THE EFFECT OF DIETARY *LYTHRUM SALICARIA* ON THE RABBIT'S PERFORMANCE AND MICROBIAL COMMUNITY

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The Effect of Dietary *Lythrum salicaria* on the Rabbit's Performance and Microbial Community

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ABSTRACT

Medical plants with anti-microbial properties have been used as an animal diet supplementation, to improve the performance and health in commercial and organic livestock productions. One of the medical plant, *Lythrum salicaria* (LS; purple loosestrife), was discovered those activities from their extracted compounds. Balanced microbial community in rabbit gastrointestinal tract have important role to provide the healthy condition. Therefore, the effect from dietary LS on performance and on gut microbiota were the objective of this research. One hundred and sixty Hycole weaned rabbits (35-day-old) were randomly decided into four groups which included one control and three treatment groups. Respectively, 0.2%, 0.4% dry powder of LS and CUNIREL (CR; the commercial phyto-additive mixture from Biotrade®, Italy, contained the LS as the main ingredient) were added in the treatment diets. Performance data were recorded at 1, 21 and 49 days after experiment beginning. For microbial diversity analysis, hard feces from 10 systemic random sampling rabbits, were collected separately at 35, 42, 49, 56, 70 and 89 days of age, whereas caecal contents were taken at slaughter day (89-day-old). After that, samples which in the same group, collected site and collected date were pooled for further molecular analysis. The microbiota were assessed at species level by using Polymerase Chain Reaction-Denaturing Gradient Gel Electrophoresis (PCR-DGGE) analysis of 16S rRNA gene. There were not statistically significant differences on performances (weight, average daily weight gain, average daily feed intake, feed conversion ratio, morbidity, mortality and health risk index) between the control and treatment groups. The dendrogram generated by using DGGE profiles of 16S rRNA fragments show that the similarity between rabbit fed with LS and commercial product were higher if compared with conventional diet. On the other hand, we found high similarity between samples obtained from caecal content and hard feces at the end of the experiment whereas no any adverse effects from dietary LS were found on rabbit's performance.

Key Words: DGGE, *Lythrum salicaria*, Microbial Community, Performance, Rabbit

INTRODUCTION

Organic livestock farming have been interested by global viewpoint because products from this husbandry are known as organic products which more safety for the consumption, higher value than other common products and less environmental contamination (Sundrum 2001). Anyways, the organic farming still has chances to suffer from health problems because the antibiotic usage is strictly limit. There was the similar situation when the European Union banned the antibiotic usage as the growth promoter in livestock animals. From that prohibition, a product yield reduction, a sharp increment of morbidity and mortality rate were discovered (Casewell et al. 2003). Any supplements which have potentiality to achieve those requirements and allow to use in the organic farming, have been investigated. Medical plants with anti-microbial and anti-oxidant properties, are receiving considerable attention (Franz et al. 2010; Christaki et al. 2012).

Lythrum salicaria (LS) with common name is purple loosestrife, is in the Lythraceae family. LS were considered as the invasive and competitive plant in the ecosystem for the special ability on environmental adaptation and faster reproduction. On the other hand, medical functions (such as anti-diarrhea, anti-dysentery, anti-haemorrhagic) were discovered and used in folk medicine. *In vitro* studies, main action compounds (*e.g.* tannins and flavanoids) which were identified by extraction, had anti-microbial, anti-fungal, anti-inflammatory and anti-oxidant activities (Becker et al. 2005; Tunalier et al. 2007; Humadi & Istudor 2009).

From those properties, LS should have capacity to apply as the additive in rabbit feed to develop rabbit's health and performance.

The microorganism in rabbit's digestive tract have important role to develop the gut immunity and prevent the pathogen overgrowth. Therefore, the microbiota community can be the criteria which evaluate gut health status (Fortun-Lamothe & Boullier 2007). Molecular methods such as polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE) are optimized for an easy comparison of profiles from related microbial assemblages and are used in many ecological studies (Michelland et al. 2010). The study on the effect from LS supplement in rabbit diet on the microbial environment and performance is the aim of this research article.

MATERIAL AND METHODS

Animals and diets

The experiment was performed at the Department of Animal Science experimental rabbitry in Carmagnola, Turin, Italy. One hundred and sixty weaned, Hycole rabbits which were 35-day-old with 934.3±118 g of the initial weight, were randomly decided into four groups with equal sex and number (one control group and three treatment groups). Normal distribution and equal variance were confirmed before experimental beginning by statistic analysis. Each animals were separated in the individual standard cage with free access of clean water. The indoor housing temperature and light-dark photoperiod cycle were maintained at 22±2°C and 12-hour light/12-hour dark along the experiment, respectively. The ingredients of the experimental diets were reported in Table 1 and conserved for the control group. Diets for treatment groups supplemented by 0.2% LS and 0.4% LS powder which provided from dry LS leaves grinding by Biotrade®, Italy. CUNIREL (CR; Biotrade®, Italy) which are the commercial mixtured of medical plants with LS are the main composition, were added in another treatment diet. A feeding strategy was *ad libitum*.

Performance and hard feces collection

Weight, feed intake, morbidity (digestive disorders, respiratory disease, weight under 2 standard deviation: SD, from group mean and others) and mortality from individual rabbits at the first day, after 3 weeks and after 7 weeks of experiment, were recorded to compute the performances which were the average daily

Table 1. Ingredients of the experimental diets

Ingredients (%)	Control	0.2% LS	0.4% LS	CR
Dehydrated alfalfa meal	29.0	29.0	29.0	29.0
Barley	19.0	18.8	18.6	18.7
Dry beet pulp	14.0	14.0	14.0	14.0
Wheat bran	20.0	20.0	20.0	20.0
Soybean seed meal	6.0	6.0	6.0	6.0
Sunflower seed meal	6.0	6.0	6.0	6.0
Soybean oil	1.0	1.0	1.0	1.0
Molasses	1.5	1.5	1.5	1.5
Dicalcium phosphate	0.5	0.5	0.5	0.5
Vitamin-mineral premix[1]	1.0	1.0	1.0	1.0
Wheat straw	1.0	1.0	1.0	1.0
Corn gluten	1.0	1.0	1.0	1.0
Supplements	0.0	0.2	0.4	0.3

LS: dry *Lythrum salicaria* powder; CR: CUNIREL (Biotrade®, Italy) mixtured of medical plants with LS were the main composition; [1] Per kg of diet: Vit. A 200 IU; α-tocopheryl acetate 16 mg; Niacine 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; Cu 0.6 mg

gain (ADG), average daily feed intake (ADFI), feed conversion ratio (FCR) and health risk index (HRi). Necropsy were performed in dead rabbits. 10 systemic random rabbits from each group were selected for the hard feces collection at 35, 42, 49, 56, 70 and 89 days of age and samples kept in -20°C until analysis will perform.

Slaughter procedures and caecal content collection

The 89-day-old rabbits (54 days after the experiment beginning) with 3126±193 g of a final weight, were killed by concussion technique without fasting at the Department of Animal Science experimental slaughter house, Carmagnola, Turin, Italy. Immediately after the end of the slaughter process, a caecal content of the same rabbit which were selected for the hard feces collection, were collected and kept in -20°C for further analysis.

DNA extraction and PCR-DGGE analysis

For DNA extraction, feces and caecal content of 10 rabbits from the same group, collected site and collected date, as previously reported, were pooled together. Samples (10 g) were homogenized in a stomacher bag with 20 ml of quarter-strength Ringer's solution (Oxoid) for 1 min; a deposit was allowed to set for 1 min, and 1 ml of the supernatant was used for the DNA extraction. The protocol described from manufacturer's instructions of Powersoil DNA kit (MO-BIO, Carlsbad, CA) were used. The DNA solution was incubated at 37°C for 30 min with 5 µl of RNAse (Promega, Milano), and then stored at -20°C. DNA was quantified by using the NanoDrop 1000 spectrophotometer (Thermo Scientific) and was standardized at 50 ng μl^{-1}. The primers 338F-GC and 518R (Muyzer et al. 1993) were used to amplify the variable V3 region of the 16S rRNA gene, giving PCR products of about 300 bp. Amplifications were performed in a programmable heating incubator (Bio-Rad, Milan, Italy) as previously described (Muyzer et al. 1993). Aliquots (2 ml) of PCR products were routinely checked on 2% agarose gels. PCR products were analyzed by denaturing gradient gel electrophoresis (DGGE) by using a Bio-Rad Dcode apparatus. Samples were applied to 8% (wt/vol) polyacrylamide gels in 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, Milano) for 30 minutes, visualized under UV-transillumination and photographed by Bio-Rad Gel Doc system (Bio-Rad, Milano, Italy).

A database of fingerprints was created by using the software Bionumerics version 5.1 (Applied Maths, Sint Marten Latem, Belgium). A dendrogram of similarity was retrieved by using the Dice coefficient and unweighted pair group method using arithmetic average (UPGMA) clustering algorithm (Vauterin & Vauterin 1992).

Statistical analysis

Continuous data were analyzed by using one-way analysis of variance (ANOVA). All of statistic analyses were performed with an SPSS 11.5.1 for Windows statistical software package (SPSS Inc., Cary, NC, USA). Categorical data (morbidity, mortality and health risk index), chi-square was used.

RESULTS AND DISCUSSION

Along the experiment, no statistic significant difference between treatment and control groups were observed from any observed parameters (Table 2).

However, Ayala et al. (2011) reported the growth parameter improvement in fattening rabbits when oregano was used as phyto-additive whereas others research did not find any effect on performance between control and treatment groups. (Botsoglou et al. 2004; Soultos et al. 2009; Szabóová et al. 2012). In general, active components from aromatic plants have potential to promote better flavour in feed that directly increase consumption from animals. Hence, the performance improvement should observe in phyto-additive animals but actually most of outcomes were given none or adverse effects (Franz et al. 2010; Christaki

Table 2. Performance of rabbit fed 0.2%LS, 0.4%LS and CR from 35 to 84 days of age[1, 2]

Performance parameters	Control	0.2%LS	0.4%LS	CR	RMSE
Weaning (35 days) to 56 days of age					
Initial weight (g)	933.78	938.78	929.38	935.25	119.09
Weight at 56 days (g)	1760.39	1761.79	1771.70	1754.69	253.58
ADG (g/day)	37.53	37.25	37.79	37.06	18.58
ADFI (g/day)	92.10	94.49	98.08	95.42	9.32
FCR	2.48	2.58	2.61	2.63	0.34
Morbidity (%)	17.50	20.00	15.00	15.00	
Mortality (%)	5.00	5.00	7.50	5.00	
56 to 84 days of age					
Weight at 84 days (g)	2925.55	2928.28	2849.75	2844.62	369.04
ADG (g/day)	41.61	41.66	38.50	38.93	23.28
ADFI (g/day)	146.25	149.41	145.95	144.29	7.75
FCR	3.54	3.69	3.99	3.91	1.04
Morbidity(%)	7.89	7.89	8.11	10.53	
Mortality(%)	0.00	0.00	0.00	0.00	
Weaning to 84 days of age					
ADG (g/day)	39.82	39.72	38.19	38.11	18.77
ADFI (g/day)	122.43	125.25	124.89	122.79	6.42
FCR	3.10	3.17	3.32	3.28	0.40
Morbidity (%)	25.00	27.50	22.50	25.00	
Mortality (%)	5.00	5.00	7.50	5.00	
HRi (%)	30.00	32.50	30.00	30.00	

LS: dry *Lythrum salicaria* powder; CR: CUNIREL (Biotrade®, Italy) mixtured of medical plants with LS were the main composition; ADG: Average daily weight gain; ADFI: Average daily feed intake; FCR: Feed conversion ratio; HRi: Health risk index= mortality+morbidity rates; [1] 40 animals in each group; [2] No statistically significant ($P<0.05$) differences were noted

et al. 2012). On the other hands, positive outputs on performances were found in the rabbit, poultry and swine production when the commercial product, mixture of essential oils or mixture of herbs, were used (Krieg et al. 2009; Franz et al. 2010; Christaki et al. 2012). Synergistic effect from different plant's essential oil provides probably stronger actions or any effects that supports on the animal performances. Anyways, more evidences from future research studies must perform.

The incidence of mortality were only found at the first three week of fattening period which no difference between any groups. After the necropsy, main gross lesions were completely without normal faecal production, gastrointestinal impaction and no inflammation in internal organs. Gastrointestinal hypomotility or gut stasis were the main suspected disorder in this experiment. Watery or mucous diarrhea and weight under 2SD from the group mean were observed and recorded as the morbidity animals. On the same pace, there were not any difference on illness and dead animals between any study groups which as similar as other articles (Ayala et al. 2011; Botsoglou et al. 2004; Soultos et al. 2009; Szabóová et al. 2012).

In this study, the overall outcome on bacterial community from PCR-DGGE analysis of DNA extracted directly from hard feces and caecal content was generated to visualize by the dendrogram (Figure 1). The age increment influenced the dynamic of the microbiota which was observed in this study. The fingerprints of control group with old age (56 and 70 days) were different if compared to the younger groups. Moreover, the relationship between age and microbial community were reported because the development of intestinal microbiota had mission to support the fiber digestion. (Combes et al. 2011).

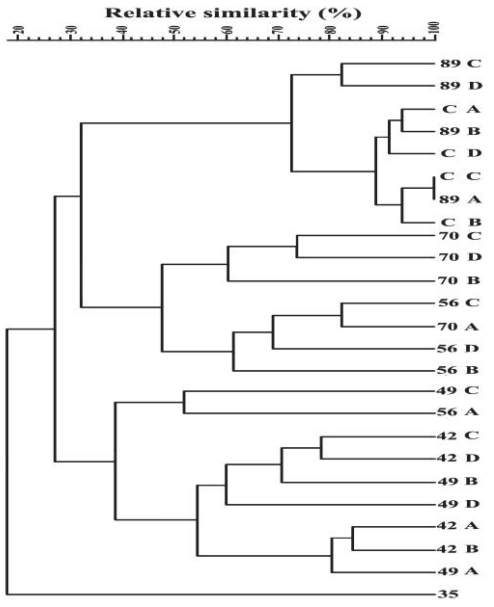

Figure 1. Dendrogram of similarity generated by the digitized DGGE fingerprints. Ages of the rabbits from beginning of the experiment (35 days) to slaughter day (89 days) are reported in the left column. C letter in the left column mean caecal content samples which collected at 89 days of age. Types of diet (right column) are also reported: control (A), CR (B), 0.2% of LS (C) and 0.4% of LS (D)

On the other hand the dendrogram showed that the similarity of microbial community between rabbits, fed with LS and CR, were higher if compared with rabbits fed with conventional diet. In addition at the end of the experiment the dendogram showed an higher similarity between samples obtained from caecal content and hard feces.

The microorganism have important role to develop the gut immunity and prevent the pathogen overgrowth. Hence, the quickly adaptation to reach the appropriate microbial ecosystem, when animals were impacted by diverse factors, was significant to provide the healthy condition in rabbits (Fortun-Lamothe & Boullier 2007). Caecum is the one of the important organ of rabbit's digestive tract and has bacterial species variety. The caecal content were collected at the slaughter day and performed the same protocol as hard feces samples. The results from this study, the bacterial community structure between caecal contents and hard feces were nearly similar. On the one hand, no significantly difference were published on the diversity index of bacterial community between caecal content and hard feces (Michelland et al. 2010).

Generally, the effect on anti-bacterial functions were originated from the essential oil in medical plants (Franz et al. 2010; Christaki et al. 2012). From this report results, LS supplement influenced on the bacterial environment transition in rabbit's gastrointestinal tract. Many types of essential oil, mainly flavanoids, tannins and terpenes, were found in LS which had the anti-bacterial activities (Becker et al. 2005). Moreover, high amount of tannins was discovered from LS extraction (Humadi & Istudor 2009) and the hexahydroxydiphenoyl ester vescalagin that was one member of hydrolyzable tannin, was considered as major active component of antibacterial activity (Becker et al. 2005). Furthermore, the results from various *in vitro* studies presented extracted LS anti-microbial ability on *Bacillus cereus*, *Escherichia coli*, *Mycobacterium smegmatis*, *Micrococcus luteus*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Rauha et al. 2000; Dulger & Gonuz, 2004; Borchardt et al. 2009). Hence, the essential oil activities were assumed that were the cause which impacted on the bacterial diversity difference.

CONCLUSION

No any adverse effects from LS supplementation were found on rabbit's performance whereas the difference on bacterial community were reported between control and treatment groups.

ACKNOWLEDGMENT

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The effect of dietary *Lythrum salicaria* on the rabbit's performance and microbial community

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Introduction

Antibiotic usage as growth promoter in animal feed

The selection of antibiotic resistant bacteria

Contamination from animals to human

Antibiotic residues in meat products

Consumers receive inappropriate dose of antibiotics.

The selection of antibiotic resistant bacteria

Antibiotics, which used as growth promoter, were banned.

(Casewell et al., 2003; Falcão-e-Cunha et al., 2007; FAO, 201

Introduction

The consequences from ban on growth-promoting antibiotics

- The reduction in some resistance bacteria.

- The deterioration in animal health and performance.
- The increment in usage of therapeutic antibiotics.

Any supplements which have potentiality to achieve - those requirements, have been studied.

One of intersting is "Phyto-additive".

(Casewell et al., 2003; Falcão-e-Cunha et al., 2007)

Introduction

Lythrum salicaria L.

- Purple loosestrife
- Family: Lythraceae
- Invasive plants
- Contained medical properties
  - Folk medicine: diarrhea, dysentery, bleeding wounds and others
  - Essential oils: phenolic compound (tannins and flavonoids)
    - *In vitro* studies discovered:
      - anti-microbial, anti-fungal and anti-oxidant activities

USDA NRCS National Plant Data Center & Louisiana State University-Plant Biology; Becker et al., 2005; Tunalier et al., 2007; Humadi and Istudor, 2009

Introduction

The aspects from phyto-additive usage in animal diets

- **Growth performance**
- **Animal health**
  - Morbidity
  - Mortality
  - Health risk index
  - Bacterial community
- **Animal products**
- **Environmental consequences**
- **Others**

(Franz, ed al., 2010; Christake et al., 2012)

Objectives

- To determine the performance influence from *L. salicaria* as supplement
- To verify the bacterial diversity from hard feces and caecal content samples

Materials and Methods

1. Animals and diets preparation

160 Hycole rabbits (35-day-old)

Rabbits were divided into 4 groups of 40.

- Control
- 0.2% LS
- 0.4% LS
- 0.3% CUNIREL (CR)

LS = Dry *Lythrum salicaria* powder from their leaves.

CR = Herbal mixture with *Lythrum salicaria* as main component (Biotrade®, Italy)

Materials and Methods

Table 1 Ingredients of the experimental diets.

Ingredients (%)	Control	0.2% LS	0.4% LS	CUNIREL
Alfalfa meal	29.0	29.0	29.0	29.0
Barley	19.0	18.8	18.6	18.7
Dry beet pulp	14.0	14.0	14.0	14.0
Wheat bran	20.0	20.0	20.0	20.0
Soybean seed meal	6.0	6.0	6.0	6.0
Sunflower seed meal	6.0	6.0	6.0	6.0
Soybean oil	1.0	1.0	1.0	1.0
Molasses	1.5	1.5	1.5	1.5
Dicalcium phosphate	0.5	0.5	0.5	0.5
Vitamin-mineral premix[1]	1.0	1.0	1.0	1.0
Wheat straw	1.0	1.0	1.0	1.0
Corn gluten	1.0	1.0	1.0	1.0
Supplements	0	0.2	0.4	0.3

LS: dry *Lythrum salicaria* powder.
CUNIREL: (Biotrade®, Italy) mixtured of medical plants with LS were the main composition.
[1] Per kg of diet: Vit. A 200 IU; α-tocopheryl acetate 16 mg; Niacine 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.

Materials and Methods

2. Animal housing

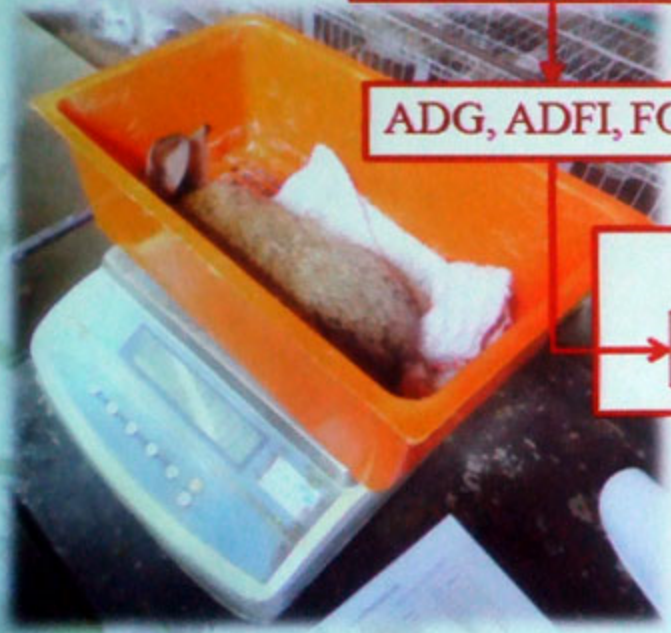
Materials and Methods

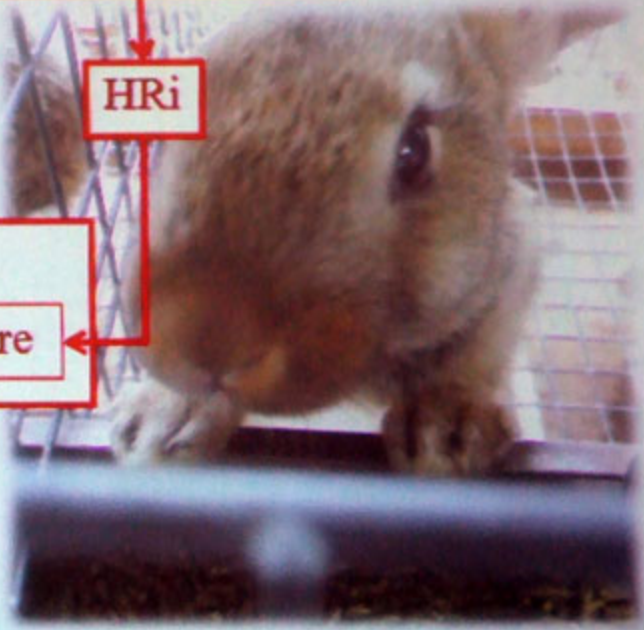
3. Performance

Performance data were collected at days 1, 21 and 49.

- Weight, Feed intake → ADG, ADFI, FCR
- Morbidity, Mortality → HRi

Statistic analysis: ANOVA, Chi-square





ADG=average daily weight gain, ADFI=average daily feed intake, FCR=feed conversion ratio, HRi=health risk index

Results and discussion

Table 2 Performance of rabbit fed 0.2%LS, 0.4%LS and CR from 35 to 84 days of age[1,2]

Performance parameters	Control	0.2%LS	0.4%LS	CR	RMSE	P-value
Weaning (35 days) to 56 days						
Initial weight (g)	933.78	938.78	929.38	935.25	119.09	0.99
Weight at 56 days (g)	1760.39	1761.79	1771.70	1754.69	253.58	0.99
ADG (g/day)	37.53	37.25	37.79	37.06	18.58	0.99
ADFI (g/day)	92.10	94.49	98.08	95.42	9.32	0.58
FCR	2.48	2.58	2.61	2.63	0.34	0.49
Morbidity (%)	17.5	20	15	15		0.92
Mortality (%)	5	5	7.5	5		0.95
56-84 days						
Weight at 84 days (g)	2925.55	2928.28	2849.75	2844.62	369.04	0.62
ADG (g/day)	41.61	41.66	38.50	38.93	23.28	0.15
ADFI (g/day)	146.25	149.41	145.95	144.29	7.75	0.81
FCR	3.54	3.69	3.99	3.91	1.04	0.23
Morbidity (%)	7.89	7.89	8.11	10.53		0.97
Mortality (%)	0	0	0	0		0.99
Weaning to 84 days of age						
ADG (g/day)	39.82	39.72	38.19	38.11	18.77	0.49
ADFI (g/day)	122.43	125.25	124.89	122.79	6.42	0.88
FCR	3.10	3.17	3.32	3.28	0.40	0.73
Morbidity (%)	25	27.5	22.5	25		0.97
Mortality (%)	5	5	7.5	5		0.95
HRi (%)	30	32.5	30	30		0.99

[1] 40 animals in each group. [2] No statistically significant ($P<0.05$) differences were noted.

Results and discussion

Table 3 Effect on rabbit's performance from plant and essential oils additive in diets.

	Treatment effects (SD difference from control group)				
Feed additives (dose)	**Feed intake**	**Body weight**	**Daily weight gain**	**Feed conversion rate**	**References**
Single plant additive					
Purple loosestrife (20mg/kg)	0	0	0	0	This study
Purple loosestrife (40mg/kg)	0	0	0	0	This study
Oregano (100mg/kg)*	0	0	0	0	Botsoglou et al., 2004
Oregano (200mg/kg)*	0	0	0	0	Botsoglou et al., 2004
Oregano (10µl/day)*	0	0	0	0	Szabóová et al., 2012
Oregano (100mg/kg)*	0	0	0	0	Soultos et al., 2009
Oregano (200mg/kg)*	0	0	0	0	Soultos et al., 2009
Echinacea (130mg/kg)*	0	+1	0	0	Arafa et al., 2010
Mixture plants additive					
CUNIREL (30mg/kg)	0	0	0	0	This study
Xtract™ (150g/kg)	0	0	0	0	Szabóová et al., 2012

Remarks: * supplement by essential oil extraction

Materials and Methods

4. Bacterial community analysis

10 rabbits from each groups

Days 1, 7, 21, 35 and 54

Days 54 (Slaughter day)

Collected hard feces

Collected caecal content

Kept in -20°C

PCR-DGGE analysis: V3 regions

Statistic analysis: Cluster analysis

PCR-DGGE = Polymerase chain reaction-denaturing gradient gel electrophoresis

Results and discussion

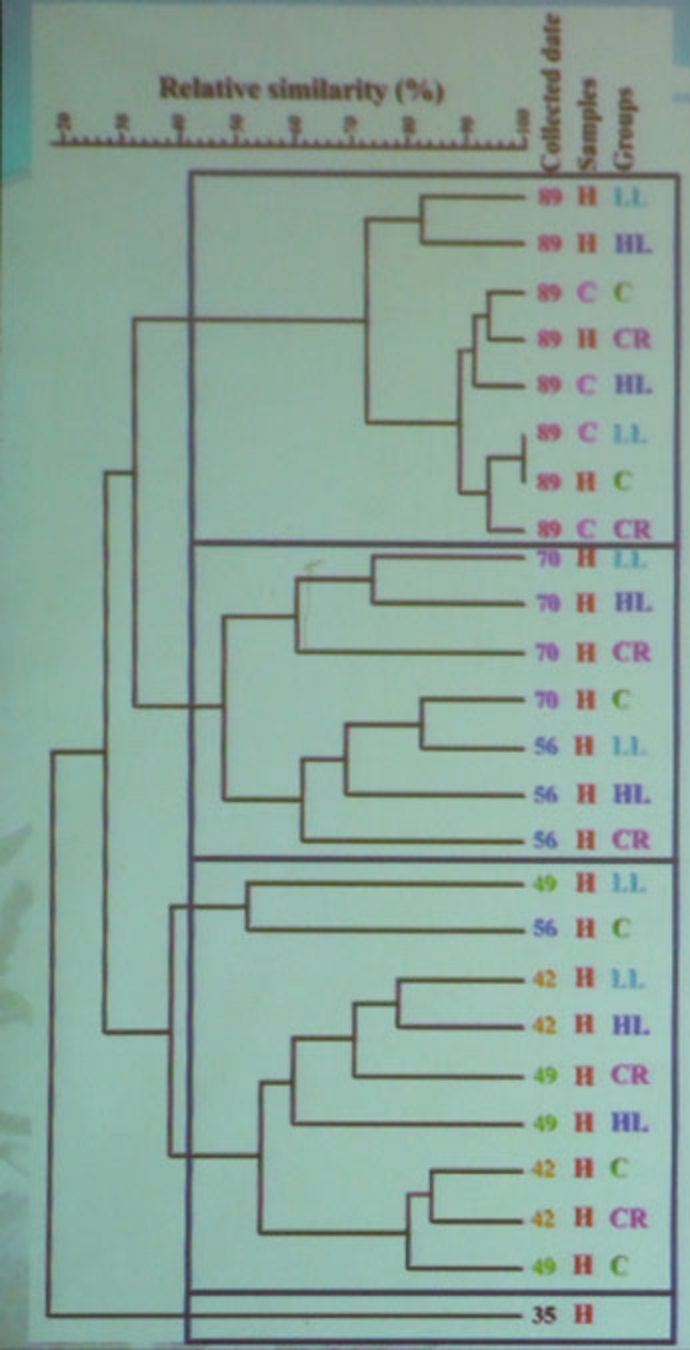


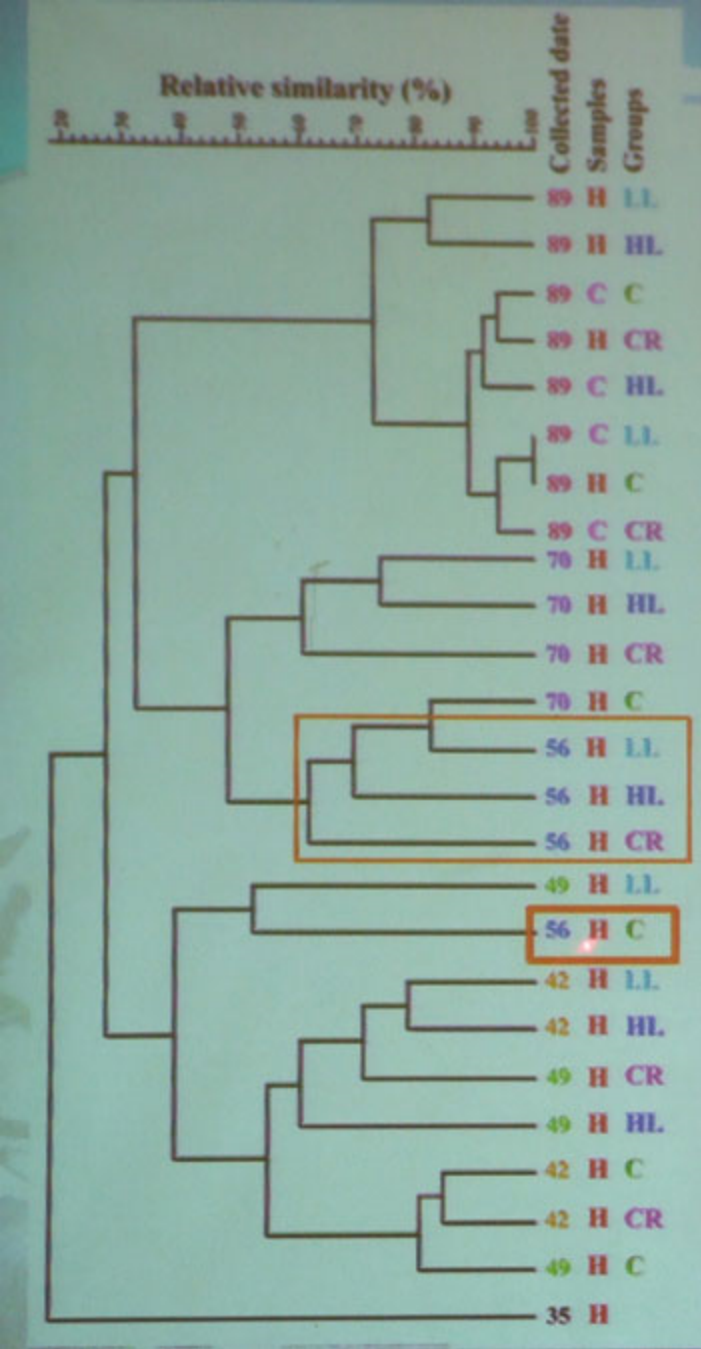
Figure 1. Dendrogram of similarity generated by the digitized DGGE fingerprints.

- Abbreviation
  - Samples
    - H = Hard feces, C = Caecal content
  - Groups
    - C = Control, LL = Low dose LS, HL = High dose LS, CR = CUNIREL

- **The age increment influenced the dynamic of the microbiota.**
- **Control group with old age was similar to the treatment group with young age.**
- **Bacterial community between caecal content and hard feces were similar.**

Fortun-Lamothe and Boullier, 2007; Michelland et al., 2010; Combes et al., 2011

Results and discussion



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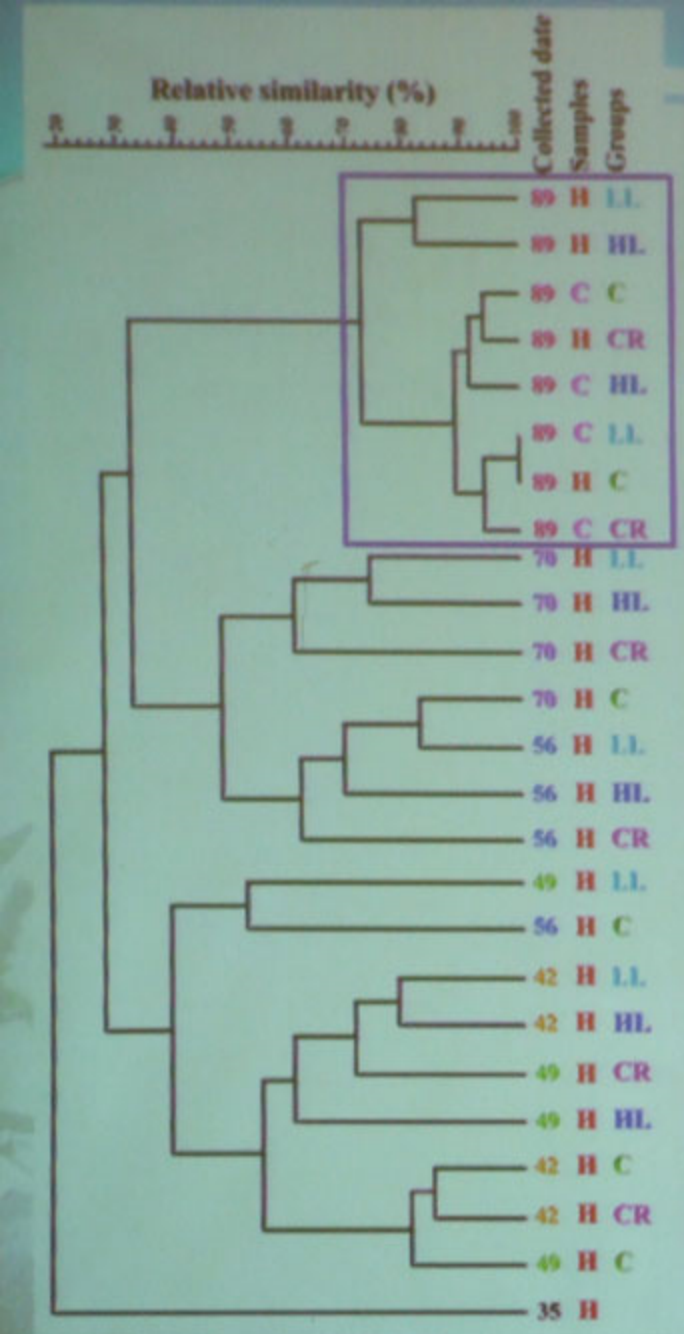
Results and discussion



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Results and discussion

Lythrum salicaria L.

- **Anti-bacterial function is mostly due to its essential oil.**
- **Flavonoids, tannins and terpenes were found in LS.**
  - **Major active component of antibacterial activity.**
    - **The hexahydroxydiphenoyl ester vescalagin (hydrolyzable tannin).**
- ***In vitro* anti-microbial ability**
  - ***Bacillus cereus*, *Escherichia coli*, *Mycobacterium smegmatis*, *Micrococcus luteus*, *Pseudomonas aeruginosa* and *Staphylococcus aureus***
- **The essential oil activities were assumed that were the cause on the bacterial diversity difference.**

Rauha et al., 2000; Dulger and Gonuz, 2004; Becker et al., 2005; Borchardt et al., 2009; Humadi and Istudor, 2009; Franz et al., 2010; Christaki et al., 2012

Conclusions

- No any adverse effects from *Lythrum salicaria* supplementation on performance
- Age and treatment affect on bacterial community.
- Bacterial diversity from hard feces and caecal content were similar.