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by:

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Abstract

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. Ten 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 crude protein digestibility than did the animals that were supplemented with the high LS levels and CR (85.7 and 84.9 vs. 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 to the control group (52.2 and 54.5 vs. 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 trials 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 vs. 8.25, 8.63 and 8.21x10^9/L; P<0.05; respectively). 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 vs. 18.9 mg/kg DM; P<0.05) and acetic acid (18.3 vs. 14.4 mg/kg DM; P<0.05), but lower ammonia levels (594 vs. 892 mg/kg DM; *P*<0.05) as compared to 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 to the control group. Moreover, only age was shown to influence microbiota diversity. In conclusion, the findings 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 of crude protein and ether extract, 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 crude protein 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 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). *Lythrum 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 anti-microbial, anti-fungal, 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). CR is a commercial mixture of herbs that contains LS as a major component. The diets were assigned to the animals (40 animals per 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**), crude protein by total nitrogen contents (**CP**), 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 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 minutes. 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 (**ADG**), average daily feed intake (**ADFI**), feed conversion ratio (**FCR**) and health risk index (**HRi**) 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

Caecal trials

The caecum from 10 animals per 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. One g of the sample was mixed with five ml of distilled deionised water at 20°C, before being centrifuged (15 minutes at 3000xg) and filtered through a Schleicher and Schull membrane filter (BA-83, 0.2 µm) for volatile fatty acid (**VFA**) determination. One µl of the extract was injected into a gas chromatography (GC 1000 DPC, Dani Instruments S.P.A., Cologno Monzese, Italy) using a wide-bore capillary column (SGE BP21 25m x 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 minutes at 3000g) of a vortexed mixture (30 s) of five g of caecal sample and 25 ml of distilled deionised water at 20°C, using an ammonia gas-sensing combination electrode (Orion, Model 95-12, Boston, MA, USA) that was connected to an ion analyser (Orion, Model 920A, Boston, MA, USA). The VFA and ammonia concentration were calculated on the dry matter 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 -20ºC until 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 µl 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/µl.
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 µl 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

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 per 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 hours 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, two g of freeze-dried meat (n=5 per 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 of 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 health risk index 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, 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 to 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 vs. 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 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 the rabbits (Liu *et al*., 2012). 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 and 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 introduction 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 to the control group (0.2%LS, 0.4%LS and CR vs. control; 8.25, 8.63 and 8.21 vs. 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 to the control group (24.1 vs. 18.9 mg/kg DM; $P<0.05$), whereas the acetic acid values were greater compared to both the control group and to animals fed with 0.2%LS (18.3 vs. 14.4 and 14.8 mg/kg DM; $P<0.05$). The ammonia level was lower in the 0.4%LS supplemented group, compared to both the control and the group treated with the addition of 0.2%LS (594 vs. 892 and 845 mg/kg DM; $P<0.05$). However, propionic and butyric acids were not influenced by the supplementation.

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 increases 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 the rabbits fed supplemented diets when compared with the control group for 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 was not influenced by dietary factors in this study. 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, 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. The pharmacokinetics of the antioxidative compounds in LS require further study in order to clarify how these compounds distribute in the active sites.

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References

Al-Mamary M, Al-Habori M, Al-Aghbari A and Al-Obeidi A 2001. *In vivo* effects of dietary sorghum tannins on rabbit digestive enzymes and mineral absorption. Nutrition Research 21, 1393-1401.

AOAC 2000. Official Method of Analysis. 17th edition. Association of Official Analytical Chemists, Arlington, VA, USA.

365 Arafa NMS, Salem SMA and Farid OAHA 2010. Influence of *Echinacea* extract pre- or
366 postnatal supplementation on immune and oxidative status of growing rabbits. Italian
367 Journal of Animal Science 9, 338-343.
368 Ayala L, Silvana N, Zocarrato I and Gómez S 2011. Use of vulgar oregano (*Origanum*
369 *vulgare*) as phytobiotic in fatting rabbits. Cuban Journal of Agricultural Science 45, 159-
370 161.
371 Becker H, Scher JM, Speakman JB and Zapp J 2005. Bioactivity guided isolation of
372 antimicrobial compounds from *Lythrum salicaria*. Fitoterapia 76, 580-584.
373 Botsoglou NA, Florou-Paneri P, Christaki E, Giannenas I and Spais AB 2004. Performance
374 of rabbits and oxidative stability of muscle tissues as affected by dietary
375 supplementation with oregano essential oil. Archives of Animal Nutrition 58, 209-218.
376 Christaki E, Bonos E, Giannenas I and Florou-Paneri P 2012. Aromatic plants as a source of
377 bioactive compounds. Agriculture 2, 228-243.
378 Combes S, Fortun-Lamothe L, Cauquil L and Gidenne T 2013. Engineering the rabbit
379 digestive ecosystem to improve digestive health and efficacy. Animal 7, 1429-1439.
380 Dabbou S, Gasco L, Gai F, Zoccarato I, Rotolo L, Dabbou FS, Brugiapaglia A, Helal AN and
381 Peiretti PG 2014. Dried artichoke bracts in rabbits nutrition: effects on the carcass
382 characteristics, meat quality and fatty-acid composition. Animal 8, 1547-1553.
383 Dalle Zotte A, Sartori A, Bohatir P, Rémignon H and Ricci R 2013. Effect of dietary
384 supplementation of Spirulina (*Arthrospira platensis*) and Thyme (*Thymus vulgaris*) on
385 growth performance, apparent digestibility and health status of companion dwarf
386 rabbits. Livestock Science 152, 182-191.
387 European Economic Community 1972. Analytical determination of starch. Official Journal of
388 European Communities 123, 7.
389 Falcão-e-Cunha L, Castro-Solla L, Maertens L, Marounek M, Pinheiro V, Freire J and
390 Mourão JL 2007. Alternatives to antibiotic growth promoters in rabbit feeding: a review.
391 World Rabbit Science 15, 127-140.

García AI, de Blas JC and Carabano R 2005. Comparison of different methods for nitrogen and amino acid evaluation in rabbit diets. Animal Science 80, 169-178.

Gidenne T, Combes S, Feugier A, Jehl N, Arveux P, Boisot P, Briens C, Corrent E, Fortune H, Montessuy S and Verdelhan S 2009. Feed restriction strategy in the growing rabbit. 2. Impact on digestive health, growth and carcass characteristics. Animal 3, 509-515.

Humadi SS and Istudor V 2009. *Lythrum salicaria* (Purple Loosestrife). Medicinal use, extraction and identification of its total phenolic compounds. Farmacia 57, 192-200.

IBM SPSS 2012. IBM SPSS Statistics 20.0 SPSS Inc., Chicago, IL, USA.

Krieg R, Vahjen W, Awad W, Sysel M, Kroeger S, Zocher E, Hulan HW, Arndt G and Zentek J 2009. Performance, digestive disorders and the intestinal microbiota in weaning rabbits are affected by a herbal feed additive. World Rabbit Science 17, 87-95.

Lahouar L, El Arem A, Ghrairi F, Chahdoura H, Ben Salem H, El Felah M and Achour L 2014. Phytochemical content and antioxidant properties of diverse varieties of whole barley (*Hordeum vulgare* L.) grown in Tunisia. Food Chemistry 145, 578-583.

Liu H, Zhou D, Tong J and Vaddella V 2012. Influence of chestnut tannins on welfare, carcass characteristics, meat quality, and lipid oxidation in rabbits under high ambient temperature. Meat Science 90, 164-169.

Maertens L and Struklec M 2006. Technical note: Preliminary results with a tannin extract on the performance and mortality of growing rabbits in an enteropathy infected environment. World Rabbit Science 14, 189-192.

Mikel UV 1994. Advanced laboratory methods in histology and pathology. Armed Forces Institute of Pathology, American Registry of Pathology, Washington, DC, USA.

Muyzer G, De Waal EC and Uitterlinden AG 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction amplified genes coding for 16S rRNA. Applied and Environmental Microbiology 59, 695-700.

418 Peeters JE, Maertens L, Orsenigo R and Colin M 1995. Influence of dietary beet pulp on
419 caecal VFA, experimental colibacillosis and iota-enterotoxaemia in rabbits. Animal
420 Feed Science and Technology 51, 123-139.
421 Perez JM, Lebas F, Gidenne T, Maertens L, Xiccato G, Parigi-Bini R, Dalle Zotte A, Cossu
422 ME, Carazzolo A, Villamide MJ, Carabaño R, Fraga MJ, Ramos MA, Cervera C, Blas
423 E, Fernandez J, Falcão-e-Cunha L and Bengala Freire J 1995. European reference
424 method for *in vivo* determination of diet digestibility in rabbits. World Rabbit Science 3,
425 41-43.
426 Phillips I 2007. Withdrawal of growth-promoting antibiotics in Europe and its effects in
427 relation to human health. International Journal of Antimicrobial Agents 30, 101-107.
428 Rotolo L, Gai F, Nicola S, Zoccarato I, Brugiapaglia A and Gasco L 2013. Dietary
429 supplementation of oregano and sage dried leaves on performances and meat quality
430 of rabbits. Journal of Integrative Agriculture 11, 1937-1945.
431 Rotolo L, Gai F, Peiretti PG, Ortoffi M, Zoccarato I and Gasco L 2014. Live yeast
432 (*Saccharomyces cerevisiae* var. *boulardii*) supplementation in fattening rabbit diet:
433 Effect on productive performance and meat quality. Livestock Science 162, 178-184.
434 Simonová MP, Chrastinová L, Mojto J, Lauková A, Szabóová R and Rafay J 2010. Quality of
435 rabbit meat and phyto-additives. Czech Journal of Food Sciences 28, 161-167.
436 Soultos N, Tzikas Z, Christaki E, Papageorgiou K and Steris V 2009. The effect of dietary
437 oregano essential oil on microbial growth of rabbit carcasses during refrigerated
438 storage. Meat Science 81, 474-478.
439 Szabóová R, Lauková A, Chrastinová L, Strompfová V, Simonová MP, Plachá I, Vasilková Z,
440 Chrenková M and Faix S 2012. Beneficial effect of plant extracts in rabbit husbandry.
441 Acta Veterinaria Brno 81, 245-250.
442 Tunalier Z, Kosar M, Kupeli E, Calis I and Baser KHC 2007. Antioxidant, anti-inflammatory,
443 anti-nociceptive activities and composition of *Lythrum salicaria* L. extracts. Journal of
444 Ethnopharmacology 110, 539-547.

Van Soest PJ, Robertson JB and Lewis BA 1991. Methods for dietary fibre, neutral detergent fibre, and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science 74, 3583-3597.

Vauterin L and Vauterin P 1992. Computer-aided objective comparison of electrophoretic patterns for grouping and identification of microorganisms. European Microbiology 1, 37-41.

Witte VC, Krause GF and Bailey ME 1970. A new extraction method for determining 2-thiobarbituric acid values of pork and beef during storage. Journal of Food Science 35, 582-585.

471 ***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
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)[5]	5.29

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

[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: Vit. A 200 IU; α-tocopheryl acetate 16 mg; Niacin 72 mg; Vit. B6 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 and 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% (crude protein); 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/100g of fresh sample were observed in the 0.2%LS, 0.4%LS, CR, dry ground LS leaves and Cunirel®, respectively.

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[1] (%)	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^{a}	60.4^{ab}	52.2^{c}	54.5^{bc}	1.4	0.02
Crude protein (%)	85.7^{A}	84.9^{A}	84.0^{B}	84.0^{B}	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 [1] Health risk index is the summation between morbidity and mortality.

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

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^{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

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.

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

[A,B] Values within a row with different superscripts differ significantly at $P<0.01$.

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^{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

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 ^{a,b} 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, µg 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_{24} and colour (n=10 per 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 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)						

TBARS (µg/kg)	297	266	335	301	13	0.38

0.2%LS = 0.2% of dry ground *Lythrum salicaria* supplementation in diets; 0.4%LS = 0.4% of dry ground *Lythrum salicaria* supplementation in diets; CR = 0.3% Cunirel® (Biotrade snc®, Modena, Italy) supplementation in diets was a mixture of medicinal plants with LS were the main composition; s.e.m. = standard error of mean; SW = Slaughter weight; CCW = Chilled carcass weight; pH_{24} = pH of *longissimus dorsi muscles* were measured after 24 h of chilling

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).*