DIETARY SUPPLEMENTATION OF SPIRULINA (*Arthrospira platensis*) AND THYME (*Thymus vulgaris*). PART 5: EFFECT ON RABBIT MEAT APPEARANCE DURING RETAIL DISPLAY

Dal Bosco A.[1*], Szendrő Zs[2], Mugnai C.[3], Kovàcs M.[2], Ruggeri S.[1], Mattioli S.[1], Castellini C.[1], Dalle Zotte A.[4],

[1]Dept. of Applied Biology, University of Perugia, Borgo XX Giugno 74, 06121 Perugia, Italy
[2]Kaposvár University, Faculty of Animal Science, Guba S. str. 40, H-7400, Kaposvár, Hungary
[3]Dept. of Food Science, University of Teramo, v.le F. Crispi 212, 64100 Teramo, Italy
[4]Department of Animal Medicine, Production and Health, University of Padova, Agripolis, Viale dell'Università 16, 35020 Legnaro (PD), Italy
[*]Corresponding author: dalbosco@unipg.it

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 rabbit meat appearance during retail display. The experiment was conducted at the experimental rabbit farm of the Kaposvár University using progeny of the Institutes' maternal line (n=294). At weaning the rabbits were randomly allocated to 7 treatments (42 rabbits/group). Rabbits of the control group (C-C) received a diet throughout the experiment (5-11 weeks of age) without supplementation of spices. The other groups were fed diets with 5% Spirulina (S), 3% Thyme (T) or by both spices (ST) for the whole (5-11 wk; treatments: S-S, T-T, ST-ST), or for part of the growing period (8-11 wk; groups: C-S, C-T, C-ST). The L*, a*, b* colour parameters, pH, water holding capacity and drip loss were determined on fresh *Longissimus dorsi* (LD) muscle and again on days 3, 6 and 9 of storage at +4 °C under continuous cool white fluorescent illumination. Spirulina and Thyme had a significant effect only on redness and yellowness of LD muscle. In particular, C-T and T-T treatments showed the lower values at the day 1 of display in the fore part, while at the end of storage the lower values were observed in C-C, C-T and T-T treatments. In the hind part C-S, C-T and T-T treatments showed lower values at the beginning of the storage. Meat samples of C-T and T-T treatments showed a significant reduction of drip loss of LD muscle during display. Based on the results of this study, Thyme seems more effective than Spirulina on improving appearance traits of rabbit meat during retail display.

Key words: Spirulina, thyme, rabbit meat, physical characteristics.

INTRODUCTION

Different studies show that the main quality aspects of consumer's choice for the purchase of meat are taste, tenderness, juiciness, freshness, 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, the meat is naturally rich in unsaturated fatty acids (Dalle Zotte, 2002) and it is likely that it can undergo lipid oxidation, mainly during processing and storage (Castellini *et al*., 1998; Dalle Zotte, 2002; Cavani *et al*., 2004), with a detrimental effect also on physical characteristics. In order to counteract this process, there has been an increasing interest in the use of antioxidants in rabbit feed formulas, because the dietary manipulation of tissue lipid composition to produce meat with a high PUFA content could decrease meat oxidative stability (Corino *et al.,* 1999; Dal Bosco *et al.,* 2004) and using appropriate modified atmosphere packaging. 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). This study is a part of an extensive research (with six papers presented in this Congress) that

had the aim to evaluate the effect of the dietary supplementation (between the ages of 5-11 weeks) of Spirulina (*Arthrospira platensis*, 5%) and Thyme (*Thymus vulgaris*, 3%) on rabbit growth performance and health status (Gerencsér *et al*., 2012), physiology (Bònai *et al*., 2012), microbial diversity in the caecum (Vàntus *et al.*, 2012), carcass and meat quality (Dalle Zotte *et al*., 2012) and oxidative status (Dal Bosco *et al.*, 2012) during retail display; specifically in this trial we investigated the main meat parameters for assessing the consumer choices (colour, drip loss during a simulated retail display) at time of purchase. The protocol was conceived to study the possibility of a reduction of the extract supplementation period and the costs, even if recently, simplified production techniques and the development of alternative nutrient sources have been adopted to obtain large quantities of spices extracts at competitive prices (Peiretti and Meineri, 2011).

MATERIALS AND METHODS

Animals and experimental design

The experiment was conducted at the experimental rabbit farm of Kaposvár University using progeny of the maternal line rabbits (n=294). The rabbits received the control diet (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 to 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 diets 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 diets 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 (%)	-	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, after 12 hours feed withdrawal, slaughtered; 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 to be analyzed. The day after, on left sample, colour parameters, water holding capacity and pH were determined as described below in fore and hind because the distribution of fibres in the rabbit's LD is heterogeneous in anterior-posterior direction (percentage of βR fibre decreases from 10 to 3% and that of αR increases from 34 to 41%).
The right sample 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 (2,300 lux). The following determinations were done at day 0 and again on days 3, 6 and 9: the colour parameters (L*, a*, b*) evaluated using a tristimulus analyser (Minolta Chroma Meter CR-200; Azuchi-Machi Higashi-Ku, Osaka 541, Japan) with the CIELAB (1976); pH measured with a Knick digital pHmeter (Broadly Corp., Santa Ana, CA,

USA) after grinding 1 g of muscle in 10 mL of distilled water for 30 sec (Korkeala *et al*., 1986) and WHC estimated, as indicated by Nakamura and Katoh (1985), by centrifuging 1 g of muscle for 4 min at 1,500 x g and determining the residual water by drying the sample at 70 °C overnight.

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

In Table 2, the physical characteristics of the fore part of LD muscle are presented. Independently on dietary treatment, storage at simulated retail display significantly increased pH. During storage, pH variation depends on two opposite events, the hydrolysis of protein with NH_3 release and the hydrolysis of lipids with liberation of fatty acids (Cabanes *et al*., 1996). We observed the maximum value in all groups after six day of storage (data not shown) and successively an inversion of trend, probably for the above mentioned release of free fatty acids. Concerning WHC it should be noted that the refrigeration for long periods should reduce it due to membrane breakage; we observed an opposite situation with an improvement in WHC during storage, probably due to the higher pH and to the lower water content of meat. The colour was affected by storage with an increase of L*, a* and b* values in accordance with the findings of Cabanes-Roiron *et al*. (1994).

Table 2. Physical characteristics of the *Longissimus dorsi* muscle fore part.

	Day 1					Day 9					P Time
	pH	WHC	L	a*	b*	pH	WHC	L	a*	b*	
C-C	5.81	56.2	56.1	3.6	1.7	5.89	57.9	58.3	4.4	1.9	***
C-S	5.86	57.1	56.0	4.4	0.8	5.91	58.2	58.7	5.2	1.6	***
C-ST	5.85	56.7	57.0	3.7	0.7	5.90	57.5	58.2	5.3	1.7	***
C-T	5.80	58.3	57.2	3.2	0.9	5.87	58.7	59.0	4.5	2.7	***
S-S	5.85	56.9	57.7	3.6	1.0	5.91	57.3	58.0	4.8	2.2	***
ST-ST	5.83	57.2	56.9	3.4	0.8	5.91	57.9	61.1	5.5	2.1	***
T-T	5.82	58.9	55.4	3.0	1.6	5.90	59.7	57.9	4.4	1.8	***
Pooled SE	0.22	4.3	3.1	1.5	0.9	0.18	3.5	4.5	1.3	0.9	-
P treatment	n.s.	n.s.	n.s.	*	**	n.s.	n.s.	n.s.	*	**	

n=35 per day; *: P<0.05; **: P<0.001; ***: P<0.0001

In Table 3 the physical characteristics are presented of the hind part of LD muscle. The observed trend was similar to that of the fore part, with slight differences related to the different fibres composition and energy metabolism of the two muscles.

Table 3. Physical characteristics of the *Longissimus dorsi* muscle hind part.

	Day 1					Day 9					P Time
	pH	WHC	L*	a*	b*	pH	WHC	L*	a*	b*	
C-C	5.68	54.1	55.6	6.1	0.5	5.76	55.2	58.8	7.1	1.3	***
C-S	5.72	56.3	56.3	4.7	1.4	5.77	56.9	60.5	5.2	1.0	***
C-ST	5.70	55.5	55.8	7.5	0.5	5.73	55.9	59.2	8.1	1.9	***
C-T	5.69	54.9	55.7	4.8	0.9	5.76	56.0	59.7	6.0	1.2	***
S-S	5.75	55.8	56.1	6.8	1.3	5.84	55.1	59.5	6.2	1.1	***
ST-ST	5.70	56.1	57.8	6.2	0.9	5.71	57.2	59.7	6.5	1.7	***
T-T	5.73	57.1	55.3	5.0	1.7	5.76	57.8	58.6	5.8	1.2	***
Pooled SE	0.12	3.5	3.1	1.5	0.9	0.13	6.8	4.5	0.9	1.1	-
P treatment	n.s.	n.s.	n.s.	**	***	n.s.	n.s.	n.s.	*	**	

n=35 per day; *: P<0.05; **: P<0,001; ***: P<0.0001

Concerning the effect of Spirulina and Thyme, a significant effect was observed only on redness and yellowness of meat. In particular, C-T and T-T treatments showed the lower values at the day 1 of display in the fore part, while at the end of storage the lower values were observed in C-C, C-T and T-T treatments. In the hind part C-S, C-T and T-T treatments showed lower values at the day 1.

In Figure 1, the effect of dietary treatments on drip loss of LD muscle during display is showed. Samples of C-T and T-T groups showed a significant reduction of this parameter along the trial. The improvement in values of water loss was probably due to the positive effect of the Thyme antioxidants on the integrity of the muscle fibre. Asghar *et al*. (1989), in poultry meat, suggested that antioxidants preserve the functionality of membranes and increases their role as a semi permeable barrier against exudative loss. According to Cheah *et al*. (1995) the beneficial effect of dietary antioxidants on drip loss is due to its ability to stabilize membranes, presumably achieved by inhibiting the phospholipase A_2 activity and lowering Ca^{++} release, determining in turn, a reduction in the rate of post-mortem glycolysis with a subsequently higher pH. An analogous positive action on drip loss was reported by Monahan *et al*. (1990) and Mitsumoto *et al*. (1995) in pork and beef meat, respectively. Contrary to our expectations, supplementing the diet with Spirulina had no substantial effect on the membrane integrity of rabbit muscle. These results are in accordance with other papers presented in this Congress on this topic (Dal Bosco *et al.*, 2012) where we found a higher antioxidant efficacy of Thyme respecting to Spirulina.

Different hypothesis could be advanced to explain such trend:

- the dietary level of Spirulina was too low to reach a significant effect on pH and consequently on physical characteristics of meat;
- the antioxidant effect of bioactive compounds of Spirulina, have a demonstrated scavenger effect in reduction of free radicals *in vivo*, but the effect in the reduction of peroxidation process in membrane phospholipidis of meat is not demonstrated;
- the possibility of a pro-oxidant effect at certain levels.

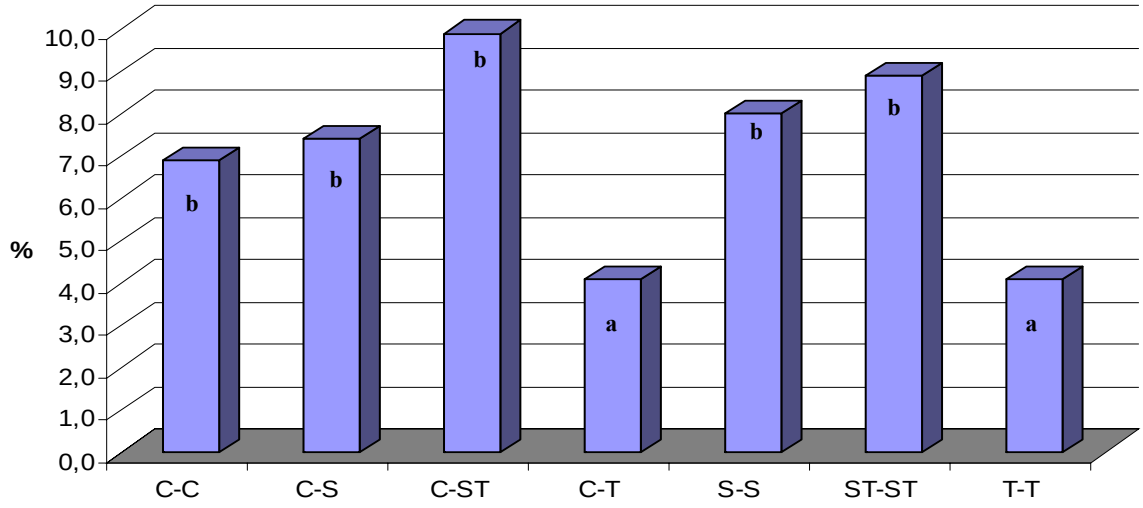

Figure 1: Effect of dietary treatments on drip loss of *Longissimus dorsi* muscle during display (n=35 per day; a..b: P<0.05).

CONCLUSIONS

Interesting results of this trial are limited to the positive effect of Thyme which improved colour parameters and exudative losses during a simulate retail display, also when fed for a short period. This situation could determine a better impact in consumer at the moment of purchase. Further research is needed to deep the mechanism of action of the two studied antioxidants.

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