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Effect of purple loosestrife (*Lythrum salicaria*) diet supplementation in rabbit nutrition on performance, digestibility, health and meat quality

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In this study, 160 Hycole weaned rabbits (35 days old) were randomly divided into four groups of 40. The rabbits were studied throughout a 54-day experimentation period in order to determine the impact of dietary supplementation from herbs composed of 0.2%, 0.4% dry ground Lythrum salicaria *leaves (LS) and 0.3% Cunirel® (CR; a commercial herb mixture containing LS as the main ingredient) on performance, digestibility, health and meat quality. The basal diet was given to the control group. No significant differences were found in performance, 10 rabbits from each group were selected for evaluation regarding apparent digestibility. The rabbits fed the control diet and the diet with the low level of LS had a higher level of CP digestibility than did the animals that were supplemented with the high LS levels and CR (85.7% and 84.9%* v. *84.0% and 84.0%, respectively;* $P < 0.05$*). The ether extract digestibility was lower in the treatment group with 0.4%LS addition and CR as compared with the control group (52.2% and 54.5%* v. *62.6%, respectively;* $P < 0.05$*). The slaughter process was performed on 89-day-old rabbits to study the carcass characteristics, meat quality, blood parameters, caecal contents and gut histology. The total leukocyte counts in the control animals were lower than they were in the rabbits fed 0.2%, 0.4%LS and CR (4.06* v. *8.25, 8.63 and 8.21* $\times 10^9$*/l, respectively;* $P < 0.05$*). For caecal fermentation, the caecal contents of the rabbits fed 0.4% of LS, showed higher concentrations of total volatile fatty acid (VFA; 24.1* v. *18.9 mg/kg dry matter (DM);* $P < 0.05$*) and acetic acid (18.3* v. *14.4 mg/kg DM;* $P < 0.05$*), but lower ammonia levels (594* v. *892 mg/kg DM;* $P < 0.05$*) as compared with the control group. PCR-denaturing gradient gel electrophoresis analyses were performed to evaluate the microbial community in hard faeces, collected at days 35, 42, 49, 56, 70 and 89, whereas the caecal contents were taken after slaughtering. The results demonstrated that between the treatment groups, the similarity of the microbial communities was higher as compared with the control group. Moreover, only age was shown to influence microbiota diversity. In conclusion, the results of this study indicated that supplementation of LS in rabbit diets leads to an increase in the total white blood cells, total VFA and acetic acid concentration, and a decrease in the ammonia levels, as well as the digestibility when CR and high level of LS were supplemented, without causing any adverse effects on other parameters.*

Keywords: blood, digestibility, *Lythrum salicaria*, rabbit, volatile fatty acid

Implications

Herbs have been used as alternative dietary supplementation in animal production since the prohibition of using of antibiotic growth promoter due to serious problems with the occurrence of antibiotic-resistant bacteria in humans. The supplementation of *Lythrum salicaria* increased in the total white blood cells and impacted caecal fermentation, which was related to animal health and was speculated to have benefits. However, a decrease in the digestibility of CP and ether extract was observed without the existence of any adverse effects on performance and meat quality.

Introduction

The long-term supplementation of animal feeds with antibiotics at subtherapeutic doses (antibiotic growth promoter, AGP) has been performed since 1951. This was often done

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without veterinary prescription, with the aim of promoting growth performance, maintaining the animal's health and reducing the mortality rate after the weaning period, which is a major problem for rabbit production (Phillips, 2007). In 2006, because of the risk of the development of drug-resistant bacteria, the European Union banned the use of AGPs. Meanwhile, a sharp deterioration in animal health and performance was observed, with a consequent decrease in profits (Phillips, 2007). Several different approaches have been tested to control or prevent diseases and improve productive performance.

Aromatic plants contain many biologically active compounds that exhibit medicinal properties (Christaki *et al*., 2012) that could improve production performance, as well as animal health and meat quality. Supplementation with alternative substances, such as probiotics, prebiotics, enzymes and organic acids, has been studied in rabbits, with interesting results emerging (Falcão-e-Cunha *et al*., 2007; Rotolo *et al*., 2014). However, the number of phyto-studies remains limited (Botsoglou *et al*., 2004; Krieg *et al*., 2009; Arafa *et al*., 2010; Simonová *et al*., 2010; Ayala *et al*., 2011; Szabóová *et al*., 2012; Rotolo *et al*., 2013). *L. salicaria* (LS) is a flowering plant, which is commonly known as purple loosestrife, belonging to the Lythraceae family. LS is considered an invasive and competitive plant in ecosystems. However, this herb has been used in traditional medicine because of its medicinal properties. In fact, several *in vitro* studies used the active compounds (e.g. tannins and flavonoids) that were extracted from LS, showing that LS also has antimicrobial, antifungal, anti-inflammatory and anti-oxidant properties (Becker *et al*., 2005; Tunalier *et al*., 2007; Humadi and Istudor, 2009). The aim of this study was, therefore, to evaluate the supplementation of feeds with LS on the performance, digestibility, health and meat quality of growing rabbits.

Material and methods

Animals, housing, diets and condensed tannin content (CTC) determination

The experiment was performed at the experimental rabbitry facility at the Department of Agricultural, Forestry and Food Sciences in Carmagnola, Turin, Italy. In this study, 160 Hycole rabbits (934 ± 118 g) were randomly housed in individual wire cages and reared from weaning (35 days) to slaughtering (89 days). A basal diet was formulated in order to cover the nutritional requirements of the growing rabbits (control). In addition, three experimental diets were set up with 0.2% (0.2%LS) or 0.4% (0.4%LS) dry ground LS leaves and 0.3% Cunirel® (CR, Biotrade snc®, Modena, Italy) in place of small fractions of barley meal in the basal diet (Table 1, Supplementary Table S1). CR is a commercial mixture of herbs that contains LS as a major component. The diets were assigned to the animals (40 animals/diet) using a completely randomised design. The feeds and clean water were provided *ad libitum* and, the facility was climate and light controlled during the whole trial in order to maintain a temperature of 22 ± 2°C and a photoperiod of 16L : 8D. The diets were analysed in triplicate for dry matter (DM), CP by total nitrogen contents, ether extract (EE), crude fibre and ash by ignition to 550°C, according to the Association of Official Analytical Chemists (AOAC, 2000). The NDF, ADF and ADL were determined according to Van Soest *et al*.'s (1991) procedures. The level of starch was determined using Ewer's polarimetric method (European Economic Community, 1972).

The CTC contents were determined in LS, CR, and the experimental diets, according to the method described by Lahouar *et al*. (2014). A 50 µl aliquot of each extract or

Table 1 *Ingredient composition and condensed tannin of the experimental diets*

	Control diet
Ingredients (%)	
Dehydrated alfalfa meal	29
Wheat bran	20
Barley[1]	19
Dried beet pulp	14
Soybean seed meal	6
Sunflower seed meal	6
Soybean oil	1
Molasses	1.5
Vitamin–mineral premix[2]	1
Wheat straw	1
Corn gluten	1
Dicalcium phosphate	0.5
Supplements[3]	0
Analysed composition on a dry matter basis (%)[4]	
Dry matter	89.7
CP	18.1
Ether extract	3.0
Ash	6.41
Crude fibre	17.5
NDF	34.2
ADF	19.1
ADL	3.71
Starch	22.6
Condensed tannin (mg catechin equivalent/100 g)[5]	5.29

0.2%LS = 0.2% of dry ground *Lythrum salicaria* (LS) supplementation in diets; 0.4%LS = 0.4% of dry ground LS supplementation in diets; CR = 0.3% of commercial supplementation in diets which is a mixture of medicinal plants with LS is the main composition (Cunirel®, Biotrade snc®, Modena, Italy).

[1]The percentage on a dry matter basis of barley in 0.2%LS, 0.4%LS and CR were 18.8, 18.6 and 18.7, respectively.

[2]Per kg of diet: vitamin A, 200 IU; α-tocopheryl acetate, 16 mg; niacin, 72 mg; vitamin B_6, 16 mg; choline, 0.48 mg; DL-methionine, 600 mg; Ca, 500 mg; Pt1, 13 920 mg; K, 500 mg; Na, 1 g; Mg, 60 mg; Mn, 1.7 mg; Cu, 0.6 mg.

[3]The percentage on a dry matter basis of supplementation in 0.2%LS, 0.4%LS and CR were 0.2, 0.4 and 0.3, respectively.

[4]Analysed composition on a dry matter basis of 0.2%LS, 0.4%LS and CR, respectively; 89.9%, 90.5% and 90.8% (dry matter); 18.2%, 18.1% and 18.2% (CP); 3.0%, 3.0% and 3.0% (ether extract); 6.52%, 6.60% and 6.11% (ash); 17.5%, 17.2% and 17.7% (crude fibre); 34.7%, 34.6% and 34.3% (NDF); 19.1%, 18.9% and 19.5% (ADF); 3.58%, 3.80% and 3.73% (ADL); 22.2%, 22.0% and 22.8% (starch).

[5]6.09, 6.16, 17.4, 27.3 and 94.9 mg catechin equivalent/100 g of fresh weight were observed in the 0.2%LS, 0.4%LS, CR, dry ground LS leaves and Cunirel®, respectively.

standard solution was mixed with 1.5 ml of 4% vanillin methanolic solution and then 750 μl of concentrated HCl was added. The well-mixed solution was incubated at ambient temperature (22°C) in the dark for 20 min. Absorbance against a blank was read at 500 nm. The concentration of CTC in the extract was quantified using a standard calibration curve at five concentration levels (0.05, 0.1, 0.25, 0.5 and 1 mg/ml), utilising a pure synthetic (+)-catechin standard (Sigma Aldrich, Milan, Italy). It was then expressed as mg of catechin equivalent/100 g fresh weight.

Performance and apparent digestibility

The rabbit's live weight and feed intake were checked weekly. Mortality and morbidity were controlled daily by the same observer, from 35 to 84 days, according to Gidenne *et al.*'s (2009) indications. The average daily weight gain, average daily feed intake, feed conversion ratio and health risk index (HRi) were calculated after the data collection. According to Rotolo *et al.*'s (2014) procedure, faeces were collected when the rabbits were 45 days old for 4 days ($n = 10$/treatment), and stored at −20°C for chemical analysis in duplicate for ash, EE and CP, according to AOAC (2000). The procedures and calculation of the apparent digestibility of the DM and nutrients were conducted according to the European standardised method (Perez *et al.*, 1995).

Slaughter procedures, carcass traits, blood parameters and digestive tract histology

A total of 10 rabbits/ treatment were stunned by concussion and slaughtered without fasting at 89 days of age. The carcasses were prepared following Dabbou *et al.*'s (2014) indications, and the data were expressed as a percentage of slaughter weight. The carcasses, including the thoracic organs, liver and kidneys, were chilled at 4°C. After 24 h of chilling, the weight of the chilled carcass (CCW) and of the aforementioned organs was recorded as percentages of CCW (Dabbou *et al.*, 2014). The cold carcasses were then kept for other analysis on meat quality.

Blood samples were collected from eight rabbits per group during the bleeding stage of the slaughter process. All of the blood haematology and serum biochemistry were performed using standard protocols (Vetlabor s.a.s., Volpiano, Italy). For gut histology, six rabbits from each group were selected in order to obtain small pieces of the caecal wall and mid-jejunum after the slaughtering procedure. The tissue samples were processed, embedded in paraffin, sectioned at 6-μm thicknesses by means of a rotary microtome (Leica RM2155; Leica Instruments GmbH, Nussloch, Germany) and stained by means of the haematoxylin and eosin method (Mikel, 1994). Villi height and crypt depth were measured under a microscope using an image analysis programme (Image Pro Plus; Media Cybernetics, Bethesda, MD, USA).

Caecal trials

The caecum from 10 animals/group was immediately separated and weighed. The pH was measured directly using a Crison MicropH 2001 pH metre (Crison Instruments, Barcelona, Spain). The caecal content was placed in sterile plastic tubes and kept at −20°C for further analysis, 1 g of the sample was mixed with 5 ml of distilled deionised water at 20°C, before being centrifuged (15 min at $3000 \times \mathbf{g}$) and filtered through a Schleicher and Schull membrane filter (BA-83; 0.2 μm Krackeler Scientific, Albany, NY, USA) for volatile fatty acid (VFA) determination, 1 μl of the extract was injected into a gas chromatograph (GC 1000 DPC; Dani Instruments S.P.A., Cologno Monzese, Italy) using a wide-bore capillary column (SGE BP21 25×0.53 mm internal diameter and 0.5 μm film thickness; P/N 054474, SGE International, Ringwood, Victoria, Australia). The testing protocol was performed according to Rotolo *et al.*'s (2014) procedures. Ammonia was measured in the supernatant after the centrifugation (10 min at $3000 \times \mathbf{g}$) of a vortexed mixture (30 s) of 5 g of caecal sample and 25 ml of distilled deionised water at 20°C, using an ammonia gas-sensing combination electrode (Model 95-12; Orion, Boston, MA, USA) that was connected to an ion analyzer (Model 920A; Orion). The VFA and ammonia concentration were calculated on the DM of the caecal content.

Faecal bacterial community

Hard faeces were collected from 10 rabbits from each group at 35, 42, 49, 56, 70 and 89 days, while the caecal content was collected after slaughtering. Samples from the same group, the same collection site and the same day were pooled together in sterilised polyethylene bags using a sterilised spatula, and were stored at −20°C until examination, 10 g of samples were homogenised in 90 ml of Ringer's solution (Oxoid, Milan, Italy) for 2 min in a stomacher (LAB Blender 400 and Sto-circul-bag stomacher bags; PBI, Milan, Italy) at room temperature. A deposit was allowed to set for 1 min, and 1 ml of the supernatant was used for the DNA extraction. The Powersoil DNA kit (MO-BIO; Carlsbad, CA, USA) was used according to the manufacturer's instructions, 5 μl of RNAse (Promega, Milan, Italy) was added to the DNA and the mixture was incubated at 37°C for 30 min before being stored at −20°C. The DNA was quantified using a NanoDrop 1000 spectrophotometer (Thermo Scientific, Milan, Italy) and was standardised at 50 ng/μl.

338F and 518R primers (Muyzer *et al.*, 1993; Invitrogen, Carlsbad, CA, USA) were used to amplify the variable V3 region of the 16S rRNA gene, and PCR products of about 250 base pairs were obtained. A GC clamp was added to the forward primers, according to Muyzer *et al.*'s (1993) procedures. Amplifications were performed in a thermal cycler (Bio-Rad, Milan, Italy) using the previously described conditions (Muyzer *et al.*, 1993), 2 μl aliquots of the PCR products were routinely checked on 2% agarose gels. The PCR products were analysed by means of denaturing gradient gel electrophoresis (DGGE) using a Bio-Rad Dcode apparatus. Samples were applied to 8% (wt/vol) polyacrylamide gels in a 1 × TAE buffer. Parallel electrophoresis experiments were performed at 60°C using gels containing a 20% to 60% urea-formamide denaturing gradient (100% corresponded to 7 M urea and 40% (wt/

vol) formamide). The gels were run for 4 h at 200 V, stained with SYBR® Gold Nucleic Acid Gel Stain (Invitrogen, Milan, Italy) for 30 min, and analysed under UV using the UVIpro Platinum 1.1 Gel Software (Eppendorf, Hamburg, Germany). A database of fingerprints was created using the Bionumerics software, version 5.1 (Applied Maths, Sint Marten Latem, Belgium). A dendrogram of similarity was retrieved using the dice coefficient and unweighted pair group method for the arithmetic average clustering algorithm (Vauterin and Vauterin, 1992).

Meat quality (pH, colour, chemical composition and lipid oxidation)

After 24 h of chilling, 10 carcasses/group were halved, and then the two *longissimus dorsi* (LD) muscles were excised. The LD muscles on both the left and the right sides were divided into the forepart and hind part. The left forepart and the left hind part were used to measure pH and establish colour, respectively. The right forepart and the right hind part were freeze-dried and kept until needed for the analyses of the proximate composition and the thiobarbituric acid reactive substances (TBARS) assay, respectively.

The pH after 24 h of chilling (pH_{24}), colour and chemical composition of the freeze-dried meat (moisture, CP, EE and ash) were determined according to Rotolo *et al.*'s (2014) procedures. After 90 days at −20°C storage in vacuum packs, 2 g of freeze-dried meat ($n = 5$/group) was homogenised with 20 ml of 10% trichloroacetic acid using a Polytron tissue homogeniser (Type PT 10–35; Kinematica GmbH, Luzern, Switzerland) to determine lipid oxidation. This was accomplished by using a modified TBARS method according to Witte *et al.*'s (1970) protocol. Analyses were performed in duplicate and the results were expressed as μg malonyldialdehyde per kilogram of fresh meat, using a standard curve that covered a concentration range from 0.5 to 10 μM 1,1,3,3-tetramethoxypropane (Sigma-Aldrich, Steinheim, Germany). The absorbance was measured at 532 nm by means of a Helios spectrophotometer (Unicam Limited, Cambridge, UK).

Statistical analysis

All of the statistical analyses were performed using the SPSS software package (IBM SPSS, 2012). The differences in morbidity rate, mortality rate and HRi among groups were tested using the Fisher exact test. The performance, digestibility, carcass traits, blood parameters, meat quality, caecal trials and digestive histology were assessed with a one-way ANOVA (with the diet as the fixed factor) using Duncan's New Multiple Range Test for *post hoc* analysis. The significance was established at $P < 0.05$.

Results and discussion

Performance, digestibility and digestive tract histology

No statistically significant difference was observed between the treatment and control groups in terms of performance,

Table 2 *Effect of phyto-additives (LS and CR) on performance, apparent digestibility and digestive tract histology in rabbits*

	Diets					
Items	Control	0.2%LS	0.4%LS	CR	SEM	*P*
Growth performance ($n = 40$/group)						
Initial BW (g)	933	938	929	935	9	0.99
Live weight at 84 days (g)	2925	2928	2849	2844	30	0.62
Daily weight gain (g/day)	39.8	39.7	38.1	38.1	0.5	0.49
Daily feed intake (g/day)	122	125	124	122	1	0.88
Feed conversion ratio	3.10	3.17	3.32	3.28	0.03	0.73
Health status ($n = 40$/group)						
Morbidity (%)	25.0	27.5	22.5	25.0	–	0.97
Mortality (%)	5.0	5.0	7.5	5.0	–	0.95
Health risk index (%)[1]	30.0	32.5	30.0	30.0	–	0.99
Apparent digestibility ($n = 10$/group)						
Dry matter (%)	68.2	65.4	63.1	61.0	1.1	0.09
Organic matter (%)	69.9	67.3	65.6	63.3	1.0	0.11
Ether extract (%)	62.6^{a}	60.4^{ab}	52.2^{c}	54.5^{bc}	1.4	0.02
CP (%)	85.7^{A}	84.9^{A}	84.0^{B}	84.0^{B}	0.2	0.001
Digestive tract histology ($n = 6$/group)						
Jejunal villus height (μm)	709	672	664	708	12	0.45
Jejunal crypt depth (μm)	129	92	85	131	12	0.37
Caecal crypt depth (μm)	88	101	100	113	4	0.12

0.2%LS = 0.2% of dry ground *Lythrum salicaria* (LS) supplementation in diets; 0.4%LS = 0.4% of dry ground LS supplementation in diets; CR = 0.3% of commercial supplementation in diets which is a mixture of medicinal plants with LS is the main composition (Cunirel®, Biotrade snc®, Modena, Italy).
^{a,b,c} or ^{A,B}Values within a row with different superscripts differ significantly at $P < 0.05$ or $P < 0.01$, respectively.
[1]Health risk index is the summation between morbidity and mortality.

morbidity, mortality, HRi and gut histology (Table 2). The CP digestibility declined significantly ($P < 0.05$) in the rabbits fed the 0.4%LS and CR, compared with the control group and the group with a low dose supplementation. There was a statistically significant difference between the rabbits fed the control diet, 0.4%LS and CR (62.6% *v.* 52.2% and 54.5%, respectively; $P < 0.05$; Table 2) in terms of EE digestibility.

In general, the active components of the aromatic plants offered the potential of better flavour, which directly increased consumption (Christaki *et al.*, 2012). Hence, an improvement in performance should have been observed in the rabbits fed phyto-additive diets. Some authors have reported these effects (Krieg *et al.*, 2009; Arafa *et al.*, 2010; Ayala *et al.*, 2011; Rotolo *et al.*, 2013). However, some studies came to a contrasting, or even completely opposite

Table 3 *Effect of phyto-additives (LS and CR) on blood parameters (blood haematology and serum biochemistry) in rabbits (*n = *8/group)*

	Diets					
Items	Control	0.2%LS	0.4%LS	CR	SEM	*P*
Haematology						
Haematocrit (%)	40.0	43.1	48.2	39.9	1.6	0.27
Erythrocytes (10^{12}/l)	5.57	5.89	6.80	5.77	0.22	0.20
Haemoglobin (g/dl)	8.33	9.00	10.35	8.65	0.36	0.22
RDW (%)	16.7	17.1	16.6	16.8	0.2	0.50
Leukocyte (10^9/l)	4.06^{A}	8.25^{B}	8.63^{B}	8.21^{B}	0.74	0.001
Neutrophils (%)	41.0	39.8	39.7	40.3	0.3	0.24
Lymphocytes (%)	41.8	43.6	42.8	44.0	1.0	0.86
Eosinophils (%)	8.71	6.59	9.43	7.08	0.75	0.51
Monocytes (%)	8.19	9.58	7.76	7.98	0.46	0.51
Serum biochemistry						
Total protein (mg/dl)	5.48	5.51	5.74	5.39	0.07	0.32
Albumin (mg/dl)	3.78	3.84	3.76	3.80	0.03	0.80
Globulin (mg/dl)	1.70	1.68	1.98	1.59	0.06	0.12
AST (U/dl)	48.5	41.2	55.7	52.5	2.7	0.27
ALT (U/dl)	31.2	33.2	38.1	39.3	1.5	0.19
Blood urea nitrogen (mg/dl)	48.1	37.9	41.2	37.9	3.6	0.27
Creatinine (mg/dl)	1.06	1.01	1.18	1.07	0.03	0.19
Cholesterol (mg/dl)	67.1	62.2	66.7	65.5	2.9	0.94
Triglyceride (mg/dl)	184	172	183	157	7	0.59

0.2%LS = 0.2% of dry ground *Lythrum salicaria* (LS) supplementation in diets; 0.4%LS = 0.4% of dry ground LS supplementation in diets; CR = 0.3% of commercial supplementation in diets which is a mixture of medicinal plants with LS is the main composition (Cunirel®, Biotrade snc®, Modena, Italy); RDW = red blood cell distribution width; AST = aspartate aminotransferase; ALT = alanine aminotransferase.
[A,B]Values within a row with different superscripts differ significantly at $P < 0.01$.

Table 4 *Effect of phyto-additives (LS and CR) on caecal traits in rabbits*

	Diets					
Items	Control	0.2%LS	0.4%LS	CR	SEM	*P*
Caecal characteristics (*n* = 10/group)						
Full caecum (%BW)	5.75	5.89	6.12	5.68	0.11	0.54
Empty caecum (%BW)	1.70	1.78	1.75	1.65	0.03	0.33
Caecal content (%BW)	4.05	4.11	4.36	4.03	0.10	0.64
Caecal pH	6.44	6.21	6.40	6.39	0.08	0.74
Caecal fermentation parameters (*n* = 10/group)						
DM content (%)	21.3	22.4	20.8	21.1	0.3	0.31
Total VFA (mg/kg DM)	18.9^{a}	19.9^{ab}	24.1^{b}	23.0^{ab}	0.8	0.04
Acetic acid (mg/kg DM)	14.4^{a}	14.8^{a}	18.3^{b}	17.2^{ab}	0.6	0.04
Propionic acid (mg/kg DM)	1.19	1.17	1.42	1.37	0.05	0.14
Butyric acid (mg/kg DM)	3.28	3.92	4.37	4.36	0.18	0.10
Ammonia-N (mg/kg DM)	892^{b}	845^{b}	594^{a}	680^{ab}	43	0.04

0.2%LS = 0.2% of dry ground *Lythrum salicaria* (LS) supplementation in diets; 0.4%LS = 0.4% of dry ground LS supplementation in diets; CR = 0.3% of commercial supplementation in diets which is a mixture of medicinal plants with LS is the main composition (Cunirel®, Biotrade snc®, Modena, Italy); DM = dry matter; VFA = volatile fatty acids.
[a,b]Values within a row with different superscripts differ significantly at $P < 0.05$.

conclusion (Botsoglou *et al*., 2004; Soultos *et al*., 2009; Dalle Zotte *et al*., 2013).

Generally, the chemical components of medicinal plants are considered cause some type of effect after usage (Christaki *et al*., 2012). Tannins have been suggested to be the main active compound in LS, but flavonoids have also been discovered to have an effect (Humadi and Istudor, 2009). The supplementation of a natural extract of chestnut wood (containing tannins) increased the daily weight gain of rabbits and poultry (Schiavone *et al*., 2008; Liu *et al*., 2012), especially at younger age (Gai *et al*., 2010). On the other hand, tannins are considered to be toxic and anti-nutritive substances, as Al-Mamary *et al*. (2001) reported a significant reduction in the CP digestibility of rabbits fed a high level of sorghum tannins (3.5% catechin equivalent in the diet). In Al-Mamary *et al*.'s (2001) study, there was a sharp decrease in intestinal enzyme activities (α-amylase, trypsin and lipase). This could help to explain the poor digestibility of EE and CP after tannin supplementation in the present study. However, this was not due to the abnormality of the jejunal or caecal histology. Moreover, excess tannin supplementation could be responsible for the negative outcomes in terms of daily weight gain (Al-Mamary *et al*., 2001), whereas the application of lower levels of tannins did not affect this study.

Blood parameters

Regarding blood haemotology, the supplements used increased the quantity of white blood cells, compared with the control group (0.2%LS, 0.4%LS and CR *v*. control; 8.25, 8.63 and 8.21 *v*. 4.06×10^9/l; $P < 0.05$; respectively). The other measured parameters were not influenced by the treatments (Table 3). The blood parameters were most likely affected by the phyto-addition, since it was reported that echinacoside and cichoric acid, which are considered to be the active compounds that induce an increase in the total white blood cells, were found in *Echinacea purpurea* (Arafa *et al*., 2010). At the moment, it is not possible to correlate this result with an improvement in animal health. A study on the action mechanism on the immune system of the active components in LS still needs to be performed.

Caecal trials

The caecal trials are reported in Table 4. The 0.4%LS supplementation increased the concentration of VFA, compared with the control group (24.1 *v*. 18.9 mg/kg DM; $P < 0.05$), whereas the acetic acid values were greater compared with both the control group and to animals fed with 0.2%LS (18.3 *v*. 14.4 and 14.8 mg/kg DM; $P < 0.05$). The ammonia level was lower in the 0.4%LS supplemented group, compared with both the control and the group treated with the addition of 0.2%LS (594 *v*. 892 and 845 mg/kg DM; $P < 0.05$). Propionic and butyric acids were not influenced by the supplementation; however, the butyric acid level increased gradually with supplementation level of LS.

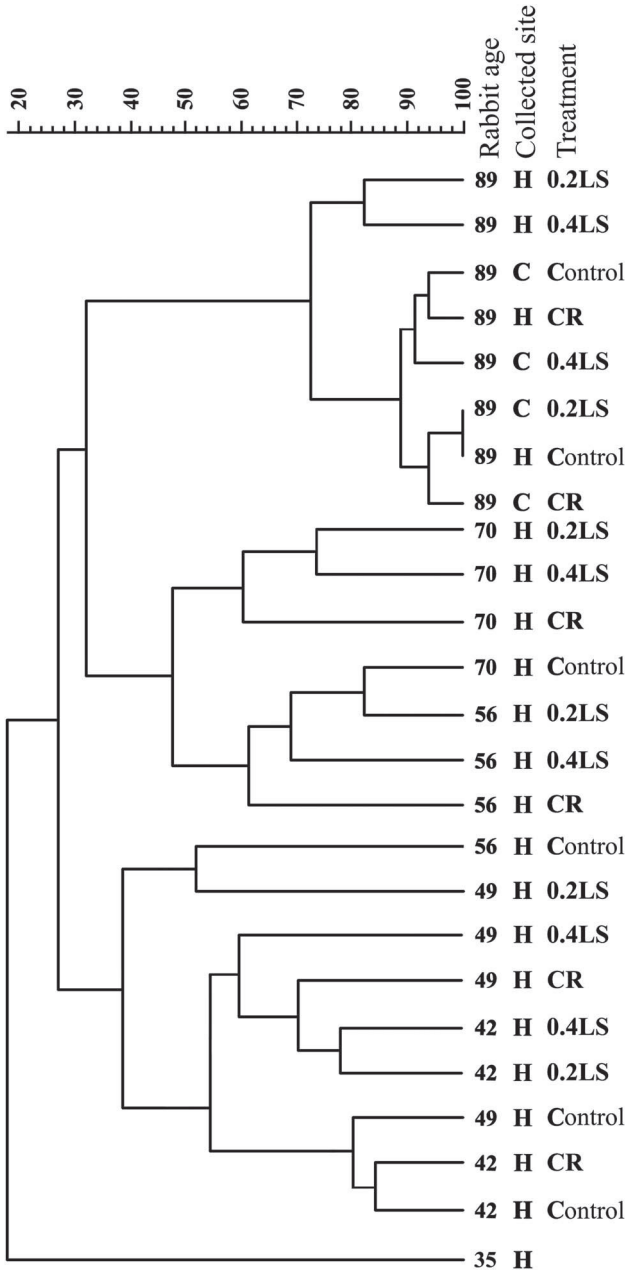

Figure 1 Cluster analysis of the denaturing gradient gel electrophoresis profile of bacterial communities in the hard faeces (H) and the caecal content (C) of rabbits that were supplemented 0.2% of dry ground *Lythrum salicaria* (0.2LS), 0.4% of dry ground *Lythrum salicaria* (0.4LS) and 0.3% Cunirel® (CR), as well as the control group (Control), from the beginning of the experiment (35 days old) to the day of slaughter (89 days old).

A high concentration of total caecal VFA in rabbits had a protective effect against enteropathogenic *Escherichia coli* infection (Peeters *et al*., 1995). Therefore, a higher level of VFA should contribute to health benefits that could prevent pathogen infection. Such benefits have been discovered after the dietary supplementation of LS in rabbits. However, more studies should be performed to confirm this theory. The nitrogenous residues are derived from the endogenous and undigested feed, which provides nitrogen sources

for caecal fermentation, providing ammonia as an end product (García *et al*., 2005). In the present study, there was less observed caecal ammonia in the group treated with the high level of LS. The lower ammonia concentration was likely due to a decrease of protein utilisation in caecum, as microbiota was unable to digest tannin-protein complexes (Maertens and Struklec, 2006). It is possible that some group of microbe may use ammonia and produce acetic acid as products which increase the amount of acetic acid and total VFA. However, it is impossible to conclude this theory until a study on the microbial mechanism and fermentation was performed.

Faecal bacterial community

The overall picture of the gut bacterial community of rabbits was generated using the PCR-DGGE analysis of DNA extracted directly from the hard faeces and from the caecal content. The results are summarised in Figure 1. The dendrogram shows a great similarity of the bacterial community for rabbits fed supplemented diets when compared with the control group of rabbits at 56 and 70 days of age. Age increments influence the dynamics of the microbiota, as a close correlation exists between digestive microbiota and diet (Combes *et al*., 2013), which was also observed in the present study. The development of gut microbiota of the control group was slower than the treated groups. As bacterial community of the control group at 56 and 70 days was clustered in the treated groups with lower age than itself. The loss of diversity may correlate with the diet and antimicrobial functions of the medicinal plants. Hexahydroxydiphenoyl ester vescalagin in LS extracts, which is one of the hydrolysable tannins, was shown *in vitro* to be the main active component in antimicrobial activity (Becker *et al*., 2005). Even though the active components that had antimicrobial properties present in LS, as well as the digestibility, were changed, the bacterial community was not affected by the supplementation in the present study.

Carcass traits, meat quality and lipid oxidation

No statistically significant difference appeared between the groups for carcass traits, meat quality (pH_{24}, colour and chemical composition) and lipid oxidation (Table 5). One of the common causes of liver enlargement is the ingestion of toxic substances, which was discovered in the rabbits fed high level of tannins (Al-Mamary *et al*., 2001). Fortunately, the low dose of tannins in our study did not induce hepatomegaly. Antioxidant substances can be used to prevent or slow down the problem of lipid oxidation. Phenolic compounds, which can be found in aromatic plants, have antioxidative properties,

Table 5 *Effect of phyto-additives (LS and CR) on carcass traits, meat quality and lipid oxidation (TBARS, μg malonyldialdehyde/kg of fresh meat) of the* longissimus dorsi *muscle in rabbits*

	Diets					
Items	Control	0.2%LS	0.4%LS	CR	SEM	*P*
Carcass traits (n = 10/group)						
SW (g)	3068	3144	3130	3163	31	0.73
Skin, paws and feet (%SW)	17.2	19.4	18.4	17.8	0.3	0.10
Full gastrointestinal tract (%SW)	17.0	17.4	18.2	17.8	0.3	0.41
CCW (g)	1853	1864	1843	1868	19	0.97
Dressing percentage (%)	60.4	59.2	58.8	59.1	0.3	0.30
Liver (%CCW)	5.22	5.61	5.42	5.86	0.14	0.41
Kidneys (%CCW)	0.95	0.95	0.99	0.88	0.04	0.79
Thoracic organs (%CCW)	1.99	2.03	2.00	1.84	0.03	0.15
pH_{24} and colour (n = 10/group)						
pH_{24}	5.65	5.66	5.65	5.68	0.02	0.94
Lightness (L^*)	55.9	54.8	55.4	54.3	0.3	0.26
Redness (a^*)	0.98	1.12	1.70	1.80	0.23	0.48
Yellowness (b^*)	7.38	7.13	7.02	7.39	0.17	0.84
Chroma (C^*)	7.54	7.32	7.79	7.70	0.18	0.81
Hue (H^*)	78.5	81.8	76.0	76.2	1.0	0.20
Chemical composition (n = 5/group)						
Moisture (%)	74.0	73.9	73.8	73.6	0.1	0.45
Protein (%)	21.7	21.7	21.7	22.0	0.1	0.23
Ether extract (%)	0.80	0.80	0.82	0.92	0.05	0.47
Ash (%)	1.04	1.06	1.08	1.08	0.01	0.41
Oxidative status (n = 5/group)						
TBARS (μg/kg)	297	266	335	301	13	0.38

0.2%LS = 0.2% of dry ground *Lythrum salicaria* (LS) supplementation in diets; 0.4%LS = 0.4% of dry ground LS supplementation in diets; CR = 0.3% of commercial supplementation in diets which is a mixture of medicinal plants with LS is the main composition (Cunirel®, Biotrade snc®, Modena, Italy); TBARS = thiobarbituric acid reactive substances; SW = slaughter weight; CCW = chilled carcass weight; pH_{24} = pH of longissimus dorsi muscles were measured after 24 h of chilling.

offering benefits in meat quality (Christaki *et al*., 2012). Previous research found that diets supplemented with 200 mg/kg of oregano essential oil, in addition to chestnut wood extracts that contained antioxidant compounds, delayed the lipid oxidation in rabbit meat (Botsoglou *et al*., 2004; Liu *et al*., 2012). Even though there were active components with antioxidant activities present in LS (Tunalier *et al*., 2007), lipid oxidation was not decreased in the present study. Conversely, Gai *et al*. (2009) as well as Liu *et al*. (2009) reported a significant decrease of lipid oxidation in rabbit fed 0.5% of chestnut tannins, and indicated a dose-related effect. The pharmacokinetics of the antioxidative compounds in LS require further studies in order to clarify how these compounds distribute in the active sites.

Conclusion

The supplementation of LS in rabbits has led to a significant increase in the total white blood cells and higher concentrations of VFA and acetic acid, but also to lower levels of ammonia, CP and EE digestibility. No adverse effects on the other studied parameters were observed. Low level of LS supplementation (<0.4%) was suggested to gain health benefits and prevent adverse effects.

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Supplementary material

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