DIETARY SUPPLEMENTATION OF SPIRULINA *(Arthrospira platensis)* AND THYME (*Thymus vulgaris)*. PART 6: EFFECT ON OXIDATIVE STATUS OF RABBIT MEAT DURING RETAIL DISPLAY

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ABSTRACT

The objective of this study was to evaluate the effect of Spirulina and Thyme supplementation (between the ages of 5-11 or 8-11 weeks) on the oxidative status of rabbit meat during a simulated retail display. The experiment was conducted at the experimental rabbit farm of the Kaposvár University (Hungary) using progeny of the Institues' maternal line (n=294). At weaning the rabbits were randomly allocated in 7 groups (42 rabbits/group). Rabbits of the control group (C) received a diet throughout the experiment (5-11 weeks of age) without any supplementation of spices. Experimental groups were fed a diet with 5% Spirulina (S) or 3% Thyme (T) or with both spices (ST) for the whole (5-11 wk; groups: S-S, T-T, ST-ST), or for part of the growing period (8-11 wk; groups: C-S, C-T, C-ST). After slaughter, *Longissimus dorsi* muscles were transported refrigerated to the Department of Applied Biology of Perugia (Italy) to determine the Thiobarbituric Reactive substances, the fatty acid profile and the antioxidant content. At the begin of storage, C-T and T-T samples showed the highest value of n-3 fatty acids, whereas S-S and ST-ST those of n-6 fatty acids. S-S group showed the highest amount of PUFA at day 1 of trial. In agreement with these results, the C-T and T-T groups showed the highest content of α-tocopherol, at the beginning and end of the storage period. C-T and T-T groups showed significantly lower lipid peroxidation, and at the same time, the lower losses of long chain fatty acids of n-3 series.

Key words: Spirulina, thyme, rabbit meat, retail display, oxidative status.

INTRODUCTION

Autocatalytic oxidative processes of lipids are the main factors responsible for altering the sensory quality of meat and decreasing its nutritional value and *shelf-life* (Jimenez-Colmenero *et al*., 2011). These processes start immediately after slaughter and their magnitude depends on the amount of free radicals present in the system and on its antioxidant capacity. The phospholipids in the cellular and subcellular membranes are very susceptible to oxidation because of their relatively high concentrations of polyunsaturated fatty acids (PUFA) and the close proximity to oxygen transition metals and peroxidases (Vladimirov *et al*., 1980). In animal production, the manipulation of diets to increase the PUFA contents of products can reduce the oxidative stability by increasing the degree of unsaturation in the muscle. For this reason, there is an increased interest in antioxidants which are widely used to improve meat quality and better sensory characteristics, extending *shelf-life* time. Synthetic antioxidants were widely used in the meat industry, but consumer concerns over safety and toxicity pressed the food industry to find natural sources (Coronado *et al*., 2002).

In rabbit, many studies were carried out in order to evaluate the effect of different antioxidants derived from: olive oil (Lopez-Bote *et al*., 1997), oats (Lopez-Bote *et al*., 1998), soy-isoflavones (Yousef *et*

al., 2004), organo-essential oils (Botsoglou *et al*., 2004), grape polyphenols (Sgorlon *et al*., 2005), grape pomace (Eid, 2008), red quebracho tannins (Dalle Zotte and Cossu, 2010), alfalfa polysaccharides (Liu *et al*., 2010), algae (Peiretti and Meineri, 2011) and green tea (Eid *et al*., 2011).
Spirulina (*Atrhrospira platensis*) is a rich source of phycocyanin, as antioxidant biliprotein pigment and carotenoids (Cheong *et al*., 2010; Belay *et al*., 1996). Thyme (*Thymus vulgaris*) essential oil contains more then 60 ingredients, which are known to have antioxidant properties and antimicrobial activity (Rota *et al*., 2008). The objective of this study was to evaluate the effect of level and length of dietary supplementation (between the ages of 5-11 or 8-11 weeks) of Spirulina (5%) and Thyme (3%) on the oxidative status of rabbit meat during a simulated retail display.

MATERIALS AND METHODS

Animals and experimental design

The experiment was conducted at the experimental rabbit farm of Kaposvár University using maternal line rabbits (n=294). The rabbits received the control pellet (C) from the age of 3 weeks. After weaning the rabbits at the age of 5 weeks were housed in wire net cages (0.61x0.32m, 16 rabbits/m^2). The weaned rabbits were randomly sorted in 7 groups (42 rabbits/group). Rabbits of the control group (C-C) received a diet throughout the experiment (5-11 weeks of age) without any supplementation. In the other groups the diet was completed by 5% Spirulina (S), 3% Thyme (T) or by both (ST) for the whole (5-11 wk; groups: S-S, T-T, ST-ST), or for part of the growing period (8-11 wk; groups: C-S, C-T, C-ST) (Gerencsér *et al*., 2012; Table 1). Water and feed were available *ad libitum* for every group. The pellets did not contain medication. The applied temperature and lighting schedule in the rabbit house were 15-18°C and 16L:8D, respectively.

Table 1: Supplementation and chemical composition of the experimental diets

	Control (C)	Spirulina (S)	Thyme (T)	Spirulina+Thyme (ST)
Supplementation				
Spirulina platensis (%)	-	5.0	-	5.0
Thyme leaves (%)	-	-	3.0	3.0
Chemical composition				
Dry matter (%)	88.6	88.8	88.7	88.9
Crude protein (%)	16.8	17.0	17.0	17.1
Ether extract (%)	2.65	2.59	2.71	2.65
Crude fibre (%)	18.5	18.9	18.4	18.7
Starch (%)	13.7	14.6	13.3	14.0
DE (MJ//kg)	10.1	9.3	9.9	9.0

Collection and management of data

At 75 days, 5 rabbits per group, with a weight close to the average of the group (+ 10%), were selected and slaughtered after 12 hours of feed withdrawal; animals did not undergo transport. Following electro-stunning, rabbits were killed by cutting the carotid arteries and jugular veins. After refrigerating the carcasses (24 h at + 4°C), the two *Longissimus dorsi* (LD) muscles were removed and carefully freed from connective and adipose tissues. The same day, samples were transported refrigerated to Department of Applied Biology of Perugia for analyses. The day after, on the left sample, the Thiobarbituric Reactive substances (TBA-Rs), fatty acid (FA) profile and antioxidant amount were determined as described below. All the samples were successively placed on plastic foam, over-wrapped with PVC film (600 cm^2) and displayed at + 4°C under continuous cool white fluorescent illumination (2,300 lux). The determinations were done again on days 3, 6 and 9, whereas the FA determination was repeated only at the end of the conservation period.
TBA-Rs were measured using the modified method of Ke *et al*. (1977). Meat lipids (samples of about 5 g) were extracted in a homogeniser with 20 ml of chloroform-methanol 2:1 (based on Folch *et al.* 1957), and then filtered through Whatman No. 1 filter paper. Fatty acids were determined as methyl esters (FAME) with a Mega 2 Carlo Erba Gas Chromatograph, model HRGC (Milano, Italy), using a D-B wax capillary column (25 mm ∅, 30 m long). Tocopherol content (α-tocopherol and its isoform β, γ, δ) and retinol of meat were quantified by HPLC according to Hewavitharana *et al*. (2004).

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) x treatment (STATA, 1990 - GLM procedure). The statistical significance of differences was assessed by a multiple t-test.

RESULTS AND DISCUSSION

The fatty acid profile of the LD meat slightly differed on the basis of the modification of the diet composition (Table 2). At the begin of storage C-T and T-T groups showed the highest value of n-3 FA, whereas S-S and ST-ST those of n-6 FA. In these former groups a significant higher amount of C18:2n-6 and C18:3n-6 was observed (data not shown), which is in agreement with Peiretti and Meineri (2011) who investigated the effects of four levels (0, 50, 100, or 150 g/kg) of Spirulina on meat quality of growing rabbit. S-S group showed the highest amount of PUFA at day 1 of trial.

Table 2: Fatty acid profile (g/100g fatty acids) of the *Longissimus dorsi* muscle at the beginning and the end of storage.

	Day 1					Day 9					*P* Time
	SFA	MUFA	PUFA	n-3	n-6	SFA	MUFA	PUFA	n-3	n-6	
C-C	41.6	28.8	29.6	3.0	26.6	43.2	27.8	29.0	2.8	26.0	***
C-S	39.4	30.3	30.3	2.2	28.2	39.7	32.9	28.2	2.0	24.6	***
C-ST	41.7	29.6	28.8	3.1	25.7	43.7	29.3	26.6	2.8	24.4	***
C-T	41.2	29.9	28.9	3.7	25.1	41.4	31.4	27.5	3.6	23.8	***
S-S	39.4	28.3	32.3	2.0	30.3	41.6	28.3	30.1	1.9	28.1	***
ST-ST	39.4	29.5	31.4	2.3	29.1	41.0	29.1	30.0	2.2	27.8	***
T-T	39.4	29.4	31.2	3.8	27.4	40.9	28.4	30.7	3.7	27.0	***
Pooled SE	3.5	2.6	1.4	0.9	4.4	4.1	4.8	4.5	0.8	2.9	-
P treatment	n.s.	n.s.	*	*	*	n.s.	*	n.s.	*	*	

N=35 per day; ***: P<0.001; *: P<0.05; n.s.: not significant.

As expected, TBA-Rs values increased during storage in all groups (Figure 1). On day 1, the highest value was recorded in the S-S group, and the lowest in the T-T one. The rate of oxidative processes during storage was similar, during the trial, for all treatments, except for T-T one. At the end of trial the highest value was observed in C-C and the lowest in T-T treatment. This is quite surprising

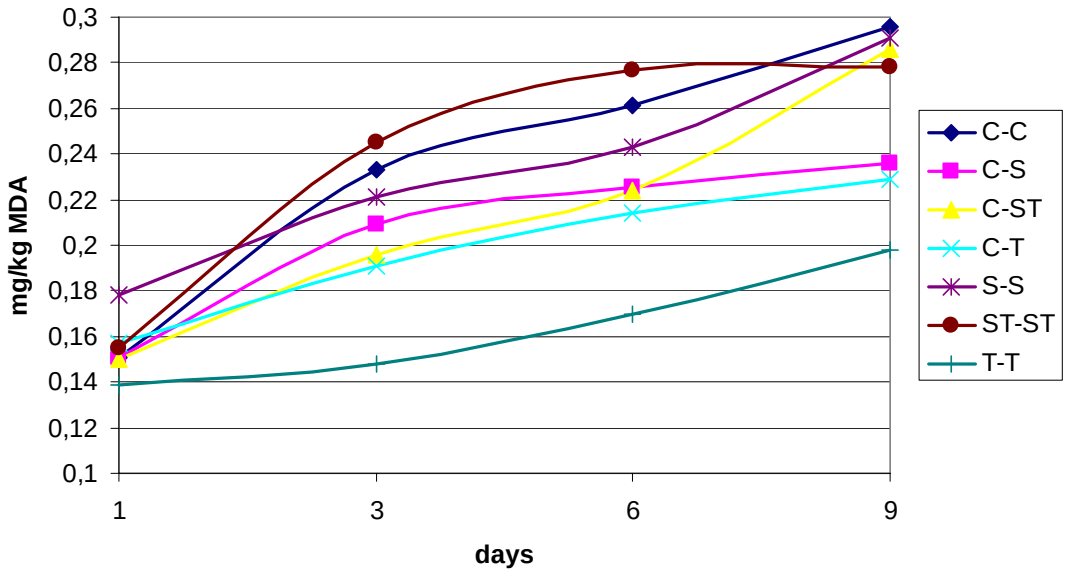

Figure 1: TBA-RS trend of the *Longissimus dorsi* muscle during retail display (N=35 per day)

because of the demonstrated *in vivo* antioxidant activity of Spirulina (Wang et al., 2007) and the reason is unclear of the lack of the same positive effect in muscle tissue. On the contrary, Eid *et al.*

(2011) reported that feeding rabbits with diets containing 0.5% of green tea (very rich in flavonoid catechins), significantly decreases TBARs of the thigh and loin rabbit meat stored for two months, but does not affect TRAP values of the rabbit *serum*. These results could confirm the hypothesis of different mechanisms of action by the different antioxidants in various vegetal essences (scavenger *in vivo*, chain-breaking in membrane, etc..).

The amount of antioxidants during the trial is reported in Table 3. Time showed a significant effect in all groups, showing a decreasing trend in antioxidants amount during the period of display. In agreement with the above mentioned results, the C-T and T-T treatments showed the highest content of α-tocopherol, at the beginning and end of the storage period.

Table 3: Antioxidant contents (ηg/g) of the *Longissimus dorsi* muscle at different storage time

Time	Day 1				Day 9				*P* Time
	α-toc	γ-toc	δ-toc	retinol	α-toc	γ-toc	δ-toc	retinol	
C-C	305.6	2.7	37.6	12.8	125.1	1.5	15.9	10.9	***
C-S	234.8	2.1	n.d.	15.9	157.4	1.1	n.d.	12.2	***
C-ST	284.0	2.4	n.d.	13.0	225.6	2.0	n.d.	12.5	***
C-T	472.3	5.1	n.d	15.2	257.9	2.9	n.d.	10.5	***
S-S	236.8	2.2	n.d.	17.2	212.5	1.3	n.d.	15.7	***
ST-ST	256.2	2.2	43.4	11.3	157.1	1.3	15.2	10.5	***
T-T	423.2	3.7	n.d.	11.8	269.9	2.7	n.d.	10.7	***
Pooled SE	109.1	1.6	10.3	8.9	95.4	1.1	6.8	4.5	-
P treatment	***	n.s.	*	n.s.	***	n.s.	*	n.s.	

N=35 per day; ***: P<0.001; *: P<0.05; n.s.: not significant.

Interesting confirmations 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 4). C-T and T-T tretments showed significantly lower increases of lipid peroxidation indicated and at the same time the lower losses of long chain FA acid of n-3 series.

Table 4: TBA-Rs increase and n-3 FA loss of the *Longissimus dorsi* muscle during retail display

	Δ TBA-Rs during display (%)	Δ n-3 FA during display (%)
C-C	48.99^{c}	$-6{,}67^{b}$
C-S	36.02^{b}	$-9{,}09^{c}$
C-ST	47.55^{c}	$-9{,}68^{c}$
C-T	31.44^{a}	$-2{,}70^{a}$
S-S	38.83^{b}	$-5{,}00^{b}$
ST-ST	44.24^{bc}	$-4{,}35^{b}$
T-T	29.80^{a}	$-2{,}63^{a}$
X^2	6.32	1.52

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

CONCLUSIONS

Results of this preliminary study indicate a positive effect of Thyme on oxidative status of rabbit meat during storage. Contrary to our expectations, dietary supplementation of Spirulina had no effect on oxidative stability. It is conceivable that the dietary level of Spirulina was not adequate for rabbit meat (too low or perhaps too high) or that the antioxidant effect of bioactive compounds of Spirulina are not effective in the reduction of peroxidation process in membrane phospholipids. Further researches are needed to deeply investigate the mechanism of action of the two studied antioxidants.

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