



# UNIVERSITÀ DEGLI STUDI DI TORINO

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Effect of Purple Loosestrife (*Lythrum salicaria*) Diet Supplementation in Rabbit Nutrition on Performance, Digestibility, Health and Meat Quality

by:

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19 **Effect of Purple Loosestrife (*Lythrum salicaria*) Diet Supplementation in Rabbit**  
20 **Nutrition on Performance, Digestibility, Health and Meat Quality**

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31

32 **Abstract**

33 In this study, 160 Hycole weaned rabbits (35 days old) were randomly divided into  
34 four groups of 40. The rabbits were studied throughout a 54-day experimentation  
35 period in order to determine the impact of dietary supplementation from herbs  
36 composed of 0.2%, 0.4% dry ground *Lythrum salicaria* leaves (LS) and 0.3% Cunirel®  
37 (CR; a commercial herb mixture containing LS as the main ingredient) on  
38 performance, digestibility, health and meat quality. The basal diet was given to the  
39 control group. No significant differences were found in performance. Ten rabbits from  
40 each group were selected for evaluation regarding apparent digestibility. The rabbits  
41 fed the control diet and the diet with the low level of LS had a higher level of crude  
42 protein digestibility than did the animals that were supplemented with the high LS  
43 levels and CR (85.7 and 84.9 vs. 84.0 and 84.0%, respectively; P<0.05). The ether

44 extract digestibility was lower in the treatment group with 0.4%LS addition and CR as  
45 compared to the control group (52.2 and 54.5 vs. 62.6%, respectively;  $P<0.05$ ). The  
46 slaughter process was performed on 89-day-old rabbits to study the carcass  
47 characteristics, meat quality, blood parameters, caecal trials and gut histology. The  
48 total leukocyte counts in the control animals were lower than they were in the rabbits  
49 fed 0.2%, 0.4%LS and CR ( $4.06$  vs.  $8.25$ ,  $8.63$  and  $8.21 \times 10^9/L$ ;  $P<0.05$ ; respectively).  
50 For caecal fermentation, the caecal contents of the rabbits fed 0.4% of LS, showed  
51 higher concentrations of total volatile fatty acid (VFA;  $24.1$  vs.  $18.9$  mg/kg DM;  
52  $P<0.05$ ) and acetic acid ( $18.3$  vs.  $14.4$  mg/kg DM;  $P<0.05$ ), but lower ammonia levels  
53 ( $594$  vs.  $892$  mg/kg DM;  $P<0.05$ ) as compared to the control group. PCR-denaturing  
54 gradient gel electrophoresis analyses were performed to evaluate the microbial  
55 community in hard faeces, collected at days 35, 42, 49, 56, 70 and 89, whereas the  
56 caecal contents were taken after slaughtering. The results demonstrated that  
57 between the treatment groups, the similarity of the microbial communities was higher  
58 as compared to the control group. Moreover, only age was shown to influence  
59 microbiota diversity. In conclusion, the findings of this study indicated that  
60 supplementation of LS in rabbit diets leads to an increase in the total white blood  
61 cells, total VFA and acetic acid concentration, and a decrease in the ammonia levels,  
62 as well as the digestibility of crude protein and ether extract, without causing any  
63 adverse effects on other parameters.

64

65 **Keywords:** Blood; Digestibility; *Lythrum salicaria*; Rabbit; Volatile fatty acid

66

67 **Implications**

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

76

## 77 **Introduction**

78 The long-term supplementation of animal feeds with antibiotics at subtherapeutic  
79 doses (antibiotic growth promoter, **AGP**) has been performed since 1951. This was  
80 often done without veterinary prescription, with the aim of promoting growth  
81 performance, maintaining the animal's health and reducing the mortality rate after the  
82 weaning period, which is a major problem for rabbit production ([Phillips, 2007](#)). In  
83 2006, because of the risk of the development of drug-resistant bacteria, the  
84 European Union banned the use of AGPs. Meanwhile, a sharp deterioration in animal  
85 health and performance was observed, with a consequent decrease in profits  
86 ([Phillips, 2007](#)). Several different approaches have been tested to control or prevent  
87 diseases and improve productive performance.

88 Aromatic plants contain many biologically-active compounds that exhibit  
89 medicinal properties ([Christaki et al., 2012](#)) that could improve production  
90 performance, as well as animal health and meat quality. Supplementation with  
91 alternative substances, such as probiotics, prebiotics, enzymes and organic acids,  
92 has been studied in rabbits, with interesting results emerging ([Falcão-e-Cunha et al.,](#)

93 2007; Rotolo *et al.*, 2014). However, the number of phyto-studies remains limited  
94 (Botsoglou *et al.*, 2004; Krieg *et al.*, 2009; Arafa *et al.*, 2010; Simonová *et al.*, 2010;  
95 Ayala *et al.* 2011; Szabóová *et al.*, 2012; Rotolo *et al.*, 2013). *Lythrum salicaria* (**LS**)  
96 is a flowering plant, which is commonly known as purple loosestrife, belonging to the  
97 Lythraceae family. LS is considered an invasive and competitive plant in ecosystems.  
98 However, this herb has been used in traditional medicine because of its medicinal  
99 properties. In fact, several *in vitro* studies used the active compounds (e.g. tannins  
100 and flavonoids) that were extracted from LS, showing that LS also has anti-microbial,  
101 anti-fungal, anti-inflammatory and anti-oxidant properties (Becker *et al.*, 2005;  
102 Tunalier *et al.*, 2007; Humadi and Istudor, 2009). The aim of this study was,  
103 therefore, to evaluate the supplementation of feeds with LS on the performance,  
104 digestibility, health and meat quality of growing rabbits.

105

## 106 **Material and methods**

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

108 The experiment was performed at the experimental rabbitry facility at the Department  
109 of Agricultural, Forestry and Food Sciences in Carmagnola, Turin, Italy. In this study,  
110 160 Hycole rabbits (934±118 g) were randomly housed in individual wire cages and  
111 reared from weaning (35 days) to slaughtering (89 days). A basal diet was formulated  
112 in order to cover the nutritional requirements of the growing rabbits (control). In  
113 addition, three experimental diets were set up with 0.2% (**0.2%LS**) or 0.4% (**0.4%LS**)  
114 dry ground LS leaves and 0.3% Cunirel® (**CR**, Biotrade snc®, Modena, Italy) in place  
115 of small fractions of barley meal in the basal diet (Table 1). CR is a commercial  
116 mixture of herbs that contains LS as a major component. The diets were assigned to  
117 the animals (40 animals per diet) using a completely randomised design. The feeds

118 and clean water were provided *ad libitum* and, the facility was climate and light  
119 controlled during the whole trial in order to maintain a temperature of  $22\pm 2^{\circ}\text{C}$  and a  
120 photoperiod of 16L:8D. The diets were analysed in triplicate for dry matter (**DM**),  
121 crude protein by total nitrogen contents (**CP**), ether extract (**EE**), crude fibre and ash  
122 by ignition to  $550^{\circ}\text{C}$ , according to the Association of Official Analytical Chemists  
123 (**AOAC**, 2000). The NDF, ADF and ADL were determined according to [Van Soest et](#)  
124 [al.'s \(1991\)](#) procedures. The level of starch was determined using Ewer's polarimetric  
125 method ([European Economic Community, 1972](#)).

126 The CTC contents were determined in LS, CR, and the experimental diets,  
127 according to the method described by [Lahouar et al. \(2014\)](#). A 50- $\mu\text{L}$  aliquot of each  
128 extract or standard solution was mixed with 1.5 mL of 4% vanillin methanolic solution  
129 and then 750  $\mu\text{L}$  of concentrated HCl was added. The well-mixed solution was  
130 incubated at ambient temperature ( $22^{\circ}\text{C}$ ) in the dark for 20 minutes. Absorbance  
131 against a blank was read at 500 nm. The concentration of CTC in the extract was  
132 quantified using a standard calibration curve at five concentration levels (0.05, 0.1,  
133 0.25, 0.5 and 1 mg/mL), utilising a pure synthetic (+)-catechin standard (Sigma  
134 Aldrich, Milan, Italy). It was then expressed as mg of catechin equivalent/100 g fresh  
135 weight.

136

### 137 *Performance and apparent digestibility*

138 The rabbit's live weight and feed intake were checked weekly. Mortality and morbidity  
139 were controlled daily by the same observer, from 35 to 84 days, according to  
140 [Gidenne et al.'s \(2009\)](#) indications. The average daily weight gain (**ADG**), average  
141 daily feed intake (**ADFI**), feed conversion ratio (**FCR**) and health risk index (**HRI**)  
142 were calculated after the data collection. According to [Rotolo et al.'s \(2014\)](#)

143 procedure, faeces were collected when the rabbits were 45 days old for four days  
144 (n=10 per treatment), and stored at -20°C for chemical analysis in duplicate for ash,  
145 EE and CP, according to [AOAC \(2000\)](#). The procedures and calculation of the  
146 apparent digestibility of the dry matter and nutrients were conducted according to the  
147 European standardised method ([Perez et al., 1995](#)).

148

#### 149 *Slaughter procedures, carcass traits, blood parameters and digestive tract histology*

150 Ten rabbits per treatment were stunned by concussion and slaughtered without  
151 fasting at 89 days of age. The carcasses were prepared following [Dabbou et al.'s](#)  
152 [\(2014\)](#) indications, and the data were expressed as a percentage of slaughter weight  
153 (**SW**). The carcasses, including the thoracic organs, liver and kidneys, were chilled at  
154 4°C. After 24 h of chilling, the weight of the chilled carcass (**CCW**) and of the  
155 aforementioned organs was recorded as percentages of CCW ([Dabbou et al., 2014](#)).  
156 The cold carcasses were then kept for other analysis on meat quality.

157       Blood samples were collected from eight rabbits per group during the bleeding  
158 stage of the slaughter process. All of the blood haematology and serum biochemistry  
159 were performed using standard protocols (Vetlabor s.a.s., Volpiano, Italy). For gut  
160 histology, six rabbits from each group were selected in order to obtain small pieces of  
161 the caecal wall and mid-jejunum after the slaughtering procedure. The tissue  
162 samples were processed, embedded in paraffin, sectioned at six µm thicknesses by  
163 means of a rotary microtome (Leica RM2155, Leica Instruments GmbH, Nussloch,  
164 Germany) and stained by means of the Haematoxylin and Eosin method ([Mikel,](#)  
165 [1994](#)). Villi height and crypt depth were measured under a microscope using an  
166 image analysis programme (Image Pro Plus, Media Cybernetics, MD, USA).

167

168 *Caecal trials*

169 The caecum from 10 animals per group was immediately separated and weighed.  
170 The pH was measured directly using a Crison MicropH 2001 pH metre (Crison  
171 Instruments, Barcelona, Spain). The caecal content was placed in sterile plastic  
172 tubes and kept at -20°C for further analysis. One g of the sample was mixed with five  
173 ml of distilled deionised water at 20°C, before being centrifuged (15 minutes at  
174 3000xg) and filtered through a Schleicher and Schull membrane filter (BA-83, 0.2  
175 µm) for volatile fatty acid (**VFA**) determination. One µl of the extract was injected into  
176 a gas chromatography (GC 1000 DPC, Dani Instruments S.P.A., Cologno Monzese,  
177 Italy) using a wide-bore capillary column (SGE BP21 25m x 0.53 mm internal  
178 diameter and 0.5 µm film thickness; P/N 054474, SGE International, Ringwood,  
179 Victoria, Australia). The testing protocol was performed according to [Rotolo et al.'s](#)  
180 [\(2014\)](#) procedures. Ammonia was measured in the supernatant after the  
181 centrifugation (10 minutes at 3000g) of a vortexed mixture (30 s) of five g of caecal  
182 sample and 25 ml of distilled deionised water at 20°C, using an ammonia gas-  
183 sensing combination electrode (Orion, Model 95-12, Boston, MA, USA) that was  
184 connected to an ion analyser (Orion, Model 920A, Boston, MA, USA). The VFA and  
185 ammonia concentration were calculated on the dry matter of the caecal content.

186

187 *Faecal bacterial community*

188 Hard faeces were collected from 10 rabbits from each group at 35, 42, 49, 56, 70 and  
189 89 days, while the caecal content was collected after slaughtering. Samples from the  
190 same group, the same collection site, and the same day were pooled together in  
191 sterilised polyethylene bags using a sterilised spatula, and were stored at -20°C until  
192 examination. Ten grams of samples were homogenised in 90 ml of Ringer's solution



193 (Oxoid, Milan, Italy) for two minutes in a stomacher (LAB Blender 400 and Sto-circul-  
194 bag stomacher bags, PBI, Milan, Italy) at room temperature. A deposit was allowed  
195 to set for one minute, and one ml of the supernatant was used for the DNA  
196 extraction. The Powersoil DNA kit (MO-BIO, Carlsbad, CA, USA) was used according  
197 to the manufacturer's instructions. Five  $\mu$ l of RNase (Promega, Milan, Italy) was  
198 added to the DNA and the mixture was incubated at 37°C for 30 minutes before  
199 being stored at -20°C. The DNA was quantified using a NanoDrop 1000  
200 spectrophotometer (Thermo Scientific, Milan, Italy) and was standardised at 50 ng/ $\mu$ l.

201 338F and 518R primers (Muyzer *et al.*, 1993) were used to amplify the  
202 variable V3 region of the 16S rRNA gene, and PCR products of about 250 base pairs  
203 were obtained. A GC clamp was added to the forward primers, according to Muyzer  
204 *et al.'s* (1993) procedures. Amplifications were performed in a thermal cycler (Bio-  
205 Rad, Milan, Italy) using the previously described conditions (Muyzer *et al.*, 1993).  
206 Two  $\mu$ l aliquots of the PCR products were routinely checked on 2% agarose gels.  
207 The PCR products were analysed by means of denaturing gradient gel  
208 electrophoresis (DGGE) using a Bio-Rad Dcode apparatus. Samples were applied to  
209 8% (wt/vol) polyacrylamide gels in a 1 x TAE buffer. Parallel electrophoresis  
210 experiments were performed at 60°C using gels containing a 20 to 60% urea-  
211 formamide denaturing gradient (100% corresponded to 7 M urea and 40% (wt/vol)  
212 formamide). The gels were run for four hours at 200 V, stained with SYBR® Gold  
213 Nucleic Acid Gel Stain (Invitrogen, Milan, Italy) for 30 minutes, and analysed under  
214 UV using the UVIpro Platinum 1.1 Gel Software (Eppendorf). A database of  
215 fingerprints was created using the Bionumerics software, version 5.1 (Applied Maths,  
216 Sint Marten Latem, Belgium). A dendrogram of similarity was retrieved using the dice

217 coefficient and unweighted pair group method for the arithmetic average clustering  
218 algorithm ([Vauterin and Vauterin, 1992](#)).

219

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

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

228         The pH after 24 hours of chilling (**pH<sub>24</sub>**), colour and chemical composition of  
229 the freeze-dried meat (moisture, CP, EE and ash) were determined according to  
230 [Rotolo, et al.'s \(2014\)](#) procedures. After 90 days at -20°C storage in vacuum packs,  
231 two g of freeze-dried meat (n=5 per group) was homogenised with 20 ml of 10%  
232 trichloroacetic acid using a Polytron tissue homogeniser (Type PT 10-35, Kinematica  
233 GmbH, Luzern, Switzerland) to determine lipid oxidation. This was accomplished by  
234 using a modified TBARS method according to [Witte et al.'s \(1970\)](#) protocol. Analyses  
235 were performed in duplicate and the results were expressed as µg  
236 malonyldialdehyde per kilogram of fresh meat, using a standard curve that covered a  
237 concentration range of 0.5 to 10 µM 1,1,3,3-tetramethoxypropane (Sigma-Aldrich,  
238 Steinheim, Germany). The absorbance was measured at 532 nm by means of a  
239 Helios spectrophotometer (Unicam Limited, Cambridge, UK).

240

241 *Statistical analysis*

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

249

## 250 **Results and Discussion**

### 251 *Performance, digestibility and digestive tract histology*

252 No statistically significant difference was observed between the treatment and control  
253 groups in terms of performance, morbidity, mortality, HRi and gut histology (Table 2).  
254 The CP digestibility declined significantly ( $P<0.05$ ) in the rabbits fed the 0.4%LS and  
255 CR, compared to the control group and the group with a low dose supplementation.  
256 There was a statistically significant difference between the rabbits fed the control  
257 diet, 0.4%LS and CR (62.6 vs. 52.2 and 54.5%, respectively;  $P<0.05$ ; Table 2) in  
258 terms of EE digestibility.

259 In general, the active components of the aromatic plants offered the potential  
260 of better flavour, which directly increased consumption (Christaki *et al.*, 2012).  
261 Hence, an improvement in performance should have been observed in the rabbits  
262 fed phyto-additive diets. Some authors have reported these effects (Krieg *et al.*,  
263 2009; Arafa *et al.*, 2010; Ayala *et al.*, 2011; Rotolo *et al.*, 2013). However, some  
264 studies came to a contrasting, or even completely opposite conclusion (Botsoglou *et*  
265 *al.*, 2004; Soutos *et al.*, 2009; Dalle Zotte *et al.*, 2013).

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

281

### 282 *Blood parameters*

283 Regarding blood haematology, the supplements used increased the quantity of white  
284 blood cells, compared to the control group (0.2%LS, 0.4%LS and CR vs. control;  
285 8.25, 8.63 and 8.21 vs.  $4.06 \times 10^9/L$ ;  $P < 0.05$ ; respectively). The other measured  
286 parameters were not influenced by the treatments (Table 3). The blood parameters  
287 were most likely affected by the phyto-addition, since it was reported that  
288 echinacoside and cichoric acid, which are considered to be the active compounds  
289 that induce an increase in the total white blood cells, were found in *Echinacea*  
290 *purpurea* (Arafa *et al.*, 2010). At the moment, it is not possible to correlate this result

291 with an improvement in animal health. A study on the action mechanism on the  
292 immune system of the active components in LS still needs to be performed.

293

#### 294 *Caecal trials*

295 The caecal trials are reported in [Table 4](#). The 0.4%LS supplementation increased the  
296 concentration of VFA, compared to the control group (24.1 vs. 18.9 mg/kg DM;  
297  $P<0.05$ ), whereas the acetic acid values were greater compared to both the control  
298 group and to animals fed with 0.2%LS (18.3 vs. 14.4 and 14.8 mg/kg DM;  $P<0.05$ ).  
299 The ammonia level was lower in the 0.4%LS supplemented group, compared to both  
300 the control and the group treated with the addition of 0.2%LS (594 vs. 892 and 845  
301 mg/kg DM;  $P<0.05$ ). However, propionic and butyric acids were not influenced by the  
302 supplementation.

303 A high concentration of total caecal VFA in rabbits had a protective effect  
304 against enteropathogenic *Escherichia coli* infection ([Peeters et al., 1995](#)). Therefore,  
305 a higher level of VFA should contribute to health benefits that could prevent pathogen  
306 infection. Such benefits have been discovered after the dietary supplementation of  
307 LS in rabbits. However, more studies should be performed to confirm this theory. The  
308 nitrogenous residues are derived from the endogenous and undigested feed, which  
309 provides nitrogen sources for caecal fermentation, providing ammonia as an end  
310 product ([García et al., 2005](#)). In the present study, there was less observed caecal  
311 ammonia in the group treated with the high level of LS. The lower ammonia  
312 concentration was likely due to a decrease of protein utilisation in caecum, as  
313 microbiota was unable to digest tannin-protein complexes ([Maertens and Struklec,](#)  
314 [2006](#)). It is possible that some group of microbe may use ammonia and produce  
315 acetic acid as products which increases amount of acetic acid and total VFA.

316 However, it is impossible to conclude this theory until a study on the microbial  
317 mechanism and fermentation was performed.

318

### 319 *Faecal bacterial community*

320 The overall picture of the gut bacterial community of rabbits was generated using the  
321 PCR-DGGE analysis of DNA extracted directly from the hard faeces and from the  
322 caecal content. The results are summarised in [Figure 1](#). The dendrogram shows a  
323 great similarity of the bacterial community for the rabbits fed supplemented diets  
324 when compared with the control group for rabbits at 56 and 70 days of age. Age  
325 increments influence the dynamics of the microbiota, as a close correlation exists  
326 between digestive microbiota and diet ([Combes et al., 2013](#)), which was also  
327 observed in the present study. The development of gut microbiota was not influenced  
328 by dietary factors in this study. The loss of diversity may correlate with the diet and  
329 antimicrobial functions of the medicinal plants. Hexahydroxydiphenoyl ester  
330 vescalagin in LS extracts, which is one of the hydrolysable tannins, was shown *in*  
331 *vitro* to be the main active component in antimicrobial activity ([Becker et al., 2005](#)).  
332 Even though the active components that had antimicrobial properties present in LS,  
333 as well as the digestibility, were changed, the bacterial community was not affected  
334 by the supplementation in the present study.

335

### 336 *Carcass traits, meat quality and lipid oxidation*

337 No statistically significant difference appeared between the groups for carcass traits,  
338 meat quality (pH<sub>24</sub>, colour and chemical composition) and lipid oxidation ([Table 5](#)).  
339 One of the common causes of liver enlargement is the ingestion of toxic substances,  
340 which was discovered in the rabbits fed high level of tannins ([Al-Mamary et al.,](#)

341 2001). Fortunately, the low dose of tannins in our study did not induce hepatomegaly.  
342 Antioxidant substances can be used to prevent or slow down the problem of lipid  
343 oxidation. Phenolic compounds, which can be found in aromatic plants, have  
344 antioxidative properties, offering benefits in meat quality (Christaki *et al.*, 2012).  
345 Previous research found that diets supplemented with 200 mg/kg of oregano  
346 essential oil, in addition to chestnut wood extracts that contained antioxidant  
347 compounds, delayed the lipid oxidation in rabbit meat (Botsoglou *et al.*, 2004; Liu *et*  
348 *al.*, 2012). Even though there were active components with antioxidant activities  
349 present in LS (Tunalier *et al.*, 2007), lipid oxidation was not decreased in the present  
350 study. The pharmacokinetics of the antioxidative compounds in LS require further  
351 study in order to clarify how these compounds distribute in the active sites.

352

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358

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

	Control diet
Ingredients (%)	
Dehydrated alfalfa meal	29
Wheat bran	20
Barley <sup>1</sup>	19
Dried beet pulp	14
Soybean seed meal	6
Sunflower seed meal	6
Soybean oil	1
Molasses	1.5
Vitamin-mineral premix <sup>2</sup>	1
Wheat straw	1
Corn gluten	1
Dicalcium phosphate	0.5
Supplements <sup>3</sup>	0
Analysed composition on a dry matter basis (%) <sup>4</sup>	
Dry matter	89.7
Crude protein	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/100g) <sup>5</sup>	5.29

472 0.2%LS = 0.2% of dry ground *Lythrum salicaria* supplementation in diets; 0.4%LS = 0.4% of dry  
473 ground *Lythrum salicaria* supplementation in diets; CR = 0.3% Cunirel® (Biotrade snc®, Modena, Italy)  
474 supplementation in diets is a mixture of medicinal plants with LS is the main composition.

475 <sup>1</sup> The percentage on a dry matter basis of barley in 0.2%LS, 0.4%LS and CR were 18.8, 18.6 and  
476 18.7, respectively.

477 <sup>2</sup> Per kg of diet: Vit. A 200 IU;  $\alpha$ -tocopheryl acetate 16 mg; Niacin 72 mg; Vit. B6 16 mg; Choline 0.48  
478 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 and  
479 Cu 0.6 mg.

480 <sup>3</sup> The percentage on a dry matter basis of supplementation in 0.2%LS, 0.4%LS and CR were 0.2, 0.4  
481 and 0.3, respectively.

482 <sup>4</sup> Analysed composition on a dry matter basis of 0.2%LS, 0.4%LS and CR, respectively; 89.9, 90.5 and  
483 90.8% (dry matter); 18.2, 18.1 and 18.2% (crude protein); 3.0, 3.0 and 3.0% (Ether extract); 6.52, 6.60  
484 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  
485 19.5% (ADF); 3.58, 3.80 and 3.73% (ADL); 22.2, 22.0 and 22.8% (Starch).

486 <sup>5</sup> 6.09, 6.16, 17.4, 27.3 and 94.9 mg catechin equivalent/100g of fresh sample were observed in the  
487 0.2%LS, 0.4%LS, CR, dry ground LS leaves and Cunirel®, respectively.

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

Items	Diets				s.e.m.	P
	Control	0.2%LS	0.4%LS	CR		
Growth performance (n=40 per group)						
Initial body weight (g)	933	938	929	935	9	0.99
Live weight at 84 d (g)	2925	2928	2849	2844	30	0.62
Daily weight gain (g/d)	39.8	39.7	38.1	38.1	0.5	0.49
Daily feed intake (g/d)	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 per 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 <sup>1</sup> (%)	30.0	32.5	30.0	30.0	–	0.99
Apparent digestibility (n=10 per 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 <sup>a</sup>	60.4 <sup>ab</sup>	52.2 <sup>c</sup>	54.5 <sup>bc</sup>	1.4	0.02
Crude protein (%)	85.7 <sup>A</sup>	84.9 <sup>A</sup>	84.0 <sup>B</sup>	84.0 <sup>B</sup>	0.2	0.001
Digestive tract histology (n=6 per 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

503 0.2%LS = 0.2% of dry ground *Lythrum salicaria* supplementation in diets; 0.4%LS = 0.4% of dry  
 504 ground *Lythrum salicaria* supplementation in diets; CR = 0.3% Cunirel® (Biotrade snc®, Modena, Italy)  
 505 supplementation in diets was a mixture of medicinal plants with LS were the main composition; s.e.m.  
 506 = standard error of mean; d = days

507 <sup>1</sup> Health risk index is the summation between morbidity and mortality.

508 a,b,c or A,B Values within a row with different superscripts differ significantly at  $P<0.05$  or  $P<0.01$ ,  
509 respectively.

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

Items	Diets				s.e.m.	P
	Control	0.2%LS	0.4%LS	CR		
Haematology						
Haematocrit (%)	40.0	43.1	48.2	39.9	1.6	0.27
Erythrocytes (10 <sup>12</sup> /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 <sup>9</sup> /L)	4.06 <sup>A</sup>	8.25 <sup>B</sup>	8.63 <sup>B</sup>	8.21 <sup>B</sup>	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

540 0.2%LS = 0.2% of dry ground *Lythrum salicaria* supplementation in diets; 0.4%LS = 0.4% of dry  
 541 ground *Lythrum salicaria* supplementation in diets; CR = 0.3% Cunirel® (Biotrade snc®, Modena, Italy)  
 542 supplementation in diets was a mixture of medicinal plants with LS were the main composition; s.e.m.



543 = standard error of mean; RDW = red blood cell distribution width; AST = aspartate aminotransferase;

544 ALT = alanine aminotransferase

545 <sup>A,B</sup> Values within a row with different superscripts differ significantly at  $P < 0.01$ .

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

Items	Diets				s.e.m.	P
	Control	0.2%LS	0.4%LS	CR		
Caecal characteristics (n=10 per 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 per group)						
DM content (%)	21.3	22.4	20.8	21.1	0.3	0.31
Total VFA (mg/kg DM)	18.9 <sup>a</sup>	19.9 <sup>ab</sup>	24.1 <sup>b</sup>	23.0 <sup>ab</sup>	0.8	0.04
Acetic acid (mg/kg DM)	14.4 <sup>a</sup>	14.8 <sup>a</sup>	18.3 <sup>b</sup>	17.2 <sup>ab</sup>	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 <sup>b</sup>	845 <sup>b</sup>	594 <sup>a</sup>	680 <sup>ab</sup>	43	0.04

574 0.2%LS = 0.2% of dry ground *Lythrum salicaria* supplementation in diets; 0.4%LS = 0.4% of dry  
575 ground *Lythrum salicaria* supplementation in diets; CR = 0.3% Cunirel® (Biotrade snc®, Modena, Italy)  
576 supplementation in diets was a mixture of medicinal plants with LS were the main composition; s.e.m.  
577 = standard error of mean; BW: body weight; DM: dry matter; VFA: volatile fatty acids.

578 <sup>a,b</sup> Values within a row with different superscripts differ significantly at  $P < 0.05$ .

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586 **Table 5** Effect of phyto-additives (LS and CR) on carcass traits, meat quality and lipid  
 587 oxidation (TBARS,  $\mu\text{g}$  malonyldialdehyde/kg of fresh meat) of the longissimus dorsi  
 588 muscle in rabbits.

Items	Diets				s.e.m	P
	Control	0.2%LS	0.4%LS	CR		
Carcass traits (n=10 per 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 <sub>24</sub> and colour (n=10 per group)						
pH <sub>24</sub>	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 per 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 per group)						



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

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