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Use of olive leaves (whether or not fortified with sodium selenate) in rabbit feeding: Effect on performance, carcass and meat characteristics, and estimated indexes of fatty acid metabolism

Simona Mattioli[a,*], Joana Margarida Machado Duarte[b], Cesare Castellini[a], Roberto D'Amato[a], Luca Regni[a], Primo Proietti[a], Daniela Businelli[a], Elisa Cotozzolo[a], Miguel Rodrigues[b], Alessandro Dal Bosco[a]

[a] *Department of Agricultural, Environmental and Food Science, University of Perugia, Borgo XX Giugno 74, Perugia, Italy*
[b] *Department of Zootechnical Sciences, University of Trás-os-Montes and Alto Douro (UTAD), Portugal*

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ABSTRACT

Sixty New Zealand White weaned rabbits were divided into three groups and subjected to different dietary treatments: a standard diet for the control (C), a standard feed supplemented with 10% of plain olive leaves (OL) and a standard feed supplemented with 10% of selenium-fortified olive leaves (100 mg/L of foliar spray sodium selenate solution; SeOL). The productive performance was recorded at the time of slaughter (after 35 days); the carcass and meat traits were determined and estimated indexes of fatty acid metabolism were calculated.

No significant differences were found on the rabbit productive performance and the physical-chemical characteristics of the meat. Both group of rabbits on the enriched diet showed leaner and thinner carcasses and a higher meat concentration of oleic acid. The estimated index of $\Delta 5 + \Delta 6$-desaturase, starting from n-6 fatty acids, was lower in both groups supplemented with leaves. The use of selenium-fortified olive leaves, positively affected the lipid oxidative stability of rabbit meat.

1. Introduction

Olive trees (*Olea europaea* L., Oleaceae) are among the most extensively cultivated crops in the world, with an annual production exceeding 15 million tons (Faostat, 2015). Mediterranean countries provide 98% of the world's olive production (Ilarioni & Proietti, 2014; Pereira et al., 2007), which are then used for oil extraction or table olives; whereas the residues from pruning (i.e. olive leaves and wood) and extraction process (i.e. olive pomace) are considered waste or by-products. The production of olive leaves from pruning is about 25 kg per olive tree plus 1 extra kg of leaves that can be collected at the oil mill (Molina-Alcaide & Yáñez-Ruiz, 2008).

For centuries, olive tree leaves have been used for different purposes: they contain several bioactive molecules (antioxidant, anti-hypertensive, anti-inflammatory, hypoglycaemic and hypocholesterolemic) that can be used as food additives. However, the procedures for extracting the bioactive compounds have an additional cost arising from the use of solvent (alcohols) or specific machinery (ultrasound, microwave, pressurized; Rosèllo-Soto et al., 2015). An alternative approach could be to use raw olive leaves as feed ingredients for animals.

The use of by-products as part of the rabbit diet can be a very effective example of recovery of healthy molecules while at the same time a way of developing a more sustainable production system (Dal Bosco et al., 2012). Compared to other meat sources, rabbit meat has a low amount of fat and cholesterol and a high content of polyunsaturated fatty acids (PUFA; Dalle Zotte, 2002), which makes it a natural functional food (Dalle Zotte & Szendrő, 2011).

Some agronomic practices, such as foliar spray administration of selenium, used to improve the water stress resistance of olive trees (Proietti et al., 2013), could further improve this whole system. It was observed that selenium applications increase the activity of some antioxidant enzymes (Djanaguiraman, Prasad, & Seppanen, 2010), therefore enabling additional benefits.

Selenium supplements have already been used by some authors to improve the performance and oxidative state in rabbits (Ebeid, Zeweil, Basyony, Dosoky, & Badry, 2013; Marounek, Dokoupilová, Volek, & Hoza, 2010). Accordingly, the idea of this research is to administer the by-product "olive leaves", to growing rabbits to improve their meat quality and partially solve the problem of waste disposal of olive tree pruning. In particular, the main goal of this study was to investigate the

* Corresponding author.
E-mail address: simona.mattioli@hotmail.it (S. Mattioli).

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Table 1
Formulation (% of ingredients), chemical composition (g/100 g d.m.), digestible energy (MJ/kg) and Se content (mg/100 g d.m.) of feed and olive leaves.

	Olive leaves		Experimental diets[1]		
	Normal	Se fortified	C	OL	SeOL
Alfa-alfa hay	–	–	45.0	38.0	38.0
Soybean meal 44%	–	–	14.5	14.5	14.5
Barley	–	–	31.0	31.0	31.0
Bran	–	–	3.00	–	–
Soybean oil	–	–	1.50	1.50	1.50
Olive leaves	–	–	–	10.0	10.0
Calcium-phosphate	–	–	1.40	1.40	1.40
Vitamin mineral premix[a]	–	–	1.20	1.20	1.20
Molasses	–	–	1.35	1.35	1.35
Salt	–	–	0.70	0.70	0.70
Calcium-Carbonate	–	–	1.00	1.00	1.00
DL-methionine	–	–	0.05	0.05	0.05
Dry matter	92.3	93.0	89.3	88.7	88.6
Ether extract	2.24	2.54	2.58	2.86	2.92
Crude protein	10.7	10.3	16.1	16.2	16.3
Ash	5.29	5.16	8.38	8.03	8.22
Crude Fiber	14.8	13.9	14.5	14.0	13.9
NDF[2]	35.0	30.9	27.6	27.0	26.9
ADF[2]	27.6	22.7	18.8	18.6	17.9
ADL[2]	15.4	12.5	3.92	4.64	4.32
Digestible energy[b]	11.2	11.2	11.9	11.9	11.9
Se	15.3	209	0.02	1.60	21.0

Values of chemical composition are the mean of three determinations.
[a]Added per kg: vit. A U.I. 11.000; vit. D_3 U.I. 2.000; vit. B1 mg 2.5; vit. B2 mg 4; vit. B6 mg 1.25; vit. B12 mg 0.01; vit. E mg 25; biotine mg 0.06; vit. K mg 2.5; niacine mg 15; folic ac. mg 0.30; D-pantotenic ac. mg 10; coline mg 600; Mn mg 60; Cu mg 3; Fe mg 50; Zn mg 15; I mg 0.5; Co mg 0.5; lysine mg 50; methionine mg 40.
[b]Estimated according to Maertens, Moermans, and Groote (1988).
[1] C: control diet; OL:control feed enriched with 10% of plain olive leaves; SeOL: control feed enriched with 10% of olive leaves fortified with selenium.
[2] NDF: Neutral Detergent Fiber; ADF: Acid Detergent Fiber; ADL: Acid Digestible Lignin.

effects of a diet enriched with a 10% of olive leaves, whether or not fortified with sodium selenate, on productive performance, carcass characteristics and meat quality of growing rabbits.

2. Materials and methods

2.1. Animals and diets

The experimental protocol was devised according to the Italian directives (Gazzetta Ufficiale, 1992) on animal welfare for experimental and other scientific purposes, and the research was carried out at the experimental farm of the Department of Agricultural, Food and Environmental Science of the University of Perugia (Italy), during the spring 2015.

Sixty New Zealand White mixed-sex rabbits were weaned at 35 days of age, allocated into three homogeneous groups (20 animals per group) in bicellular wire net cages (60 × 25 cm length × 35 cm height) and subjected to three different isoenergetic dietary treatments (Table 1) for 35 days and until the slaughter:

- Control feed (C);
- Control feed enriched with 10% of plain olive leaves (OL);
- Control feed enriched with 10% of olive leaves fortified with selenium (SeOL).

The olive leaves used in the diets were of the *Leccino* variety, from trees that are approximately 30 years old, located near Deruta (Umbria, Italy). At the end of April, 30 randomly selected trees were sprayed with a solution containing 100 mg/L of selenium. This solution was obtained by dissolving sodium selenate (Sigma-Aldrich, Milan, Italy) in water. For each treatment, 0.50% of the wetting agent “Bagnante” (Albamilagro International S.p.A., Parabiago, MI, Italy) was added. Each plant was treated with 10 L selenium solution and wrapped with filter paper to prevent the solution from dripping onto the soil. The paper was weighed before and after spraying in order to calculate the amount of solution that was absorbed by the plant, which amounted to 7.30 ± 1.40 L. As a control, another batch of twenty randomly selected trees were sprayed using the same technique, but with a water solution containing only the wetting agent (D'Amato et al., 2017).

The leaves were collected after pruning (November), dried in a ventilated stove at 60 °C for 24 h and subsequently grinded. The feeding program of the rabbits was adjusted according to previous studies (de Blas & Mateos, 2010; Martens & Villamide, 1998). Water was supplied ad libitum and the applied temperature and lighting schedules in the rabbit house were 15–18 °C and 16 L:8 D, respectively.

2.2. Productive performance

The feed intake of the rabbits was recorded on a daily basis and the live weight was recorded weekly to determine the growth and the feed efficiency (which was calculated as the ratio between the daily intake and the daily weight).

2.3. Slaughtering, carcass dissection and meat sampling

At 70 days of age, 10 rabbits per group were stunned and slaughtered under veterinary supervision of the University of Perugia, in the departmental processing plant, 12 h after feed withdrawal; the animals did not undergo transport. Specifically, the rabbits were killed by cutting the carotid artery and jugular veins.

In slaughtered animals, the caecal pH was measured in situ by direct insertion of a pH glass electrode. The slaughtering and carcass dissection procedures followed the World Rabbit Science Association (WRSA) recommendations described by Blasco and Ouhayoun (1993). The carcasses sizes and weight were recorded. The interscapular and perivisceral fat, liver and kidneys weights were also registered. The *longissimus thoracis and lumborum* muscles (LTL; between the 1st and 7th lumbar vertebrae) were excised from the two sides of refrigerated carcasses (24 h at 4 °C), trimmed of all external fat and epimisyal connective tissue, divided in two aliquots and stored at −80 °C. Subsequently, an aliquot was used for fatty acids profile and lipid oxidative status evaluation, whereas the other one was freeze-dried for 24 h for analyses of chemical characteristics.

2.4. Physical characteristics of the LTL meat

The pH was measured in duplicate at 24 h post mortem with a pH Meter Knick digital (Broadly Corp. Santa Ana CA, USA) by stabbing at the 7th thoracic vertebra. The water holding capacity (WHC) was estimated (Nakamura & Katoh, 1985) after centrifugation of 1 *g* of muscle for 4 min at 1500 ×*g*. The remaining water after centrifugation was quantified by drying the sample overnight at 70 °C. The sample WHC was calculated as follows: (weight after centrifugation − weight after drying) × 100 / initial weight.

The colour parameters of the muscle were determined using an analyzer tristimulus (Minolta Chroma Metre CR-200, Azuchi-Macgi Higashi-Ku, Osaka 541, Japan; light source of D65, standard observer of 10°, 45°/0° geometry, in light surface, calibrated against a standard white tile) with CIELAB colour system (1976), which gives the average of three subsequent measurements of brightness (L*), the parameter of the red (a*) and yellowness (b*).

Approximately 10 g of the LTL sample were kept for 15 min in the oven at 198 °C, and after this time, the weight difference was calculated in order to determine the weight loss of the sample because of cooking (Dal Bosco et al., 2012).