World Rabbit Sci. 2014, 22: 1-9
doi:10.4995/wrs.2014.1351
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EFFECT OF DIETARY SUPPLEMENTATION OF SPIRULINA (*ARTHROSPIRA PLATENSIS*) AND THYME (*THYMUS VULGARIS*) ON APPARENT DIGESTIBILITY AND PRODUCTIVE PERFORMANCE OF GROWING RABBITS

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Abstract: The aim of this study was to evaluate the effect of dietary supplementation with spirulina (*Arthrospira platensis*) or/and thyme (*Thymus vulgaris*) on total tract apparent digestibility of nutrients and the performance of growing rabbits. At weaning (5 wk of age) the rabbits were randomly allotted to 7 groups (42 rabbits/group, 3 rabbits/cage). Rabbits in the control group (C-C) received a control pelleted feed throughout the experiment (5-11 wk of age) without any supplementation (crude protein: 176 g CP/kg, neutral detergent fibre: 325 g NDF/kg). In the other groups, the control diet was supplemented with 5% spirulina (S, mainly in substitution of soybean meal), or 3% thyme (T, mainly in substitution of alfalfa meal) or by both 5% S and 3% T (ST) for the whole (5-11 wk of age; groups: S-S, T-T, ST-ST) or part of the growing period (8-11 wk of age; groups: C-S, C-T, C-ST). Supplementations had no effect on apparent digestibility of dry matter, organic matter, acid detergent fibre, gross energy and digestible energy. The CP TTAD was lowest in rabbits fed the S diet, whereas it was highest in C fed rabbits, the other 2 treatments being intermediate (P<0.001). The starch TTAD was lowest for S fed rabbits (98.3%) and highest for ST fed rabbits (99.4%), the other 2 dietary groups being intermediate (P<0.001). In contrast, the ether extract TTAD was higher in T than ST and C dietary groups (on av. 70.4 *vs.* 67.7% respectively; P<0.001), with S fed rabbits showing an intermediate value (69.1%). The NDF TTAD of the ST diet was lower than that of the other 3 groups (16.4 *vs.* 21.0% respectively; P<0.001). The TTAD of Ca reached the lowest value for the S diet (53.5%) compared with the other 3 diets (on av. 59.1%; P<0.001). The S diet also had the lowest digestibility (P<0.001) for K and P, but in this case the C group always showed the highest values (P<0.001), with T and ST rabbits exhibiting intermediate results. Spirulina and/or thyme dietary supplementation had no effect on feed intake (133 g/d), daily weight gain (38.3 g/d), morbidity (9.9 %) or mortality (1.8 %). Significant differences were only found for feed conversion ratio, which was lower for the C-T group (3.39) than for the C-C group (3.54; P<0.05). Based on these results, spirulina and thyme included separately or combined in growing rabbit diets did not exhibit substantial effects on growth performance or health status.

Key Words: Spirulina, thyme, rabbits, growth performance, total tract apparent digestibility.

INTRODUCTION

Spirulina *(Arthrospira platensis)* is generally regarded as a rich source of protein, vitamins, essential amino acids, minerals, essential fatty acids (FA) and antioxidant pigments like carotenoids (Belay *et al.*, 1996; Dalle Zotte and Szendrő, 2011). Feeding spirulina-enriched diets (5, 10 and 15%) to rabbits from 9 to 13 wk of age (Peiretti and Meineri, 2008, 2011) did not result in significant effects on productive performance, despite the fact that the meat lipids content increased and their FA profile changed (polyunsaturated FA and n-6/n-3 increased; total n-3 FA

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http://dx.doi.org/10.4995/wrs.2014.1351

decreased). As no mortality or health problems were observed (Peiretti and Meineri, 2008), the effect of spirulina supplementation on health status could not be assessed.

Thyme *(Thymus vulgaris)* is rich in essential oils (2.17-4.73%) which are known to have antioxidant properties and antimicrobial activity against food-borne pathogens (Jordán *et al.*, 2006; Rota *et al.*, 2008). Özkan *et al.* (2010) examined the effect of a methanol extract (essential oil) from wild thyme *(Thymus serpyllum)* on coccidiosis in rabbits. The treatment (100 mg/kg body weight) decreased the faecal oocyst counts and increased growth rate until 24 d post treatment.

Research into animal feeding tested the inclusion of thyme essential oils widely and found beneficial effects on gut microflora (Hernández *et al.*, 2004; Amad *et al.*, 2011) and growth performance (Abd El-Hakim *et al.*, 2009) in growing broiler chickens. Dried thyme leaves are also supposed to have a certain effect on health and growth performance. In rabbits, the only study where dry thyme leaves at 2.5% of dietary inclusion level was used did not find beneficial effects either on live performance or health status (Dalle Zotte *et al.*, 2013), likely due to the low inclusion level or the optimal health status of rabbits in this trial.

Available data on mineral digestibility, especially in rabbits, are scarce. Spirulina is a good source of minerals but the effect of its supplementation on mineral digestibility has been tested only once, in companion dwarf rabbits (Dalle Zotte *et al.*, 2013). Thyme has never been evaluated in this sense: the presence of tannins (polyphenols, plant materials) may negatively affect mineral digestibility, as reported by Al-Mamary *et al.* (2001) when evaluating the effects of dietary sorghum tannins on rabbit digestive enzymes and mineral absorption.

Therefore, this study aimed to evaluate the effect of dietary supplementation and the length of supplementation (between the ages of 5-11 or 8-11 wk) of spirulina (5%) or/and thyme leaves (3%) on total tract apparent digestibility of nutrients, productive performance and health status in growing rabbits.

MATERIAL AND METHODS

Animals and experimental design

The experiment was conducted at the experimental rabbit farm of Kaposvár University (Hungary) using a maternal rabbit line (selected for litter size, adult body weight: 4.0 to 4.5 kg). All animals were treated according to the principles stated by EC Directive 86/609/EEC regarding the protection of animals used for experimental and other scientific purposes. All rabbits received a pelleted control diet (C) from 3 wk of age onwards. After weaning (at 5 wk of age) the individually marked rabbits were housed in wire net cages (0.61×0.32 m, 3 rabbits/cage). The environmental temperature was kept from 15 to18 and daily lighting in the rabbitry was 16 h long (6:00-22:00).

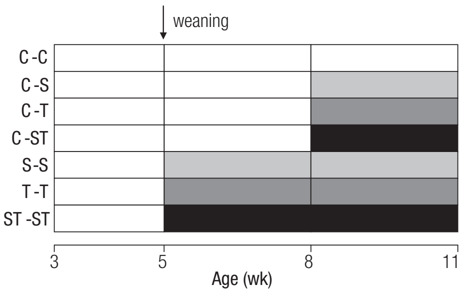

Figure 1: Experimental design. ☐ C-Basic feed; ■ S-Spirulina supplementation (5%); ■ T- Thyme supplementation (3%); ■ ST-Spirulina+Thyme supplementation (S:5%; T:3%).

Two hundred and ninety-four weaned rabbits weighing 952±81 g were randomly allotted to 7 groups (42 rabbits/group, no littermates were allowed within the groups, 3 rabbits/cage, 14 cages/treatment). Rabbits in the control group (C-C) received a pelleted diet without any supplementation throughout the experiment (5-11 wk of age). In the other groups, the diet was supplemented with 5% spirulina (*Arthrospira platensis*) (S diet, mainly in substitution of soybean meal), or with 3% thyme (*Thymus vulgaris*) leaves (T diet, mainly in substitution of alfalfa meal) or by both supplements (ST diet) before pelleting and fed to rabbits for the whole experimental period (5-11 wk of age; groups: S-S, T-T, ST-ST, respectively) or during the last 3 wk of the growing period (8-11 wk of age; groups: C-S, C-T, C-ST, respectively, Figure 1).

Table 1: Chemical composition (g/kg) of spirulina (*Arthrospira platensis*) and thyme (*Thymus vulgaris*).

	Arthrospira platensis	*Thymus vulgaris*
Dry matter	944	889
Crude protein	658	52.3
Ether extract	8.6	31.9
Crude fibre	n.d.	181
Ash	65.1	65.9
Neutral detergent fibre	2.4	298
Acid detergent fibre	4.8	210
Acid detergent lignin	0.6	68.1
Starch	35.6	58.4
Ca	2.2	13.6
P	9.2	0.7
Ca/P	0.2	18.7
Gross energy (MJ/kg)	19.5	15.7

n.d.: not detected.

Supplements (spirulina powder and dried thyme leaves) were purchased from a commercial supplier. To obtain diets balanced in energy and nutrient content, the chemical composition and energy content of the supplements were analysed beforehand (Table 1). Then, in order to formulate isonitrogenous, iso-energetic and isofibrous diets, soybean meal represented the main ingredient that was replaced by the supplements studied.

The diets contained no medication and no coccidiostat. Water and feed were available *ad libitum*. The ingredients and the chemical composition and mineral profile of the experimental diets are shown in Table 2 and 3, respectively.

Digestibility trial

The total tract apparent digestibility (TTAD) of dry matter (DM), organic matter, crude protein (CP), ether extract (EE), starch, neutral detergent fibre (NDF), acid detergent fibre (ADF), cellulose, hemicelluloses, gross energy (GE), Ca, K and P of the experimental diets (C, T, S and TS) was measured in an *in vivo* digestibility trial conducted at the experimental farm of Padova University (Italy). The digestibility trial was approved by the Italian Ministry of Education,

Table 2: Ingredients of the experimental diets.

	Experimental diets			
	C	S	T	ST
Ingredient, as-fed basis (g/kg)				
Dehydrated alfalfa meal 170 g/kg CP	400	398	370	398
Barley meal	247	262	237	262
Soybean meal	130	55	140	60
Wheat straw	120	110	120	90
Dried apple pomace	40	40	40	40
Spirulina	-	50	-	50
Thyme leaves	-	-	30	30
Fat powder[1]	35	35	35	35
Sodium Chloride	5.0	5.0	5.0	5.0
Monocalcium phosphate	3.0	3.0	3.0	3.0
DL-methionine	1.0	1.0	1.0	1.0
L-lysine	4.0	6.0	4.0	6.0
Premix[2]	5.0	5.0	5.0	5.0
Zeolite	10	30	10	15

C: Basic feed; S: Spirulina supplementation (5%); T: Thyme supplementation (3%); ST: Spirulina+Thyme supplementation (S:5%; T:3%).

[1] Fat content: 40%.

[2] 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; Fe, 100 mg; Cu, 20 mg; Mg, 50 mg; Co, 2 mg; I, 1mg; Zn, 100 mg; Se, 0.1 mg.

Table 3: Chemical composition of experimental diets.

	Experimental diets			
	C	S	T	ST
Analysed composition (g/kg as fed)				
Dry matter	896	898	898	896
Crude protein	176	170	175	172
Ether extract	25	26	27	28
Ash	86	75	84	77
Crude Fibre	160	162	157	158
Neutral detergent fibre (NDF)	323	316	314	301
Acid detergent fibre (ADF)	212	205	208	195
Acid detergent lignin (ADL)	53	45	53	46
Hemicelluloses (NDF-ADF)	111	111	106	106
Cellulose (ADF-ADL)	158	160	155	149
Starch	163	181	170	178
NFE[1]	463	478	471	471
NNCC[2]	325	352	341	356
Gross energy (MJ/kg)	16.3	16.5	16.4	16.4
Ca	9.6	9.3	9.4	10.6
P	4.0	3.9	4.0	4.3
K	10.5	9.4	9.7	10.6
Na	2.7	2.6	2.4	2.7
Fe	0.52	0.48	0.53	0.54

C: Basic feed; S: Spirulina supplementation (5%); T: Thyme supplementation (3%); ST: Spirulina+Thyme supplementation (S:5%; T:3%).

[1]NFE: Nitrogen Free Extracts.

[2]NNCC: Non nitrogenous cellular content=Organic matter–CP–NDF.

University and Research and the Ethical Committee of the Padova University. The digestibility trial was carried out on 32 (8 animals per diet) 62 d-old hybrid rabbits according to the European standardised method (Perez *et al.,* 1995). Rabbits were equally distributed by gender and live weight into the 4 dietary groups and individually caged. After 1 wk of adaptation to the new diets, faeces were collected over a 4-d period.

Data collection and management

Body weight (BW) was measured at 5, 8 and 11 wk of age and feed intake (FI) for 5-8 and 8-11 wk period, daily weight gain (DWG) and feed conversion ratio (FCR) were then calculated. BW and DWG were evaluated based on individual data (n=42 rabbits/group), whereas FI and FCR were assessed based on the cage unit (n=14 cages/group). When calculating FI, it was assumed that morbid rabbits did not consume any pellets for the 2 d preceding their death. Morbidity and mortality were recorded weekly and daily, respectively. Rabbits suffering from diarrhoea and/or with a negative or very low DWG during a 1-wk period were considered as morbid. When the same individual was registered with diarrhoea at several subsequent examinations, morbidity was registered only once within the same period.

Chemical analyses

Analyses of spirulina and thyme supplements as well as those of the experimental diets and faeces were carried out in duplicate using AOAC (2000) methods to determine the concentrations of DM (934.01), CP (2001.11), crude fibre (CF; 978.10), ash (967.05) and starch (amyloglucosidase- α-amylase method, 996.11). EE was determined after acid-hydrolysis (EC, 1998). NDF (without sodium sulphite), ADF, and acid detergent lignin (ADL) were analysed according to Mertens (2002), AOAC (2000, procedure 973.187) and Van Soest *et al.* (1991), respectively, using the sequential procedure and the filter bag system (Ankom Technology, New York). The GE was measured with an adiabatic bomb calorimeter (ISO, 1998). Mineral analyses were performed on spirulina and thyme supplements (Ca, P), on the experimental diets (Ca, P, K, Na, Fe) and faeces (Ca, P, K) by ICP-OES (Spectro Ciros Vision EOP) after microwave digestion (AOAC 2000, 999.10).

Statistical analysis

The productive traits and digestibility data were analysed by one-way ANOVA; morbidity and mortality by chi-square test using SPSS 10.0 software package, with diet as fixed effect. Probability values were considered significant when <0.05.

RESULTS AND DISCUSSION

Total tract apparent digestibility

Dietary supplementation of spirulina, thyme and the combination of the 2 natural supplements did not affect rabbits' DM intake (Table 4). In contrast, TTAD of nutrients showed great differences among dietary groups (Table 4). The CP TTAD was lowest in rabbits fed the S diet, whereas it was highest in C fed rabbits, the other 2 treatments being intermediate (P<0.001). The low levels of CP TTAD for S and ST diets might be related to a lower CP digestibility of spirulina and thyme compared to soybean meal (Table 3).

The starch TTAD was lowest for S-fed rabbits (98.3%) and highest for ST fed rabbits (99.4%), the other 2 dietary groups being intermediate (P<0.001). On the other hand, the EE TTAD was higher in T than ST and C dietary groups (on av. 70.4 *vs.* 67.7% respectively; P<0.001), with S fed rabbits showing an intermediate value (69.1%).

As far as fibre fractions TTAD are concerned, only the TTAD of NDF and hemicelluloses were found to be different among dietary groups. The value of the ST diet was lower than that of the other 3 groups (16.4 *vs.* 21.0% for NDF; P<0.01, and 29.2 *vs.* 38.8% for hemicelluloses; P<0.001.

The TTAD of the macroelements Ca, K and P also differed among dietary treatments (Table 4). The TTAD of Ca reached the lowest value for the S diet (53.5%) compared with the other three diets (on av. 59.1%; P<0.001); analogous results were found for K and P (P<0.001). Regarding these latter 2 macroelements, the C group always showed the highest value, with T and ST rabbits exhibiting intermediate digestibility coefficients (P<0.001). The only other study on mineral TTAD in rabbits fed both supplements was recently conducted by Dalle Zotte *et al.* (2013) on dwarf rabbits

Table 4: Effect of dietary spirulina (*Arthrospira platensis*) and thyme (*Thymus vulgaris*) on total tract apparent digestibility (TTAD) and nutritive value (8 rabbits/diet).

	Experimental diets					
	C	S	T	ST	RSD	P-value
Live Weight (g)	1812	1897	1842	1778	223	ns
Dry matter (DM) intake, (g/d)	128.6	139.1	139.6	127.8	17.8	ns
TTAD (%)						
DM	61.4	60.6	60.7	61.0	1.49	ns
Organic matter	62.5	62.0	62.2	62.0	1.44	ns
Crude protein	76.0^{c}	73.1^{a}	74.6^{bc}	74.4^{ab}	0.97	<0.001
Ether extract	67.8^{a}	69.1^{ab}	70.4^{b}	67.7^{a}	1.18	<0.001
Neutral detergent fibre (NDF)	23.2^{b}	19.1^{b}	20.9^{b}	16.4^{a}	3.07	0.002
Acid detergent fibre (ADF)	13.58	9.69	11.92	9.39	3.39	ns
Hemicelluloses (NDF-ADF)	41.5^{b}	36.5^{b}	38.5^{b}	29.2^{a}	2.47	<0.001
Cellulose (ADF-ADL)	19.3	16.5	17.1	15.6	3.16	ns
Starch	99.1^{b}	98.3^{a}	99.2^{b}	99.4^{c}	0.04	<0.001
Gross energy	62.3	61.6	62.0	61.7	1.45	ns
Ca	60.7^{b}	53.5^{a}	58.0^{b}	58.7^{b}	1.60	<0.001
P	45.6^{c}	36.1^{a}	41.5^{b}	43.2^{b}	2.22	<0.001
K	86.6^{d}	80.5^{a}	82.9^{b}	85.4^{c}	0.62	<0.001
Nutritive value:						
Digestible protein (DP), (g/kg)	133.3^{d}	124.2^{a}	130.5^{c}	127.6^{b}	1.72	<0.001
Digestible energy (DE), (MJ/kg)	10.14	10.19	10.19	10.11	0.21	ns
DP to DE ratio (g/MJ)	13.16^{c}	12.19^{a}	12.81^{b}	12.62^{b}	0.13	<0.001

C: Basic feed; S: Spirulina supplementation (5%); T: Thyme supplementation (3%); ST: Spirulina+Thyme supplementation (S:5%; T:3%).
RSD: Residual Standard Deviation; ns: no significant.
[a,b,c,d] Means not sharing superscript in the same row are significantly different at P<0.05.

supplemented with 3% spirulina and/or 2.5% thyme and no significant effect was evidenced. However, based on the results obtained in these 2 studies, some differences emerged. In our work the TTAD of Ca was 57.7% on average (Table 4), definitely higher than that (36.8%) found by Dalle Zotte *et al.* (2013). A similar pattern was noted for the average TTAD of K and P in the 2 studies (83.9 *vs.* 69.0% and 41.6 *vs.* 13.3% for K and P, respectively). In the 2 studies, the age of the rabbits was similar and they were in perfect sanitary condition during the digestibility trial, but the dietary EE content differed, being lower in the current study. However, no negative influence on mineral TTAD attributable to dietary EE content has been reported in the literature (Fernández *et al.,* 1994).

As a direct consequence of the CP TTAD trend, the dietary digestible protein (DP) content was highest for rabbits fed C diet and lowest for those fed S diet ($P<0.001$). Thus, the DP to digestible energy (DE) ratio also exhibited the same trend ($P<0.001$).

Our results did not confirm those reported by Peiretti and Meineri (2009), who observed an improvement in the TTAD of the CP caused by dietary inclusion of 1% spirulina. However, in the study by Peiretti and Meineri (2009) the inclusion level of the ingredients was not provided, the rabbits were much older than ours, they were feed-restricted, and the dietary CP content varied between control and supplemented diets. Thus, the 2 studies followed very different protocols and the results obtained for nutrients digestibility are scarcely comparable.

However, it is assumed that the CP TTAD seems to be dependent on the inclusion levels of spirulina. In fact, whereas the 3% spirulina inclusion produced no adverse effect on CP TTAD (Dalle Zotte *et al.*, 2013), its increase to 10% and 15% (in substitution of soybean meal and alfalfa meal) resulted in a negative TTAD trend for CP but also for the other nutrients (Peiretti and Meineri, 2008).

Another hypothesis for such results on TTAD of nutrients could be deducted from zeolite presence in the diets, especially for S diet, where a higher inclusion level was found. This natural clay was added in order to produce diets with similar chemical composition. Several studies evaluated the effect of dietary zeolite supplementation on growth, digestive metabolism and nutrient digestibility of livestock, especially ruminants and pigs. In a study on growing pigs, retention of Ca, P, Mg, Na, K and Fe was linearly reduced by increasing dietary supplementation levels of zeolite (Shurson *et al.*, 1984).

Based on the results obtained so far, the effect of dietary spirulina and/or thyme supplementation on nutrient digestibility in rabbits is still unclear. The results were especially surprising for spirulina, as Alvarenga *et al.* (2011) showed that *Arthrospira platensis* had a higher nutritional value compared to soybean meal in terms of crude protein content, mineral matter, apparent metabolisable energy and amino acids profile. Further investigation is therefore needed to elucidate the mode of action of S and T in the rabbit digestive system, and the feed processing technology should be taken also into account, as it probably plays a role as a source of variation in feed digestibility. Caecal microbiota and fermentation of rabbits fed S and T supplements were also considered in another study (Vántus *et al.*, 2012), but no substantial effects were observed.

Dietary thyme inclusion level of 3% slightly impaired nutritive value, whereas synergic effect of S and T supplements was not evidenced; additional research is thus needed, as this was the first scientific attempt to test this combination in rabbits reared for meat purposes.

Productive performance

Despite the fact that the digestive efficiency of some nutrients was different among control and treated groups, separated or combined dietary inclusion of spirulina and thyme did not affect BW, average DWG or FI of growing rabbits throughout the trial (Table 5). In contrast, FCR from 8 to 11 wk of age and that of the whole period (5-11 wk of age) were affected by dietary treatments ($P<0.05$). Specifically, C-T rabbits exhibited better FCR than C-C ones ($P<0.05$), whereas the other feeding groups presented intermediate values.

Similar to the results of the present trial, other studies did not observe differences in rabbit live performance when fed spirulina supplements at levels of 0.5% (Colla *et al.*, 2008), 1% (Peiretti and Meineri, 2009), 3% (Dalle Zotte *et al.,* 2013) or 5, 10 and 15% (Peiretti and Meineri, 2008).

Table 5: Effect of dietary supplementation with spirulina (*Arthrospira platensis*) and thyme (*Thymus vulgaris*) on live performance of rabbits from 5 to 11 weeks of age.

	Experimental groups								
	C-C	C-S	C-T	C-ST	S-S	T-T	ST-ST	SEM	*P*-value
No. rabbits	42	42	42	42	42	42	42		
Daily weight gain (g/d)									
5-8 wk	41.9	42.2	40.5	43.2	42.2	43.9	41.7	0.36	ns
8-11 wk	33.6	33.0	35.9	34.5	34.9	34.3	34.5	0.33	ns
5-11 wk	37.8	37.8	38.2	38.8	38.6	39.1	38.1	0.23	ns
Feed intake (g/d)									
5-8 wk	115	114	109	119	113	118	115	0.96	ns
8-11 wk	152	148	151	154	152	151	154	1.02	ns
5-11 wk	134	131	130	137	133	135	134	0.80	ns
Feed conversion rate									
5-8 wk	2.75	2.72	2.69	2.76	2.69	2.68	2.76	0.01	ns
8-11 wk	4.57^{b}	4.50^{ab}	4.21^{a}	4.50^{ab}	4.37^{ab}	4.42^{ab}	4.49^{ab}	0.03	0.033
5-11 wk	3.54^{b}	3.48^{ab}	3.39^{a}	3.52^{ab}	3.44^{ab}	3.44^{ab}	3.53^{ab}	0.01	0.032
Live weight at 11 wk (g)	2542	2539	2551	2582	2569	2594	2555	11.6	ns

C-C: Basic feed without any supplementation (5-11 wk of age); S: Spirulina supplementation (5%); T: Thyme supplementation (3%); ST: Spirulina+Thyme supplementation (S:5%; T:3%); S-S, T-T, ST-ST: supplemented feed (5-11 wk of age); C-S, C-T, C-ST: rabbits fed with C diet till 8 wk of age, and with supplemented diets between 8 and 11 wk of age. SEM: Standard error of the mean; No. of replicates:14 cages/treatment for feed intake and feed conversion ratio and 42 rabbits/treatment for body weight and daily weight gain; ns: no significant.

^{a,b} Means not sharing superscript in the same row are significantly different at $P<0.05$.

In poultry, sewage-grown spirulina was first tested as a replacement for ground nut cake (Saxena *et al.*, 1983) and results were promising because the 6-week weight gain and FI were improved by increasing the level of dietary spirulina inclusion. In another study in weanling pigs (Grinstead *et al.*, 2000), dietary supplementation with 0.2, 0.5 and 2% spirulina (in substitution of soybean meal) showed slight improvements in growth performance and feed efficiency, but only if considering certain phases of the cycle.

The effect of thyme supplementation on rabbit performance has not been clearly demonstrated yet, so further research is required. In one study (Ibrahim *et al.*, 2000), thyme supplementation promoted an increase in BW, DWG and FI in rabbits. In contrast, a more recent experiment by Dalle Zotte *et al.* (2013), reported that 2.5% thyme supplementation in diets fed to dwarf rabbits for 14 wk did not lead to any difference in live performance. The only exception was in the 8-9 wk of age period, when rabbits fed a diet supplemented with thyme showed higher daily weight gain compared to those fed simultaneously with spirulina and thyme.

Table 6: Effect of dietary supplementation with spirulina (*Arthrospira platensis*) and thyme (*Thymus vulgaris*) on rabbit morbidity and mortality rates (%) from 5 to 11 wk of age.

	Experimental groups							
	C-C	C-S	C-T	C-ST	S-S	T-T	ST-ST	*P*-value
Morbidity:								
5-8 wk	7.1	11.9	14.3	4.8	9.5	4.8	14.3	ns
8-11 wk	0.0	0.0	0.0	0.0	0.0	2.4	0.0	ns
5-11 wk	7.1	11.9	14.3	4.8	9.5	7.1	14.3	ns
Mortality:								
5-8 wk	2.4	0.0	2.4	0.0	2.4	0.0	0.0	ns
8-11 wk	0.0	4.8	0.0	0.0	0.0	0.0	0.0	ns
5-11 wk	2.4	4.8	2.4	0.0	2.4	0.0	0.0	ns

C-C: Basic feed without any supplementation (5-11 wk of age); S: Spirulina supplementation (5%); T: Thyme supplementation (3%); ST: Spirulina+Thyme supplementation (S:5%; T:3%); S-S, T-T, ST-ST: supplemented feed (5-11 wk of age); C-S, C-T, C-ST: rabbits fed with C diet till 8 wk of age, and with supplemented diets between 8 and 11 wk of age.
ns: no significant.

Rabbit morbidity and mortality were satisfactory throughout the study period and no effects attributable to the dietary treatments were observed (Table 6). Morbidity ranged from 4.8% (C-ST group) to 14.3% (C-T and ST-ST group) in the whole growth period (5-11 wk of age). Mortality was also very low, ranging from zero (C-ST, T-T, ST-ST groups) to 4.8% (C-S group). These results support those in the literature (Peiretti and Meineri, 2008). In both studies, the favourable environmental conditions and excellent health status of the rabbits did not allow us to verify the assumed positive effects of spirulina and/or thyme in preventing digestive disorders.

Optimal DP to DE ratio for growing rabbits, suggested in order to guarantee both high performance and good sanitary conditions, ranges from 9.5 to 10 g/MJ (Carabaño *et al.*, 2009; De Blas and Mateos, 2010). However, even if the DP to DE ratio obtained in our study was higher and varied between 12.19 and 13.16 g/MJ, no negative effects either on growth performance (Table 5) or on morbidity and mortality (Table 6) were observed.

When growing rabbits' diet was supplemented with a blend of formic acid, lactic acid and essential oil of rosemary, thyme and cinnamon, mortality was lower compared to those fed only with formic and lactic acid, but similar to that of a control group without any additive and to another one supplemented with antibiotics (Cesari *et al.*, 2008). Dietary inclusion of *Thymus serpyllum* decreased the faecal oocyst count in rabbits, which could be considered a possible indicator of the rabbits' health (Özkan *et al.*, 2010).

In a recent study (Vántus *et al.*, 2012), the S and/or T dietary supplementations had no substantial effect on the volatile FA content of caecal digesta, even though by means of Quantitative PCR it was demonstrated that ST supplementation exerted an antimicrobial effect on the bacterial groups investigated in the caecum.

CONCLUSIONS

Supplementing diets with 5% spirulina (in substitution of soybean meal) or 3% thyme (in substitution of alfalfa meal) in growing rabbits for 3 or 6 wk reduced the nutritive value of the diets. Particularly, spirulina lowered CP, starch and mineral TTAD, suggesting a lower protein digestibility than soybean meal. Despite this, no substantial effect on rabbit performance and health status was observed. Spirulina and thyme in combination did not provide the advisable synergistic effect. Future studies should take into consideration feed processing technology, pelleting, storage and packaging conditions, as they may reduce or nullify the availability of nutrient and functional compounds. The effects of spirulina and/or thyme on health status should be tested under poorer sanitary conditions.

Acknowledgements: Research funded by Padova University research funds (Progetti di Ricerca di Ateneo 2011) code: CPDA117509/11, and the GOP-1.3.1-11/B-2011-0045.

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