UNIVERSITY OF STUDY OF MOLISE

Department of Agriculture, Environmental and Food

INTERNATIONAL PhD PROGRAM IN

WELFARE, BIOTECHNOLOGY AND QUALITY OF ANIMAL PRODUCTION

(XXIV CYCLE)

General Coordinator: Prof. Giuseppe Maiorano

Doctorate Thesis

ASSESSMENT OF VERBASCOSIDE-BASED DIETARY SUPPLEMENT ON SOME BLOOD AND PHYSIO-PRODUCTIVE PARAMETERS IN INTENSIVELY-REARED *LEPUS CORSICANUS* HARES

PhD Student:

Dr. Vizzarri Francesco

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DECLARATION

I hereby declare that the thesis is based on my original work except for citations which have been duly acknowledged. I also declare that this thesis has not been previously or concurrently submitted for any degree or any other institution.

Dr. Francesco Vizzarri

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Campobasso, 14th March 2013

DEDICATION

I dedicate this Thesis to my Family ..... my mother, my father and my sister ..... I was supported in difficult moments, but mostly they shared with me the satisfaction and the beautiful feelings that training envolves.

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After these 4 years of PhD, I kindly thank ...

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CONTENTS

ABSTRACT

Over the past decade, researchers and food manufacturers have significantly focused attention on polyphenols. The key reason for this interest is the recognition of the antioxidant properties of these substances, their great abundance in our diet and the probable role in preventing diseases associated with oxidative stress. The great class of polyphenols consists the phenylpropanoids glucosides, distributed in many medicinal plants, which are responsible for cytostatic, cardiac and hepatic effects. One of the major representatives of the phenylpropanoids glucosides is verbascoside, retrieved from different plants belonging to the Verbenaceae family and featured a rhamnose units linked to glucose, which serves as a bridge. The literature on the biological activity of verbascoside is very wide: it possesses an anti-inflammatory, anti-spasmodic, immunomodulatory, antioxidant, cholesterol-lowering, cardiotonic, epato-protective effect, photoprotective, analgesic and neuroprotective activity. The study focused on the evaluation of the dietary verbascoside supplement administration effect on reproductive parameters, on some blood parameters and plasma oxidative status of *Lepus corsicanus* hares intensively-reared. In addition, during two-years trial, some productive performance on growing hares were evaluated.

The test was performed on 75 couples of Italian hare (*Lepus corsicanus*) and took place in 2 years, of which it was 210 days for the first year and 260 days for the second year. At the same time the performance of 300 growing hares were also evaluated, of which 150 the first year and 150 the second year.

All animals, couples of hares and growing hares, into 3 homogeneous groups were divided. One was a control group (CON) and the other two experimental which were administered *ad libitum* during the whole trial period, a feed containing a dietary supplement of verbascoside at 5 mg/kg feed in the LVB

group and 10 mg/kg feed in HVB group. The food was specially prepared by adding to the amount of feed natural extract, standardized to 0.5% verbascoside, equal to 1 kg/tonne of feed supplement for the experimental LVB group and 2 kg/tonne of feed supplement for the experimental HVB group. The daily diet was completed by the addition alfalfa hay that was administered *ad libitum*.

The couples of hares were subjected, during the 2-year-trial period, to the following experimental controls: body weight of hares at the beginning and at the end of each reproductive cycle, feed intake, number of leverets born alive or died at birth, number of leverets weaned/birth, kindling interval, percentage of superfetation and pseudo-gestation for each trial-year, blood samplings for each reproductive cycle.

During the two years of experimentation, animals were tested at the beginning of each reproductive cycle (4 ± 2 days from partum) by blood samplings, for a total of 4 in the first year (0d, 70d, 140d, 210d) and 3 withdrawals during the second year (0d, 130d, 260d) for the determination of triglycerides, total cholesterol, HDL cholesterol, bilirubin, ROMs, TBARS, vitamin A and vitamin E.

The growing hares were subjected, during the 2-year-trial period, the following experimental controls: born body weight, weaning body weight (28d), half-trial (60d) and final (90d) body weight of growing hares for the determination of growth rate; daily feed intake for the determination of conversion index.

The experimental treatment, on blood and in both years of experimentation, a significant ($P<0,05$) reduction of triglycerides, total cholesterol and bilirubin and an increase in HDL cholesterol in couples of hares has produced.

The verbascoside also improved the oxidative status and plasma homeostatic stability through an important decrease in concentrations of ROMs ($P<0,05$)

and TBARS ($P<0,01$) and increase concentrations of vitamin A ($P<0,05$) and vitamin E ($P<0,01$); these results were found in both test periods.

Dietary verbascoside supplementation on growing treated hares a higher ($P<0,05$) average daily gain resulted, in 60-90 d period of the first year. This trend also was found in the 2nd test-year without reaching, however, the statistical significance. Treatment with verbascoside has also resulted, in the second experimental year, an improvement of food conversion index in HVB growing hares.

Therefore, the results of the present research have shown an important role of dietary supplements with antioxidant activity on some blood parameters and plasma oxidative status in couples of hares and on productive performance in growing hares.

RIASSUNTO

Negli ultimi dieci anni, i ricercatori e le industrie alimentari hanno notevolmente focalizzato l'attenzione sui polifenoli. La ragione chiave di questo interesse è il riconoscimento delle proprietà antiossidanti di queste sostanze, della loro grande abbondanza nella nostra dieta e del probabile ruolo nella prevenzione di malattie associate allo stress ossidativo. Alla grande classe dei polifenoli appartengono i fenilpropanoidi glucosidi responsabili di avere effetti citostatici, cardioattivi ed epatocitari. Uno dei maggiori rappresentanti dei fenilpropanoidi glucosidi è il verbascoside, estratto da diverse piante appartenenti alle famiglie delle Verbenaceae e caratterizzato da una unità di ramnosio legata al glucosio, che funge da ponte. Le attività biologiche che caratterizzano la molecola di verbascoside sono molteplici: possiede, infatti, attività anti-infiammatoria e anti-spasmodica, immunomodulatrice, antiossidante, ipocolesterolemizzante, antitumorale, cardiotonica, epatoprotettiva, fotoprotettiva, analgesica e neuroprotettiva.

Si è inteso, quindi, valutare l'effetto dell'aggiunta, nel mangime di allevamento, di un integratore alimentare a base di verbascoside sui parametri riproduttivi, su alcuni parametri ematici e sullo stato ossidativo plasmatico di coppie di *Lepus corsicanus* allevate intensivamente. Inoltre, nei due anni di sperimentazione, sono state valutate le performance produttive di leprotti in accrescimento. La prova è stata eseguita su 75 coppie di lepre italica (*Lepus corsicanus*) e si è svolta in 2 anni la cui durata è stata, per il primo anno, di 210 giorni e, per il secondo anno, di 260 giorni. Nello stesso arco di tempo sono state valutate anche le performance produttive di 300 leprotti in accrescimento, di cui 150 il primo anno e 150 il secondo anno. Tutti gli animali, coppie di riproduttore e leprotti in accrescimento, sono stati suddivisi in tre gruppi, omogenei tra loro, di cui uno di controllo (CON) e gli altri due

sperimentali ai quali è stato somministrato, *ad libitum*, durante l'intero periodo di prova, un mangime di allevamento contenente un integratore alimentare a base di verbascoside nella misura di 5 mg/kg di mangime nel gruppo LVB e di 10 mg/kg di mangime nel gruppo HVB. L'alimento è stato preparato appositamente aggiungendo al mangime una quantità di estratto naturale, titolato in verbascoside allo 0,5%, pari a 1kg di integratore/t di mangime per il gruppo sperimentale LVB e a 2kg di integratore/t di mangime per il gruppo sperimentale HVB. La razione alimentare è stata completata dall'aggiunta, a tutti i soggetti, di fieno di erba medica che veniva somministrata *ad libitum*.

Le coppie di lepri sono state sottoposte, durante i periodi di prova, ai seguenti controllo sperimentali:

-peso vivo dei riproduttori all'inizio e alla fine di ogni ciclo riproduttivo;

-consumo alimentare giornaliero delle coppie;

-numero dei figli nati vivi e/o morti al parto;

-numero dei figli portati vivi e vitali allo svezzamento/parto;

-durata interparto;

-percentuale di superfetazione e pseudo-gestazione per ciascun periodo di prova;

-prelievi ematici per ogni ciclo riproduttivo.

Nel corso dei due anni di sperimentazione gli animali sono stati sottoposti all'inizio di ogni ciclo riproduttivo (4±2 giorni dal parto), a prelievi ematici, per un numero complessivo di 4 prelievi, per il primo anno (0d, 70d, 140d, 210d) e 3 prelievi durante il secondo anno (0d, 130d, 260d) per valutare le variazioni di alcuni parametrici ematici e dello stato ossidativo plasmatico: colesterolo totale, colesterolo HDL, trigliceridi, bilirubina, ROMs, TBARS, vitamina A, vitamina E.

Controllo sperimentale sui leprotti in accrescimento.

Gli animali in accrescimento sono stati sottoposti, durante il periodo di 2 anni di prova, ai seguenti controlli sperimentali:

- peso vivo alla nascita;
- peso vivo allo svezzamento (28d), a metà (60d) e a fine sperimentazione (90d) e determinazione dei relativi accrescimenti;
- rilievo del consumo alimentare giornaliero e dei relativi indici di conversione.

Il trattamento sperimentale ha prodotto, a livello ematico e in entrambi gli anni di sperimentazione, nelle coppie di riproduttori, una riduzione significativa ($P<0,05$) dei trigliceridi, del colesterolo totale e della bilirubina ed un aumento del colesterolo HDL.

Il verbascoside ha fatto evidenziare anche un miglioramento dello stato ossidativo e della stabilità omeostatica plasmatica attraverso un significativo decremento delle concentrazioni dei ROMs ($P<0,05$) e dei TBARS ($P<0,01$) ed un incremento delle concentrazioni di vitamina A ($P<0,05$) e vitamina E ($P<0,01$); tali risultati sono stati riscontrati in tutti e due i periodi di prova.

L'integrazione alimentare a base di verbascoside ha determinato, nel I anno di prova, sui leprotti in accrescimento, un più elevato incremento giornaliero ($P<0,05$) nei soggetti dei gruppi trattati nel periodo 60-90d. Tale andamento si è riscontrato anche nel II anno di prova senza raggiungere, però, la significanza statistica. Il trattamento con verbascoside ha, altresì, determinato un miglioramento, solo nel II anno di sperimentazione, dell'indice di conversione alimentare nei leprotti del gruppo HVB.

Dai risultati della presente ricerca, emerge, pertanto, un importante ruolo degli integratori alimentari, ad attività antiossidante, su alcuni parametri ematici, sul controllo dello stato ossidativo plasmatico nelle coppie di lepri e sulle performance produttive dei leprotti in accrescimento.

INTRODUCTION

Information note on Hare

Hares are placental mammals belonging to the family Leporidae, included in the order Lagomorphs.

Lagomorphs retained many primitive characters and did not develop special morphological adaptations and behavioural differences between the different species, despite their ancient origin (about 55 million years ago) and wide distribution, which originally included the Palaearctic and Ethiopian regions and the Americas. Currently they are also present in Australia and New Zealand as a result of recent introductions.

They are plantigrade terrestrial animals and they are of medium size and slender shape, with small head, big eyes and long ears, highly developed hind legs designed for running and jumping; front limbs are equipped with five toes, and back four.

The diet is essentially vegetarian; common features are the presence of four incisors with no roots in the upper jaw and the lack of canines.

It is known different species belonging to the family Leporidae:

- *Lepus corsicanus* (Italian hare). The Italian hare, or Apennine hare, was described in 1898 by W.E. de Winton as a distinct species from Lepus europaeus, based on some morphological characters observed on specimens in museum collections. The Italian hare, which was probably widely distributed in the past in central-southern Italy and in Sicily, and which was introduced in the 16th century in Corsica (Vigne, 1992), was later downgraded to a subspecies of L. europaeus. In the middle of last century, because of hunting pressure and restocking with the European hare also in central and southern Italy, the subspecies corsicanus was considered extinct (Toschi, 1965). The description of diagnostic morphological characters (Palacios, 1996), and the

results of recent genetic studies (Pierpaoli et al., 1999), have confirmed the status of species and have shown the presence of residual populations of hares in different areas of central-southern Italy and Sicily.

- *Lepus europaeus* (European brown hare). The current Eurasian distribution of Lepus europaeus extends from the northern provinces of Spain, to introduced populations in the United Kingdom and southern regions of Scandinavia, south to northern portions of the Middle East, and has naturally expanded east to sections of Siberia (Flux and Angermann, 1990). This species has been extensively introduced as a game species into several countries across the globe. These countries are: Argentina, Australia, Barbados, Brazil, Canada, Chile, Falkland Islands, New Zealand (North and South Island), Rèunion, the United Kingdom, Ireland and the United States (Flux and Angermann, 1990). In Italy the species has been subject to massive repopulation in the last century, that have led to the release of animals imported from abroad, or, in small part, raised in the peninsula. The populations of the subspecies L. europaeus meridiei, originally distributed throughout north-central Italy, have been replaced by introduced non-native hares and probably belonging to different subspecies.

- *Lepus timidus varronis* (Mountain hare). Lepus timidus has a widespread distribution and there are currently 15 recognized subspecies; we consider the subspecies varronis, distributed in the Alps. Historical hybridization events and genetic introgression with L. europaeus, recently documented in Scandinavia, in the Iberian Peninsula and in Russia (Thulin et al., 1997; Melo-Ferreira et al., 2005; Waltari and Cook, 2005; Thulin et al., 2006; Melo-Ferreira et al., 2007), have made more complicated the identification of the genetic structure of populations.

- *Lepus capensis* (Cape hare). The geographic range of Lepus capensis (in Arabia) includes isolated populations scattered across the entire peninsula and

extends east into India. It is also found on the islands of Sardinia (ssp. Lepus capensis mediterraneus, but taxonomy is still uncertain (Suchentrunk et al., 1998) and Cyprus. Geographic range in Africa is extensive and separated into two distinct regions of non-forested areas (Boitani et al., 1999). The southern distribution includes the following countries: South Africa, Lesotho, Swaziland, Namibia, Botswana, Zimbabwe, southern portions of Angola, Mozambique, and Zambia (Boitani et al., 1999). The northern distribution includes: Tanzania, Kenya, Uganda, Eritrea, Sudan, Egypt, Libya, Chad, Niger, Tunisia, Algeria, Burkina Faso, Mali, Morocco, Western Sahara, Mauritania, and Senegal.

- *Lepus granatensis* (Iberian hare). The geographic range of Lepus granatensis includes Portugal and nearly the entire Spain (Alves et al., 2003). It is absent from northern regions of Spain where L. castroviejoi and europaeus exist (Alves et al., 2003). In most of the northern provinces (Navarra, Asturias, Cantabria, Aragon, Catalunya, and Basque Country), L. europaeus and L. granatensis exist in parapatry, the Iberian hare inhabits the southern region and the Brown hare can be found to the north (Fernandez et al., 2004). L. granatensis is also located on the island of Mallorca of the Balearic chain (Schneider, 2001). It has been introduced in southern France and Corsica (Perpignan) (Alves et al., 2003).

- *Lepus castroviejoi* (Broom hare). The distribution of L. castroviejoi is limited to the Cantabrian Mountains in the northwest of Spain (Flux and Angermann, 1990).

Lepus corsicanus

In this century, the distribution area of the species has been subjected to a substantial contraction accompanied by a significant reduction in density of populations. The most important risk factors have been identified in the

fragmentation of the distribution area, isolation and low population density, deterioration of the habitat, introduction of L. europaeus and over-hunting.
Lepus corsicanus may be considered a typical Italian endemism, because in Corsica the species was introduced by humans: it is important to adopt as soon as possible measures for the conservation and management.
Currently, the distribution area of the Italian hare recognizes as the northern limit Monte Amiata in the province of Grosseto, on the Tyrrhenian coast, and a small area near the National Park of Abruzzo, in the province of L'Aquila, on the Adriatic coast. South of these areas, the taxon is still present in all peninsular regions up to the province of Reggio Calabria, but with relict populations, often isolated in protected or inaccessible mountainous areas (Angelici and Luiselli, 2001). On the contrary, in Sicily the species is relatively widespread and is also observed in hunting areas far from protected parks (for example, in the province of Enna, where there aren't protected areas). Despite the identification of several tens of hares taken in recent years in the territory where hunting is practiced, it was not possible to confirm the presence of the Italian hare on the Island of Elba, but only the European Hare (introduced for hunting purposes).

Morphological aspects of Lepus corsicanus

The Italian hare, as all Leporidae, shows a laterally compressed head, very long auricles, narrow and elongated body usually kept bent, hind legs much longer and stronger than the front legs and suitable for jumping, short tail. The fur is reddish-gray on the neck, shoulders, hips, grayish-black on the back, white on the belly; long ears are black-tipped, black is also the top of the queue, and eyes are big and brown. There isn't sexual dimorphism.
Although similar in general to the European hare, the Italian hare has a relatively more slender shape, in fact the head-body length, the back foot and

the ears are proportionally longer, the average weight of adults is about 800 g lower. The morphological characteristics of Lepus corsicanus may imply a greater potential for thermal regulation and adaptation to the warm climate of the Mediterranean regions, whereas it is known that the European hare is well adapted to open environments with a continental climate.

The distinction between the two species in nature is not easy, especially with the naked eye and with animals moving. The coat colour of the Italian hare differs from that of the European hare for tawny shades and for the clear transition between the reddish fur of the hip and the white belly. The ecological distribution of L. corsicanus confirms the adaptation to habitats characterized by a Mediterranean climate (Tomaselli et al., 1973; Blondel and Aronson, 1999), although it is present from sea level up to 1900 m above sea level in the Apennines and 2400 m above sea level on Mount Etna. Favourite habitats seems to be those with alternating clearings, also grown, bushy areas and broad-leaved woods; can also occupy areas with dense cover of Mediterranean vegetation, including dune environments.

The species seems to have a sedentary behaviour with relatively small living spaces, attending after sunset and for the entire night almost the same areas of pasture. In areas of sympatry with the European hare they were observed frequenting the same pastures. The diet of L. corsicanus, studied in Sicily, varies seasonally as the available vegetation changes. Monocotyledones, Cyperaceae and Juncaceae, are ingested year round, while Gramineae and Labiatae are consumed during spring and summer, respectively (De Battisti et al., 2004). Dicotyledones ingested year round by L. corsicanus are Leguminosae and Compositae (De Battisti et al., 2004). The sexual rest period is relatively short (about sixty-seventy days), between October and December and for the other months the species doesn’t know practically sexual activity stops, although it is more intense in summer season. The species is

polygamous and doesn't form stable pairs, for the possession of the females, males often fight with aggression and violence, hitting with the front legs, and rarely, trying to bite. Mating takes place mostly at dusk or at night and the act of copulation is often preceded by a sort of courtship; the female prepares a special haven where giving birth to leverets (the number of births varies from one to five), which born after a gestation of about 41-42 days. A female can reproduce an average of three or four times a year, but as the breeding season is more or less long in relation to latitude, in regions with a warmer climate also occur five births. Hares have therefore a relatively high reproductive potential and this condition is well suited to a medium-sized herbivore that is subjected to a strong predation by several species of carnivores.

Threats

There are several conservation problems about the Italian hare that make this species threatened with extinction. Listed below are the main ones:

- Fragmentation and isolation of the distribution areas. The genetic differences observed between the haplotypes of specimens of L. corsicanus coming from central Italy, from south Italy and Sicily (Pierpaoli et al., 1999) reflect an evolutionary history with the presence of ancient subdivisions in the distribution area and consequently long periods of reproductive isolation. Current distribution data show an important fragmentation that must necessarily be attributed to anthropogenic causes, with very small populations isolated from each other, within an environmental matrix became increasingly unfavourable. The erosion and fragmentation of habitat due to human impacts are the major causes of isolation of the populations.

- Interspecific competition. The protracted restocking with L. europaeus for hunting purposes may have led to interspecific competition and the transmission of infectious diseases (Guberti et al., 2000). Competition may

occur mainly through the use of the same food resources or breeding sites and shelters; this may affect the coexistence of the populations concerned, in terms of changes in their size, distribution and structure.

- Genetic pollution. In the genus Lepus hybridization between species has already been documented; in Sweden hybrids were observed between the native form L. timidus and introduced L. europaeus (Thulin et al., 1997), and in Spain the three Iberian species of hares (L. granatensis, L. castroviejoi, L. europaeus) (Melo Ferreira et al., 2005).

The absence of observation of intermediate phenotypes and the lack of introgression in mitochondrial haplotypes of a species in the other leads to the belief that hybridization between the European and the Italian hare is an unlikely event. More concretely, however, is the risk of genetic pollution from translocated individuals (often from breeding station) in areas where genetically and morphologically different populations live (Pierpaoli et al., 1999; Riga et al., 2001).

- Hunting activity. Although the species is not included in the list of hunted species (L. n. 157/92) in the peninsula, the hunting exercise can be a real limiting factor: this is a complex issue because of the coexistence in the same areas of L. corsicanus and L. europaeus, of the difficulties in the recognition in nature, of the lack of a specific tradition in hares management and of the knowledge basis for sustainable management. These difficulties are reflected in a high impact on the residual populations of Italian hare and a practical impossibility in the implementation of conservation strategies, different between the two species.

- Poaching. In central and southern Italy and Sicily poaching on hares is traditional and widespread, encouraged by the lack of supervisory activities.

- Habitat degradation. Reforestation in general represents a threat to the habitat of the hare. Moreover, the intensification of cultivation occurred since

the war has led to a series of very heavy impact on the agricultural environment and adjacent natural areas, as well as for wildlife directly. They are also various consequences about the use of chemicals products (fertilizers and pesticides): direct consequences for acute and chronic toxicity, and indirect consequences for trophic sources significant reduction.

Legal protection

In 2008 the species was classified as “vulnerable” according to the criteria of the IUCN Red List. In 2001 the National Action Plan for the Italian has been published, which contains guidelines for conservation actions for the species. The DPCM 07.05.2003 (Official Gazette. July 3, 2003, No. 152) introduced this species among those hunted ("Only population living in Sicily" for the period October 15-November 30), of which art. 18, paragraph 1, letter e) of National Law 157/1992.

Vegetable natural extract with antioxidant activity

The use of traditional medicine is widespread and plants still represent a large source of natural antioxidants that might serve as leads for the development of novel drugs. All the Mediterranean countries are extremely rich in native plants, many of which are cultivated systematically, others are spontaneous.

The populations in the Mediterranean region, according to the epidemiological studies, have the lowest prevalence of many degenerative diseases, including cancers (Leighton et al., 1999), which have been ascribed to the reactive oxygen species damage (Lorenz et al., 2003). This phenomenon seems to be associated with the healthy plant-based diet comprising complex polyphenols as well as individual flavonoids (Martinez-Valverde et al., 2000). Polyphenols belong to the most potent anti-oxidants and because of that may protect

against cancer through inhibition of oxidative damage, which is likely to be an important cause of mutation (Feng et al., 2001). Other proposed mechanisms for explaining their action include antiproliferation, estrogenic/antiestrogenic activity, induction of cell-cycle arrest and apoptosis, modulation of activities of many enzymes, induction of detoxification enzymes, and changes in cellular signaling (Lee et al., 2002).

The antioxidative property of polyphenols is a predominant feature of their radical-scavenging capacity (Yang et al., 2001). However, their metal-chelating potential can not be ignored (Brown et al., 1998). The plant species contain several thousand polyphenols, but most probably only a limited number of them is important for human health. Flavonoids are the best known among them. The estimation of the polyphenol content in plants is very difficult, mainly because of the polyphenol structural diversity. In most of the studies, which refer to the polyphenol content, the total phenols were estimated by reduction of the Folin-Ciocalteu reagent (Kujala et al., 2001). The antioxidative power of the individual compound depends on their chemical structure, which is also responsible for the stability of the reactive flavonoid radicals. The less stable radicals, formed during the redox-cycle reaction, can propagate the harmful events through the radical attack. Thus, plant extracts can act as an anti-oxidant or pro-oxidant, depending on the structure and composition of different classes of polyphenols. The interaction between individual polyphenols may decide about the final outcome. Because of that, the results obtained when the crude plant extracts are used have to be considered with special precaution. Since the oxidative DNA damage can play a significant role in mutagenesis, cancer, aging, and other human pathologies (Aviram, 2000), the decrease of the oxidative stress seems to be the best strategy possible to achieve by eating food rich in antioxidants and/or

by taking supplements containing polyphenols, for example, plant extracts (Halliwell, 1996).

It is commonly accepted that, in a situation of oxidative stress, reactive oxygen species (ROS), such as superoxide (O_2, OOH^-), hydroxyl (OH^-) and 2 peroxyl (ROO^-) radicals, are generated. The ROS plays an important role in the pathogenesis of various serious diseases, such as neuro-degenerative disorders, cancer, cardiovascular diseases, atherosclerosis, cataracts, and inflammation (Aruoma, 1998; Kris-Etherton et al., 2004). Several anti-inflammatory, digestive, antinecrotic, neuro-protective, and hepatoprotective drugs have recently been shown to have an antioxidant and/or radical-scavenging mechanism as part of their activity (Lin and Huang, 2002; Repetto and Llesuy, 2002).

The mechanism of inflammation injury is attributed, in part, to release of reactive oxygen species from activated neutrophils and macrophages. This over production leads to tissue injury by damaging macromolecules and lipid peroxidation of membranes. In addition, ROS propagate inflammation by stimulating release of cytokines, such as interleukin-1, tumour necrosis factor-a, and interferon-c, which stimulate recruitment of additional neutrophils and macrophages. Thus, free radicals are important mediators that provoke or sustain inflammatory processes and, consequently, their neutralisation by antioxidants and radical scavengers can attenuate inflammation (Delaporte et al., 2002; Geronikaki and Gavalas, 2006).

Most clinically important medicines are steroidal or non-steroidal anti-inflammatory chemical therapeutics, for treatment of inflammation-related diseases. Though these have potent activity, long-term administration is required for the treatment of the chronic disease. Furthermore, these drugs have various and severe adverse effects. Therefore, naturally occurring agents with very few side-effects are required to substitute the chemical therapeutics.

Epidemiological and experimental studies reveal a negative correlation between the consumption of diets rich in fruit and vegetables and the risks for chronic diseases, such as cardiovascular diseases, arthritis, chronic inflammation and cancers (Chen et al., 2005; Zhang et al., 2005). These physiological functions of fruits and vegetables may be partly attributed to their abundance of phenolics.

There is a growing interest in phenolic components of fruits and vegetables, which may promote human health or lower the risk of disease. Recent studies have focused on health functions of phenolics, including flavonoids from fruit and vegetables (Chen et al., 2006; Saleem et al., 2002).

In the search for sources of natural antioxidants, in recent years some medicinal plants have been extensively studied for their antioxidant activity and radical-scavenging activity (Conforti et al., 2009).

Lippia citriodora (Ort.) HBK (Verbenaceae) is a herbal species mainly used as a spice and medicinal plant. It grows spontaneously in South America and is cultivated in northern Africa and southern Europe. The leaves of this species are reported to possess digestive, antispasmodic, antipyretic, sedative, and stomachic properties (Newall et al., 1996) as well as antioxidant properties (Valentão et al., 2002).

Due to these properties, which are frequently evaluated in vitro, the application of verbascoside in animal production could have an antioxidant effect as well as a growth promoting effect in vivo.

INTRODUCTION TO THE RESEARCH WORK

Over the past decade, researchers and food manufacturers have significantly focused attention on polyphenols. The key reason for this interest is the recognition of the antioxidant properties of these substances, their great abundance in our diet and the probable role in preventing diseases associated with oxidative stress (Manach et al., 2004). As is known, the oxidation reactions are an essential part of a normal cell metabolism, characterized by an appropriate antioxidant and pro-oxidant balance (Somogyi et al., 2007). In fact the normal presence, at the cellular level, of ROS plays positive roles as energy production, phagocytosis, cell growth and regulation of intercellular signals. An excessive accumulation of ROS can be highly harmful as responsible for the attack of biological macromolecules (lipids, proteins, nucleic acids), oxidation induction of cell membranes, inactivation of enzymes and DNA damage (Halliwell and Gutteridge, 1999; Valdo et al., 2004). Then, when the ROS level exceeds the antioxidant capacity of the cell, the intracellular redox homeostasis alters and hence arises a state of oxidative stress (Halliwell, 1999).

The latter plays a key role in the pathogenesis of aging and in many degenerative diseases, such as atherosclerosis, type II diabetes, cancer and cardiovascular diseases (Masella et al., 2005; Storz, 2005). It is precisely the latter that turn out to be the highest cause of death in developed countries like United States and several European countries, in spite of the substantial progress achieved by medicine. In the coming decades is expected an increase of these diseases, primarily due to a sedentary lifestyle and an increased ageing population, which will result in a higher incidence of various diseases related to cardiovascular disorders. Most applicants strategies to reduce the risk of cardiovascular disease, are focused on increasing HDL-cholesterol

levels because it is not enough to merely lowering total cholesterol and LDL-cholesterol (Geamann et al., 2012). Therefore, more attention is paid to the role of diet on human health. It is indicated, particularly the Mediterranean one, as a milestone in the prevention of such diseases; however we must emphasize that if on one side has discovered the importance of antioxidant compounds, it cannot say the same of the mechanisms of action involved, which are still not completely understood (Carluccio et al., 2003).

In order to oppose the excessive radicals in stressful situations, living organisms have developed sophisticated systems to maintain the homeostatic redox balance. These protective mechanisms block the production of ROS, intercept transition metals that are responsible for the formation of free radicals and activate antioxidant enzymatic defences (Yao et al., 2004; Porrini et al., 2005). Natural polyphenols have been extensively studied for their strong antioxidant capacity and recently for additional properties such as the regulation of certain activities. Most of the polyphenols are present in food in the form of esters, glycosides (including the verbascoside) or polymers that cannot be absorbed in their original form and must be hydrolyzed by enzymes or by intestinal microflora of the colon before being absorbed. During the absorption polyphenols are conjugated in the small intestine and then into the liver while those circulating are conjugated derivatives, mainly related to albumin. Polyphenols have the ability to penetrate into tissues, especially in those where they are metabolized, but their ability to accumulate in target tissues requires further investigation. Polyphenols and their derivatives are eliminated primarily via urine and bile and are excreted into the duodenum via the bile ducts, which are subject to the action of bacterial enzymes, particularly β-glucuronidase, only to be reabsorbed. This recycling entero-hepatic causes a greater presence of polyphenols in the organism (Manach et al., 2004).

The great class of polyphenols consists the phenylpropanoids glucosides, distributed in many medicinal plants, which are responsible for cytostatic, cardiac and hepatic effects (De Pascual et al., 1978). One of the major representatives of the phenylpropanoids glucosides is verbascoside, retrieved from different plants belonging to the Lamiaceae, Oleaceae, Buddlejaceae, Laminaceae and Scrophulariaceae family (Scorgin, 1997; Kupeli et al., 2007) and featured a rhamnose units linked to glucose, which serves as a bridge. Only the aglycones and some glucosides can be absorbed in the small intestine, while polyphenols related to units of rhamnose, as verbascoside, must reach the colon and be hydrolyzed by microflora-ramnosidasi before being absorbed.

The literature on the biological activity of verbascoside is very wide: it possesses an anti-inflammatory and anti-spasmodic activity (Lau et al., 2004; Penido et al., 2006; Kupeli et al., 2007; Korkina et al., 2007; Hausmann et al., 2007; Fleer and Verspohl, 2007), immunomodulatory (Akbay et al., 2002), antioxidant (Lee et al., 2005; Corino et al., 2007a; Bilia et al., 2008; Casamassima et al., 2011) cholesterol-lowering (Corino et al., 2007a), cancer (Zhang et al., 2002), cardiotonic (Pennacchio et al., 1996), epato-protective effect (Lee et al., 2004; Casamassima et al., 2011), photoprotective (Avila et al., 2005), analgesic (Nakamura et al., 1997; Calvo, 2006; Backhouse et al., 2008) and neuroprotective (Sheng et al., 2002).

The use in dietary administration of natural extracts and phyto-derivatives both promote increased digestive secretions, improving food ingestion, and stimulates a better immune, anti-bacterial, antiviral, anti-inflammatory and antioxidant response, increasing the status of welfare (Corino et al., 2007b).

In our previous research (Casamassima et al., 2010; Casamassima et al., 2011), using verbascoside as a dietary supplement, conducted on sheep, we found a general improvement of the lipid blood profile and plasma oxidative

status with regard to a marked reduction of reactive oxygen metabolites and tiobarbituric acid reactive substances, and a significant increase in antioxidant endogenous reserves of the organism into vitamin A and E as well as, a significant increase in milk yield in sheep.

Administration of dietary supplements, verbascoside-based, in small ruminants, it might be helpful in minimizing some forms of stress, both in the sheep peripartum period, both during the period of milking-feed in lambs, thanks to its anti-inflammatory, anti-bacterial, anti-viral, immunomodulatory and antioxidant activity. The verbascoside actions in postpartum recovery, it also protects the gastro-intestinal tract from some forms of colitis (Esposito et al., 2010), with a protective effect on the cellular phospholipid membranes, limiting the access of hydrophilic surface-membrane oxidant (Manach et al., 2004) and by modulating the plasma antioxidant activity in *in vivo* experiments (Funes et al., 2010).

In the animal field, as well as in human, oxidative stress is influenced by several pathological conditions and is very relevant to the general state of animal welfare and for their production capacity. For example, common diseases like pneumonia in pigs (Lauritzen et al., 2003) and respiratory obstruction in horses (Deaton et al., 2004) are determined by redox/oxidative imbalance. Although its use remains controversial, the supplemention with antioxidants to treat these diseases, targeted at the improvement of ROS systems scavengers, turns out to be an economical alternative and potentially important. The interest of the Scientific Community to experiment with the use of nutraceuticals that contain natural antioxidants, due to public attention to hygiene and sanitary quality of animal origin foods and welfare conditions in factory farms, is also normative support after the emanation of the Regulation 1804/99/EC and 91/2092/CE and the White Paper on food safety.

Oxidative stress is a particular condition induced by an accentuation in pro-oxidant sense of dynamic equilibrium between oxidative and reductive processes in every cell with the production of several radical species (Sies, 1991).

Free radicals are highly unstable and reactive molecules, characterized by the presence of an unpaired electron in their outer orbital. They are chemical compounds that occur naturally in the organism when oxygen from the atmosphere through respiration, is used to produce energy by metabolic processes. Most popular free radicals are oxygen-content (ROS) such as superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) and are due to environmental factors (UV rays, pollution, drugs, etc.) and to endogenous factors (electron transport in mitochondria, phagocytosis cell activity). Due to their high reactivity, they quickly interact with the environment in the organism, thus starting a chain reaction that ultimately harm the biological structures, and in particular:

- the lipids of cell membranes, with functional alterations of cells and tissue;
- low-density lipoproteins (LDL), leading the development and progression of atherosclerotic lesions;
- nucleic acids (DNA and RNA), resulting in damage to the genetic material;
- proteins, both structural (hyarulonic acid, collagen, etc.) and regulatory ones (enzymes, hormones, etc.) resulting in damage of structural and functional cell.

The human organism has developed sophisticated mechanisms to maintain redox homeostasis. They include endogenous antioxidant defenses both enzymatic (catalase, superoxide dismutase, glutathione peroxidase) and not (glutathione, co-enzyme Q, uric acid etc.), which are linked by exogenous defenses, mostly represented by antioxidants taken with diet including polyphenols, found in plant-based foods, such as fruits, vegetables, olive oil,

wine and tea (Benzie et al., 1999; Porrini et al., 2005). Carluccio et al. (2003) have shown, in particular on oil and wine, that trans-resveratrol contained in grape skins and red wine range from 1.5 to 7 mg/L, and oleuropein, found in olive oil (range from 50 to 800 mg/kg) and olives (about 2 g/100 g of dry matter), show antiaterogenic activities, such as the inhibition of LDL oxidation and TFE (tissue factor expression) in vascular cells and platelet aggregation.

Many experimental data show that cellular systems treated with polyphenols have a higher concentration of glutathione GSH, main radical scavenger, and enzymatic activities related thereto. Studies have shown that the oleuropein and protocatechuic acid, phenolic compounds contained in extra virgin olive oil, increase significantly the activity of glutathione reductase (GRed) and especially of glutathione peroxidase (GPx) in murine macrophage J774 A.1 and that this increase is linked to the ability of these polyphenols to induce the transcription of GRed and even more of the GPx (Masella et al., 2004). Among dietary antioxidants include vitamins A, E and C and carotenoids. Vitamin A, or retinol (active form of vitamin A), produced by beta-carotene through enzyme action of intestinal mucosa (Chew, 1987; NRC, 2001), plays a key role in the block of radicals normally produced during cellular metabolism (Bendich, 1993). Vitamin E is the generic term used to describe a group of fat-soluble compounds known as tocopherols and tocotrienols. In nature are present in different forms: α-, β-, γ-, and δ-tocopherols and α-, β-, γ-, and δ-tocotrienol (Kayden and Traber, 1993) by which the α-tocopherol is the most abundant foods stereoisomer. The best characterised role of vitamin E is being biological antioxidant in mammalian cell membranes, protecting polyunsaturated fatty acids (PUFAs) from attack by the radicals which forms a complex until their metabolites (Putnam and Comben, 1987). The water-soluble vitamin C, is especially effective against the hydroxyl radical,

superoxide radical and singlet oxygen (Sauberlich, 1990). Changes in plasma concentration of these components are used to evaluate the antioxidant status of an organism; for the global assessment of the pro-oxidant component of oxidative stress, the d-ROMs test is used for the quantification of hydroperoxides, reactive oxygen metabolites (ROMs) produced in cells from oxidative attack of ROS. Frequently used biomarkers of lipid peroxidation are the malondialdehyde (MDA) and thiobarbituric acid reactive substances (TBARS). Many naturally occurring substances in plants have the ability to neutralize the action of free radicals. Some of them break the chain reactions that lead to the formation of more radicals, thus preventing the spread of cellular damage; others play a scavenger of free radicals, oxidizing in turn and requesting to be regenerated to regain their function (Halliwell, 1997; Nijveldt et al., 2001). Flavonoids, flavonols, catechins and proanthocyanidins, content in extracts of some plants characteristics of Mauritius, have shown modulatory effects on the activities of various antioxidant enzymes promoter in renal tubular cells of monkey COS7 (Toyokuni et al., 2003). Plant extracts have demonstrated their *in vitro* antimicrobial effect, but their influence on growth performances of animals of zootechnical interest has not yet been sufficiently documented.

Another important aspect about the hare breeding is the reproductive one. Hare has a potential life span in the wild of 13 years; it reaches sexual maturity at 6 to 7 months (Stott and Wight, 2004) and give birth from one to four times per year. Pregnancy lasts for 41 days, but the interval between two successive parturitions is often shorter, ranging between 35 and 40 days. This is due to conception occurring at a fertile oestrus some days before parturition, and this phenomenon is known as superfetation (Caillol and Martinet, 1976). Consequently, the increase of reproductive performances is of remarkable scientific and commercial interest because hare farming in

Europe is developing due to an increase of market request, particularly for restocking. In recent years, there has been considerable concern about an apparent decline in populations of European hare (Lepus europaeus) in Western and Central Europe where an important haunting is carried out (Homolka and Zima, 1999).

RESEARCH OBJECTIVE

The study focused on the evaluation of the dietary verbascoside supplement administration effect on reproductive parameters, on some blood parameters and plasma oxidative status of *Lepus corsicanus* hares intensively-reared. In addition, during two-years trial, some productive performance on growing hares were evaluated.

MATERIALS AND METHODS

Research n°1 on Italica hare couples

The test was performed on 75 couples of Italian hare (*Lepus corsicanus*) and took place in 2 years: it was 210 days for the first year and 260 days for the second year. The couples of hares, reared in cages like "french model" (Castiglione et al., 1996), were divided into three groups of 25 couples each, homogeneous by age, body weight. One was a control group (CON) and the other two experimental which were administered *ad libitum* during the entire trial period, a feed containing a dietary supplement of verbascoside at 5 mg/kg feed in the LVB group and 10 mg/kg feed in HVB group. The food was specially prepared by adding to the amount of feed natural extract, standardized to 0.5% verbascoside, equal to 1 kg/tonne of feed supplement for the experimental LVB group and 2 kg/tonne of feed supplement for the experimental HVB group; the food was provided by the company Martini S.p.A (Budrio di Longiano, Italy) and the Table 1 lists the components of the feed and the chemical composition of the same (AOAC, 2000).

The daily diet was completed by the addition alfalfa hay that was administered *ad libitum*.

The couples of hares were subjected, during the 2-year-trial period, to the following experimental controls:

-body weight of hares during the trial;

-feed intake of hare couples;

-number of leverets born alive or died at birth;

-number of leverets weaned/birth;

-kindling interval;

-percentage of superfetation and pseudo-gestation for each year reproductive cycle.

Throughout the trial, blood samples were taken at the beginning of each reproductive cycle (4 ± 2 days post partum), for a total of 4 samplings (0 d, 70 d, 140 d, 210 d) in the first year and a total of 3 samplings (0 d, 130 d, 260 d) in the second year of experiment to evaluate changes in some blood parameters and plasma oxidative status: triglycerides, total cholesterol, high density lipoprotein (HDL) cholesterol, bilirubin, ROMs, TBARS, vitamin A and vitamin E.

The blood was taken from the ear vein with vacutainers method, by immobilizing the animal in a tissue bag, from which only the ears protruded through the slots. The bag, made to fit the animal, maintained their stillness with darkness to keep them calm. The samples were quickly centrifuged in the lab, for 15 minutes at 3000 rpm and on plasma were immediately determined the following parameters: triglycerides, total cholesterol, HDL cholesterol and bilirubin through the use of a semi-automatic analyzer model ARCO. The TBARS determination was made by using spectrophotometric method and a standard curve with 1, 1, 3, 3-tetramethoxypropane (Sigma Aldrich, St. Louis) was built. The plasma samples was added 10% trichloroacetic acid to assist the precipitation of proteins and the mixture obtained was incubated for 15 minutes on ice. After centrifugation at 2200 rpm for 15 minutes at 4° C, the supernatant was added 0.67% thiobarbituric acid and incubated at 90° C water bath for 10 minutes; the spectrophotometer absorbance was read at 532 nm wavelength. The results were expressed as μmol of thiobarbiturico acid per liter of plasma. The ROMs were spectrophotometrically determined with colorimetric method proposed by Diacron at a wavelength of 505 nm, using a specific commercial kit (Cesarone et al., 1999). Vitamin A and E were extracted from plasma samples with chloroform (Zhao et al., 2004) and the separation was performed by HPLC (Kontron Instruments-Italy) consisting of an autosampler (HPLC

Autosampler 360) with a loop from 20 µl, two pumps (HPLC Pump 422), C18 column, 5 um 250 x 4.60 mm (Phenomenex, Torrance, Ca, USA), using a mobile phase at 25% methanol and 75% nitrile acid (flow 1.0 ml/min.), a fluorimeter detector (SFM) and a computer equipped with software. The concentration of vitamins A and E have been determined on the basis of those internal standard and elution times of pure standards.

Research n°2 on growing hares

The test was carried out over a period of 2 years and performed on 300 growing little hares Hare italica (*Lepus corsicanus*): 150 animals in the first year and 150 animals in the second. In both years the experimental phase had a duration of 60 days, from the age of weaning (28d) at the age of 90 days.

The growing hares into 3 groups (50 animals each) were divided, homogeneous by age and body weight. One group was control (CON) and the others 2 were experimental groups (LVB and HVB group). The characteristics of livestock feed in the control group and experimental groups are the same as already described previously for hare couples. The growing hares were subjected, during the 2-year-trial period, the following experimental controls:

- birth body weight;
- body weight at weaning (28d), half-trial (60d) and final (90d) for the determination of growth rate at 28-60d, at 60-90d and whole trial (28-90d);
- daily feed intake at 28-60d, at 60-90d and whole trial (28-90d) for the determination of conversion index.

STATISTICAL ANALYSIS: RESEARCH N°1 AND RESEARCH N°2

All variables were subjected to analysis of variance, after evaluating the normality of frequency distribution, using one-way model to the productive and reproductive parameters while it was adopted the procedure GLM repeated measures of statistical package SPSS (2011) for blood parameters.
The fixed effect of dietary treatment (CON, LVB, HVB), time and their interaction were included in the model. The hare couple or single growing animal formed the experimental unit. The data were presented as mean values of each group and with its standard error; the differences were considered significant at $P<0.05$ with the Scheffé test.

RESULTS AND DISCUSSION

Research n°1 on Italica hare couples

Productive and reproductive parameters

The table 2 provides data on doby weight and feed intake of hare couples used in experimentation. It shows that the parameters considered, in two years of trial, were not affected by dietary treatment. The verbascoside did not alter either body weights and feed intakes, which were attested on average values of 543 g/couple/d for the control group and 534 and 535 g/couple/d, respectively, for experimental groups LVB and HVB.

The table 3 shows reproductive performance data of hare couples in the two years of trial; all considered parameters were not influenced by the administration of verbascoside supplement. In particular, the number of births between a minimum value of 4.40 in LVB group, to a maximum value of 4.80 in the CON group have varied in the first year; while, in the second year, the values have varied between 5.00 in the HVB group, to 5.30 in the LVB group. Relative to live total borns per couple per births, the values were attested on 2.42 in the first year and 2.20 in the second year of trial. Assessing the maternal capacity, with reference to weaned hare per birth, values of 1.76 and 1.97, respectively in the first and second year of trial, were noticed.

Relative to the kindling interval period and the number of births per couple, data are in accordance with what has been reported by Castiglione et al. (1996) and Mertin et al. (2010) in European hare bred in captivity. Tufarelli et al. (2010), by feeding European hare couples raised in cages, a dietary supplementation of ϖ3 and ϖ6 polyunsaturated fatty acids, showed a reduction in the kindling interval, an increase in the number of births per couple, the number of live born per birth and the number of weaned hares per birth. According to these Authors, the administered PUFA have influenced

the composition of cell membrane phospholipids of the reproductive apparatus.

Blood parameters

The table 4 lists the blood parameters related to triglycerides, total cholesterol, HDL cholesterol and bilirubin of the two years of trial.

The experimental treatment, in both years, a marked reduction in triglycerides in experimental groups LVB and HVB has determined.

Triglycerides, in the first year of trial, a significant ($P<0.05$) reduced values have shown in experimental LVB and HVB groups compared to CON group at140d respectively (-4.3 and -4.1%) and at 210d (-6.9 and -8.5%).

The duration of treatment has resulted in a marked decrease in triglycerides ($P<0.01$), which, from the beginning to the end of the test, were reduced by 7.4% in the LVB group and 8.2% in the HVB group; the control group did not show significant variations in the same time period.

This parameter, in the second year of trial, highlighted a significant treatment effect ($P<0.01$), sampling made at 130d and 260d, compared to CON group. In particular at 260d, LVB and HVB groups values decreased compared to the CON group by 23.1% and 28.8%, respectively. The treatment time has influenced the values of triglycerides; they, from the beginning to the end of the test, decreased significantly ($P<0.01$) in the LVB and HVB groups by 16.9% and 22.0% respectively, while the control group in the same time period, has presented a significant increase of the values of 9.0%.

In addition, the treatment with verbascoside, for both years of trial, a statistical decrease ($P<0.05$) of serum total cholesterol has produced.

In the first year, the decrease has been highlighted in the sampling at 140d and continued even at 210d, in which, respectively in the LVB and HVB groups, a reductions of 12.5% and 11.7% were observed. The time effect was

significant ($P<0.01$) on total cholesterol production; the values have decreased, in the LVB and HVB groups, from the first to the fourth sampling, 9.1% and 8.9% respectively. The control group, however, did not show noticeable variations in the same time period.

Total cholesterol level, in the 2nd year of trial, was influenced by the experimental treatment, only at the end of the test (260d), highlighting, compared to CON group, a significant decrease ($P<0.05$) of values by 8.4% and 17.2% in LVB and HVB groups respectively. The parameter, in these two experimental groups, decreased statistically ($P<0.05$) with the duration of treatment; the values have decreased, from 0 to 260d, of 15.5% in LVB group and 21.8% in HVB group, while in the CON group the values have remained almost unchanged.

HDL cholesterol, in the two years of experimentation, was influenced statistically ($P<0.01$) by the integration with verbascoside. The parameter, in the first year of trial, noted statistical differences from the second sampling to day 70, continuing with those made at 140d and 210d, and in the latter, in the experimental LVB and HVB groups compared to the control group, higher values respectively of 20.1% and 22.3% were found.

The duration of treatment has resulted also in the first year of trial, a significant increase ($P<0.01$) of HDL cholesterol content, highlighting over time, from beginning to the end of the test, an increase of 20.1% and 22.3% respectively in LVB and HVB group. The control group at the same time did not show variations.

HDL cholesterol content, in the 2nd year of experiment, since sampling at 130d, increased by 39.3% in of the LVB group and 41.7% in HVB group. At the end of the test, the increase in HDL cholesterol reached values of 64.9% and 57.1% in the LVB and HVB groups, compared to CON group. The duration of treatment has resulted also from the beginning to the end of the

test, a significant increase ($P<0.05$) of HDL cholesterol, to the extent of +21.5% and +26.0%, respectively in LVB and HVB groups, whereas in the CON group the values decreased by 20.6%.

Lowering-cholesterol effects of verbascoside can be attributed to the ability of phenolic compounds to inhibit the absorption of cholesterol in the intestine, causing therefore a reduction in levels of cholesterol in the liver and plasma. In fact, phenolic components have probably acted on lipid metabolism in a similar manner to statins (simvastatin and pravastatin), used in the treatment of ipercholesterol diet, which reduce cholesterol synthesis and induce increased expression of the LDL receptor, acting on HMG-CoA, enzyme which is involved in cholesterol synthesis in the liver. Inhibition of cholesterol synthesis in the liver results in a up-regolation of the expression of LDL receptor which are invoked by the peripheral circulation to the liver with decline in their plasma concentration.

Even Lee et al., (2003) a decrease in triglycerides and phospholipids in blood of 12% and 7% respectively have noted, by feeding broiler a diets enriched with carvacrol which a lower cholesterol biosynthesis has produced. Similar results also by Fidan and Dundar (2008) were obtained that, feeding diabetic rats Yucca Schidigera and Quillaja Saponaria extracts, a significant decrease in triglycerides and cholesterol have showed. This could be probably due to the fact that saponins contained in these plants, have influenced the cholesterol micelles stability, through the formation of micelles containing bile acids, fatty acids and triglycerides, reducing its penetration through the intestinal cells.

Choi and Hwang (2005), by experimenting on rats that were fed the flowers and/or fruits of certain medicinal plants (Piper cubeba, Physalis angolata and Rosa Hybrida) showed an increase in HDL cholesterol and a decrease in total cholesterol, LDL and triglycerides even if they have not reached statistical

significance, probably according to the Authors, because of the short treatment period (3 weeks). Radwan et al. (2008), in laying hens whose diets were supplemented with vitamin E, oil of thyme, oregano, rosemary and turmeric, have highlighted in the groups receiving oil of thyme and rosemary, a significant decrease of total lipids, compared to the control group, by 17.15% and 27.15%, respectively, while the total cholesterol and LDL cholesterol did have a tendency to decrease without reaching statistical significance. Even Yousef. (2003) a significant decrease in blood concentration of total lipids, triglycerides and total cholesterol and increased HDL cholesterol have showed in New Zealand White rabbits who had been given 2 mg/kg of body weight of isoflavones.

In table 4 is shown that the serum bilirubin level decreased statistically ($P<0.05$), in two years of experimentation, using the experimental treatment. In the first year, the differences between compared groups were observed only in the sampling at 210d, in which the groups LVB and HVB have presented lower values of 15.9% and 19.0% respectively, compared to the control group. The time treatment has affected the reduction of values which, from the beginning to the end of the test, decreased by 11.7% and 15.5%, respectively, in the LVB and HVB groups. The CON group has not presented significant variations in the same period of time.

Even in the second year of testing, serum bilirubin levels have decreased only in the last sampling at 260d, where experimental LVB and HVB groups have presented the lowest values of 14.8% and 18.0% respectively, compared to CON group. The time treatment has resulted in a significant ($P<0.05$) reduction in values, in both experimental groups LVB (-8.8%) and HVB (-12.3%), while the control group at the same time, no significant differences have presented.

All parameters listed in table 4, in both years of experimentation, a significant interaction between dietary treatment and the time of administration ($P<0.05$) have presented, suggesting a sensible modifications of the blood parameters increasing the time of administration.

The decrease in bilirubin, which we highlighted in the experimental groups could be likely attributed to the antioxidant activity of polyphenols that interfere with inhibition of biochemical mechanisms involved in the formation of the same (Aliyu et al., 2007). In this regard, also Yousef et al. (2003) in rabbits that received a diet enriched with antioxidants such as ascorbic acid, a decrease of bilirubin have noted, after a daily ingestion of doses of aflatoxins B1. Arhan et al. (2009) by administering to New Zealand White rabbits an aqueous extract of garlic, no significant variation in the values of total bilirubin have found.

Table 5 shows the data of plasma oxidative markers, such as ROMs, TBARS, vitamin E and vitamin A, which we considered in the two years of trial.

The experimental treatment has significantly influenced ($P<0.01$) the values of the ROMs, during the two years. The values of this parameter, in the last sampling at 210d of the first experimental year, have been lower in the LVB and HVB groups compared to control (131.9 and 125.6 U/Carr vs 254.1 U/Carr, respectively).

In particular, differences are highlighted since sampling made at 140d and continued until the end of the test (210d) with lower values of 48.1% in the l LVB group and of 50.6% in HVB group, compared to the control.

Within each group and during the test, the ROMs have shown a significant reduction of the values ($P<0.01$), in both LVB and HVB groups, in the exent of 37.6% and 36.3% from the beginning to the end of the test; however the control group a statistically significant increase of 29.2% ($P<0.01$) has shown from the beginning to the end of the trial.

In the 2nd year of trial, the values of the ROMs decreased, at 130d and 260d sampling, in LVB and HVB groups compared to CON group, showing, at 260 d, a decrease of 18.8% and 21.4%, respectively. The duration of treatment has also resulted, from the beginning to the end of the test, a significant ($P<0.05$) decrease of ROMs values by 17.9% and 18.8%, respectively in LVB and HVB group, whereas in the CON group the values, at the same time, remained unchanged.

The experimental treatment has significantly influenced ($P<0.01$), in two years of trial, TBARS values. During the first year, the TBARS values were lower of 35.7% in the LVB group and 36.6% in the HVB group compared to the control group, as early as 70 days trial; statistical variations observed for the experimental groups compared to control, have expanded afterwards until the end of the test (210d) with a reduction in percentage values, respectively, of 70.8% and 73.4%.

Within each group, TBARS values decreased significantly ($P<0.01$), from the beginning to the end of the test, in the experimental LVB and HVB groups in the exent of 39.2% and 40.8%, respectively, while in the control group at the same time, they increased of 108.1%.

In the 2nd year of trial, TBARS values have declined, in the two experimental LVB and HVB groups, compared to CON group, of 32.2%, and 41.6%, respectively. The time effect, from the beginning to the end of the test, a significant decrease of values ($P<0.05$), to the extent of 14.4% in LVB group and 25.1% in the HVB group have also resulted; the CON group, at the same time, did not highlight variations.

These results are most likely due to the antioxidant properties of the polyphenols present in verbascoside that, acting as redox-active molecules, i.e. able to oxidize and shrink without becoming in turn highly reactive radical, perform a preventive function towards ROS, resulting in reduction of

lipo-peroxidation highlighted by decrease of plasma levels of MDA. The reduction of ROMs and lipid peroxidation (TBARS) can be attributed both to a direct action on free radicals capture, made by the verbascoside for its antioxidant activity, during the propagation of chain reaction and to a block of the initiation phase of oxidative process, through the inhibition of pro-oxidant enzymes that would produce free radicals (Miller et al., 1993; Kamiloglu et al., 2006). These results are consistent with that encountered by Susca et al. (2005) in Charolaise breed bovine, under transport stress, which showed a decrease in the concentration of MDA in the group of animals which had received a food ration supplemented with high antioxidant plant derivatives. Even Li et al. (1999), in a study on skeletal muscle of rats subjected to strain, whose diet was supplemented with verbascoside for a period of 10 days, have observed a decrease in concentration of ROMs than untreated rats. Similar results were also observed by Fidan and Dundar (2008) in diabetic rats, after administration of Yucca Yucca and Quillaja Saponaria extracts, both rich in saponins, a decrease of MDA due to a protective effect of phenolic antioxidant present in these extracts, such as resveratrol.

These results are consistent with that encountered by Corino et al. (2007a) and Pastorelli et al. (2012) in previous experiences on weaned pigs fed with dietary supplement with verbascoside. In our previous research (Casamassima et al., 2012b, 2012c), we pointed out an improvement of plasma oxidative markers in ovine, fed with dietary verbascoside supplement.

The levels of vitamin E, in the two years of trial, have statistically grew up ($P<0.01$) in the two experimental groups compared to control. In the first year of test, statistical variations of vitamin E, as early as sampling made at 70 days, were observed between the experimental HVB group and control; to be later extended, at 140d and 210d in both experimental groups, with a

percentage increase of the values, in the last sampling, of 34.0% in the LVB group and 39.3% in HVB group, compared to the control group.

The content of vitamin E, during the trial, have significantly increased ($P<0.01$) by passing from initial concentrations of 0.847, and 0.850 μmol/L to final values of 1.126 and 1.170 μmol/L, respectively in LVB and HVB groups, while in the control group the concentrations remained unchanged.

The experimental treatment, even in the 2nd year of trial in experimental HVB and LVB groups, a marked increase of vitamin E has determined, in samplings at 130d and 260d, compared to CON group. In particular at 260d, values of LVB and HVB groups were increased by 243.3%, and of 245.9%, respectively, compared to CON group. The treatment time has influenced the values of vitamin E; they, from the beginning to the end of the test, have significantly grown ($P<0.01$) in the LVB and HVB groups of 223.1% and of 255.6%, respectively, while in the control CON group, the concentrations were, at the same time, almost unchanged.

From the examination of table 5, Vitamin A has been significantly affected ($P<0.05$) in the first year, by the experimental treatment; in fact, in the sampling at 210d, a significant increase in the concentration of the vitamin in the two LVB and HVB experimental groups, compared to CON group, were noted (0.347 and 0.329 vs 0.232 μmol/L, respectively). In particular statistical differences of vitamin A were already observed at 140 days-trial for the experimental groups compared to control and continued thereafter until the end of the same (210d), reporting a percentage increase of 41.8% and 49.6% respectively.

In addition, during the test and within each group, a significant increase ($P<0.05$) of the vitamin A concentration was observed in LVB and HVB groups, beginning at the end of the test, of 37.7% and 49.6% respectively, while in the control group remained unchanged.

In the 2nd year of test, in sampling at 130d and the 260d, a significant increase of the values of vitamin A in LVB and HVB groups was noted, compared to CON group. These values, in the sampling at 260d, were higher in the experimental groups compared with the control group, in the extent of 95.4% and 112.9%, respectively. Blood concentrations of vitamin A, have statistically grown ($P<0.01$) in experimental groups, even during administration time; the values, from 0 to 260d, have risen of 89.7% in LVB group and 112.7% in HVB group, whereas in the CON group the values have remained the same.

Increasing of serum vitamin A and E could be attributed to the capacity of verbascoside to enhance and save the endogenous antioxidant system, controlling oxidative metabolism by reducing the production of reactive species and induction of antioxidant enzymes (Princen et al., 1998; Zhu et al., 1999; Liao and Yin, 2000).

Contrary to what we found in this research, Capper et al. (2005) by administering vitamin E and fatty acids to pregnant sheep, have not detected a statistical increase in vitamin E content in plasma and tissues of born lambs.

As determinated in this study, similar results were obtained even in previous studies on naturally fed lambs and ewes, whose diet was supplemented with verbascoside (Casamassima et al., 2009, 2012b, 2012c).

Research n°2 on Italica growing hares

Productive parameters

Table 6 shows productive performance data of growing hares.

From the examination of the table, body weights of growing hares were not statistically influenced by dietary verbascoside, in the two years of treatment. Average values were attested, in the first year of trial and at the age of 90 days, on 2.39kg in the control group and 2.43kg and 2.47kg respectively in the experimental LVB and HVB groups. In the second year of test, on the other hand, body weights were attested on average values of 2.83kg, 2.88kg, and 3.04kg, respectively in the control group, LVB and HVB. These results, weights at weaning and 60d, are consistent with those reported by Mertin et al. (2010) and by Paci et al. (1999) on European hare raised in cage.

Even the average daily gain were not statistically affected, throughout the two-years-trial, by the experimental treatment; they were, in the first year of experimentation (0-90d), on average of 24.91g in CON group and 25.10g and 24.20g, respectively in the experimental LVB and HVB groups. In the second year, the average daily gain were more than satisfactory results, reaching values of 30.65g, 31.22g and 33.03g respectively in CON groups, LVB and HVB group.

In particular, in the first year of trial, it was observed a significant improvement of growth ($P<0.05$), between 60-90d, in favor of the experimental LVB and HVB groups, compared to the control group (27.43g/d and 27.67g/d vs 24.79 g/d, respectively). Also the duration of verbascoside administration, in the first year of test, significantly influenced ($P<0.05$) the growth of the animals, in both LVB and HVB groups, by percentage increments of 16.3% and 14.3%, from the second to the third trial-period,

respectively; in the same period, the growth of the control group animal remained substantially unchanged.

In the second year of experimentation, between different periods and groups, no statistical differences were found, in the growth of the animals; the duration of treatment, within each group, was irrelevant.

The average daily gain can be considered fully satisfactory in relation to the growth results achieved by other authors (Paci et al. 2000) on European growing hares, reared in cages, from the age of weaning (25-28d) up to 270 days. These Authors have found, in the period 25-90 d, a growth of 18.1g/d, decidedly lower than that detected by us at the same time-period. A positive effect of this dietary supplement on growth, in our previous research on naturally fed lambs (Casamassima et al., 2012b) was observed, and by other Authors on piglets during the post-weaning (Corino et al., 2007a). Erdelyi et al. (2008), feeding an antioxidant dietary supplement in Pannon white rabbits, based on essential oil of rosemary (*Rosmarinus officinalis*) and garlic (*Allium sativum*), an improvement of growth have detected, compared to the control group. Mehrez et al. (2011), New Zealand white rabbits fed with flour and seeds of black sesame (*Nigella sativa*), rich in antioxidant substances, such as sesamoline and sesamine, at the end of the test, an improvement of body weight and daily gain have highlighted in treated animals, although not statistically significant. Ibrahim et al. (2000; 2002), on rabbits weaned and fed with dietary supplement based on basil and/or oregano and/or sage extracts, have showed a significant increase of body weight, everage daily gain and feed intake, with a slight improvement in feed conversion index. Botsoglou et al. (2004) and Omer et al. (2010), however, have not shown significant changes on growth performance of weaned rabbits, fed an enriched feed with extracts for antioxidant activity. Corino et al. (2007a) in weaned pigs, fed with integrated feed verbascoside-based, statistically positive and significant

effects on growth of the animals, which received the integration, have detected.

Feed intake, shown in table 6, were not statistically affected by dietary treatment; the average values were attested on, in the first year (0-90d), between a minimum value of 144.4 g/animal/d and a maximum value of 150.1 g/animal/d in compared groups; while in the 2nd year of trial, they were on average between a minimum value of 123.3 g/animal/d and a maximum value of 136.0 g/animal/d. Feed intake, during the trial, has shown a time effect in both years of experimentation, whose values have significantly increased ($P<0.05$), in the year, from the first (28-60d) to the second period (60-90d) of 12.9%, 26.9% and 29.9%, in CON, LVB and HVB groups, respectively; while, in the second year and in the same groups, they increased by 23.8% in the CON group, 11.7% in the LVB group and 15.7% in HVB group.

The feed conversion index in the forst year, has not been affected by the experimental treatment; average values, tend to favour of experimental HVB and LVB group, compared to control, were attested on 5.62, 5.96 and 6.13 kgDM/kgGain, respectively. This parameter, on the other hand, was statistically influenced ($P<0.05$), in the 2nd year of trial, by highlighting more favourable values in HVB (3.61) and LVB (3.87) group, compared to control (4.30).

Feed intake values are in accordance with the risults provided by Paci et al. (2000) on European growing hares raised in cages. In our previous experiences, carried out on naturally fed lambs and daily supplemented with a verbascoside based product, we found only a favorable trend of feed conversion index, in treated animals which had received the integration (Casamassima et al., 2012b). Other Authors (Erdelyi et al., 2008), in Pannon white rabbits, weaned at 23d and fed until the age of 77d using an enriched-

feed with essential oils of rosemary and garlic, have shown an improved trend of feed conversion index in treated rabbits. El-Manylawi and Ali (2009), by feeding to New Zealand white rabbits a dietary supplement with seeds and essential oils of cumin, a significant improvement of feed conversion index have detected, in experimental groups compared to the control group.

The improvement of food efficiency, observed in the animals of the HVB group in the 2nd year of trial, compared to control, could be due to the effect of verbascoside which would result in a general improvement in the health status of the growing hares with a positive effects on the gastrointestinal and digestive processes and absorption of nutrients ingested with the feed.

CONCLUSIONS

The dietary verbascoside supplementation, in blood parameters of hare couples, in both years of trial, a significant reduction of triglycerides, total cholesterol and bilirubin and an increase of HDL cholesterol have determinated; this supplement has also highlighted an improvement of oxidative stability through a significant decrease in plasma concentrations of ROMs and TBARS and increased concentrations of vitamin A and vitamin E.

However, the use of the dietary supplement has not produced a statistical effects on productive and reproductive parameters of hare couples and growing hares, except for the feed conversion index that has resulted in more favourable values in HVB group of the 2nd year-test, compared to the control group.

Therefore, these results indicate a positive effect of the dietary supplement on blood parameters, but has not translated into improved productive performance of animals.

Table 1. Chemical composition and feed ingredients for *Lepus corsicanus*.

Item	Chemical composition	
	Fresh Feed (%)	Dry Matter (%)
Humidity	10.50	-
Crude Protein	16.50	18.43
Ether extract	3.20	3.57
Crude fiber	16.70	18.66
Nitrogen free extract	45.40	50.74
Ash	7.70	8.60
Natural extract[a]	0 or 0.1 or 0.2	

Components/100kg feed: dry fodder (30%), products and by-products of cereal grains (28%), products and by-products of seeds oil (15%), cereal grains (8%), products and by-products of sugar (2%), toasted carob bean pulp flour (9%), soybean oil (0.5%), oats (5.3%), additives (1.2%), calcium carbonate (0.7%), sodium chloride (0.3%).
Supplied per kg of feed: 13,500 I.U. vitamin A (trans-retinyl acetate); 800 I.U. vitamin D3 (cholecalciferol); 35 mg vitamin E (α-tocopherol min 91%), 35 mg copper (cupric sulphate pentahydrate), mg 150 aminoside sulphate.

Natural extract[a]: respectively 0, 0.1 and 0.2 kg for Control (CON), Low (LVB) and High (HVB) level of feed supplement inclusion groups.

[a]PPGs and benzoic acid content of dietary supplement, g /kg:

Gallic acid	1.755 ± 0.07
3.4-dihydroxybenzoic acid	0.45 ± 0.04
Methyl gallate	1.915 ± 0.09
Isoverbascoside	0.435 ± 0.04
Verbascoside	4.470 ± 0.08

Table 2. Body weight and feed intake of Italica hare couples.

Parameters		CON	LVB	HVB	SEM	*P values*		
						D	T	DxT
			I year of trial (0-210d)					
Hare couples	***n.***	***25***	***25***	***25***				
Body weight of hare couples								
males:								
0d	kg	3,42	3,41	3,50	0,051			
70d	"	3,25	3,29	3,33	0,051			
140d	"	3,17	3,37	3,32	0,048			
210d	"	3,28	3,37	3,36	0,059	0,850	0,755	0,366
females:								
0d	kg	3,87	3,86	3,92	0,052			
70d	"	3,84	3,83	3,77	0,061			
140d	"	3,70	3,73	3,82	0,068			
210d	"	3,82	3,74	3,77	0,066	0,895	0,456	0,268
Feed intake/couple/d								
0-70d	g	546,91	538,33	536,31	15,04			
70-140d	"	548,45	537,01	530,67	12,16			
140-210d	"	542,82	533,44	526,98	21,28			
Whole trial (0-210d)	"	546,06	536,26	531,32	14,35	0,566	0,488	0,745
			II year of trial (0-260d)					
Hare couples	***n.***	***25***	***25***	***25***				
Body weight of hare couples								
males:								
0d	kg	3,52	3,61	3,41	0,049			
130d	"	3,30	3,45	3,39	0,039			
260d	"	3,32	3,39	3,45	0,050	0,795	0,256	0,159
females:								
0d	kg	3,75	3,81	3,76	0,042			
130d	"	3,74	3,72	3,68	0,078			
260d	"	3,79	3,85	3,81	0,073	0,356	0,348	0,598
Feed intake/couple/d								
0-130d	g	556,31	539,74	543,37	20,22			
130-260d	"	523,75	524,62	532,33	22,24			
Whole trial (0-260d)	"	540,03	532,18	537,85	15,22	0,128	0,566	0,847

D= fixed effect of dietary supplementation; T=fixed effect of time; DxT= interaction dietary supplementation x time

Table 3. Reproductive paramentes in Italica hare couples.

Parameters		CON	LVB	HVB	SEM	P values
			I year of trial (0-210d)			
Hare couples	***n.***	***25***	***25***	***25***		
Reproductive parameters						
Birth interval	d	39	40	40	1,250	0,442
Super-fetation	%	60,00	71,40	50,00		
Pseudo-gestation	%	0,00	7,10	0,00		
Birth/couple	n.	4,80	4,40	4,70	0,680	0,080
Live born/couple	n.	2,78	2,14	2,33	0,290	0,125
Stillborn/couple	n.	0,29	0,49	0,46	0,009	0,065
Total live born and stillborn	n.	3,07	2,63	2,79	0,850	0,128
Weaned hare/birth						
Values	n.	2,08	1,61	1,59	0,720	0,155
Percentage values	%	74,82	75,23	68,24		
Dead animals during milking period	%	25,18	24,77	31,76		
			II year of trial(0-260d)			
Hare couples	***n.***	***25***	***25***	***25***		
Reproductive parameters						
Birth interval	d	43	41	46	1,320	0,325
Super-fetation	%	40,00	60,00	30,00		
Pseudo-gestation	%	20,00	10,00	20,00		
Birth/couple	n.	5,10	5,30	5,00	0,750	0,069
Live born/couple	n.	2,21	2,29	2,10	0,210	0,111
Stillborn/couple	n.	0,48	0,37	0,41	0,006	0,128
Total live born and stillborn	n.	2,69	2,66	2,51	0,125	0,356
Weaned hare/birth						
Values	n.	2,07	1,96	1,89	0,322	0,089
Percentage values	%	93,67	85,59	90,00		
Dead animals during milking period	%	6,33	14,41	10,00		

Table 4. Blood parameters in Italica hare couples.

Items	CON	LVB	HVB	SEM	P values		
					D	T	DxT
			I year of trial (0-210d)				
Hare couples n.	***25***	***25***	*25*				
Triglycerides, mmol/l							
0 d	1.306	1.346[a]	1.334[a]	0.009			
70 d	1.312	1.321	1.322	0.009			
140 d	1.326[1]	1.269[2b]	1.272[2b]	0.013			
210 d	1.339[1]	1.246[2b]	1.225[2b]	0.010	0.039	0.003	0.035
Total cholesterol, mmol/l							
0 d	0.643	0.648[a]	0.652[a]	0.006			
70 d	0.659	0.621	0.608	0.009			
140 d	0.668[1]	0.601[2]	0.602[2]	0.011			
210 d	0.673[1]	0.589[2b]	0.594[2b]	0.009	0.044	0.003	0.028
HDL cholesterol, mmol/l							
0 d	0.138	0.139[a]	0.139[a]	0.002			
70 d	0.140[1]	0.160[2b]	0.164[2b]	0.003			
140 d	0.140[1]	0.166[2b]	0.167[2b]	0.003			
210 d	0.139[1]	0.167[2b]	0.170[2b]	0.003	0.001	0.004	0.035
Bilirubin, µmol/l							
0 d	10.09	10.26[a]	10.26[a]	0.171			
70 d	10.26	9.75	9.75	0.171			
140 d	10.60	9.58	9.41	0.205			
210 d	10.77[1]	9.06[2b]	8.72[2b]	0.188	0.005	0.028	0.042
			II year of trial (0-260d)				
Hare couples n.	***25***	***25***	***25***				
Triglycerides, mmol/l							
0d	1.174[a]	1.183[a]	1.167[a]	0.058			
130d	1.210[1]	1.055[2b]	1.007[2b]	0.042			
260d	1.279[1b]	0.983[2b]	0.911[2c]	0.048	0.005	0.009	0.049
Total cholesterol, mmol/l							
0d	0.554[a]	0.567[a]	0.554[a]	0.056			
130d	0.552[1]	0.541[2]	0.513	0.049			
260d	0.523[1b]	0.479[2b]	0.433[2b]	0.054	0.049	0.035	0.029
HDL cholesterol, mmol/l							
0 d	0.194[a]	0.209[a]	0.192[a]	0.039			
130 d	0.168[1]	0.234[2]	0.238[2b]	0.040			
260 d	0.154[1b]	0.254[2b]	0.242[2b]	0.039	0.008	0.048	0.033
Bilirubin, µmol/l							
0 d	9.75	9.74[a]	9.76[a]	0.181			
130 d	10.43	9.41	9.39	0.172			
260 d	10.43[1]	8.89[2b]	8.55[2b]	0.144	0.042	0.015	0.048

D= fixed effect of dietary supplementation; T=fixed effect of time; DxT= interaction dietary supplementation x time
[(1,2)] Means with different superscripts within a row are different P<0.05
[(a,b,c)] Means with different superscripts within a column are different P<0.05

Table 5. Plasma oxidative markers in Italica hare couples.

Parameters		CON	LVB	HVB	SEM	P values		
						D	T	DxT
			I year of trial (0-210d)					
Hare couples	***n.***	***25***	***25***	***25***				
ROMs:								
0d	U/Carr	196,6^{a}	211,3^{a}	197,0^{a}	4,145			
70d	"	210,8^{a}	199,2^{a}	184,8^{a}	4,415			
140d	"	231,2^{1}	172,6 ^{2b}	164,8 ^{2b}	5,617			
210d	"	254,1^{1b}	131,9^{2c}	125,6 ^{2c}	7,825	0,002	0,001	0,028
TBARS:								
0d	µg/ml	0,186^{a}	0,186^{a}	0,174^{a}	0,006			
70d	"	0,235^{1b}	0,151^{2b}	0,149^{2b}	0,007			
140d	"	0,285^{1c}	0,143^{2b}	0,129^{2b}	0,009			
210d	"	0,387^{1d}	0,113^{2c}	0,103^{2c}	0,009	0,026	0,001	0,035
Vit E:								
0d	µmol/L	0,838	0,847^{a}	0,850^{a}	0,011			
70d	"	0,847^{1}	0,926^{a}	1,070^{2b}	0,009			
140d	"	0,845 ^{1}	0,996^{2}	1,107^{2b}	0,010			
210d	"	0,840 ^{1}	1,126^{2b}	1,170^{2b}	0,010	0,003	0,001	0,033
Vit A:								
0d	µmol/L	0,237	0,239^{a}	0,232^{a}	0,006			
70d	"	0,231	0,245^{a}	0,264^{ba}	0,007			
140d	"	0,235^{1}	0,291^{2b}	0,307^{2b}	0,007			
210d	"	0,232^{1}	0,329^{2b}	0,347^{2bc}	0,009	0,035	0,029	0,045
			II year of trial (0-260d)					
Hare couples	***n.***	***25***	***25***	***25***				
ROMs:								
0d	U/Carr	192,3	196.7^{a}	192.4^{a}	8,566			
130d	"	192,2^{1}	176.8^{2b}	169.2^{2b}	2,736			
260d	"	198.8^{2}	161.4^{2c}	156.2^{2b}	8,478	0,004	0,022	0,036
TBARS:								
0d	µg/ml	0,199	0.202^{a}	0.199^{a}	0,049			
130d	"	0,231^{1}	0,199	0.165^{2b}	0,061			
260d	"	0,255^{1}	0.1732^{b}	0.149^{2b}	0,062	0,005	0,022	0,025
Vit E:								
0d	µmol/L	1,567	1.612^{a}	1.476^{a}	0,149			
130d	"	1,509^{1}	2.927^{2b}	2.386^{2b}	0,055			
260d	"	1,517^{2}	5.208^{2c}	5.248^{2c}	0,955	0,001	0,002	0,039
Vit A:								
0d	µmol/L	0,909	0.926^{a}	0.900^{a}	0,172			
130d	"	0,924^{1}	1.243^{2b}	1.278^{2b}	0,238			
260d	"	0,899^{1}	1.757^{2c}	1.914^{2c}	0,256	0,008	0,001	0,045

D= fixed effect of dietary supplementation; T=fixed effect of time; DxT= interaction dietary supplementation x time

(^{1,2}) Means with different superscripts within a row are different P<0.05

($^{a, b,c}$) Means with different superscripts within a column are different P<0.05

Table 6. Productive performance of Italica growing hares.

Parameters		CON	LVB	HVB	SEM	P values		
						D	T	DxT
			I year of trial					
Animals	***n.***	***50***	***50***	***50***				
Body weight:								
at birth (0d)	g	151,86	172,21	171,37	3,59			
at weaning (28d)	"	908,37	852,05	864,13	13,08			
60d of age	"	1641,50	1606,79	1638,53	15,17			
90d of age	"	2385,34	2429,81	2468,51	23,21	0,921	0,724	0,521
Average daily gain:								
from 0d to 28d	g	27,02[1]	24,28[2]	24,74	0,45			
from 28d to 60d	"	22,91	23,59[a]	24,20[a]	0,35			
from 60d to 90d	"	24,79[1]	27,43[2b]	27,67[2b]	0,42			
whole trial: from 0d to 90d	***"***	***24,91***	***25,10***	***24,20***	***0,36***	***0,125***	***0,039***	***0,044***
Feed daily intake:								
from 28d to 60d	g	137,79	132,27[a]	125,59[a]	4,85			
from 60d to 90d	"	155,62	167,83[b]	163,18[b]	4,14			
whole trial: from 28d to 90d	***"***	***146,70***	***150,05***	***144,39***	***3,44***	***0,720***	***0,048***	***0,045***
Feed convertion index:								
from 28d to 60d	n	5,97	5,78	5,35	0,25			
from 60d to 90d	"	6,31	6,16	5,90	0,17			
whole trial: from 28d to 90d	***"***	***6,13***	***5,96***	***5,62***	***0,17***	***0,155***	***0,388***	***0,655***
			II year of trial					
Animals	***n.***	***50***	***50***	***50***				
Body weight:								
at birth (0d)	g	159,62	160,00	160,55	2,66			
at weaning (28d)	"	964,52	1002,86	1021,25	28,19			
60d of age	"	1896,94	1940,00	2028,75	35,43			
90d of age	"	2829,35	2877,14	3036,59	60,44	0,633	0,255	0,425
Average daily gain:								
from 0d to 28d	g	28,73	30,10	30,76	1,00			
from 28d to 60d	"	31,08	31,24	33,58	1,04			
from 60d to 90d	"	32,15	32,32	34,74	1,07			
whole trial: from 0d to 90d	***"***	***30,65***	***31,22***	***33,03***	***0,36***	***0,054***	***0,058***	***0,061***
Feed daily intake:								
from 28d to 60d	g	121,55[a]	116,25[a]	114,36[a]	2,15			
from 60d to 90d	"	150,53[b]	129,85[b]	132,29[b]	6,53			
whole trial: from 28d to 90d	***"***	***136,04***	***123,05***	***123,32***	***8,55***	***0,055***	***0,048***	***0,056***
Feed convertion index:								
from 28d to 60d	n	3,91[a]	3,72	3,41	0,29			
from 60d to 90d	"	4,68[1b]	4,02	3,81[2]	0,35			
whole trial: from 28d to 90d	***"***	***4,30 [1]***	***3,87***	***3,61 [2]***	***0,48***	***0,045***	***0,049***	***0,056***

D= fixed effect of dietary supplementation; T=fixed effect of time; DxT= interaction dietary supplementation x time

([1,2]) Means with different superscripts within a row are different P<0.05

([a, b]) Means with different superscripts within a column are different P<0.05

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1. PALAZZO M., **VIZZARRI F.,** CINONE M., CORINO C., CASAMASSIMA D. Assessment of dietary natural extract, titrated in polyphenols, on some blood parameters in Italica hare intensively reared. In proceeding **International PhD Workshop on "Welfare, biotechnology and quality of animal production". Zielonka (Poland),** 2010, 3rd - 7th, July.
2. **VIZZARRI F**, MASSANYI P, ONDRUSKA L, PALAZZO M, CASAMASSIMA D. (2010). Assessment of dietary natural extract, titrated in polyphenols, on rabbit spermatozoa motility using Casa system: preliminary study. **In: X. Risk Factors of Food Chain. Nitra, Slovakia***,* 2010, 13th -14th September.
3. CASAMASSIMA D., PALAZZO M.**,** MARTEMUCCI G., **VIZZARRI F**., CORINO C. (2010). Effetto del verbascoside sui parametri ematici e produttivi in pecore Lacaune. In: **XIX CONGRESSO NAZIONALE S.I.P.A.O.C. Società Italiana di Patologia e Allevamento degli Ovini e dei Caprini** Pesaro, 2010, 22nd-25th September.
4. CASAMASSIMA D., **VIZZARRI F.,** PALAZZO M., MASSANYI P., ONDRUSKA L., CORINO C. 2011. Effect of the addition of a verbascoside-based food supplement in feed, on some blood parameters

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5. **VIZZARRI F.,** CASAMASSIMA D., PALAZZO M., MASSANYI P., ONDRUSKA L., CORINO C. Effect of dietary supplementation with natural verbascoside-based extracts on productive performance of White New Zealand rabbits. In proceeding **International PhD Workshop on "Welfare, biotechnology and quality of animal production". Rackova Dolina (Slovak Republic),** 2011, 30th – 31st August
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2. 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 Ruminant Research,** 105, p. 1-8.
3. CASAMASSIMA D., PALAZZO M., **VIZZARRI F**., CINONE M., CORINO C. (2012). Effect of dietary phenylpropanoid glycoside-based natural extracts on blood parameters and productive performance in intensively-reared young hare. **Czech Journal of Animal Science**, *Accepted.*
4. CASAMASSIMA D., PALAZZO M., D'ALESSANDRO A.G., COLELLA G.E., **VIZZARRI F.**, CORINO C. (2012). Effect of plant extracts, phenylpropanoid glycoside based, on productive performance, plasma oxidative status and some blood metabolites in suckling lambs. **Journal of Animal and Feed Sciences**, *Submitted.*
5. CASAMASSIMA D., **VIZZARRI F.**, PALAZZO M., MASSANYI P.,

ONDRUSKA L., CORINO C. (2012). Effect of a Verbascoside-based food supplement on some blood parameters and plasma oxidative status in New Zealand White rabbit does. **World Rabbit Science,** *Submitted.*

LIST OF ABBREVIATIONS

DM= dry matter

DNA= deoxyribonucleic acid

GPx= glutathione peroxidase

Gred= glutathione reductase

GSH= glutathione

HDL= high density lipoprotein

HVB= high verbascoside dose

LDL= low density lipoprotein

LVB= low verbascoside dose

MDA= malondialdehyde

PUFA= poly-unsaturated fatty acid

RNA= ribonucleic acid

ROMs= reactive oxygen metabolites

ROS= reactive oxygen species

TBARS= substances reactive thiobarbituric acid

TFE= tissue factor expression