Productive performance and meat quality traits in growing rabbit following the dietary supplementation of *Lippia citriodora*, *Raphanus sativus* and *Solanum lycopersicum* extracts

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Abstract

Consumers particularly appreciate rabbit meat for its low lipid content, rich of polyunsaturated fatty acids, for the high biological value and content of protein, and for its low cholesterol content. The high degree of unsaturation of fatty acids makes meat particularly susceptible to oxidative processes during storage; so in the effort to increase its oxidative stability and improve quality traits, a study was carried out to assess the effect of dietary supplementation of *Lippia citriodora, Raphanus sativus* and *Solanum lycopersicum* plant extract in intensively-reared growing rabbits. The experiment was performed on 160 weaned rabbits for 80 days, from which one group was control (CON) receiving a fattening-feed without any supplements. The other three experimental groups received an integration of *Lippia citriodora* extract (VB group, containing 5 mg of verbascoside per kg of feed), of *Solanum lycopersicum* extract (LIC group, containing 5 mg of lycopene per kg of feed), and of *Raphanus sativus* extract (RAF group, containing 350 mg per kg of feed) respectively. The plant extracts have determined in the muscle *Longissimum lumborum* of rabbit meat a significant reduction in cholesterol, saturated fatty acids and thiobarbituric reactive substances, as well as a marked increase in polyunsaturated fatty acids and in alpha-tocopherol and retinol content. The use of plant extracts have not produced a meat chemical composition change a productive performance and dressing percentage increase; however feed additives favoured improving the nutritional and nutraceuticals quality of meat, with beneficial effects on the health of the consumer. In addition, the use of plant extracts has permitted obtaining meat with greater oxidative stability with evident positive implications on its shelf-life.

Keywords: rabbit; plant extract; growing performance; meat quality

1. Introduction

Rabbit meat is considered a Mediterranean food (Gai et al., 2010), particularly appreciated by consumers for its low lipid content, rich of polyunsaturated fatty acids (PUFA), for its high biological value proteins content (20-21%), and because of its low cholesterol content (Dalle Zotte and Szendrő, 2011). The high degree of unsaturation of fatty acids makes meat particularly susceptible to oxidative processes during storage (Wood et al., 2003; Dal Bosco et al., 2014), with negative effects on smell, colour, texture and nutritional value and possible formation of toxic compounds (Del Rio et al., 2005; Czauderna et al., 2011). Oxidative stability of rabbit meat can be improved through dietary strategies, which aim to integrate feed with natural plant extracts with antioxidant activity (Dal Bosco et al., 2014; Rossi et al., 2013). Many polyphenols-rich plants have interesting antioxidant properties (Korkina et al., 2007) as they are able to increase endogenous antioxidant defences of animals, especially in critical periods of breeding as weaning. In several studies were evaluated the antioxidant activity of plant-extract, based on lycopene and green tea polyphenols (Tedesco et al., 2005), oats (Lopez-Bote et al., 1998), oregano and rosemary (Cardinali et al., 2015), to improve lipid stability and shelf life of the meat (Marzoni et al., 2014). Botsoglou et al. (2004) observed an improving of oxidative stability of Japanese quail meat, following the dietary supplementation of 5% of dry tomato pulp, with lycopene and beta-carotene as main components. Among many plant extracts, found in nature and used as dietary supplements in farm animals, include those based on *Lippia citriodora*, radish (*Raphanus sativus*) and *Solanum lycopersicum*. The *Lippia citriodora* belongs to the family of *Verbenaceae* and contains verbascoside as a major component. It is a water soluble compound of phenylpropanoids glycosides, including caffeic acid and hydroxytyrosol bound to a beta-(D)-glucopyranoside. Its high antioxidant activity were studied both *in vitro* (Afanasev, 2005) and *in vivo*, with the increase of blood content of retinol and alfa-tocopherol (Casamassima et al., 2013a; Palazzo et al., 2011), and also in meat of different species, such as rabbits (Palazzo et al., 2015), pig (Rossi et al., 2013) and hares (Vizzarri et al., 2014) in order to improve oxidative stability. Radish belongs to the family of *Brassicaceae*, and has been showed to possess antioxidant properties due to the presence of phenolic compounds, as triterpenoids, alkaloids, flavonoids, both *in vitro* (Agarwal and Varma, 2015; Suh et al., 2006), and *in vivo* (Lugasi et al., 2005). Lycopene is an antioxidant compound present in many plants, including tomato (*Solanaceae* family) widely used as a dietary supplement in livestock production, to improve the quality of the meat (Englmaierova et al., 2011) and eggs (Karadas et al., 2006; Ali et al., 2014). Lycopene is known for its free-radical scavenger activity and its dietary use could be

useful in reared intensively livestock, such as rabbits and poultry farms, with high reproductive growth and maintained often in stressful environments with high breeding density (Englmaierova et al., 2011). When used in the right dosage, lycopene improves endogenous antioxidant defense system, administered alone or in combination with other carotenoids (Sahin et al., 2008; Ševčíková et al., 2008). Otherwise over the maximum threshold of tolerance, it could show possible negative effects. In this regard, Pozzo et al. (2013) adding doses of 500 mg of lycopene per kg of feed in broilers, showed degenerative disorders in liver, spleen and bursa of *Fabricius*. In order to deepen the knowledge on the use of natural feed supplements, the present study was conducted to evaluate the effect of plant extracts *Lippia citriodora*, radish (*Raphanus sativus*) and *Solanum lycopersicum* on productive performance and meat quality traits in intensively-reared growing rabbits.

2. Material and Methods

2.1 Animals and experimental design

All husbandry and management procedures concerning the protection of animals for scientific purposes were conducted in accordance with European directives 2010/63/EU. Rabbits used in the experiment were clinically healthy and their physiological *status* was considered good throughout the experimental period. The experiment lasted 80 days and was conducted on 160 weaned male-rabbits (White New Zealand x Californian) during one whole breeding cycle. The animals have been reared in cages (2 for each cage) equipped with feeders and automatic watering. Rabbitry temperature and relative humidity were continuously recorded using a digital thermograph positioned at the height of the animal cages; the average values throughout the trial were 18 ± 4 °C and 70 ± 5%, respectively. The rabbits were divided into 4 groups of 40 animals each, matched by age (38 ± 2 days) and body weight (1.49 ± 0.07 kg). The control group (CON) received a fattening-feed without any nutritional supplements whereas the experimental groups received:

- VB group, supplemented with 1g of *Lippia citriodora* extract per kg of feed, standardized in 5.0 mg of verbascoside (main component)
- LIC group, integrated by tomato extract with 5 mg of lycopene per kg of feed
- RAF group, integrated by 350 mg of radish extract per kg of feed.

Animal feed were provided by Agri-zoo s.n.c company (Miranda, Isernia, Italy) and administrated *ad libitum*. *Lippia citriodora* extract (standardized to 0.5% verbascoside), radish extract (*Raphanus sativus* root dry extract with Inuzyme ® enzyme complex) and lycopene (standardized to 2% of the tomato fruit, *Solanum lycopersicum*) were provided by Sintal Zootecnica (Isola Vicentina, Vicenza, Italy), Erba Vita Italy s.p.a. (Montegrimano Terme, Perugia, Italy) and Erbamea s.r.l. (San Giustino, Perugia, Italy).

2.2 Feed chemical composition

Animal feed were analyzed in triplicate using the Official Methods of Analysis (AOAC, 2000) following the recommendation of the European Group on Rabbit Nutrition (EGRN, 2001). Fatty acids were determined in triplicate with direct derivatization as described by O'Fallon et al. (2007). The fatty acids were identified by comparing the retention time of methyl esters FAMEs (NLEA Mix, Rt-2560, Resteck Corporation, Bellefonte, PA, USA). Methyl esters were analyzed using a gas chromatograph ThermoQuest TRACE 2000 (SAC^{tm} column 5 x 0.25 mm, 300 cm, Supelco, USA). Data on ingredients, chemical composition and fatty acid of feed are given in Table 1.

2.3 Controls in vita

During the experimental, all animals were subjected to control of body weight and feed intake at the beginning (0 d), half (40 d) and at the end of the test (80 d), in order to determine, the daily weight gain and feed conversion ratio respectively, in the period 0-40 d, 40-80 d and whole test (0-80 d).

2.4 Carcass traits

At the end of the test (80 d), after the record of body weight gross and body weight subsequently 12 h of fasting, a statistically representative sample of 15 animals per group was slaughtered into experimental slaughterhouse. Rabbits were subjected to electrical stunning and sacrificed by bleeding according with the guidelines established by the European Community (1099/2009/EC) and recommended by the Italian Ministry of health (Law n. 116/92), in accordance with national laws on the protection of animals during the slaughter or killing. The carcasses of rabbits were prepared according to Blasco and Ouhayoun (1996), discarding the skin, the distal part of the limbs, genitals, bladder and gastrointestinal tract. Carcasses were weighed and the slaughter yield calculated. The dissection of the meat carcasses was made after the storage at 4° C for 24 h, and on the right side meat samples were taken from *Longissimus lumborum* (LL), in order to perform the chemical composition according to AOAC method (2000) and to analyse the first measurement of oxidative stability (0 h). The remaining part of the muscle sample was storage under vacuum at -20 °C for the further determination of fatty acids and cholesterol.

2.5 Oxidative stability measurements

Meat oxidative stability was measured by evaluating the thiobarbituric acid reactive substances (TBARS) content on 4 °C chilling LL meat samples at 0 h, and then at 24 h and 72 h, in accordance with Meineri et al. (2010). The implemented method was as follow: 500 mg of meat was

homogenized with 10 mL of distilled water using a Homogenizer Ultra Turrax T25 (IKA, Cincinnati, USA). The homogenized sample were added with 2.5 mL of 25% trichloroacetic acid, cooled at 4 °C for 15 min, and then centrifuged at 4000 g, at 4 °C for 5 minutes. The supernatant was filtered through Whatman 52 filter paper, an aliquot of 3.5 mL was added 1.5 mL of 0.67% thiobarbituric acid and incubated at 70 °C for 30 minutes. Immediately after cooling the absorbance of the sample was read in a spectrophotometer at 532 nm and compared to a standard curve of malondialdehyde (MDA; Sigma Aldrich, St. Louis, USA). All analyses were performed in duplicate and the result was expressed as mg of MDA per kg of meat. Retinol and alfa-tocopherol were extracted from meat samples according to Zaspel and Csallany (1983) and then analyzed by HPLC system (Kontron Instruments, Milan, Italy) consisting of an auto sampler (HPLC Autosampler 360, Kontron Instruments, Milan, Italy) with a loop of 20µl, a high-pressure pump and a C18 column 5µm, 250 × 4.60 mm (Phenomenex, Torrance, CA, USA). The mobile phase consisted of acetonitrile and methanol (75:25 v/v) with a flow of 1 ml/min. Retinol and tocopherol, carefully stored and manipulated under low light condition, were identified by comparing the retention time of the samples with the retention time pure standards provided by Sigma Aldrich (St. Louis, USA). The quantification was performed using the Gyminix system (version 1.8.1) by comparing the peak of the sample area with that of the reference standard curve.

2.6 Fatty acid composition in intramuscular fat and cholesterol determination in meat

The fatty acid composition of intramuscular fat samples was determined after chloroform-methanol extraction (Folch et al., 1957), and fatty acids were determined as methyl esters (FAME) (Dal Bosco et al., 2004), using a gas chromatograph ThermoQuest TRACE 2000 (SAC^{tm}-5 column 3000cm×0.25mm, Supelco, USA). Fatty acids were identified on the basis of elution times corresponding to the standard (FAME PUFA2, Supelco, Bellefonte, PA, USA). Meat cholesterol content was determined in accordance with the procedure of Du and Ahn (2002) and, briefly lipids were extracted from 1.5 g of meat with chloroform and methanol (2:1 v/v), then treated with 33% KOH with ratio of 94:6. Cholesterol was extracted with 5 ml of hexane and 1 µl was injected into the gas chromatograph. Identification of cholesterol was made based on the retention time of the standard (Sigma Aldrich, St. Louis, USA) and quantification was made with software Chrom Card Data System (version 1.17) by comparing the peak area with the reference standard curve.

2.7 Statistical analysis

Statistical analysis was performed with SPSS statistical package (version 18.0, 2009, SPSS Inc., USA). Data concerning the productive performances, slaughter controls, and chemical composition,

fatty acid and vitamins (alpha-tocopherol and retinol) content, were analysed with the one-way ANOVA, with the dietary treatment (CON, VB, RAF and LIC) as fixed factor. The differences between the mean values were compared using the Tukey's test and considered significant at least for $P < 0.05$. TBARS data were processed with the GLM (global linear model) for repeated measures with dietary treatment, the sampling time and their interaction as fixed factors. The means were compared using Duncan's test. Relative to the *in vivo* parameters, the cage was the experimental unit, whereas for the *post-mortem* parameters individual animal constituted the experimental unit.

3. Results and Discussion

3.1 In vita and post mortem measurements

Evaluating Table 2, it is clear that the dietary plant extracts, *Lippia citriodora*, *Solanum lycopersicum* and *Raphanus sativus*, produced no effects on productive parameters. Even Abedo et al. (2012), supplementing growing-rabbits feed with 1.5% of radish seeds, observed no effects on body weight, daily weight gain and on slaughter performance, compared with the control group. However, these authors reported a marked improvement of total digestibility of feed components, with a significant reduction in the feed conversion index. Considering a shorter experimental period, these results on rabbits agree with findings of Tedesco et al. (2005), after supplementation with lycopene and green tea extracts, and with other authors (Chrenkova et al., 2013), using a dry extract of Siberian ginseng (*Eleutherococcus senticosus*) integrated in the feed. Salih et al. (2015), adding the 0.5% horseradish seed to broilers feed, found higher body weight and better feed conversion ratios. Pozzo et al. (2013), in experiences conducted on broilers, observed no effects on the growth performance following the dietary supplementation with high dose of lycopene (500 mg/kg feed). Similar results were obtained in broilers by Leal et al. (1999), after the administration of 25 mg of lycopene/kg body weight per day, and by Englmaierova et al. (2011) adding 75 mg of lycopene/kg feed. In previous experiences on growing rabbits and hares (Palazzo et al., 2015; Vizzarri et al., 2014; Casamassima et al., 2013a), feeding animals with *Lippia citriodora* extract, with verbascoside as main component, there were no significant effects on growing performance, except for the daily weight gain in hares that resulted increased in the last period of research (Casamassima et al., 2013a). Feeding natural suckling-lambs with *Lippia citriodora* liquid-extract a significant improvement of milk consumption and the daily weight gain was observed (Casamassima et al., 2013b).

3.2 Chemical composition of meat

205 The chemical composition of LL meat (Table 3) was not affected by dietary treatment, in
206 accordance with Waheed (2005) that in rabbits feeding supplemented growing doses of dry extract
207 of tomato pulp (0%, 8%, 16% and 24%). Our results agree with what we found in our previous
208 study on young rabbits fed with two different doses of *Lippia citriodora* extract (Palazzo et al.,
209 2015). Also Dal Bosco et al. (2004), integrating rabbit feed with vitamin E, did not reported any
210 effect on chemical composition of meat.
211
212 *3.3 Oxidative stability markers in meat*
213 Table 4 summarizes data on retinol, alpha-tocopherol and cholesterol of LL muscle samples. The
214 content of the two fat-soluble vitamins were positively affected ($P < 0.05$) by dietary inclusion with
215 plant extracts. In particular, the increased concentration of retinol was higher in LIC meat
216 (+141.8%), followed by VB group (+76.7%) and RAF (+53.5%) compared with the control group;
217 whereas, the content of alpha-tocopherol was higher in the VB group (+303.3%) followed by the
218 RAF (+272.8%) and LIC (+246.1%). Increased vitamins content is consistent with that experienced
219 by other authors and in our previous studies on *Lippia citriodora*. Moreover, the obtained results
220 suggest that supplementation with plant extracts reduces the muscle fat peroxidation, by increasing
221 the content of fat-soluble vitamins in meat. This was recently evidenced by Rossi et al. (2013) in
222 swine and by our research group in hares and rabbit species (Vizzarri et al., 2014; Palazzo et al.,
223 2015). Cholesterol content was influenced by dietary treatment with a significant decrease ($P <$
224 0.01) of 16.9% and 13.1%, only in VB and LIC groups than in the control. The decrease in
225 cholesterol found in VB and LIC experimental groups could be attributed to antioxidant effect
226 exerted by the molecules of verbascoside and lycopene, which would inhibit the activity of HMG-
227 CoA reductase, an enzyme that controls cholesterol synthesis (Kowalska and Bielanski, 2009). Also
228 Englmaierova et al. (2011) have experienced a reduction in cholesterol in broiler leg meat
229 supplementing lycopene in feed, with and without the addition of vitamin E.
230 The TBARS content of LL muscle samples (Table 5), after 72 hours of storage at 4 °C, was
231 significantly lower ($P < 0.01$) in animals that were fed with *Lippia citriodora* (-91.3%), radish (-
232 42.4%) and lycopene (-42.0%), than those in the control group. The time of storage (from 0 h to 72
233 h) obviously determined a significant increase ($P < 0.01$) of the contents of malondialdehyde in
234 meat samples, even if the values in the three experimental groups were much smaller compared to
235 the control group. This development led to a significant interaction ($P < 0.05$) between dietary
236 supplementation with plant extracts and the storage time of meat samples in the fridge. Some
237 studies, conducted in the rabbit species, showed an improvement of the shelf-life of meat, following
238 administration of plant supplements, both based on alfalfa (Dal Bosco et al., 2014) and based on

Lippia citriodora (Palazzo et al., 2015). Some researchers (Botsoglou et al., 2003) showed a reduced amount of MDA in turkey meat, due to the antioxidant activity possessed by polyphenolic compounds, contained in the essential oils of oregano. In fact, following the inclusion into the feed and administration to animals, the natural compounds reach the bloodstream, and successively distribute and accumulate in the muscle and other tissues. Natural antioxidants are able to avoid or even inhibit the lipid oxidation of meat, leading as direct consequence a longer shelf-life, since they have the ability to block the oxidizing propagation of chain reactions. Polyphenols have a high antioxidant activity that is carried out with three different mechanisms: as a scavenger of free radicals (Zheng et al, 2009), as transition metal chelators (Andjelkovic et al., 2006) and as quencher of free singlet oxygen (Mukai et al., 2005).

3.4 Fatty acid composition in meat

Saturated fatty acids (SFA) in LL muscle were significantly affected ($P < 0.01$) by dietary treatment with lower values of 11.3% in VB group and of 14.1% in the LIC group (Table 6), when compared to the CON group. Polyunsaturated fatty acids (PUFA) showed in the three experimental groups VB, RAF and LIC a significant increase ($P < 0.01$) in values of 12.8%, 9.6% and 16.2%, respectively, when compared to the control group. This increase is mainly attributable to a significant increase ($P < 0.01$) of linoleic acid in experimental groups, in the extent of 13.4% (VB) of 14.4% (RAF) and 18.8% (LIC), respectively, when compared to CON group. The lycopene feed supplement showed a significant increase ($P < 0.05$) of 21.0% in arachidonic acid, of 36.0% in eicosapentaenoic acid (EPA) and of 16.0% in docosahexaenoic acid (DHA), compared with the control group and VB and RAF experimental groups. The n-6 fatty acids were significantly higher ($P < 0.01$) of 13.3% in VB, 14.2% in the RAF and 18.9% in LIC group, as compared to the CON group. As it is well known some essential fatty acid (like dietary alfa-linolenic and linoleic acid) are precursor of n3 and n6 PUFA family in meat, since the latters can be synthesized through a series of elongation and desaturation reactions. The enzymatic system involved in these reactions consisting of fatty acil-CoA synthetases 6- and 5- desaturases and the respective elongases (Garcia 2011). The plant extracts of *Lippia citriodora*, radish and tomato lycopene have positively influenced the fatty acid composition of rabbit meat, leading to a reduction of SFA and increased of PUFA content. Those results are in agreement with our previous studies on supplementation with *Lippia citriodora*, both in rabbit (Palazzo et al., 2015) and hares (Vizzarri et al., 2014). Also other research conducted by Meineri et al. (2010) on growing rabbits, fed with seeds of sages (*Salvia hispanica* L.), resulted in a dose-dependent increase of C18:2 (n-6) and C18:3 (n-3) fatty acid. Jung et al. (2010) found a reduction of the SFA and MUFA with an increase in PUFA meat content in broiler fed a diet

supplemented with a mixture of polyphenols and linoleic acid. These results could be due to a reduced synthesis of MUFA, as a consequence of the inhibiting activity of 9-desaturase, key enzyme that converts the SFA in MUFA.

4. Conclusion

The use of herbal *Lippia citriodora*, radish and tomato lycopene plant extracts has determined a reduction of cholesterol, SFA and TBARS in the *Longissimus lumborum* rabbit muscle. At the same time a significant increase of content in retinol and alpha-tocopherol, and PUFA was observed. The three dietary extracts did not produce an improvement on chemical composition of meat and growing performance; nevertheless the dietary treatment improved the nutritional and nutraceuticals quality of rabbit meat with an improved oxidative stability. Therefore, dietary supplementation with the three plant extracts may be a useful strategy to be used in rabbit meat production both from the consumer's health point of view, and the improved nutraceutical quality of meat, and from the viewpoint of product marketing for an andoubted increased shelf-life.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Table 1 Ingredients and chemical composition of diet feed of rabbit

	Experimental diets[1]			
	CON	VB	RAF	LIC
Ingredients (g/kg diet)				
Soybean meal	230	227	229	230
Alfalfa hay	220	221	219	221
Wheat bran	208.8	209.8	210.45	207.795
Alfalfa meal dehydratated, 17% CP	100	100	100	100
Beet pulp	100	100	100	100
Barley	78	78	78	78
Wheat	20	20	20	20
Lippia citriodora extract	0	1	0	0
Lycopene	0	0	0	0.005
Raphanus sativus	0	0	0.350	0
Calcium carbonate	20	20	20	20
Soybean oil	10	10	10	10
Sodium Chloride	4	4	4	4
Dicalcium phosphate	2	2	2	2
Vitamin and mineral premix[a]	2.5	2.5	2.5	2.5
Methionine (99%)	2.3	2.3	2.3	2.3
Lysine (78.5%)	1.4	1.4	1.4	1.4
Choline (75.0%)	1	1	1	1
Chemical composition (g/kg as-feed)[2]				
Dry matter (DM)	909.0±1.0	911.3±1.5	912.0±2.6	912.3±1.5
Crude protein	165.8±0.6	166.6±0.7	165.5±0.9	166.3±0.9
Crude fiber	171.4±1.1	174.8±1.6	175.4±1.1	172.7±1.0
Ether extract	26.6±0.3	29.4±0.9	31.6±0.4	31.7±0.4
Crude ash	61.8±0.3	59.2±0.4	59.2±0.7	60.9±0.6
NDF	366.3±1.1	362.6±2.2	348.7±1.1	355.5±1.2
ADF	207.1±1.7	197.4±1.1	192.7±1.2	196.6±0.9
FA composition (% of total fatty acids)[3]				
C14:0	1.5±0.39	1.36±0.25	1.24±0.31	1.29±0.09
C16:0	25.87±0.15	21.84±1.32	19.13±0.97	19.28±0.84
C16:1	0.21±0.14	0.21±0.10	0.25±0.10	0.23±0.05
C18:0	2.79±0.31	2.49±0.11	2.62±0.24	2.53±0.13
C18:1 n-9	21.39±2.39	22.76±0.19	23.93±1.13	23.92±1.29
C18:1 n-7	0.26±0.12	0.30±0.11	0.38±0.17	0.41±0.07
C18:2 n-6	42.85±2.87	43.85±1.36	45.68±1.28	46.79±1.66
C18:3 n-3	4.88±0.42	6.31±0.23	6.24±0.15	4.96±0.19
C20:0	0.60±0.20	0.89±0.04	0.53±0.03	0.58±0.04
∑SFA	29.99±0.61	26.33±1.53	23.17±1.18	23.30±0.77
∑MUFA	21.87±2.16	23.27±0.10	24.56±0.99	24.56±1.28
∑PUFA	47.73±2.54	50.15±1.50	51.92±1.41	51.76±1.70

[1]CON= control diet; VB= experimental diet supplemented with 5mg of verbascoside per kg of feed; RAF= experimental diet supplemented with 350mg of radish extract per kg of feed; LIC= experimental diet supplemented with 5mg of lycopene per kg of feed.

SFA, saturated fatty acids [C14:0+C16:0+C18:0+C20:0]; MUFA, monounsaturated fatty acids [C16:1+C18:1n-9+C18:1n-7]; PUFA, polyunsaturated fatty acids [C18:2n-6+C18:3n-3].

[2]Supplied per kg of feed: vitamin A 2000 I. U., vitamin D3 320 I. U., vitamin E 4.0 mg, vitamin B2 0.52 mg, vitamin B6 0.40 mg, vitamin B12 0.006 mg, vitamin K 0.32 mg, vitamin H 0.020 mg, vitamin PP 3.2 mg, folic acid 0.10 mg, D-pantotenic acid 2.4 mg, copper 5.6 mg, manganese 4.0 mg, iron 12.0 mg, zinc 16.0 mg, iodine 0.060 mg, selenium 0.040 mg.

[3]n = 3; mean±sd.

454 **Table 2** Productive performance in rabbit.

	Experimental diets[1]				
	CON	VB	RAF	LIC	*P*-value[3]
No rabbits	40	40	40	40	
Body weight, kg					
0d	1.45±0.05	1.50±0.02	1.50±0.02	1.49±0.02	0.505
40d	2.78±0.06	2.86±0.04	2.79±0.04	2.78±0.04	0.433
80d	3.98±0.12	4.14±0.06	3.97±0.07	4.07 ±0.04	0.189
Daily weight gain, g/day					
0d-40d	33.35±1.21	33.96±1.03	32.43±1.07	32.26±0.91	0.601
40d-80d	28.83±2.11	32.05±0.83	29.45±1.10	32.23±0.38	0.114
0-80d	31.59±1.48	33.01±0.85	30.94±0.86	32.24±0.48	0.321
Feed intake, g/day					
0-40d	117.75±0.89	122.90±1.36	122.55±0.90	121.32±1.12	0.054
0-60d	161.23±1.16	158.46±3.89	157.79±2.17	153.78±2.86	0.462
40-80d	139.49±0.57	140.68±2.30	140.17±1.50	137.55±1.78	0.621
FCR[2]					
0-40d	3.55±0.16	3.65±0.13	3.82±0.14	3.79±0.11	0.557
40-80d	5.51±0.38	4.00±0.21	5.42±0,21	3.79±0.10	0.067
0-80d	4.46±0.23	4.29±0.15	4.56±0.14	4.27±0.07	0.359

455
456 [1]CON= control diet; VB= experimental diet supplemented with 5mg of verbascoside per kg of feed; RAF= experimental diet
457 supplemented with 350mg of radish extract per kg of feed; LIC= experimental diet supplemented with 5mg of lycopene per kg of
458 feed. [2]FCR = feed conversion ratio, calculated as g of dry matter intake on g of daily weight gain. [3]Experimental unit 20 cages (2
459 rabbits per cage).

Table 3 Chemical composition of rabbit meat

	Experimental diets[1]				
	CON	VB	RAF	LIC	*P*-value
Moisture, %	71.8±0.4	71.9±0.9	72.0±0.9	72.2±0.7	0.97
Crude protein, %	22.7±0.2	22.9±0.7	23.5±0.8	23.2±0.5	0.58
Ash, %	1.1±0.9	1.2±0.6	1.1±0.8	1.3±0.5	0.68
Ether extract, %	3.4±0.5	3.3±0.9	3.5±0.1	3.6±0.3	0.41

[1]CON= control diet; VB= experimental diet supplemented with 5mg of verbascoside per kg of feed; RAF= experimental diet supplemented with 350mg of radish extract per kg of feed; LIC= experimental diet supplemented with 5mg of lycopene per kg of feed.

Table 4 Retinol, □lpha-tocopherol and cholesterol content in rabbit meat

	Experimental diets[1]				
	CON	VB	RAF	LIC	*P*-value[2]
No rabbits	15	15	15	15	
Retinol, mg/100g	0.043±0.006[1]	0.076±0.014[2]	0.066±0.006[2]	0.104±0.011[3]	0.021
Alpha-tocopherol, mg/100g	0.180±0.004[1]	0.726±0.127[2]	0.671±0.138[2]	0.623±0.061[3]	0.025
Cholesterol, mg/100g	55.08±0.58[1]	47.10±0.87[2]	52.37±0.68[1,2]	48.71±1.12[2]	0.001

[1]CON= control diet; VB= experimental diet supplemented with 5mg of verbascoside per kg of feed; RAF= experimental diet supplemented with 350mg of radish extract per kg of feed; LIC= experimental diet supplemented with 5mg of lycopene per kg of feed. [2] Different numbers within the same row indicate significant differences ($p < 0.05$) [(1,2,3)].

Table 5 Oxidative stability of rabbit meat

	Experimental diets[1]				P-value[2]		
	CON	VB	RAF	LIC	G	T	GxT
No rabbits	15	15	15	15			
TBARS (MDA), mg/kg							
a 0h	0.708±0.010[1a]	0.409±0.013[2a]	0.569±0.008[1,2a]	0.417±0.025[2a]			
a 24h	0.924±0.022[1b]	0.470±0.052[2b]	0.699±0.008[3b]	0.518±0.012[4b]			
a 72h	1.102±0.025[1c]	0.576±0.098[2c]	0.774±0.020[3c]	0.776±0.030[3c]	0.001	0.001	0.029

[1]CON= control diet; VB= experimental diet supplemented with 5mg of verbascoside per kg of feed; RAF= experimental diet supplemented with 350mg of radish extract per kg of feed; LIC= experimental diet supplemented with 5mg of lycopene per kg of feed. [2] G = effect of diet; T = effect of time; G x T = interaction diet x time; Different numbers within the same row indicate significant differences ($p < 0.05$) [(1,2,3)]; Different letters within the same column indicate significant differences ($p < 0.05$) [(a,b,c)]

Table 6 **Fatty acid profile of rabbit meat (expressed as % on total fatty acids)**

	Experimental diets[1]				
	CON	VB	RAF	LIC	P-value[2]
No rabbits	15	15	15	15	
Saturated fatty acids (SFA)					
C14:0	1.70±0.07[1]	2.03±0.14	2.20±0.06[2]	1.86±0.08[1]	0.001
C16:0	32.20±0.58[1]	28.39±0.82[2]	29.60±0.65[2]	27.21±0.54[3]	0.001
C18:0	7.52±0.02	7.50±0.02	7.54±0.01	7.57±0.07	0.880
C20:0	0.69±0.07	0.67±0.07	0.62±0.03	0.61±0.04	0.653
C22:0	0.59±0.02	0.56±0.04	0.59±0.02	0.58±0.02	0.887
Others	1.42±0.07[1]	0.48±0.09[2]	0.65±0.08[2]	0.84±0.05[3,2]	0.001
Total SFA	44.12±0.51[1]	39.64±0.95[2]	41.21±0.65[1,2]	38.68±0.52[2]	0.001
Mono-unsaturated fatty acid (MUFA)					
C14:1	0.30±0.06	0.31±0.05	0.43±0.05	0.36±0.06	0.386
C16:1	0.21±0.01	0.17±0.01	0.28±0.01	0.32±0.13	0.689
C18:1	23.59±0.42	23.95±0.21	22.89±0.19	23.60±0.35	0.173
C20:1	0.16±0.03[1]	0.14±0.02[1]	0.72±0.19[2]	0.12±0.15[1]	0.001
Others	0.56±0.06[1]	0.73±0.07[3]	0.42±0.04[2]	0.59±0.06[1]	0.012
Total MUFA	24.81±0.44	25.31±0.19	24.74±0.23	24.99±0.25	0.683
Poly-unsaturated fatty acid (PUFA)					
C18:2 n-6	23.86±0.57[1]	27.05±0.38[2]	27.29±0.65[2]	28.35±0.37[2]	0.001
C18:3 n-3	4.16±0.94	4.68±0.27	3.65±0.32	3.89±0.28	0.224
C20:3 n-3	0.25±0.02	0.31±0.03	0.31±0.01	0.26±0.02	0.064
C20:3 n-6	0.17±0.01	0.20±0.02	0.21±0.01	0.19±0.01	0.247
C20:4 n-6	0.43±0.07[1]	0.44±0.09[1]	0.42±0.06[1]	0.52±0.07[2]	0.042
C20:5 n-3	0.10±0.02[1]	0.10±0.03[1]	0.10±0.01[1]	0.46±0.29[2]	0.014
C21:5 n-3	0.36±0.05	0.44±0.07	0.38±0.04	0.44±0.04	0.548
C22:5 n-3	0.97±0.16	1.05±0.22	0.87±0.12	1.07±0.16	0.749
C22:6 n-3	0.52±0.09[1]	0.53±0.12[1]	0.52±0.07[1]	0.68±0.12[2]	0.029
Others	0.27±0.04	0.24±0.03	0.31±0.02	0.27±0.03	0.533
Total PUFA	31.07±0.53[1]	35.06±0.90[2]	34.05±0.74[2]	36.12±0.49[2]	0.001
n-3	6.35±0.50	7.12±0.52	5.83±0.50	6.80±0.57	0.422
n-6	24.45±0.53[1]	27.69±0.47[2]	27.92±0.63[2]	29.06±0.33[2]	0.001
n-6/n-3 ratio	4.06±0.31	3.98±0.27	5.22±0.44	4.73±0.43	0.155

[1]CON= control diet; VB= experimental diet supplemented with 5mg of verbascoside per kg of feed; RAF= experimental diet supplemented with 350mg of radish extract per kg of feed; LIC= experimental diet supplemented with 5mg of lycopene per kg of feed. [2] Different numbers within the same row indicate significant differences ($p < 0.05$) [(1,2,3)].