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Original study

Effect of dietary *Lippia citriodora* extract on productive performance and meat quality parameters in hares (*Lepus europaeus Pall.*)

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Abstract

Three different dietary doses of *Lippia citriodora* natural extract were tested in order to verify their effect on productive performance and the meat quality of intensively-reared hare (*Lepus europaeus Pall.*). The 240 day-trial was conducted on 20 male hares, divided into 4 homogeneous groups of 5 animals each. A control group received a basal diet without any supplementation, and the other three experimental groups received a natural extract of *Lippia citriodora*, titrated in verbascoside, in the following amounts: 1 g, 1.5 g and 2 g of natural extract/kg feed in the low (LNE), medium (MNE), and high natural extract (HNE) groups, respectively. Natural extract enhanced the quality of the meat: there was a significant decrease ($P<0.05$) in saturated fatty acids and a significant increase ($P<0.05$) in mono- and poly-unsaturated fatty acids. The oxidative stability of the meat improved, thus highlighting a possible link between the decrease ($P<0.05$) in TBARS values and an increase ($P<0.05$) in lipid vitamin content. The cholesterol content of the meat decreased markedly ($P<0.01$) after the dietary verbascoside treatment, thus improving the health benefits of the meat. These results clearly show the important role of *Lippia citriodora* extract, titrated in verbascoside, in improving the quality of the meat of intensively-reared hares. In addition the present paper underlines how the use of natural antioxidant in the animal feeding system may provide healthier and low-oxidized products to the final consumer.

Keywords: *Lippia citriodora* extract, productive performance, meat quality, hare (*Lepus europaeus Pall.*)

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Abbreviations: HNE: high natural extract, LNE: low natural extract, MNE: medium natural extract, TBARS: thiobarbituric acid reactive substances

Introduction

Natural antioxidants in food are able to scavenge the free radicals that lead to the oxidation of cellular lipids (Di Benedetto *et al*. 2010). These compounds also prevent the lipid oxidation of meat, which is one of the primary causes of the deterioration of muscle tissue (Buckley *et al*. 1995). This is because they reduce the action of oxygen, slow the development of odours and improve the oxidative stability of the meat. Synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), are widely used for the control of meat lipid peroxidation. However their use insecure (Kahl & Kappus 1993), integration of natural antioxidants in the animal diet is a good strategy to enhance the shelf life of meat (Djenane *et al*. 2004). The use of antioxidants can increase the oxidative stability of polyunsaturated fatty acids thus influencing meat storage and its susceptibility to oxidation (Meineri *et al*. 2010). Several studies carried out on laboratory animals have shown that many natural compounds (such as polyphenols, carotenoids, anthocyanidins, vitamin C) are also interesting immunostimulants (Katiyar 2002). In fact, they integrate endogenous antioxidant defences especially under stress, particularly during weaning, pre-acclimatization and restocking young hares into the natural environment. Among plant extract compounds, *Lippia citriodora* extract (containing verbascoside or acteoside) have shown the highest scavenger capacity in the group of phenylpropanoids glycosides (Wang *et al*. 1996) and the highest antioxidant activity compared to other phenyl compounds. Previous studies have shown that dietary supplementation of *Lippia citriodora* extract, positively influenced the plasma oxidative status in Lacaune ewes during the peripartum period (Casamassima *et al*. 2012) and in intensively reared *Lepus corsicanus* hare (Palazzo *et al*. 2011). A dietary *Lippia citriodora* supplement could thus be used in animal feeding to improve animal welfare, meat quality and also have health benefits for consumers. The use of dietary natural extracts can also be useful when the hares are slaughtered as the extracts are rich in antioxidant substances (flavonoids, tannins, polyphenols, terpenes), which then has a positive impact on the shelf life and on food safety (Sánchez-Escalante *et al*. 2003). These antioxidant substances act through many various physiological mechanisms. Rosemary, for example, chelates metal ions such as Fe^{-2}, resulting in a reduction in the formation of the activated oxygen (Fang & Wada 1993). The inclusion of antioxidants in food can have positive effect on human health because they protect important cellular components such as DNA, proteins and lipid membranes by reactive oxygen substances (Elmastas *et al*. 2007). The literature is poor regarding the effect of *Lippia citriodora* extract on meat quality (Rossi *et al*. 2013), and there are no data regarding the effect of this extract on hare meat. Skrivanko *et al*. (2008) recommend the addition of hare meat in the diet, due to its sensory characteristics, its high protein content, its low fat content and its energetic value which is similar to other meats. In order to verify a possible antioxidant effect on animal products using a feed strategy, a dietary *Lippia citriodora* supplement was evaluated on productive parameters and various meat quality traits of intensively-reared hare.

Material and methods

Animals, diet and experimental protocol

The 240 day-trial was conducted on 20 male hares (*Lepus europaeus Pall.*), divided into 4 groups of 5 animals each, homogeneous by age (210 ± 5 days) and body weight (2.90 ± 0.315 kg). A control group received a basal diet without any supplementation, and the other three experimental groups received a natural extract of *Lippia citriodora* leaves (Verbenaceae), titrated in verbascoside (5 mg/g of natural extract), according to the following amounts: 1 g, 1.5 g and 2 g of natural extract/kg feed in the low natural extract (LNE), medium natural extract (MNE), and high natural extract (HNE) groups, respectively. The natural extract supplement did not contain vitamin E and the composition of the natural extract, according to a certificate of analysis provided by the manufacturer (Lombarda Trading, Casalbuttano ed Uniti, Italy), was: gallic acid, 1.75 ± 0.07; 3.4-dihydroxybenzoic acid, 0.45 ± 0.04; methyl gallate, 1.91 ± 0.09; isoverbascoside, 0.43 ± 0.04 and verbascoside, 4.47 ± 0.08 g/kg. The concentrated pellets were provided by Martini S.p.A (Budrio di Longiano, Italy) and the chemical composition of the feed (AOAC 2000) were (per kg of dry matter): crude protein 154 g; crude fat 33 g; crude fibre 195 g; Neutral Detergent Fibre 385 g; Acid Detergent Fibre 240 g; ashes 85 g, moisture 111 g. The feed concentrate and alfalfa hay were administered daily *ad libitum*. All the hares were reared in a weaning-fattening cage with feeders and automatic water dispensers until slaughter. At slaughter, the relief of body weight and weight of the carcass were registered, and the killing percentages were determined. In addition the following quality parameters were performed: chemical composition, meat fatty acidic profile, cholesterol content, thiobarbituric acid reactive substances (TBARS) content, vitamin A, and vitamin E concentrations of *longissimus lumborum* muscle. Non-fasting animals were slaughtered at the age of 450 ± 5 d in an experimental slaughterhouse, and immediately before slaughter the body weight of all the hares was recorded. The hares were stunned electrically and sacrificed by bleeding, following the guidelines established by the European Community (n. 86/609/EEC) and approved by the Italian Ministry of Health (Law 116/92), in accordance with Italian laws on the slaughter and killing of animals. The carcasses were prepared as reported by Blasco & Ouhayoun (1993) for rabbits by removing the skin, the distal part of the limbs, genital organs, bladder and gastrointestinal tract. Warm carcasses were weighed and the killing percentage was calculated. After the carcasses had chilled at 4 °C for 48 h, the content of TBARS and chemical composition of meat were determined in a sample of *longissimus lumborum*, cut between the first and the seventh right-side lumbar vertebra. The determination was made following Meineri *et al*. (2010), and complied with ASPA recommendations (1996). The rest of the *longissimus lumborum* muscle samples were vacuum packaged and frozen at –20 °C until analysis. The fatty acid composition of intramuscular fat 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 300 cm × 0.25 mm, Supelco, USA). The fatty acid percentages were calculated with Chrom-Card software v. 1.17. Cholesterol content was determined according to Du & Ahn (2002) while vitamin A and E contents were determined according to Oriani *et al*. (2001).

Statistical analysis of the data

After the normal evaluation of frequency distribution, analysis of variance was performed on all variables using the GLM procedure of the statistical package SPSS v. 18 (SPSS Inc., Chicago, IL, USA). The productive data were processed using one-way ANOVA with the dietary treatment as a main effect. All data are expressed as mean ± standard error. The differences were considered significant at $P<0.05$.

Results and discussion

The dressing percentage and chemical composition data of hare meat are reported in Table 1. The dietary natural extract supplementation did not affect any of the parameters shown in the table. Table 2 reports various qualitative traits and the oxidative stability of *longissimus lumborum* muscle. Total saturated fatty acids were affected by natural extract ($P<0.05$), resulting in lower values in groups LNE, MNE and HNE by 12.3 %, 12.6 % and 15.4 % respectively, compared to the control group. Monounsaturated fatty acids were also influenced by dietary treatment with an increase of 9.5 %, 13.5 % and 11.1 % respectively in the LNE, MNE and HNE groups compared to the control group. *Lippia citriodora* extract significantly influenced ($P<0.05$) the polyunsaturated fatty acids values, which increased in the LNE, MNE and HNE groups, by 12.4 %, 9.7 % and 16.1 % respectively, compared to the control group. Dietary supplementation naturally led to a variation in the n-3 and n-6 polyunsaturated fatty acids, by significantly decreasing ($P<0.05$) the n-6/n-3 ratio, compared to the control group. In fact, the ratio decreased by 33.0 %, 14.3 % and 23.7 %, respectively in the LNE, MNE and HNE groups. The meat TBARS content was lower ($P<0.05$) by 25.4 %, 27.7 % and 37.5 % in the LNE, MNE and HNE groups compared to the control group. Regarding the meat vitamin concentration, vitamin E increased significantly ($P<0.01$) by 28.4 %, 21.4 % and 64.1 % in the three experimental groups (LNE, MNE and HNE respectively). Vitamin A on the other hand increased significantly ($P<0.05$) only between the control group and HNE group (37.5 %). The meat cholesterol content was positively influenced ($P<0.01$) by the dietary treatment, which in the experimental groups was lower by 23.5 % in the LNE group, by 26.4 % in the MNE group and 31.8 % in the HNE group compared to the control group.

The productive data and chemical composition of hare meat (Table 1) resulting from our test, are in agreement with the literature (Vicenti *et al.* 2003); also the meat fatty acidic profile and the content of individual fatty acids (Table 2) are in line with Vicenti *et al.* (2003). Table 2 highlights an improvement in the quality traits of the experimental animal groups. In fact, the natural extract reduced the saturated fatty acids and increased the mono- and poly-unsaturated fatty acids with an improvement in the n-6/n-3 ratio. This is particularly important considering that polyunsaturated fatty acids, especially the n-6 and n-3 series, are not synthesized by the human body (Kulasek & Bartnikowska 1994). Our results are in line with Bernardini *et al.* (1999) who in growing rabbits fed with 160 g flax seeds per kg of food, reported an increase in polyunsaturated fatty acids with a decrease in the n-6/n-3 ratio. Recently, researchers focused on the use of plant-based substances to improve the cholesterol content and to modify the acidic profile in meat. In fact, Habibian Dehkordi *et al.* (2010) have shown a decrease in cholesterol and lipoproteins in chicken meat when extracts of garlic are used in feed. The decrease in TBARS content and the increase of vitamin E in treated-animal meat, highlight that dietary natural

Table 1
Effect of *Lippia citriodora* extract on dressing percentage and some meat quality parameters in intensively-reared hares

Parameters	Diet groups (n=5 per each diet, mean ± SE)				
	Control	LNE, 1 g NE	MNE, 1.5 g NE	HNE, 2 g NE	D
Performance					
Gross body weight, kg	3.41 ± 0.12	3.54 ± 0.17	3.28 ± 0.40	3.21 ± 0.08	0.369
Hot weight carcass, kg	2.17 ± 0.09	2.26 ± 0.11	2.10 ± 0.28	2.15 ± 0.06	0.513
Killing percentage, %	63.7 ± 2.00	63.8 ± 0.21	64.0 ± 2.20	66.9 ± 1.09	0.054
Meat chemical composition, %					
Moisture	72.4 ± 1.96	73.0 ± 2.01	72.5 ± 1.83	72.3 ± 1.95	0.408
Crude protein	23.9 ± 1.33	22.9 ± 1.42	23.4 ± 1.28	23.0 ± 0.99	0.547
Crude lipid	1.85 ± 0.38	1.91 ± 0.34	1.77 ± 0.42	1.86 ± 0.33	0.629
Ashes	1.06 ± 0.15	1.15 ± 0.11	1.16 ± 0.15	1.11 ± 0.18	0.320

NE: natural extract, D: fixed effect of dietary supplementation

Table 2
Effect of *Lippia citriodora* extract on meat quality parameters in intensively-reared hares

Parameters	Diet groups (n=5 per each diet, mean ± SE)				
	Control	LNE, 1 g NE	MNE, 1.5 g NE	HNE, 2 g NE	D
Meat fatty acid, %					
Total saturated FAs	47.5 ± 3.24[a]	41.6 ± 2.88[b]	41.5 ± 1.40[b]	40.2 ± 1.28[b]	0.048
Total mono-unsaturated FAs	22.8 ± 2.52[a]	24.9 ± 1.38[b]	25.8 ± 0.99[b]	25.3 ± 1.53[b]	0.047
Total poly-unsaturated FAs	29.8 ± 1.60[a]	33.5 ± 0.31[b]	32.7 ± 1.54[b]	34.6 ± 0.40[b]	0.029
n-3	4.77 ± 0.36[a]	7.64 ± 0.97[b]	5.77 ± 0.15[b]	6.91 ± 0.47[b]	0.020
n-6	25.0 ± 1.48[a]	26.8 ± 0.60[b]	25.9 ± 2.29	27.6 ± 0.34[b]	0.043
n-6/n-3	5.24 ± 0.65[a]	3.51 ± 0.22[c]	4.49 ± 0.19[b]	4.00 ± 0.52[b]	0.039
Meat parameters, mg/100 g meat					
Cholesterol	140.6 ± 4.80[a]	107.6 ± 9.14[b]	103.5 ± 5.53[b]	95.9 ± 1.05[c]	0.001
TBARS	2.56 ± 1.11[a]	1.91 ± 0.53[b]	1.85 ± 0.70[b]	1.60 ± 0.18[c]	0.004
Vitamin E	0.13 ± 0.01[a]	0.17 ± 0.01[b]	0.16 ± 0.02[b]	0.22 ± 0.03[c]	0.001
Vitamin A	0.016 ± 0.003[a]	0.018 ± 0.004	0.017 ± 0.002	0.022 ± 0.003[b]	0.048

NE: natural extract, D: fixed effect of dietary supplementation, FAs: fatty acids, [a, b, c]Values within a row with different superscripts differ significantly at *P*<0.05.

extract supplement increased the shelf life of the meat. In fact, many dietary spices, including oregano (*Origanum vulgare L.*), rosemary (*Rosmarinus officinalis L.)* and sage (*Salvia officinalis L.*) have a high antioxidant capacity (Wojdyło *et al.* 2007). Natural antioxidants, in fact, delay or inhibit the oxidation of other substances, because of inhibition chain reactions oxidant propagation. Polyphenols, including verbascoside, have a high antioxidant activity through three mechanisms: as a scavenger of free radicals (Zheng *et al.* 2009), a chelator of transitionmetal (Andjelković *et al.* 2006), and/or through the ability to detect singlet oxygen (Mukai *et al.* 2005). Some researchers (Botsoglou *et al.* 2003) reported that the low MDA formation in meat by feeding animals with oregano essential oils, is probably due to the presence of antioxidant compounds which pass to the blood, and are distributed and stored in muscles and other tissues. The decrease in cholesterol content found in the experimental groups, can be attributed to the effect of antioxidant substances which can stimulate or inhibit the hepatic action of HMG-CoA reductase, an enzyme that controls the synthesis of cholesterol (Kowalska & Bielanski 2009).

In conclusion, the use of *Lippia citriodora* extract positively affected the quality of the meat, with a significant decrease in saturated fatty acid content and a significant increase in mono- and poly-unsaturated fatty acids in treated meat. The oxidative stability of the meat improved, highlighting a possible link between the decrease in TBARS values and the increase in vitamins content. In addition, the cholesterol content of the meat decreased markedly after the dietary natural extract treatment, thus improving the health benefits of the meat. These results (cholesterol and TBARS content, vitamin E concentration) could suggest that the best dose of natural extract supplementation is the highest (2 g/kg feed), but further investigations need to confirm this conclusion. This experiment clearly shows the important role of natural extract in enhancing the health and welfare of intensively-reared hares and also in improving the quality of the meat.

References

Andjelković M, Van Camp J, De Meulenaer B, Depaemelaere G, Socaciu C, Verloo M, Verhe R (2006) Iron-chelation properties of phenolic acids bearing catechol and galloyl groups. Food Chem 98, 23-31

AOAC (2000) Official Methods of Analysis. Vol. 2, 18th edition, Association of Analytical Chemists, Arlington, VA, USA

ASPA (1996) [Methods for the determination of the quality features of meat]. Associazione Scientifica di Produzione Animale, Centro Stampa, Università degli Studi di Perugia, Italy [in Italian]

Bernardini M, Dal Bosco A, Castellini C (1999) Effect of dietary n-3/n-6 ratio on fatty acid composition of liver, meat and perirenal fat in rabbits. Anim Sci 68, 647-654

Blasco A, Ouhayoun J (1993) Harmonization of criteria and terminology in rabbit meat research. Revised proposal. World Rabbit Sci 4, 92-99

Botsoglou NA, Govaris A, Botsoglou EN, Grigoropoulou SH, Papageorgiou G (2003) Antioxidant Activity of Dietary Oregano Essential Oil and α-Tocopheryl Acetate Supplementation in Long-Term Frozen Stored Turkey Meat. J Agric Food Chem 51, 2930-2936

Buckley DJ, Morrissey PA, Gray JI (1995) Influence of dietary vitamin E on the oxidative stability and quality of pig meat. J Anim Sci 73, 3122-3130

Casamassima D, Palazzo M, Martemucci G, Vizzarri F, Corino C (2012) Effects of verbascoside on plasma oxidative status and blood and milk production parameters during the peripartum period in Lacaune ewes. Small Rumin Res 105, 1-8

Dal Bosco A, Castellini C, Bianchi L, Mugnai C (2004) Effect of dietary α-linolenic acid and vitamin E on the fatty acid composition, storage stability and sensory traits of rabbit meat. Meat Sci 66, 407-413

Di Benedetto R, Attorri L, Chiarotti F, Eusepi A, Di Biase A, Salvati S (2010) Effect of Micronutrient-Enriched Sunflower Oils on Plasma Lipid Profile and Antioxidant Status in High-Fat-Fed Rats. J Agric Food Chem 58, 5328-5333

Djenane D, Martínez L, Sánchez-Escalante A, Beltrán JA, Roncalés P (2004) Antioxidant effect of carnosine and carnitine in fresh beef steaks stored under modified atmosphere. Food Chem 85, 453-459

Du M, Ahn DU (2002) Simultaneous Analysis of Tocopherols, Cholesterol, and Phytosterols Using Gas Chromatography. J Food Sci 67, 1696-1700

Elmastas M, Isildak O, Turkekul I, Temur N (2007) Determination of antioxidant activity and antioxidant compounds in wild edible mushrooms. J Food Compos Anal 20, 337-345

Fang X, Wada S (1993) Enhancing the antioxidant effect of a-tocopherol with rosemary in inhibiting catalyzed oxidation caused by Fe2+ and hemoprotein. Food Res Int 26, 405-411

Folch J, Lees M, Stanley GHS (1957) A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem 226, 497-509

Habibian Dehkordi S, Zamani Moghadam A, Maghsoudi N, Aali E, Gerami R, Dehsadeghi E (2010) The effects of fresh garlic on the serum concentration of total cholesterol, total triglyceride and adipose tissues of broilers. Comp Clin Pathol 19, 363-365

Kahl R, Kappus H (1993) Toxicology of the synthetic antioxidants BHA and BHT in comparison with the natural antioxidant vitamin E. Z Lebensm Unters Forsch 196, 329-338

Katiyar SK (2002) Treatment of silymarin, a plant flavonoid, prevents ultraviolet light-induced immune suppression and oxidative stress in mouse skin. Int J Oncol 21, 1213-1222

Kowalska D, Bielański P (2009) Meat quality of rabbits fed a diet supplemented with fish oil and antioxidant. Anim Sci Pap Rep 27, 139-148

Kulasek G, Bartnikowska E (1994) [Importance of unsaturated fatty acids in human and animal nutrition. Part I. Sources, metabolism and requirement]. Magazyn Weterynaryjny 3, 39-44 [in Polish]

Meineri G, Cornale P, Tassone S, Peiretti PG (2010) Effects of Chia (*Salvia hispanica L.*) seed supplementation on rabbit meat quality, oxidative stability and sensory traits. Ital J Anim Sci 9, 45-49

Mukai K, Nagai S, Ohara K (2005) Kinetic study of the quenching reaction of singlet oxygen by tea catechins in ethanol solution. Free Radic Biol Med 39, 752-761

Oriani G, Corino C, Pastorelli G, Pantaleo L, Ritieni A, Salvatori G (2001) Oxidative status of plasma and muscle in rabbits supplemented with dietary vitamin E. J Nutr Biochem 12, 138-143

Palazzo M, Vizzarri F, Cinone M, Corino C, Casamassima D (2011) Assessment of a natural dietary extract, titrated in phenylpropanoid glycosides, on blood parameters and plasma oxidative status in intensively reared Italian hares (*Lepus corsicanus*). Animal 5, 844-850

Rossi R, Pastorelli G, Cannata S, Tavaniello S, Maiorano G, Corino C (2013) Effect of long term supplementation with plant extract on carcass characteristics meat quality and oxidative stability in pork. Meat Sci 95, 542-548

Sánchez-Escalante A, Djenane D, Torrescano G, Beltrán JA, Roncales P (2003) Antioxidant Action of Borage, Rosemary, Oregano, and Ascorbic Acid in Beef Patties Packaged in Modified Atmosphere. J Food Sci 68, 339-344

Skrivanko M, Hadziosmanovi M, Cvrtila Z, Zdolec N, Filipovic I, Kozacinski L, Florijancic T, Boskovic I (2008) The hygiene and quality of hare meat (*Lepus europaeus Pallas*) from Eastern Croatia. Arch Lebensmittelhyg 59, 180-184

SPSS (2010) Version 18.0 Package program, User's Guide, SPSS Inc., Chicago, IL, USA

Vicenti A, Ragni M, di Summa A, Marsico G, Vonghia G (2003) Influence of Feeds and Rearing System on the Productive Performances and the Chemical and Fatty Acid Composition of Hare Meat. Food Sci Technol Int 9, 279-284

Wang P, Kang J, Zheng R, Yang Z, Lu J, Gao J, Jia Z (1996) Scavenging effects of phenylpropanoid glycosides from *Pedicularis* on superoxide anion and hydroxyl radical by the Spin trapping method(95)02255-4. Biochem Pharmacol 51, 687-691

Wojdyło A, Oszmiański J, Czemerys R (2007) Antioxidant activity and phenolic compounds in 32 selected herbs. Food Chem 105, 940-949

Zheng W, Miao K, Zhang Y, Pan S, Zhang M, Jiang H (2009) Nitric oxide mediates the fungal-elicitor-enhanced biosynthesis of antioxidant polyphenols in submerged cultures of *Inonotus obliquus*. Microbiology 155, 3440-3448