mise between Thyme's efficacy and farmers demand to limit produc- tion costs.

Contrarily to our expectations, also considering the encouraging results obtained in our related study on the effect of these supple- ments on bacterial community in the caecum and caecal fermentation of rabbits (Vántus et al., 2012), dietary supplementation of Spirulina had no effect on oxidative stability of rabbit meat, maybe for the poor absorption from the gut as a result of interference on uptake of antioxidants by spirulina.

It is conceivable that the dietary level of Spirulina was not ade- quate for rabbit requirements for the reduction of tocopherol deposi- tion and its failure to decrease TBARs and drip loss. However, from the scientific viewpoint the mechanism of the disconnect between TBARs and protection of $n-3$ FA is worthy of further investigations.

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Table 1
Ingredients (g/kg), chemical composition (g/kg) and gross energy (MJ//kg) of the experimental diets.

	Control (C)	Spirulina (S)	Thyme (T)	Spirulina + Thyme (ST)
Dehydrated alfalfa meal	379.0	390.0	355.0	380.0
Barley	260.0	275.0	250.0	260.0
Soybean meal	145.0	65.0	155.0	70.0
Wheat straw	120.0	130.0	120.0	120.0
Dried beet pulp	56.2	56.2	56.2	56.2
Spirulina	0.0	50.0	0.0	50.0
Thyme leaves	0.0	0.0	30.0	30.0
Sunflower seed oil	10.0	10.0	10.0	10.0
Dicalcium phosphate	4.0	4.0	4.0	4.0
NaCl	5.0	5.0	5.0	5.0
DL-Methionine	1.8	1.8	1.8	1.8
L-Lysine	3.0	3.0	3.0	3.0
Vit.-Min. premix[1]	5.0	5.0	5.0	5.0
Zeolite	11.0	5.0	5.0	5.0
Chemical composition:				
Dry matter	895.72	897.94	898.16	895.87
Crude protein	175.79	169.87	174.96	171.51
Ether extract	25.39	26.46	26.87	27.68
Ash	85.62	75.26	84.01	76.62
Starch	163.31	180.93	169.81	178.02
Gross Energy, MJ/kg	16.32	16.53	16.44	16.40

[1] Premix provided per kg of complete diet: vitamin A, 12,000 IU; vitamin D3, 1000 IU; vitamin E acetate, 50 mg; vitamin K3, 2 mg; biotin, 0.1 mg; thiamin, 2 mg; riboflavin, 4 mg; vitamin B6, 2 mg; vitamin B12, 0.1 mg; niacin, 40 mg; pantothenic acid, 12 mg; folic acid, 1 mg; choline chloride, 300 mg; iron, 100 mg; copper, 20 mg; manganesium, 50 mg; cobalt, 2 mg; iodine, 1 mg; zinc, 100 mg; selenium, 0.1 mg.

Table 2
Effect of the dietary Spirulina (*Arthrospira platensis*) and Thyme (*Thymus vulgaris*) supplementation on some physical characteristics of the *Longissimus dorsi* muscle during display.

	Experimental groups							*P*-value	Pooled SE
	C	C–S	C–T	C–ST	S	T	ST		
Day 1									
pH	5.81	5.86	5.80	5.85	5.85	5.82	5.83	ns	0.22
L*	56.1	56.0	57.2	57.0	57.7	55.4	56.9	ns	3.10
a*	3.61	4.42	3.24	3.74	3.64	3.02	3.41	0.042	1.46
b*	1.72	0.89	0.90	0.78	1.08	1.64	0.89	0.003	0.88
WHC (%)	56.2	57.1	58.3	56.7	56.9	58.9	57.2	ns	4.3
Day 3									
pH	5.88	5.89	5.85	5.89	5.90	5.86	5.86	ns	0.28
L*	56.9	56.5	57.3	57.6	58.2	56.3	57.5	ns	2.92
a*	3.80	4.50	3.35	3.70	3.80	3.28	3.50	0.031	1.02
b*	1.79	1.24	1.50	1.26	1.50	1.66	1.46	ns	0.54
WHC (%)	56.8	57.6	58.0	56.1	57.4	59.0	57.6	0.028	1.85
Day 6									
pH	5.92	5.94	5.90	5.92	5.94	5.93	5.95	ns	0.19
L*	56.9	55.9	58.8	57.6	58.6	56.1	58.4	ns	4.52
a*	3.80	4.95	3.12	3.51	4.08	3.12	4.95	0.046	0.98
b*	1.79	1.55	1.50	1.65	1.91	1.74	1.89	ns	0.61
WHC (%)	56.8	58.5	58.0	57.9	57.0	59.9	56.9	0.005	1.78
Day 9									
pH	5.89	5.91	5.87	5.90	5.91	5.90	5.92	ns	0.18
L*	58.3	58.6	59.0	58.2	58.0	58.9	61.1	ns	4.51
a*	4.85	5.23	4.50	5.32	4.81	4.44	5.54	0.036	1.30
b*	1.90	1.65	1.71	1.74	2.25	1.80	2.25	0.004	0.92
WHC (%)	57.9	58.2	58.7	57.5	57.3	59.7	57.8	ns	3.50

N = 35 per day; ns: not significant.

Table 3
Effect of the dietary Spirulina (*Arthrospira platensis*) and Thyme (*Thymus vulgaris*) supplementation on antioxidant contents (μg/g) and TBARs level (mg MDA/100 g) of the*Longissimus dorsi* muscle during display.

	Experimental groups					*P*-value	Pooled SE
	C	C–S	C–T	C–ST	S		
Day 1							
α-toc	305.6	234.8	472.3	284.0	236		
γ+β-toc	2.70	2.12	5.14	2.46	2.25		
δ-toc	37.6	n.d.	n.d	n.d.	n.d.		
Retinol	12.8	15.9	15.2	13.0	17.2		
TBARs	0.15	0.15	0.15	0.15	0.18		
Day 3							
α-toc	250.2	213.0	372.3	261.8	225		
γ+β-toc	2.14	2.00	4.85	2.40	2.20		
δ-toc	27.0	n.d.	n.d	n.d.	n.d.		
Retinol	12.7	15.1	14.8	13.2	17.0		
TBARs	0.23	0.21	0.19	0.20	0.22		
Day 6							
α-toc	175.8	184.2	350.1	239.1	216		
γ+β-toc	1.78	1.52	3.26	2.26	1.65		
δ-toc	15.4	n.d.	n.d	n.d.	n.d.		
Retinol	11.1	13.0	11.9	12.7	15.7		
TBARs	0.26	0.22	0.20	0.22	0.24		
Day 9							
α-toc	125.1	157.4	257.9	225.6	212		
γ+β-toc	1.51	1.13	2.90	2.08	1.35		
δ-toc	15.9	n.d.	n.d.	n.d.	n.d.		
Retinol	10.9	12.2	10.5	12.5	15.7		
TBARs	0.30	0.24	0.23	0.28	0.29		

N = 35 per day; ns: not significant.

Table 4
Effect of the dietary Spirulina (*Arthrospira platensis*) and Thyme (*Thymus vulgaris*) supplementation on fatty acid profile (% total FAME) of the *Longissimus dorsi* muscle during display.

	Experimental groups							*P*-value	Pooled SE
	C	C–S	C–T	C–ST	S	T	ST		
Day 1									
Saturated	41.62	39.01	41.20	41.67	39.37	39.38	39.43	ns	4.30
C14:0	2.49	2.33	2.63	2.59	2.07	2.13	2.05	ns	0.22
C16:0	29.62	28.88	30.40	30.02	29.94	29.85	30.09	ns	0.58
C18:0	7.56	6.33	6.39	7.16	5.90	6.04	6.01	ns	1.41
Others	1.95	1.47	1.78	1.90	1.46	1.36	1.28	ns	0.18
Monounsaturated	28.43	30.31	29.93	29.56	28.39	29.38	29.44	ns	2.62
C16:1 *n*-7	5.31	5.93	5.57	5.63	5.08	5.14	4.93	ns	0.59
C18:1 *n*-9	22.63	23.76	23.88	23.39	23.00	23.42	23.87	ns	2.10
Others	0.49	0.62	0.48	0.54	0.31	0.82	0.64	ns	0.14
Polyunsaturated	29.60	30.52	28.93	28.87	32.32	31.20	31.30	0.0066	3.35
C18:2 *n*-6	22.15	23.40	21.55	21.60	25.19	23.53	24.29	b0.001	3.09
C18:3 *n*-6	0.25	0.29	0.33	0.58	0.79	0.22	0.54	b0.001	0.73
C20:4 *n*-6	3.51	3.72	2.91	3.15	3.60	2.99	3.39	ns	0.34
C18:3 *n*−3	2.10	1.58	2.50	2.03	1.29	2.72	1.49	b0.001	0.44
C20:5 *n*−3	0.12	0.12	0.23	0.14	0.19	0.69	0.48	b0.001	0.05
C22:5 *n*−3	0.57	0.43	0.54	0.51	0.17	0.14	0.11	b0.001	0.07
C22:6 *n*−3	0.01	0.04	0.28	0.30	0.28	0.25	0.14	ns	0.05
Others	0.90	0.94	0.59	0.56	0.81	0.77	0.86	0.0245	0.25
Σ *n*-6	25.91	27.41	24.79	25.33	29.58	26.74	28.22	b0.001	3.62
Σ *n*−3	3.00	2.17	3.69	3.09	1.96	3.79	2.26	b0.001	0.81
Day 9									
Saturated	43.24	39.70	41.36	43.73	41.66	40.90	41.03	0.0009	3.21
C14:0	2.97	2.86	3.14	3.52	3.84	3.39	3.26	ns	0.60
C16:0	30.33	29.92	30.58	31.19	30.29	28.61	29.65	ns	1.94
C18:0	7.59	5.04	5.78	6.28	5.81	7.30	6.30	b0.001	1.36
Others	2.35	1.88	1.86	2.74	1.72	1.60	1.82	0.0287	0.35
Monounsaturated	27.36	32.90	31.25	29.32	28.29	28.41	28.89	b0.001	4.25
C16:1 *n*-7	4.74	7.37	6.74	6.14	5.38	5.52	5.82	0.0001	1.10
C18:1 *n*-9	22.23	24.91	24.02	22.79	22.66	22.64	22.74	0.0219	2.06
Others	0.39	0.62	0.49	0.39	0.25	0.25	0.33	0.0421	0.14
Polyunsaturated	28.76	27.10	27.43	27.15	29.77	30.77	30.9	b0.001	3.40
C18:2 *n*-6	21.38	20.95	20.15	20.62	24.08	23.88	23.64	b0.001	3.97
C18:3 *n*-6	0.59	0.33	0.52	0.41	0.57	0.11	0.63	ns	0.11
C20:4 *n*-6	3.45	3.15	2.81	2.97	2.95	2.53	3.09	ns	0.26
C18:3 *n*−3	2.33	1.50	2.86	2.20	1.28	2.92	1.88	b0.001	0.64
C20:5 *n*−3	0.10	0.08	0.14	0.08	0.11	0.47	0.12	b0.001	0.01
C22:5 *n*−3	0.22	0.29	0.39	0.33	0.14	0.12	0.08	0.0031	0.15
C22:6 *n*−3	0.00	0.01	0.01	0.02	0.01	0.10	0.08	0.0039	0.002
Others	0.69	0.79	0.55	0.52	0.63	0.64	1.38	b0.001	0.30
Σ *n*-6	25.42	24.43	23.48	24.00	27.60	26.52	27.36	b0.001	4.14
Σ *n*−3	2.80	1.96	3.56	2.75	1.84	3.66	2.21	b0.001	0.92

N = 35 per day. ns: not significant.

Table 5
Trend of TBARs, *n* − 3 fatty acids and drip loss (%) in the *Longissimus dorsi* muscle during retail display (Day 1 *vs* Day 9).

	Δ TBARs	Δ *n*− 3 FA during display	Drip loss
C	50.0^{c}	−6.67^{b}	6.87^{b}
C–S	37.5^{b}	−9.68^{c}	7.05^{b}
C–T	34.8^{a}	−3.52^{a}	3.96^{a}
C–ST	46.4^{c}	−11.00^{c}	9.69^{c}
S	37.9^{b}	−6.12^{b}	7.80^{b}
T	30.0^{a}	−3.43^{a}	3.90^{a}
ST	40.1^{bc}	−2.21^{a}	8.80^{bc}
X^2	6.06	1.85	2.13

N = 35 per day; a...c: *P* < 0.05.