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Dietary effects of plant extracts, based on verbascoside, lycopene and horseradish on several blood variables and plasma oxidative status in growing rabbits

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ABSTRACT

Oxidative stress can cause damage to lipids, proteins and nucleic acids in several biological systems, leading to the functional and structural impairment of individual molecules that may be involved in various diseases. The aim of study was to evaluate the effect of dietary plant extract supplementation, based on *Lippia citriodora*, horseradish (*Raphanus sativus* L.) and lycopene (*Solanum lycopersicum* L.), on several blood parameters and plasma oxidative status in growing rabbits. The experiment lasted 80 days and was conducted on 160 weaned-rabbits, divided into four 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 weaning-fattening feed without any feed additives, while of the other three experimental groups, the first group received a supplement of plant extract based on *Lippia citriodora*, containing 5 mg of verbascoside/kg feed (VB group), the second group received 5 mg of lycopene/kg feed, tomato fruit extract (LIC group), and the third received 350 mg of *Raphanus sativus* root extract/kg feed (RAF group). The feed additives, based on verbascoside (*Lippia citriodora*), horseradish (*Raphanus sativus* L.) and lycopene (*Solanum lycopersicum* L.) resulted in a marked decrease in blood content of low density lipoprotein cholesterol and bilirubin. In addition, only when verbascoside was supplemented, were improvements in high density lipoprotein cholesterol and AST enzyme levels observed. Plasma oxidative markers significantly improved for all three extract groups. There was a significant reduction in ROMs and TBARS values, as well as an increase in the content of retinol and alfa-tocopherol, confirming the strong antioxidant ability of the plant extracts used. A dietary supplementation with phyto-extracts, based on *Lippia citriodora*, horseradish and lycopene can thus be effectively used in rabbit feeding due to the positive effects observed on the blood parameters and plasma oxidative status, with possible beneficial effects on the welfare of livestock animals.

1. Introduction

Oxidative stress is an imbalance between antioxidant and pro-oxidant molecules, with a predominance of the latter. This stress can cause oxidative damage to lipids, proteins and nucleic acids in several biological systems, leading to structural and functional impairment of compounds involved in various diseases (Sahin et al., 2006, 2008). The main compounds involved in oxidative stress are reactive oxygen species (ROS) molecules which have a short life, are highly reactive, and are derived from the oxygen metabolism at the cellular and tissue levels (Sies, 1997).

In our previous studies, we highlighted the positive role of dietary plant extracts, which are rich in polyphenols on blood antioxidant capacity in several livestock animal species (Palazzo et al., 2011; Casamassima et al., 2013a, 2017). Plant phenolic compounds are gaining increasing attention from the scientific community due to their antioxidant and anti-inflammatory properties (Luo and Wu, 2011). Among the natural plant extracts, which can be used as feed additives in animal feeding, those based on verbascoside (*Lippia citriodora*), horseradish (*Raphanus sativus* L.), and lycopene (*Solanum lycopersicum* L.) are especially interesting.

Lippia citriodora (*Verbenaceae*) is an herbaceous species that grows wild in South America. However, in North Africa and Southern Europe it is cultivated. In all these regions, it is used as a spice and/or as a medicinal plant. Antipyretic, sedative, digestive, antispasmodic, stomata and antioxidant activities have been highlighted in the leaves of

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this plant (Valentäo et al., 2002). Phenylpropanoids are chemical compounds that abound in extracts of *Lippia citriodora*, of which the main component is verbascoside (VB).

The VB is a water soluble phenylpropanoid glycoside (Funes et al., 2010), whose chemical structure is formed from one molecule of caffeic acid (CA) linked with a (D)-glucopyranoside to a molecule of hydroxytyrosol (HT), with a higher antioxidant activity than CA and HT molecules taken individually (Born et al., 1996). The VB appears to be more active in preventing oxidative stress, compared to CA and HT molecules in equimolar mixtures (Obied et al., 2008), thanks to the scavenger activity of catechol groups, and to the solubility and reducing properties of sugar residue (Rice-Evans et al., 1996). In our previous study on supplemented feed with verbascoside-based extracts, we found hepatoprotective and hypolipidemic properties in lambs (Casamassima et al., 2013a) and in growing hares (Casamassima et al., 2013b). Pastorelli et al. (2012) obtained the same results in weaned piglets.

Horseradish belongs to the family of Brassicaceae and since ancient times it has been considered as a medicinal plant. In healthy non-diabetic rats, dietary supplemented with the horseradish extract, Taniguchi et al. (2007) found that this plant increases the plasma content of high density lipoprotein (HDL) cholesterol. On the other hand in rats, fat diets positively influenced the activity of colon mucosa and protected cell membranes from lipid oxidation (Sipos et al., 2002). In diabetic rats, Dehghani et al. (2011) showed a significant decrease in blood triglycerides but no effect on cholesterol and glucose, following a 14-day administration of a horseradish aqueous extract (1600 mg/kg/d). It has also been observed that the horseradish extract is free from toxicity and has a high hepatoprotective activity (Salah-Abbès et al., 2009). The ethanol leaf extract of this plant led to a decrease in transaminases, lactate dehydrogenase, alkaline phosphatase and total bilirubin in rabbits (Anwar and Ahmad, 2006).

Lycopene is a carotenoid that is found mainly in tomatoes and tomato-based products. It has received considerable attention due to its biologically active protection (Palozza et al., 2010). Lycopene is an acyclic isomer of beta-carotene, with 11 conjugated double bonds, and characterized by a higher antioxidant power than normal beta-carotene and vitamin E (Di Mascio et al., 1989; Liu et al., 2005). Lycopene is an important compound for hypocholesterolemic activity (Fuhrman et al., 1997; Heber and Lu, 2002; Riso et al., 2006), hypoglycemic and hepatoprotective properties (Selvan et al., 2011).

Based on the existing literature and on previous experience evaluating the dietary effect with plant extracts, *Lippia citriodora*, horseradish (*Raphanus sativus* L.) and lycopene (*Solanum lycopersicum* L.) on productive performance and the quality of meat in growing rabbits (Vizzarri et al., 2017), this study examined on the same animals various blood parameters and plasma oxidative status.

2. Materials and methods

2.1. Animals and experimental design

All breeding procedures and the management of animals were conducted in compliance with the European directive 2010/63/EU, concerning the protection of animals used for scientific purposes. The Ethic and Scientific Committee of the University of Molise approved full experimental design. The experiment was conducted on 160 weaned-male-rabbits (New Zealand white x Californian) and lasted 80 days. The animals were reared in cages (two per cage) equipped with feeders and automatic watering. Temperature and relative humidity of the rabbitry were recorded continuously using a digital thermograph located at the height of the cages. The rabbitry was equipped with an environmental microclimate control system in order to maintain a temperature of 18 ± 4 °C and a relative humidity of 70 ± 5% throughout the experimental period.

Randomly rabbits were divided into four 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 natural supplements, whereas of the other experimental groups, the first group received a supplement of *Lippia citriodora* extract, containing 5 mg of verbascoside/kg feed (VB group), the second received 5 mg of lycopene, extracted from tomato fruit/kg feed (LIC group), and the third received 350 mg of root extract of *Raphanus sativus*/kg feed (RAF group).

The feed was administered ad libitum and was produced by Agrizoo s.n.c (Miranda, Isernia, Italy). Feed additives based on *Lippia citriodora* (0.5% of verbascoside as the main component), horseradish (*Raphanus sativus* dry root extract with enzyme complex Inuzyme®) and lycopene (2% of *Solanum lycopersicum* extract fruit) were provided by Sintal Zootecnica (Isola Vicentina, Vicenza, Italy), Erba Vita Italia SpA (Montegrimano Terme, Perugia, Italy) and Erbamea srl (San Giustino, Perugia, Italy), respectively.

2.2. Feed chemical composition

The animal feed was analyzed in triplicate using the AOAC (2000) method, as recommended by the European Group on Rabbit Nutrition (EGRN, 2001). Fatty acids were analyzed in triplicate by performing direct derivatization, as described by O'Fallon et al. (2007). The individual fatty acids were identified by comparing the retention time with the methyl esters FAME standard (NLEA mix) Rt-2560 Resteck Corporation (Bellefonte, PA, USA). Methyl esters were analyzed using a gas chromatograph ThermoQuest TRACE 2000 (SAC^{tm-5} column 30 m × 0.25 mm × 0.25 µm, Supelco, USA). Data relating to the ingredients, chemical composition and fatty acid of the feed are shown in Table 1.

2.3. Sampling and laboratory analysis of blood

Individual samples were taken from the *auricolaris marginalis* vein with the vacutainer method (Venoject, Terumo Europe N.V., Leuven, Belgium), using tubes with lithium heparin for plasma production. Blood samplings were performed at the beginning (weaning age, 0d), at the half (40d) and at the end of the experiment (80d). Blood was centrifuged for 20 min at 3000 rpm, and the following parameters were determined on the plasma: glucose, triglycerides, total cholesterol, LDL (low density lipoprotein) cholesterol, HDL cholesterol, AST (aspartate aminotransferase), ALT (alanine aminotransferase), bilirubin and creatinine, using a semi-automatic clinical chemistry Analyzer Arco model (Biotechnical Instruments, S.p.A., Italy). Reactive oxygen metabolites (ROMs) were spectrophotometrically determined with the colorimetric method proposed by Diacron at a wavelength of 505 nm, using a specific commercial kit (Cesarone et al., 1999). The results were expressed in Carr units (1 Carr corresponds to 0.024 mmol/l of H_2O_2). The determination of thiobarbituric acid reactive substances (TBARS) was spectrophotometrically performed according to Esterbauer and Zollner (1989), using a standard curve with 1,1,3,3-tetramethoxypropane (Sigma Aldrich, St. Louis, USA). The results were expressed as µmol of malondialdehyde (MDA)/l of plasma. Vitamins A and E were extracted from plasma samples with chloroform, according to Zhao et al. (2004). Vitamins amount was detected by HPLC (Kontron Instruments, Italy) which consisted of an automatic auto-sampler (HPLC Autosampler 360) with a loop of 20 µl, pump system (HPLC Pump 422), a column C18, 5 µm, 250 × 4.60 mm, (Phenomenex, Torrance, Ca, USA). The mobile phase consisted of a mixture of acetonitrile and methanol (85:15 v/v) with a flow value of 1 ml/min. Vitamins A and E were identified by comparing the retention time of the samples with the retention time of pure standards (> 97%) purchased by Sigma Aldrich (St. Louis, USA). The quantification was performed using the Gyminix system (version 1.8.1) by comparing the peak of the area with that of the reference standard curve.

2.4. Statistical analysis of data

After the evaluation of the normality of frequency distribution, all