DIETARY SUPPLEMENTATION OF SPIRULINA (*Arthrospira platensis*) AND THYME (*Thymus vulgaris*). PART 4: EFFECT ON RABBIT CARCASS AND MEAT QUALITY

Dalle Zotte A.[1], Sartori A.[1], Cullere M.[1]*, Gerencsér Zs.[2], Matics Zs.[2], Dal Bosco A.[3], Kovàcs M.[2], Szendrő Zs.[2]

[1]Department of Animal Medicine, Production and Health, University of Padova, Agripolis, Viale dell'Università 16, 35020 Legnaro (PD), Italy
[2]Faculty of Animal Science, Kaposvár University, 40, Guba S. str., H-7400, Kaposvár, Hungary
[3]Department of Applied Biology, University of Perugia, Borgo XX Giugno 74, 06121 Perugia, Italy
*Corresponding author: cux@hotmail.it

ABSTRACT

The aim of the study was to evaluate the effect of the dietary supplementation of 5% Spirulina (S) and 3% Thyme (T) leaves and the duration of their supplementation on carcass traits and meat quality of fattening rabbits. Maternal line rabbits (n=252) of both sexes were used. Spirulina and Thyme were supplemented to isonitrogenous and isoenergy diets formulated to cover the requirements of meat producing rabbits. All diets did not include coccidiostatic. Rabbits received the S and T supplementation separately or combined (TS), during the whole growing-fattening period (5th-11th week of age: S-S; T-T; ST-ST) or only during fattening (8th-11th week of age: C-S; C-T; C-ST). Seven groups containing 36 rabbits each were used for carcass evaluation: Control (C-C; not supplemented diet) C-S, S-S, C-T, T-T, C-ST, ST-ST. Rabbits were slaughtered at 77 days of age. Carcasses were dissected, carcass yield, fatness and meatiness were determined and pHu and L*a*b* colour were measured on Longissimus dorsi (LD) muscle. LD muscle of 15 rabbits per treatment were dissected and frozen; WHC, heme iron and vitamin B12 were analyzed on raw meat whereas proximate composition and cholesterol content were analyzed on both raw and cooked meat. Carcass traits were not affected by dietary treatments, except the scapular fat incidence which showed its lowest value in C-T group (0.39%) and its highest value in S-S (0.56%; $P<0.05$). Spirulina and Thyme supplementations didn't affect the rheological traits as well, even though a tendency towards a lower total water loss was found in S-S and C-S (33.1 and 33.4%, respectively; n.s.) compared to the other groups (35.2±0.86 %). Consequently, cooked LD meat of S-S group contained more water (67%) than the other groups (66.1±0.27 %; n.s.). S-S and T-T diets reduced the protein content in the cooked LD meat, compared to the C-C diet (31.3 and 31.5 vs 32.4%; $P=0.051$), and the lowest value for cholesterol content was found in S-S group, in raw and cooked LD meat as well (49.7 and 76.0 mg/100 g). In this study the effect of Spirulina supplementation as lipid lowering wasn't confirmed. Based on our results, Spirulina supplement seems promising in enhancing WHC and vitamin B12, and in reducing cholesterol content of rabbit meat, but its inclusion level should be further increased.

Key words: Rabbit, spirulina, thyme, feed, carcass traits, meat quality.

INTRODUCTION

Spirulina (*Arthrospira platensis*) is a blue-green microalga, rich source of protein (over 60%), β-carotene, α-tocopherol, vitamin B12 and essential FAs, mainly γ-linolenic acid (GLA; Ramadan *et al*., 2008), pigment (C-Phycocyanin) and enzymes like the superoxide dismutase. Spirulina has recently attracted attention due to its potential health benefits, such as antiobesity, lipid lowering, hypocholesterolemic action (Belay, 2002; Cheong *et al.,* 2010), antioxidant effect, and anemia amelioration (Selmi *et al*., 2011). In rabbits, its dietary inclusion (5-10-15%) was tested on live performances, meat proximate composition and FA profile (Peiretti & Meineri, 2008; 2011; Meineri *et al*., 2009) and its supplementation was effective in increasing the GLA content and in reducing the

atherogenic and thrombogenic indexes of the meat. Scientific evidence is accumulating that many herbs and spices do have medicinal properties that alleviate symptoms or prevent disease. Among them, Thyme (*Thymus vulgaris* L.) essential oil contains more than 60 constituents, which are known to have antioxidant properties, antimicrobial activity (Rota *et al*., 2008), blood cholesterol and abdominal fat lowering action (Abdulkarimi *et al*., 2011). Even so, the effect of dietary supplementation of Thyme leaves to growing-fattening rabbit carcass and meat hasn't been studied until now. This study is a part of an extensive research (with six papers presented in this Congress) that aimed 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, meat appearance (Dal Bosco *et al*., 2012a) and oxidative status (Dal Bosco *et al*., 2012b) during retail display. Considering the higher feeding costs derived from the use of these 2 raw materials, this protocol aimed to combine both the nutritional advantages and low costs for the producers, by considering also a shorter supplementation length (8-11 weeks of age). Specifically, in this paper we studied the dietary inclusion of Spirulina and Thyme leaves on carcass traits and meat quality of fattening rabbits.

MATERIALS AND METHODS

Animals and experimental design

Maternal line rabbits of the Pannon breeding program (n=252) were used. Animals were reared by 3 in wire cages sized 61 x 32 cm. Experimental rabbitry temperature and photoperiod were 15-18 °C and 16L:8D, respectively. Spirulina (5%) and Thyme (3%) leaves were supplemented to isonitrogenous and isoenergy diets for meat producing rabbits. All diets did not include any coccidiostatic. Rabbits of both sexes received the Spirulina (S) and Thyme (T) supplementation separately or combined (ST), during the whole growing-fattening period (5th-11th week of age: S-S; T-T; ST-ST) or only during fattening (8th-11th week of age: C-S; C-T; C-ST). Seven groups of 36 rabbits each were used: Control (C-C; not supplemented diet) C-S, S-S, C-T, T-T, C-ST, ST-ST.

Slaughter, carcass dissection and meat sampling

At 11 weeks of age rabbits were transported to a slaughterhouse located 200 km from the experimental rabbitry (n=35, 34, 34, 36, 35, 36 and 36 rabbits for C-C, C-S, C-T, C-ST, S-S, T-T and ST-ST groups, respectively). Carcasses were chilled for 24 h at 3 °C, dissected, and then carcass yield and fatness were determined. Fore, mid and hind part of the carcass were dissected as described by Blasco and Ouhayoun (1996). *Longissimus dorsi* (LD) muscle was dissected from 15 rabbits per dietary treatment and pHu and L*a*b* colour values (CIE, 1976) were measured at the 5th lumbar vertebra level of the right part of the LD. Afterwards, right and left sides of LD were individually packed in food PVC bags and frozen at -80°C for further physicochemical analysis.

Chemical analyses

Chemical composition of the experimental diets is reported elsewhere (Gerencsér *et al.,* 2012). Frozen left parts of LD were first thawed overnight at room temperature, then removed from plastic bags and individually vacuum-packed in new plastic bags, finally cooked at 80 °C for 1h in a water bath. Thawing and cooking losses were calculated from sample weighing before and after thawing, and after cooking.

On raw (right part of LD) and cooked (left part of LD) meat samples the proximate composition was determined (AOAC, 1995), with protein content calculated by difference, cholesterol content according to Casiraghi *et al.* (1994), heme iron content (Hornsey, 1956) and vitamin B12 determination (AOAC, 2006).

Statistical Analysis

Data were analysed using GLM procedures of SAS (2004) with diet as the fixed effect.

RESULTS AND DISCUSSION

Carcass traits were not affected (P>0.05) by the dietary S and T supplementation (Table 1). Only the scapular fat incidence statistically differed (P=0.029), The C-T group had less scapular fat than S-S group (0.39 *vs* 0.56 % CC; P<0.05). This finding is in contrast to the literature where Spirulina caused lipid lowering and so a related antiobesity property; however the lipid lowering effect of Spirulina was effective on rabbits fed high fat diets only and not on low fat diet group (Meineri *et al*., 2009).

Table 1. Effect of the dietary Spirulina and Thyme supplementation on rabbit carcass traits.

Traits	Experimental groups							*P* value	SE
	C-C	C-S	C-T	C-ST	S-S	T-T	ST-ST		
n.	35	34	34	36	35	36	36		
Slaughter weight (SW), g	2474	2471	2480	2516	2497	2536	2492	0.814	12.6
Chilled carcass (CC), g	1502	1502	1504	1525	1527	1543	1514	0.791	7.95
Reference carcass (RC), g	1228	1226	1233	1248	1250	1268	1238	0.663	6.73
Carcass yield, % SW	60.7	60.8	60.7	60.6	61.1	60.9	60.8	0.744	0.08
Reference carcass yield, % CC	81.7	81.6	82.0	81.8	81.9	82.2	81.7	0.361	0.07
Head, % CC	9.35	9.52	9.40	9.25	9.32	9.30	9.25	0.328	0.03
HLTTO, % CC (1)	1.69	1.62	1.62	1.57	1.62	1.26	1.66	0.308	0.02
Liver, % CC	5.66	5.65	5.20	5.72	5.57	5.38	5.75	0.134	0.06
Kidneys, % CC	1.07	1.03	1.09	1.10	1.03	1.02	1.05	0.067	0.01
Perirenal fat, % CC	1.51	1.47	1.35	1.60	1.55	1.59	1.57	0.395	0.03
Scapular fat, % CC	0.46^{ab}	0.51^{ab}	0.39^{a}	0.51^{ab}	0.56^{b}	0.48^{ab}	0.45^{ab}	0.029	0.01
Dissectable fat, % CC	1.97	1.98	1.74	2.11	2.11	2.07	2.03	0.149	0.04
Fore part, % RC	28.0	28.4	28.4	28.5	28.0	28.2	28.1	0.152	0.06
Mid part, % RC	31.7	31.4	31.7	31.4	31.5	31.3	31.7	0.521	0.07
Hind part, % RC	37.9	37.8	37.8	37.5	37.9	37.9	37.8	0.829	0.07
Perirenal fat, % RC	1.85	1.81	1.65	1.95	1.90	1.94	1.93	0.405	0.04

(1)HLTTO: Heart, lung, thymus, trachea and oesophagus

Rheological traits of LD muscle were not affected by the dietary treatment (Table 2). Contrarily to what was observed by Dal Bosco *et al*.(2012), our results didn't show differences in a* and b* colour values among dietary treatments. Again, whereas Dal Bosco *et al*. (2012) found a significant reduction of drip loss during 9 days display on C-T and T-T groups, in our trial it was observed that the average total loss of the groups in which S was supplemented to the diet seemed to be lower (34%) compared to the groups without S supplementation (35.5%) and that the groups S-S and C-S presented even lower values (33.06 and 33.42% for S-S and C-S, respectively); despite this, statistical evidence didn't support our assumption (P=0.324). These different results could depend on how the samples were stored, refrigerated in the study of Dal Bosco *et al*. (2012) and frozen in our study. Spirulina may preserve the cell membrane disruption during freezing-thawing phases, improving thus the WHC of the cooked meat (Table 4). Proximate composition of raw and cooked LD meat is reported in Table 3 and 4, respectively. Whereas the water content of the raw meat was slightly higher in the C-T group (75.4%) compared to the other feeding groups (on average 75.05; P<0.10), in the cooked meat the water content was the highest in S-S group (67%) due to its lower water loss during thawing and cooking (33.06%; Table 2).

Table 2. Effect of the dietary Spirulina and Thyme supplementation on rheological traits of LD muscle.

Traits	Experimental groups							*P* value	SE
	C-C	C-S	C-T	C-ST	S-S	T-T	ST-ST		
n.	15	15	15	15	15	15	15		
pHu	5.90	5.97	5.94	5.88	5.92	5.84	5.84	0.339	0.16
L* value(1)	50.8	50.2	50.4	51.2	50.0	51.2	51.1	0.938	0.34
a* value(1)	3.17	3.87	3.61	3.57	3.32	4.93	3.36	0.084	0.17
b* value(1)	0.74	0.85	0.58	0.72	0.59	1.13	0.83	0.870	0.11
Thawing losses, %	11.40	11.33	11.77	12.10	10.42	12.06	11.23	0.718	2.73
Cooking losses, %	24.42	22.09	22.56	22.79	22.63	24.31	23.37	0.268	2.76
Total losses, %	35.81	33.42	34.33	34.90	33.06	36.37	34.60	0.324	3.84

[1] L*a*b* colour values measured 18h *post mortem* on LD muscle of all the slaughtered rabbits.

The difference in protein content among dietary treatments (P=0.051) of cooked LD meat is difficult to explain and further studies are needed to elucidate this finding. Our data didn't show a lipid lowering effect of Spirulina on the intramuscular lipids. On the contrary, C-T diet seemed to exhibit leaner carcasses and meat even if values didn't differ statistically. Even though the hypocholesterolemic action of Spirulina is supported by several studies (Nakaya et al., 1988; Belay, 2002; Cheong et al., 2010) the cholesterol content of raw and cooked meat wasn't significantly affected by the 5% Spirulina supplementation, but a slight reduction was evidenced when compared to the average value of the other dietary treatments (49.7 vs 51.1 mg/100 g for raw and 76.0 vs 77.7 mg/100 g for cooked LD meat; Table 3). Also the cholesterol and abdominal fat lowering effects of Thyme reported in chickens (Abdulkarimi et al., 2011) wasn't shown on rabbits at 3% supplementation level.

Table 3. Effect of the dietary Spirulina and Thyme supplementation on proximate composition, Heme iron, cholesterol and Vitamin B12 contents of raw LD meat.

Traits	Experimental groups							*P* value	SE
	C-C	C-S	C-T	C-ST	S-S	T-T	ST-ST		
n.	15	15	15	15	15	15	15		
Water, %	75.0	75.0	75.4	75.1	75.1	75.1	75.0	0.089	0.04
Protein, %	23.1	23.2	22.9	23.2	23.1	23.1	23.1	0.299	0.03
Lipids, %	0.48	0.40	0.34	0.40	0.40	0.50	0.47	0.287	0.02
Ash, %	1.37	1.41	1.40	1.36	1.35	1.36	1.40	0.864	0.01
Cholesterol, mg/100 g	50.9	51.1	51.2	51.2	49.7	51.0	51.1	0.607	0.34
Heme iron, mg/kg(1)	1.68	1.61	1.72	1.58	1.49	1.67	1.70	0.861	0.40
Vitamin B_{12}, mcg/100 g(2)	0.6620[a]	-	-	-	0.9539[b]	-	0.8051[ab]	0.012	0.03

(1)n=10 samples/dietary treatment; (2)n=6 samples/dietary treatment

Table 4. Effect of the dietary Spirulina and Thyme supplementation on proximate composition and cholesterol content of cooked LD meat.

Traits	Experimental groups							*P* value	SE
	C-C	C-S	C-T	C-ST	S-S	T-T	ST-ST		
n.	15	15	15	15	15	15	15		
Water, %	65.8	66.2	66.6	66.0	67.0	66.2	66.3	0.198	0.13
Protein, %	32.4	32.1	31.8	32.1	31.3	31.5	32.0	0.051	0.09
Lipids, %	0.56	0.59	0.45	0.60	0.50	0.54	0.57	0.731	0.02
Ash, %(1)	1.72	1.57	1.57	1.73	1.48	1.62	1.60	0.690	0.04
Cholesterol, mg/100 g	78.9	78.8	76.8	78.9	76.0	79.1	77.9	0.449	0.47

(1)n=6 samples/dietary treatment

Spirulina is reported to be rich in iron (Belay, 2002) and for this reason useful to ameliorate anaemia (Selmi *et al*., 2011) but in our study the heme iron content in meat of S-S groups was even lower than that of the other feeding groups (Table 3). Spirulina is also reported to be rich in vitamin B12. Its amount in diets was 1.853, 2.828 and 2.109 mcg/100 feed for C, S and ST diets, and its content in raw meat significantly increased when diets contained Spirulina supplement not combined with Thyme (0.662 *vs* 0.954 mcg/100 g meat for C-C and S-S groups, respectively; P<0.05), whereas the meat of ST-ST group exhibited intermediate values (Table 3).

CONCLUSIONS

In this study the effect of Spirulina supplementation as lipid lowering wasn't confirmed. Spirulina seems promising in enhancing WHC and vitamin B_{12}, and in reducing cholesterol content of rabbit meat, but further research is needed to confirm our findings, testing higher inclusion levels of both S and T supplements.

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REFERENCES

Abdulkarimi R., Daneshyar M., Aghazadeh A. 2011. Thyme (*Thymus vulgaris*) extract consumption darkens liver, lowers blood cholesterol, proportional liver and abdominal fat weights in broiler chickens. *Italian J. of Anim. Sci., 10, 101-105.*

AOAC 1995. Official Methods of Analysis. (15th ed.). *Washington, DC, USA: Association of Official Analytical Chemists.*

AOAC 2006. Official Methods of Analysis, *Association of Official Analytical Chemist Inc., Gaithesbourg.*

Belay A. 2002. The potential application of Spirulina (*Arthrospira*) as a nutritional and therapeutic supplement in health management. *JANA, 5, 27-48.*

Blasco A., Ouhayoun J. 1996. Harmonization of criteria and terminology in rabbit meat research. *World Rabbit Sci., 4, 93–99.*

Bónai A., Dalle Zotte A., Vántus V., Morsy W.A., Kametler L., Matics Zs., Dal Bosco A., Szendrő Zs., Kovàcs M. 2012. Dietary supplementation of Spirulina (*Arthrospira platensis*) and Thyme (*Thymus vulgaris*). Part 2: Effect on gastrointestinal growth, caecal microbiota and fermentation in rabbits. *In: Proc. 10th World Rabbit Congress, Sharm El-Sheikh, Egypt, 3-6 September 2012.*

Casiraghi E., Lucisano M., Pompei C., Dellea C. 1994. Cholesterol determination in butter by high performance chromatography. *Milchwissenschaft, 49(4) 194-196.*

Cheong F.H., Kim F.Y., Sok D., Hwang S., Kim J.H., Kim H., Lee J.H., Kim Y., Kim M. 2010. Spirulina prevents atherosclerosis by reducing hypercholesterolemia in rabbits fed a high-cholesterol diet. *J. Nutr. Sci. Vitaminol., 56, 34-40.*

CIE, Commission Internationale de l'Eclairage 1976. *Official recommendations on uniform colour spaces, colour differences equations and metric colour terms. Paris, France.*

Dal Bosco A., Szendrő Zs., Mugnai C., Kovàcs M., Ruggeri S., Mattioli S., Castellini C., Dalle Zotte A. 2012a. Dietary supplementation of Spirulina (*Arthrospira platensis*) and Thyme (*Thymus vulgaris*). Part 5: Effect on rabbit meat appearance during retail display. *In: Proc. 10th World Rabbit Congress, Sharm El-Sheikh, Egypt, 3-6 September 2012.*

Dal Bosco A., Szendrő Zs., Mugnai C., Kovàcs M., Ruggeri S., Mattioli S., Castellini C., Dalle Zotte A. 2012b. Dietary supplementation of Spirulina (*Arthrospira platensis*) and Thyme (*Thymus vulgaris*). Part 6: Effect on oxidative status of rabbit meat during retail display. *In: Proc. 10th World Rabbit Congress, Sharm El-Sheikh, Egypt, 3-6 September 2012.*

Gerencsér Zs., Szendrő Zs., Matics Zs., Radnai I., Kovács M., Nagy I., Dal Bosco A., Dalle Zotte A. 2012. Dietary supplementation of Spirulina (*Arthrospira platensis*) and Thyme (*Thymus vulgaris*). Part 1: Effect on productive performance of growing rabbits. *In: Proc. 10th World Rabbit Congress, Sharm El-Sheikh, Egypt, 3-6 September 2012.*

Hornsey H.C. 1956. The colour of cooked cured pork. I-Estimation of the Nitric oxide-Haem Pigments. *J. Sci. Food Agric., 7, 534-540.*

Meineri G., Ingravalle F., Radice E., Aragno M., Peiretti P.G., 2009. Effects of high fat diets and *Spirulina platensis* supplementation in New Zealand White Rabbits. *J. Anim. Vet. Adv. 8 (12), 2735–2744.*

Nakaya N., Homma Y., Goto Y. 1988. Cholesterol lowering effect of Spirulina. *Nutr. Rep. Int., 37: 1329-1337.*

Peiretti P.G., Meineri G., 2008. Effects of diets with increasing levels of *Spirulina platensis* on the performance and apparent digestibility in growing rabbits. *Livest. Sci. 118, 173–177.*

Peiretti P.G., Meineri G., 2011. Effects of diets with increasing levels of *Spirulina platensis* on the carcass characteristics, meat quality and fatty acid composition of growing rabbits. *Livest. Sci. 140, 218–224.*

Ramadan M.F., Asker M.M.S., Ibrahim Z.K. 2008. Functional bioactive compounds and biological activities of *Spirulina platensis* lipids. *Czech J. of Food Sci., 26(3), 211−222.*

Rota M.C., Herrera A., Martínez R.M., Sotomayor J.A., Jordán M.J. 2008. Antimicrobial activity and chemical composition of *Thymus vulgaris*, *Thymus zygis* and Thymus hyemalis essential oils. *Food Control, 1, 681–687.*

SAS 2004. SAS/STAT User's Guide (Release 9.1) *SAS Inst. Inc., Cary NC, USA.*

Selmi C., Leung P.S.C., Fischer L., German B., Yang C.Y., Kenny T.P., Cysewski G.R., Gershwin M.E. 2011. The effects of Spirulina on anemia and immune function in senior citizens. *Cellular & Molecular Immunology 8, 248-254.*

Vàntus V., Dalle Zotte A., Kovàcs M., Dal Bosco A., Szendrő Zs., Zolnai A. 2012. Dietary supplementation of Spirulina (*Arthrospira platensis*) and Thyme (*Thymus vulgaris* L.). Part 3: Effect on caecal bacterial community in growing rabbits. *In Proc. 10th World Rabbit Congress, Sharm El-Sheikh, Egypt, 3-6 September 2012.*