# EFFECT OF DIETARY SUPPLEMENTATION OF SPIRULINA (*ARTHROSPIRA PLATENSIS*) AND THYME (*THYMUS VULGARIS*) ON CARCASS COMPOSITION, MEAT PHYSICAL TRAITS, AND VITAMIN B$_{12}$ CONTENT ON GROWING RABBITS

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**Abstract:** The aim of this study was to compare the effect and duration of dietary inclusion of 5% spirulina (*Arthrospira platensis*) and/or 3% thyme (*Thymus vulgaris*) on growing rabbit carcass composition, meat and bone rheological traits, and the vitamin B$_{12}$ content of *Longissimus dorsi* (LD) meat. The study involved 294 maternal line growing rabbits from the Pannon breeding programme. At weaning (5 wk), animals were randomly divided by dietary treatment into 7 groups of 42 rabbits each. A control group (C-C) received a pellet with no supplementation throughout the trial (5-11 wk of age), whereas the other groups were fed diets supplemented with 5% spirulina (S), 3% thyme (T) or with both ingredients (ST) for either the entire growing period (5-11 wk of age; groups: S-S, T-T, ST-ST, respectively), or its final part only (8-11 wk of age; groups: C-S, C-T, C-ST, respectively). Results showed that regardless of the duration of supplementation, spirulina and thyme provided no effect on the traits examined, except for scapular fat content, whose value was higher in the S-S group than in the C-T group (*P*<0.05). Spirulina was confirmed as a rich source of vitamin B$_{12}$ that was successfully transferred into LD meat, thus demonstrating its value as an effective natural supplement in producing food fortified with this vital element. Further studies are necessary to clarify the effect of spirulina on carcass fat deposition, bone development, and mineralisation.

**Key Words:** *Spirulina platensis*, *Thymus vulgaris*, rabbit meat, vitamin B$_{12}$.

## INTRODUCTION

Since the European Union first limited and then definitively banned the use of antibiotics as growth promoters in animal feeding (Anadón, 2006), public opinion on antibiotic use by humans in the USA has changed progressively and scientific studies have increasingly focused on natural alternatives (Montesissa and Calini, 2006; Falcão-e-Cunha, 2007; Franz *et al.*, 2010; Hashemi and Davoodi, 2011). The EU decision stemmed from the concern that low-continuative dosage of antibiotics to either enhance animal performance or for simple prophylaxis purposes could lead to the formation of resistant strains of human pathogens that pose a real sanitary risk to the population (Wegener, 2003).

Furthermore, the growing need to reduce the environmental impact of livestock combined with higher consumer pressure for more natural food production systems has increased the industry's interest in natural feed supplements. In this context, essential oils and aromatic plants have become more and more widely used as natural feed additives to increase feed palatability, positively affect gastrointestinal flora, exert a coccidiostatic effect, ensure optimal productive performance and achieve antimicrobial action on chilled meat (Dickens *et al.*, 2000; Hernández *et al.*, 2004; Cross *et al.*, 2007).

Thyme (*Thymus vulgaris*) is a well known Mediterranean shrub traditionally used as an appetising substance, sensory additive, and flavouring agent. Most studies conducted as yet have investigated its antimicrobial and antioxidant actions with thymol and carvacrol as its major phenolic compounds (Yanishlieva *et al.*, 2006; Al-Turki, 2007; Solomakos *et al.*, 2008; Hoffman-Pennesi and Wu, 2010).

Another set of natural products that might prove useful in animal production to enhance the nutritional value of conventional food and improve the health status of consumers through diet are microalgae (Gouveia *et al.*, 2008), one of the most promising of which is spirulina (*Arthrospira platensis*), a filamentous blue-green microalgae once consumed by the Aztecs in Mexico and still consumed in the Lake Chad area in Africa (Belay, 2002). Today, spirulina is widely known and appreciated for its high protein content and as an important source of β-carotene, vitamin $B_{12}$, whose dietary deficiency in vegetarians represents a growing concern (Stabler and Allen, 2004), and minerals. The organic source of Ca and P provided by spirulina suggests its use in poultry and rabbit feeding to guarantee correct lifelong bone development and higher bone strength, thus reducing carcass downgrade. Moreover, it has been shown to be anticarcinogenic and to have many positive health properties, such as the mitigation of hyperlipidemia and the control of hypertension and high serum glucose levels (Belay *et al.*, 1993). Despite having a higher production cost than common animal feeds, it represents an interesting alternative thanks to its ability to grow under alkaline and saline conditions that are unsuitable for most traditional crops (Carlos *et al.*, 2004). Spirulina is normally produced in outdoor ponds that leave small environmental footprints and minimise the use of land, which can be given over to other purposes (Belay, 2002). Research has also shown that spirulina can prove useful in recycling nutrients through organic waste treatment processes (Ahsan *et al.*, 2008). Thanks to all these positive aspects, spirulina is currently being produced worldwide, with half of its production used in feeding fish and livestock.

In poultry feeding, incorporation with spirulina has provided satisfactory results in terms of productive performance and as a substitute for mineral-vitamin premixes (Venkataraman *et al.*, 1994; Belay *et al.*, 1996). It has also proven effective in improving carcass colour and lowering total cholesterol content when its effect on egg quality was tested (Holman and Malau-Aduli, 2013). However, research in species such as pigs and rabbits is still in the earliest phases, so a wider scope of research is required before the previous results can be confirmed (Grinstead *et al.*, 2000).

The aim of this study was therefore to evaluate the effect of dietary thyme and spirulina supplementation on growing rabbit carcass composition, vitamin $B_{12}$ absorption into meat cuts, meat rheological traits, and bone development. The results presented in this article are part of a wider study that has involved productive performance, the health status and apparent digestibility of the diets (Gerencsér *et al.*, 2014), microbial diversity in the caecum and caecal fermentation (Vàntus *et al.*, 2012), the fatty acid profile of the meat and its oxidative status during retail display (Dal Bosco *et al.*, 2013). To our knowledge, this is the first study that evaluates the synergic effect of spirulina and thyme on animal productive performance, health, and meat quality.

## MATERIALS AND METHODS

### Animals and experimental design

For this study, a total of 294 maternal line growing rabbits from the Pannon breeding programme were used. Animals were reared at the experimental farm of Kaposvár University (Hungary) and received a control pelleted diet (C) from the age of 3 wk. At weaning (5 wk of age), animals were randomly divided by dietary treatment into 7 groups and housed in wire net cages (0.61×0.32 m). Control group rabbits (C-C) received a pelleted diet with no supplementation throughout the trial (from 5 to 11 wk of age). The other groups received pelleted diets supplemented with 5% spirulina (S diet, mainly in substitution of soybean meal), 3% thyme leaves (T diet, mainly in substitution of alfalfa meal) or with both ingredients (ST) for the entire period (groups: S-S, T-T, ST-ST) or for only the last 3 wk of fattening (8-11 wk of age; groups: C-S, C-T, C-ST, Figure 1). These 2 different durations of supplementation were planned in a perspective of cost reduction. Experimental diets were isonitrogenous and isoenergetic and did not include coccidiostatics. Water and feed were available *ad libitum* and the temperature and photoperiod in the rabbitry were 15-18 °C and 16L:8D, respectively. Ingredients and chemical composition of the experimental diets are reported in this same issue by Gerencsér *et al.* (2014).

### Slaughter, carcass dissection and meat sampling

At 11 wk of age, the rabbits were transported to a slaughterhouse located 200 km from the experimental farm (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) and slaughtered by cutting the carotid arteries and jugular veins after electro-stunning. The slaughtering and carcass dissection procedures followed the World Rabbit Science Association (WRSA) recommendations described by Blasco and Ouhayoun (1996), and all the steps taken to obtain offal (head, heart+lung+thymus+ trachea+oesophagus –HLTTO– liver, kidneys, perirenal and scapular fat), weights (slaughter weight –SW–, chilled carcass –CC– weight and reference carcass –RC– weight), and yields (carcass yield and reference carcass yield) are detailed in a previous study (Dalle Zotte *et al.*, 2009). CC was recorded after 24 h chilling in a ventilated room at 4 °C.



**Figure 1:** Experimental design and dietary treatment. □ C-Control; ▨ S-Spirulina; ▨ T-Thyme; ■ ST-Spirulina+Thyme.

Subsequently, the *Longissimus dorsi* (LD) muscle and hind legs (HL) were dissected from 15 and 10 rabbits per dietary group, respectively, and then weighed. Once pHu (pH measured at 24 h *post mortem*) was measured at the 5th lumbar vertebra level, the right and left sides of the LD muscle were individually packed and frozen at –80 °C until further analysis. HL were individually frozen at –80 °C immediately after dissection.

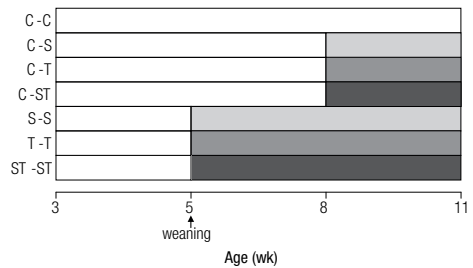### Thawing, cooking loss, bone trait and vitamin B$_{12}$ determination

Left LD were allowed to thaw overnight at room temperature, weighed, and subsequently used for thawing and cooking loss measurements. Samples were individually vacuum-packed in polyvinyl chloride (PVC) bags and cooked in a water bath at 80 °C for 1 h. Right LD from the animals belonging to C-C, S-S and ST-ST groups (6 samples per group) were ground in frozen state with a Retsch Grindomix GM 200 grinder at 4000 rpm for 10 s, then freeze-dried and subsequently used for vitamin B$_{12}$ determination (AOAC 2006, Method no. 952.20). Diets C, S and ST were also analysed for vitamin B$_{12}$ content. Right and left HL were thawed overnight and, after weighing, right legs were deboned to determine the meat/bone ratio (Blasco and Ouhayoun, 1996). Femur and tibia were separately weighed, then length and minor diameter were measured with a digital calliper (JUWEL *Digital-Schieblehre Rostfrei* H4215/5X A12). Femur fracture toughness (FT) was calculated at the average bone length point using a dynamometer Texture TA-HD (SMS- *Stable Micro System*) with a 6 cm wide cell and a load rate of 0.5 mm/s. Left HL were individually vacuum-packed in PVC bags and cooked in a water bath at 80 °C for 2.5 h for cooking loss determination.

### Statistical Analysis

Data were analysed using the General Linear Model procedures of SAS (2004). A one-way analysis of variance (ANOVA) tested the diet as fixed effect and the significance level was calculated at the 5% confidence level. Normality of data was analysed with a Shapiro-Wilk confidence level of 85%.

## RESULTS AND DISCUSSION

Dietary supplementation with 5% spirulina, 3% thyme or both had no affect on rabbit slaughter weight, carcass yields or retail cut percentages (Table 1). The only exception was scapular fat content, which differed between S-S and C-T groups (0.56 *vs.* 0.39% of the CC, $P<0.05$). In literature, spirulina has been reported to possess hypotriglyceridemic action by stimulating lipoprotein lipase activity (Belay, 2002). This property has been observed under pathogenic conditions, however, such as in rabbits fed high cholesterol diets in which 0.5 g/d of spirulina increased serum levels of high density lipoproteins (HDL), the latter representing a protective factor against atherosclerosis (Colla *et al.*, 2008). In a similar trial, supplementations of 1% and 5% spirulina were shown to be effective in reducing serum total

Table 1: Effect of the dietary spirulina (*Arthrospira platensis*) and thyme (*Thymus vulgaris*) supplementation on carcass traits.

| | Experimental groups | | | | | | | Significance | SEM |
|---|---|---|---|---|---|---|---|---|---|
| | C-C | C-S | C-T | C-ST | S-S | T-T | ST-ST | | |
| No. of rabbits | 35 | 34 | 34 | 36 | 35 | 36 | 36 | | |
| Slaughter weight (SW), (g) | 2474 | 2471 | 2480 | 2516 | 2497 | 2536 | 2492 | NS | 12.6 |
| Chilled Carcass (CC), (g) | 1502 | 1502 | 1504 | 1525 | 1527 | 1543 | 1514 | NS | 7.95 |
| Reference Carcass (RC), (g) | 1228 | 1226 | 1233 | 1248 | 1250 | 1268 | 1238 | NS | 6.73 |
| Carcass yield (% SW) | 60.7 | 60.8 | 60.7 | 60.6 | 61.1 | 60.9 | 60.8 | NS | 0.08 |
| RC yield (% CC) | 81.7 | 81.6 | 82.0 | 81.8 | 81.9 | 82.2 | 81.7 | NS | 0.07 |
| Drip loss (%) | 2.33 | 2.26 | 2.17 | 2.32 | 2.21 | 2.18 | 2.27 | NS | 0.23 |
| As % of chilled carcass: | | | | | | | | | |
| Head | 9.35 | 9.52 | 9.40 | 9.25 | 9.32 | 9.30 | 9.25 | NS | 0.03 |
| HLTTO | 1.69 | 1.62 | 1.62 | 1.57 | 1.62 | 1.26 | 1.66 | NS | 0.02 |
| Liver | 5.66 | 5.65 | 5.20 | 5.72 | 5.57 | 5.38 | 5.75 | NS | 0.06 |
| Kidneys | 1.07 | 1.03 | 1.09 | 1.10 | 1.03 | 1.02 | 1.05 | NS | 0.01 |
| Perirenal fat | 1.51 | 1.47 | 1.35 | 1.60 | 1.55 | 1.59 | 1.57 | NS | 0.03 |
| Scapular fat | 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 |
| Dissectible fat | 1.97 | 1.98 | 1.74 | 2.11 | 2.11 | 2.07 | 2.03 | NS | 0.04 |
| As % of reference carcass: | | | | | | | | | |
| Fore part | 28.0 | 28.4 | 28.4 | 28.5 | 28.0 | 28.2 | 28.1 | NS | 0.06 |
| Intermediate part | 31.7 | 31.4 | 31.7 | 31.4 | 31.5 | 31.3 | 31.7 | NS | 0.07 |
| Hind part | 37.9 | 37.8 | 37.8 | 37.5 | 37.9 | 37.9 | 37.8 | NS | 0.07 |
| Perirenal fat | 1.85 | 1.81 | 1.65 | 1.95 | 1.90 | 1.94 | 1.93 | NS | 0.04 |

C: Basic feed; S: Spirulina supplementation (5%); T: Thyme supplementation (3%); ST: Spirulina+Thyme supplementation (S:5%; T:3%). SEM: Standard Error of the Least Squares Means; HLTTO: Heart, lung, thymus, trachea and oesophagus. Significance: No significant (NS; *P*>0.05); when significant *P*-value is given.
[a,b] Means in the same row not sharing superscripts differ at *P*<0.05.

cholesterol and low density lipoproteins (LDL) levels in hypercholesterolemic rabbits (Cheong *et al.*, 2010). On the other hand, contrary to that observed in serum parameters, spirulina did not appear to lower carcass fatness in rabbits fed high fat diets when compared to those fed low-fat diets (Meineri *et al.*, 2009). In fact, the addition of 150 mg spirulina/kg to the diet of growing rats increased the visceral fat content compared to a Control group (Sixabela *et al.*, 2011). Generally speaking, spirulina seems effective in improving serum cholesterol status in pathogenic conditions, and although this suggests its use as a protective factor against atherosclerosis, its effect at lipid deposit level requires further investigation, as the results obtained thus far preclude definitive assumptions.

As regards thyme, our results support those found in the literature, which considered its essential oil. Abdominal fat content was significantly reduced when thyme essential oil was supplemented to Japanese quails diets either at 60 or 200 mg/kg (Denli *et al.*, 2004; Khaksar *et al.*, 2012) and to broiler chick diets at 1 g/kg inclusion level (Al-Kassie, 2009). These results might be attributable to the positive effect of the thyme compounds on digestive efficiency, which leads to improved feed conversion rate, as observed in our study (Gerencsér *et al.*, 2014). However, in another recent study on growing dwarf rabbits, a lower thyme leave dietary inclusion level (2.5%) was unable to modify digestive efficiency and animal growth (Dalle Zotte *et al.*, 2013), thus hypothesising a dose-related effect.

Overall carcass weight, yields, dissectible fat, and meatiness results (Table 1) were satisfactory and comparable to results provided in literature (Dal Bosco *et al.*, 2002; Metzger *et al.*, 2003; Gondret *et al.*, 2005; Gidenne *et al.*, 2009).

Neither the dietary inclusion of spirulina and/or thyme nor the duration of their supplementation influenced the traits of LD and HL meat portions (Tables 2 and 3, respectively), thus confirming previous results that considered dietary inclusions of 5, 10 and 15% of spirulina (Peiretti and Meineri, 2011).

The ratio of LD and HL portions on the reference carcass (RC) were on average 10.9% and 34.9%, respectively. Comparing these ratios with those obtained in a study that used the same genetic line as we did (Metzger *et al.*, 2006), the rabbits in our study had lighter LD and heavier HL, thus resulting in different incidences on RC, which were, however, most likely attributable to the successful CT based selection scheme carried out over the years to increase hind leg muscle volume (Szendrő *et al.*, 2009).

Table 2: Effect of the dietary spirulina (*Arthrospira platensis*) and thyme (*Thymus vulgaris*) supplementation on traits of *Longissimus dorsi* (LD) muscle.

| | Experimental groups | | | | | | | Significance | SEM |
|---|---|---|---|---|---|---|---|---|---|
| | C-C | C-S | C-T | C-ST | S-S | T-T | ST-ST | | |
| No. of samples | 15 | 15 | 15 | 15 | 15 | 15 | 15 | | |
| LD[1] (g) | 133 | 132 | 136 | 134 | 140 | 138 | 139 | NS | 1.65 |
| LD[1] (% RC) | 10.9 | 10.7 | 11.0 | 10.7 | 11.2 | 10.8 | 11.1 | NS | 0.08 |
| pHu | 5.90 | 5.97 | 5.94 | 5.88 | 5.92 | 5.84 | 5.84 | NS | 0.08 |
| Thawing losses (%) | 11.4 | 11.3 | 11.8 | 12.1 | 10.4 | 12.1 | 11.2 | NS | 0.02 |
| Cooking losses (%) | 24.4 | 22.1 | 22.6 | 22.8 | 22.6 | 24.3 | 23.4 | NS | 0.27 |
| Total losses (%) | 35.8 | 33.4 | 34.3 | 34.9 | 33.1 | 36.4 | 34.6 | NS | 0.27 |

Experimental groups as defined in Table 1. SEM: Standard Error of the Least Squares Means; RC: Reference Carcass; NS: no significant.
[1] Two LD muscles.

Rabbit LD pHu was unaffected by dietary treatment, and the average value of 5.9 observed was within the range reported in literature (Ouhayoun and Dalle Zotte, 1993; Hernández and Dalle Zotte, 2010). Even though differences were not statistically significant, the S-S group showed a numerically lower thawing loss percentage than the average for the other dietary treatments (10.4 *vs.* 11.7%, respectively) and thus numerically lower total losses (33.1 *vs.* 34.9%). An initial explanation of this trend suggests that spirulina might have positively affected cell membrane integrity during freezing-thawing phases, but this theory was not confirmed by HL thawing losses.

Spirulina and/or thyme dietary supplementation did not affect HL bone traits (Table 4), and mean values were in accordance with those reported in literature (Dalle Zotte *et al.*, 2009). Femur and tibia presented average lengths of 91.4 and 70.2 mm, respectively, and the meat/bones ratio was 5.65, which was higher than the value reported by Metzeger *et al.* (2003) in a study on 13 wk-old New Zealand White rabbits.

As reviewed by Holman and Malau-Aduli (2013), spirulina is an important source of Ca (1200 mg/kg) and P (13000 mg/kg), so the mineral content of the experimental diets used in our study were balanced by taking this aspect into account. Unlike the mineral premix, however, spirulina is an organic source, and for this reason we hypothesised a higher mineral bioavailability in S-supplemented diets than in the others, as well as a possible effect on rabbit bone traits. Unexpectedly, the results of the total tract apparent digestibility of the diets showed S diet mineral digestibility to be the lowest (Gerencsér *et al.*, 2014) and no differences in bone traits were observed as a result.

Tibia length, which is an indicator of linear growth (Masoud *et al.*, 1986; Fritton *et al.*, 2005), was found to be greater in rats fed 150 and 1500 mg/kg supplemented with spirulina than those fed a control diet (Sixabela *et al.*, 2011). In another work on ovariectomised rats and hindlimb-unloaded mice, 0.08, 0.8, and 4 g/kg body weight and day of spirulina determined trabecular bone loss, although the component responsible for this depletion has yet to be identified (Ishimi *et al.*, 2006).

Spirulina is known to be an important source of vitamins, especially vitamin $B_{12}$, which is an almost exclusive prerogative of animal-origin foods (Dalle Zotte and Szendrő, 2011). Rabbit meat naturally contains this micronutrient, as may be seen in the LD meat of the C-C group (Table 5). In this study, spirulina was shown to be an effective fortifier,

Table 3: Effect of the dietary spirulina (*Arthrospira platensis*) and thyme (*Thymus vulgaris*) supplementation on hind legs (HL) traits.

| | Experimental groups | | | | | | | Significance | SEM |
|---|---|---|---|---|---|---|---|---|---|
| | C-C | C-S | C-T | C-ST | S-S | T-T | ST-ST | | |
| No. of rabbits | 10 | 10 | 10 | 10 | 10 | 10 | 10 | | |
| HL[1] (g) | 427.4 | 437.5 | 442.7 | 446.0 | 451.3 | 459.3 | 442.9 | NS | 3.94 |
| HL[1] (% RC) | 34.9 | 35.2 | 35.2 | 34.8 | 34.6 | 35.4 | 34.4 | NS | 0.12 |
| Thawing loss (%) | 4.15 | 4.05 | 3.93 | 4.04 | 3.95 | 4.35 | 3.74 | NS | 0.10 |
| Cooking loss (%) | 18.1 | 17.9 | 18.3 | 19.0 | 19.0 | 19.9 | 18.9 | NS | 0.20 |
| Total losses (%) | 26.2 | 26.1 | 26.9 | 27.0 | 27.1 | 28.9 | 27.4 | NS | 0.28 |

Experimental groups as defined in Table 1. SEM: Standard Error of the Least Squares Means; RC: Reference Carcass.
[1] Two hind legs.

Table 4: Effect of the dietary spirulina (*Arthrospira platensis*) and thyme (*Thymus vulgaris*) supplementation on rabbit hind leg (HL) bones traits.

| | Experimental groups | | | | | | | Significance | SEM |
|---|---|---|---|---|---|---|---|---|---|
| | C-C | C-S | C-T | C-ST | S-S | T-T | ST-ST | | |
| No. of samples | 10 | 10 | 10 | 10 | 10 | 10 | 10 | | |
| HL bones (g) | 30.6 | 31.4 | 31.8 | 30.5 | 31.2 | 31.7 | 31.9 | NS | 0.31 |
| Femur (g) | 13.0 | 13.5 | 13.8 | 13.1 | 13.3 | 14.0 | 13.3 | NS | 0.11 |
| Femur length (mm) | 91.1 | 91.8 | 92.4 | 91.0 | 91.7 | 90.6 | 91.3 | NS | 0.29 |
| Femur minor Ø (mm) | 6.48 | 6.50 | 6.60 | 6.54 | 6.54 | 6.72 | 6.45 | NS | 0.04 |
| Femur fracture toughness (kg) | 25.3 | 26.8 | 31.3 | 29.0 | 27.2 | 27.8 | 28.3 | NS | 0.56 |
| Tibia (g) | 7.56 | 7.54 | 7.61 | 7.57 | 7.86 | 7.88 | 7.78 | NS | 0.07 |
| Tibia minor Ø (mm) | 5.38 | 5.54 | 5.58 | 5.28 | 5.45 | 5.77 | 5.27 | NS | 0.05 |
| Tibia length (mm) | 70.5 | 70.0 | 71.4 | 70.5 | 70.6 | 68.9 | 69.7 | NS | 0.35 |
| HL bones (% HL) | 15.3 | 15.3 | 15.2 | 14.7 | 14.7 | 15.1 | 15.3 | NS | 0.08 |
| Meat to bones ratio | 5.56 | 5.55 | 5.61 | 5.83 | 5.80 | 5.66 | 5.57 | NS | 0.04 |

Experimental groups as defined in Table 1. SEM: Standard Error of the Least Squares Means; Ø=diameter; NS: no significant.

given that the LD meat of rabbits fed the S-S diet presented a significantly ($P<0.05$) higher vitamin $B_{12}$ content compared to rabbits fed the C-C diet, whereas the ST-ST fed animals showed an intermediate value (0.662, 0.954 and 0.805 µg vitamin $B_{12}$/100 g meat for C-C, S-S and ST-ST groups, respectively). The group-dependent trend of vitamin $B_{12}$ presence in LD meat corresponded directly to that of the diets (0.509, 0.841 and 0.697 µg/100 g feed, for C-C, S-S and ST-ST groups, respectively).

In accordance with the literature, vitamin $B_{12}$ content in the feed and its absorption percentage are inversely proportional (95, 85 and 86% for C-C, S-S and ST-ST groups, respectively), even if rabbits showed higher absorption capacity than what has been reported for humans (Allen, 2009).

This represents the first scientific evaluation of the dietary fortification of vitamin $B_{12}$ and its absorption and consequent content in rabbit meat by means of dietary raw materials. Although other examples of vitamin $B_{12}$ fortification in pig-meat products via dietary vitamin $B_{12}$ supplementation are reported in the literature (Sahlin and House, 2006), said supplementation consisted of synthetic vitamin $B_{12}$. Vitamin $B_{12}$ content of meat and meat products is not often reported in studies evaluating meat quality, and when it is quantified, great differences can be noted: vitamin $B_{12}$ content of lean beef meat ranges between 0.8 and 3.9 µg/100 g meat, that of lean pork meat between 0.3 and 2 µg/100 g, and that of lamb between 0.9 and 3.5 µg/100 g (Giguère *et al.*, 2005; Ortigues-Marty *et al.*, 2005; Sahlin and House, 2005; Truswell, 2007; Williams *et al.*, 2007; Schönfeldt *et al.*, 2011). Rabbit meat is reported to be very rich in vitamin $B_{12}$, ranging from 8.7 to 11.9 µg/100 g (review by Dalle Zotte and Szendrő, 2011), which is clearly higher than the values obtained in our study. The reason for these wide ranges could be attributed to the fact that vitamin $B_{12}$ exists in different forms (cobalamins and cobalamin analogues), so the sample preparation method and the analysis technique applied are crucial to final content quantification. Several methods are reported in the literature (Baker and Miller-Ihli, 2000; Heudi *et al.*, 2006; Indyk *et al.*, 2002), all of which present different sensitivity, detection specificity, precision, selectivity and reliability, and for this reason divergent results are not surprising. For example, one study comparing a microbial and a chemiluminescence method in estimating the vitamin $B_{12}$ content of spirulina tablets (Watanabe *et al.*, 1998) obtained extremely different results (147.5 *vs.* 17.35 µg/100 g for the

Table 5: Vitamin $B_{12}$ content in feeds (µg/100 g feed) supplemented with spirulina (*Arthrospira platensis*) and its effect on vitamin $B_{12}$ content (µg/100 g meat) in raw *Longissimus dorsi* (LD) meat.

| | Experimental groups | | | *P*-value | SEM |
|---|---|---|---|---|---|
| | C-C | S-S | ST-ST | | |
| No. of samples | 6 | 6 | 6 | | |
| Vitamin $B_{12}$ in feeds | 0.509 | 0.841 | 0.697 | - | - |
| Vitamin $B_{12}$ in LD meat | 0.662[a] | 0.954[b] | 0.805[ab] | 0.012 | 0.03 |

Experimental groups as defined in Table 1. SEM: Standard Error of the Least Squares Means.

[a,b] Means in the same row not sharing superscripts differ at $P<0.05$.

microbial and chemiluminescence methods, respectively), thus suggesting that the commonly-known and accepted values for this microelement could easily be over- or under-estimated. The development of laboratory techniques that allow reliable and comparable vitamin $B_{12}$ quantification in food for more precise nutritional information to consumers is therefore desirable.

## CONCLUSIONS

Dietary supplementation with 5% spirulina and/or 3% thyme and the duration of treatment had no effect on rabbit carcass composition, LD and HL traits, or HL bone traits. Spirulina was effective in fortifying vitamin $B_{12}$ content of LD meat, even if the absorption percentage decreased as dietary vitamin $B_{12}$ increased. Weak signs of carcass fatness change were observed with dietary spirulina/thyme supplementation, thus requiring further studies to demonstrate if and how these supplements affect lipid metabolism.

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