Effect of dietary phenylpropanoid glycoside-based natural extracts on blood parameters and productive performance in intensively-reared young hares

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ABSTRACT: Two different doses of a dietary verbascoside-based supplement were evaluated on various blood parameters and on productive performance in young weaned hares reared intensively from 28 to 90 days of age. The study lasted for 62 days and it was conducted on 210 young hares divided into three homogeneous groups of 70 animals each, consisting of a control group and two experimental groups. Each group received the dietary supplement in the feed, titrated to 0.5% verbascoside, 1 kg/t of concentrate (LVB group) and 2 kg/t of concentrate (HVB group). The experimental reliefs included the measurement of blood parameters such as triglycerides, total cholesterol, high density lipoprotein cholesterol, and bilirubin, along with some markers of oxidative status in plasma such as reactive oxygen metabolites, thiobarbituric acid-reactive substances, and vitamins A and E. Various productive parameters were also measured such as body weight and growth rate, food consumption, and feed conversion. The experimental treatment resulted in a significant decrease in triglycerides ($P < 0.01$) and bilirubin ($P < 0.05$) and an increase in high density lipoprotein cholesterol ($P < 0.01$) both in the low verbascoside (LVB) and high verbascoside (HVB) experimental groups. In addition, the oxidative plasma stability in blood also improved, with a significant decrease in the concentration of reactive oxygen metabolites ($P < 0.01$) and thiobarbituric acid-reactive substances ($P < 0.01$), along with increased levels of vitamin E ($P < 0.05$). The productive performance was not statistically influenced by the experimental treatment, except for the growth rate which increased significantly ($P < 0.05$) in the experimental LVB and HVB groups: 6.9% and 8.7% respectively compared with the control group.

Keywords: leverets; plant extract; verbascoside; plasma metabolites; growth performances

The use of natural antioxidants may be a good alternative to synthetic additives for improving the productive performance and welfare of animals without leaving residues, either in the animals or in the environment. Plant extracts with antioxidant activities, such as food supplements, can effectively replace the antibiotics that were once used widely for the auxin purposes, and which were subsequently banned by the European Union (Regulation CE 1831/2003). Verbascoside, which is a phenylpropanoid glycoside, is one of the numerous plant extracts used for this purpose. It has strong antioxidant, antimicrobial, anti-inflammatory activities and is a scavenger of free radicals. It has also been found to have a positive effect on the health and welfare of farm animals (Friedman et al., 2002; Rababah et al., 2004).

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Experiments conducted on weaned piglets (Corino et al., 2007a, b) and suckling lambs (Casamassima et al., 2009) revealed an improvement in growth, blood lipid profile, and oxidative plasma stability. The better oxidative profile was highlighted by a significant decrease in the concentration of thiobarbituric acid reactive substances (TBARS) and reactive oxygen metabolites (ROMs) and, in lambs, also by a marked increase in blood concentrations of vitamins E and A. In similar experiments, by feeding pairs of Italian hares (*Lepus corsicanus*) a concentrate supplemented with verbascoside, Palazzo et al. (2011) found an increase in high density lipoprotein cholesterol (HDL-cholesterol) and a significant reduction in blood triglycerides, total cholesterol, and bilirubin. The authors also reported an improvement in oxidative plasma stability resulting in an increased content of vitamins A and E and a decrease in the concentrations of TBARS and ROMs. In rats, the administration of verbascoside reduced lipid peroxidation in muscular tissue and in rabbits it produced strong antioxidant and protective effects on the membranes of erythrocytes in plasma (Liu et al., 2003).

Few studies have been carried out on the postnatal growth of the European brown hare (*Lepus europaeus*) (Pepin, 1979; Bray et al., 2002; Hacklander et al., 2002) and there have been even fewer studies on the Italian hare (Spagnesi, 1972; Paci et al., 2000). The growing interest in this species for hunting purposes has prompted farmers to breed the brown hare in captivity, to repopulate the marginal hilly and mountainous areas, especially in central-southern Italy, in order to preserve animal biodiversity. In order to contribute to the knowledge on the topic, the effect of a verbascoside-based dietary supplement of a water-soluble plant extract of Verbenaceae (*Lippia citriodora*) was evaluated on various blood parameters and the growth of young Italian hares (*Lepus corsicanus*), in order to restock the natural areas in central-southern Italy.

MATERIAL AND METHODS

Animals and diets

The 62-day trial carried out on 210 young hares (*Lepus corsicanus*) reared after weaning to day 90 took place on the Allevamenti Roger farm in the countryside of Isernia, in the region of Molise. The animals were distributed in groups of five into French model type cages (Castiglione et al., 1996). They were divided into three groups of 70 hares, homogeneous in age (28 ± 2 days) and body weight (875 ± 29.7 g), a control group (CON) that did not receive the dietary supplement in the feed, and two experimental groups with a verbascoside based natural extract of leaves of Verbenaceae (*Lippia citriodora*) devoid of essential oils in the feed. An amount of 5 mg of active compound per kg of concentrate was given to the low verbascoside experimental group (LVB) and 10 mg of active compound per kg of concentrate to the high verbascoside experimental group (HVB). The content of phenylpropanoid glycosides and benzoic acid in the natural extract is shown in Table 1.

Table 1. Phenylpropanoid glycosides and benzoic acid content of feed supplement

Items	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

The feed was prepared by adding the natural extract, standardized to 0.5% verbascoside, as 1 kg of supplement per t of concentrate for the experimental group LVB and 2 kg of supplement per t of concentrate for the experimental group HVB. The pelleted feed mixtures were provided by Martini S.p.a. (Budrio di Longiano FC, Italy). Table 2 shows the components and the chemical composition of the feed (AOAC, 2000). The daily diet of the hares consisted of the concentrate and alfalfa hay, *ad libitum*.

Young hares were healthy and their condition was judged as good at the beginning of the experiment. All procedures involving the animals were performed in accordance with European Community guidelines and approved by the Italian Ministry of Health.

The following parameters were checked during the trial period: body weight at the beginning (day 28), in the middle (day 60), and at the end (day 90) of the trial; growth rate in the periods of 28–60 and 60–90 days; feed intake and feed conversion during the two periods (28–60 and 60–90 days); blood

Table 2. Ingredients and chemical composition of diet (% as-fed basis unless otherwise indicated)

Item	
Ingredients	
Sunflower meal	23
Alfalfa hay	22
Wheat bran	20.83
Alfalfa meal dehydrated (17% CP)	10
Beet pulp	10
Barley	7
Wheat	1.5
Calcium carbonate	1.5
Cane molasses	1.5
Palm oil	0.6
Soybean oil	0.7
Sodium chloride	0.4
Dicalcium phosphate	0.2
Vitamin and mineral premix[a]	0.25
Methionine (99%)	0.23
Lysine (78.5%)	0.14
Choline (75.0%)	0.1
Coccidiostatic	0.05
Natural extract[b]	0/0.1/0.2
Analyses[c]	
Moisture	11.0 ± 0.11
Crude protein (% DM)	15.4 ± 0.30
Ether extract (% DM)	3.3 ± 0.32
Crude fibre (% DM)	19.5 ± 0.14
NDF (% DM)	38.5 ± 0.42
ADF (% DM)	24.0 ± 0.35
ADL (% DM)	6.5 ± 0.43
Ash (% DM)	8.5 ± 0.28
Starch (% DM)	10.0 ± 0.92

DM = dry matter, CP = crude protein, NDF = neutral detergent fibre, ADF = acid detergent fibre, ADL = acid detergent lignin

[a]supplied per kg of diet: vitamin A (trans-retinyl acetate) 7000 I.U., vitamin D3 (cholecalciferol) 1600 I.U., vitamin E (all-rac-tocopherol-acetate) 20 I.U., vitamin K3 (bisulphate menadione complex) 1.0 mg, thiamin (thiamine-mononitrate) 0.7 mg, riboflavin 3.0 mg, pantothenic acid (D-Ca pantothenate) 9 mg, vitamin B_3 (niacin) 15 mg, choline (choline chloride) 100 mg, pyridoxine (pyridoxine HCl) 1 mg, vitamin B_{12} (cobalamin) 0.016 mg, Cu ($CuSO_4 \cdot 5H_2O$) 16.5 mg, Fe ($FeSO_4 \cdot 7H_2O$) 75 mg, Mn (MnO_2) 40 mg, Zn (ZnO) 110 mg, Co ($CoSO_4$) 0.1 mg, Se (Na_2SeO_3) 0.3 mg, I ($Ca(IO_3)_2$) 0.8 mg, ethoxiquin 125 mg

[b]0, 0.1, and 0.2 kg for control (CON), low (LVB), and high (HVB) level of feed supplement inclusion groups, respectively

[c]n = 2; mean ± SD

parameters in 10 animals of each group on days 28 (beginning of trial) and 90 (end of trial).

Blood analyses

Blood samples were collected in the morning from the external ear vein by immobilizing the animal in a fabric bag, with only the ears protruding through the slots. The bag was designed to fit the animal, to keep it in darkness in order to stay calm. The blood was collected into tubes with sodium heparin and centrifuged at 3000 rpm for 20 min. Triglycerides, total cholesterol, HDL-cholesterol, and bilirubin on plasma were immediately determined with a specific commercial kit using an Automatic Clinical Chemistry Analyzer (Arco model) (both Biotecnica Instruments S.p.A., Roma, Italy). ROMs were determined on plasma with a spectrophotometer using a specific commercial kit (Diacron srl, Grosseto, Italy) at a wavelength of 505 nm (Cesarone et al., 1999). The results were expressed in arbitraty units called "Carratelli Units" (CARR U) where 1 CARR corresponds to 0.024 mmol/l of H_2O_2). TBARS were determined on plasma according to Esterbauer and Zollner (1989), where a standard curve was generated using 1,1,3,3-tetramethoxypropane (Sigma Aldrich, St. Louis, USA). 10% (v/v) trichloroacetic acid (Sigma Aldrich, St. Louis, USA) was added to the plasma sample promoting precipitation of proteins.

The resulting mixture was incubated on ice for 15 min. After centrifugation at 2200 rpm at 4°C for 15 min, 0.67% thiobarbituric acid (Sigma Aldrich, St. Louis, USA) was added to the supernatant, which was then incubated in a water bath at 90°C for 10 min and the absorbance was read at 532 nm in a spectrophotometer. The results were expressed as mol of thiobarbituric acid per litre of plasma.

Vitamins A and E were extracted from plasma samples with chloroform (Zhao et al., 2004, lightly modified) and analyzed on HPLC system (Kontron Instruments S.p.A., Milano, Italy) consisting of an autosampler (HPLC Autosampler 360) with a 20 ml loop, a high-pressure mixing pump (HPLC Pump 422), and a 5 mm × 250 mm × 4.60 mm C18 column (Phenomenex, Torrance, USA). The mobile phase was 100% methanol at flow rate 1.0 ml/min. A fluorimeter detector (SFM) and a computer with Kroma System 2000 software were used. The concentrations of vitamins A and E were determined using retinyl acetate and α-tocopheryl

acetate as internal standard and the time of elution of pure standards.

Statistical analysis

After assessing whether the frequency distribution was normal, all variables were subjected to a variance test using the GLM procedure for repeated measures in the SPSS program (Version 18.0, 2010). The data obtained were processed through One-Way ANOVA, and are presented as means and standard errors of groups; differences were considered significant at $P < 0.05$. An individual animal was considered as the experimental unit.

RESULTS AND DISCUSSION

Blood parameters

Table 3 reports on the blood parameter values of the leverets. The table shows that the verbascoside treatment influenced almost all the parameters considered, except total cholesterol and vitamin A.

The triglyceride values in the LVB and HVB groups showed a significant reduction of 5.5 and 9.4%, respectively, compared with the CON group. The length of treatment from the first to the second sampling highlighted a significant decrease in values in the LVB and HVB groups of 6.8 and 10.0% ($P < 0.05$), respectively. The control group did not show any appreciable changes within the same time period.

Table 3. Blood parameters of leverets ($n = 10$ per each group) fed control diet (CON) and diets supplemented with two levels of a natural extract titrated in verbascoside (LVB and HVB supplemented with 1 and 2 kg/t feed respectively)

Items	Experimental group			SEM	Effect *P*
	CON	LVB	HVB		
Triglycerides (mmol/l)					
Day 28	1.32	1.37[a]	1.36[a]	0.01	
Day 90	1.36[1]	1.28[2b]	1.23[2b]	0.02	1.001
Total cholesterol (mmol/l)					
Day 28	0.64	0.64[a]	0.65[a]	0.01	
Day 90	0.63	0.58[b]	0.60[b]	0.01	0.344
HDL cholesterol (mmol/l)					
Days 28–60	0.13	0.14[a]	0.14[a]	0.02	
Days 60–90	0.14[1]	0.17[2b]	0.17[2b]	0.03	0.003
Bilirubin (mmol/l)					
Days 28–60	10.02	10.70[a]	10.09[a]	0.22	
Days 60–90	10.15[1]	9.03[2b]	8.93[2b]	0.17	0.032
ROMs (CARR U)					
Days 28–60	180.96	184.97[a]	186.63[a]	5.73	
Days 60–90	198.79[1]	138.00[2b]	124.92[2b]	7.94	0.009
TBARS (mmol/l)					
Days 28–60	0.17[a]	0.21[a]	0.18[a]	0.09	
Days 60–90	0.231[b]	0.16[2b]	0.15[2b]	0.01	0.003
Vitamin E (mmol/l)					
Days 28–60	0.42	0.37	0.36[a]	0.02	
Days 60–90	0.39[1]	0.41	0.45[2b]	0.01	0.0450
Vitamin A (mmol/l)					
Days 28–60	0.25	0.26	0.24	0.01	
Days 60–90	0.22	0.23	0.26	0.01	0.382

[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 ($P < 0.05$)

Total cholesterol was not statistically influenced by the experimental treatment, as the values were similar in all the groups. Note that there was a significant decrease in total cholesterol values ($P < 0.05$) in the LVB and HVB groups between the start of weaning and the end of sampling; no change was observed in the control group.

HDL cholesterol was statistically influenced ($P < 0.01$) by the experimental treatment; it increased significantly (by 22.9 and 23.6%, respectively) in the LVB and HVB groups compared to the control. The time effect of the treatment significantly ($P < 0.05$) affected the HDL cholesterol values showing an increase in values of 25.5 and 20.1% in the LVB and HVB groups, respectively. The control group did not show any appreciable changes within the same time period.

These results agree in part with the findings from previous research on the use of verbascoside (Casamassima et al., 2011, 2012; Palazzo et al., 2011) in pairs of Italian hare (*Lepus corsicanus*), in rabbits and sheep. There was a significant improvement in some parameters of the lipid profile with a decrease in triglycerides, total cholesterol, and low-density lipoprotein cholesterol (LDL cholesterol) and an increase in HDL cholesterol. The increase in HDL cholesterol concentration in plasma using verbascoside could be due to the effect of polyphenols involved in the regulation of lipid and glucose metabolism which, according to some authors (Norata et al., 2003; van Raalte et al., 2004; Zambon et al., 2006; Bursill and Roach, 2007), activate the PPARα receptor with modulation effects of the expression of key proteins involved in the metabolism of high density lipoprotein (HDL) in the liver. Triglycerides are also involved in the same activation of PPARα by polyphenols, with an induction in the peripheral tissues of the enzyme lipoprotein lipase (LPL) leading to increased lipolysis, which would appear to result in a reduction in circulating triglycerides and a very low density lipoprotein (VLDL).

At the end of the trial, serum levels of bilirubin statistically decreased ($P < 0.05$) using verbascoside; LVB and HVB groups showed by 11.0 and 12.0% lower values, respectively, compared to the CON group. Treatment time had an influence on the reduction of bilirubin values, which decreased by 15.6 and 11.5%, respectively in the LVB and HVB groups; no change was found in the control group.

The decrease in bilirubin, in agreement with findings from our previous studies (Casamassima et al., 2011, 2012; Palazzo et al., 2011), could most likely be attributed to the antioxidant activity of polyphenols, which would seem to inhibit the biochemical mechanisms responsible for making the same bilirubin (Aliyu et al., 2007). Palanivel et al. (2008) also noted a decrease in serum bilirubin following the administration of an alcohol extract of *Pisonia aculeata* and silymarin in rats receiving a hepatotoxic compound daily for 10 days. This compound is based on carbon tetrachloride, and resulted in a loss of membrane integrity of hepatocytes and liver cell function.

The experimental treatment significantly ($P < 0.01$) influenced ROMs values, which in the LVB and HVB groups decreased by 30.6 and 37.2%, respectively, compared with the control. The effect of time on ROMs was also significant from day 28 to day 90. Values decreased markedly by 25.4 and 33.1% ($P < 0.01$) in the LVB and HVB groups, respectively; whereas the CON group did not produce any significant changes in the same time period.

At the end of the experiment, TBARS were statistically lower by 30.0 and 33.0% ($P < 0.01$), respectively in the LVB and HVB groups compared to the CON group. The time of treatment, from the beginning to the end of the trial, resulted in a reduction of 23.9 and 16.5% in the TBARS values ($P < 0.05$) in the LVB and HVB groups, while in the same time span the CON group showed a significant increase of 31.2%.

At the end of the trial the vitamin E content was influenced only by the highest level of verbascoside (HVB). The values were by 14.5% ($P < 0.05$) higher in the HVB group than in the CON group. The time effect ($P < 0.05$) was also significant for this group, whose values increased by 25.1% during the trial, whereas the LVB group did not show statistically significant variations, both compared to the CON group and in relation to the duration of the treatment. Vitamin E concentrations in the CON group remained almost unchanged throughout the trial. Vitamin A content was not affected by dietary treatment.

In blood the experimental treatment resulted in an improvement in plasma oxidative status, evidenced by a significant decrease in plasma concentration of ROMs and TBARS and an increase in vitamin E, as was already observed in previous studies (Casamassima et al., 2011, 2012; Palazzo et al., 2011). These results could well be attributed to the ability of polyphenols to neutralize the produc-

tion of free radicals in the propagation phase of the chain reaction (Funes et al., 2009). Alternatively they may inhibit the activity of pro-oxidant enzymes, which are responsible for their production, with a protective effect of LDL from the attack of free radicals (Cos et al., 2002), or even saving α-tocopherol of LDL or the regeneration of the radical forms of α-tocopherol (Zhou et al., 2005). Corino et al. (2007) also showed a significant reduction in TBARS in weaned piglets fed a supplemented concentrate with verbascoside, as did Yousef et al. (2004) in rabbits fed with isoflavones supplement. This was most likely due to the ability of phenolic compounds to provide hydrogen atoms to free radicals, leading to a protective effect from oxidative damage at the cellular level of DNA, proteins, and membrane lipids.

Productive performance

Table 4 reports on the productive performance of young hares. It shows that the treatment had no effect on most of the productive parameters. Similar values were recorded in body weight both at day 60 (P = 0.587) and at the end of the trial (day 90; P = 0.344). Growth rates were significantly different (P < 0.05) throughout the trial (days 28–90), with higher values of 6.9 and 8.7% in the LVB and HVB groups, respectively, compared to the control. Due to a general improvement in the welfare of the leverets, this result could be due to the effect of the verbascoside treatment, that a significant increase (P < 0.01) in growth rate values in the LVB and HVB groups during the 60–90-day period had determined (27.43 and 27.67 g/day vs. 24.79 g/day, respectively). The length of the trial also significantly (P < 0.05) influenced the growth rate of the animals both in the LVB and HVB groups with percentages of 16.3 and 14.3% from the first (days 28–60) to the second (days 60–90) period, respectively; no time effect on growth rate in the CON group was observed.

The growth rate observed is in accordance with the results achieved by other authors (Paci et al., 2000) in previous research on the European brown hare, naturally fed with milk and reared in cages from weaning (days 25–28) up to 270 days. During the period of 25–90 days, Paci et al (2000) found a growth rate of 18.1 g/day, which is significantly lower than that found in the same breeding period in our experiment. A positive effect

Table 4. Productive performances of leverets (n = 70 per each group) fed control diet (CON) and diets supplemented with two levels of a natural extract titrated in verbascoside (LVB and HVB supplemented with 1 and 2 kg/t feed respectively)

Items	Experimental group			SEM	Effect P
	CON	LVB	HVB		
Body weight (kg)					
Initial (day 28)	0.91	0.85	0.86	13.08	0.191
Day 60	1.64	1.61	1.64	15.17	0.587
Day 90	2.39	2.43	2.47	23.21	0.344
Growth rate (g/day)					
Days 28–60	22.91	23.59^{a}	24.20^{a}	0.35	0.329
Days 60–90	24.79^{1}	27.43^{2b}	27.67^{2b}	0.42	0.007
Whole trial (days 28–90)	23.85^{1}	25.50^{2}	25.93^{2}	0.36	0.044
Feed intake (g/day)					
Days 28–60	137.79	132.27^{a}	125.59a	4.85	0.248
Days 60–90	155.62	167.83^{b}	163.18^{b}	4.14	0.187
Whole trial (days 28–90)	146.70	150.05	144.39	3.44	0.530
Feed conversion (kg of DM/kg growth rate)					
Days 28–60	5.36	5.18	4.72	0.22	0.382
Days 60–90	5.58	5.43	5.21	0.15	0.279
Whole trial (days 28–90)	5.47	5.30	4.96	0.15	0.427

^{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)

of verbascoside supplement on growth rate was observed in our previous research into suckling lambs (Casamassima et al., 2009) and piglets in the post-weaning period (Corino et al., 2007).

In White Pannon rabbits fed a rosemary (*Rosmarinus officinalis*) and garlic (*Allium sativum*) essential oil supplement with antioxidant activity, Erdelyi et al. (2008) found an improvement in growth rate compared to the control group. In New Zealand White rabbits fed meal and seeds of black sesame (*Nigella sativa*), rich in substances with antioxidant activities such as sesamolin and sesamin, Merez et al. (2011) found an improvement (although not statistically significant) in body weight and average daily gain in treated animals. Ibrahim et al. (2000, 2002) reported a significant increase in body weight, average daily gain, and feed intake, with a slight improvement in feed conversion index in weaned rabbits fed a dietary supplement with basil (*Ocimum basilicum*), oregano (*Origanum vulgare*) and/or sage (*Salvia officinalis*) extracts.

Botsoglou et al. (2004) and Omer et al. (2010), however, did not find any significant variations in growth rate in weaned rabbits given a feed supplemented with antioxidant activity extracts.

Feed intake, as reported in Table 4, was not statistically influenced by dietary treatment; the average values varied between 144.4 and 150.1 g per animal per day among the groups compared during the trial. Time effect, however, was reflected in the values that significantly increased (by 26.9 and 29.9%; $P < 0.05$) in the LVB and HVB groups, respectively, from the first to the second trial period; no significant changes were reported for the CON group in the same period. The increased feed intake over time in the experimental LVB and HVB groups could be due to a stimulatory effect of verbascoside that provided the young hares with a greater feed intake by improving the ability of ingestion and, therefore, growth rate. The feed conversion index was not statistically influenced by experimental treatment; average values, better in the HVB and LVB groups than the control, were 4.96, 5.30, and 5.47 kg of DM/kg growth rate, respectively. In our previous studies (Casamassima et al., 2009) carried out on naturally suckling lambs, daily fed a verbascoside-based supplement, the same trend of feed conversion index was found. Other authors (Erdelyi et al., 2008) have reported a trend improvement in feed conversion ratio in White Pannon rabbits, weaned at day 23 and reared until the age of 77 days, given a feed supplemented with essential oils of rosemary and garlic. El-Manylawi and Ali (2009), on the other hand, reported a significant improvement in feed conversion rate compared to the control group after feeding New Zealand White rabbits a feed supplemented with seeds and essential oils of cumin (*Cuminum cyminum* L.).

CONCLUSION

A dietary verbascoside-based supplement, given as 1 kg/t of concentrate in the LVB group and 2 kg/t of concentrate in the HVB group, produced a better blood profile in young hares, causing a significant reduction in triglycerides and bilirubin values and an increase in HDL cholesterol. Plasma oxidative stability also improved, highlighted by a significant decrease in concentrations of ROMs and TBARS along with a marked increase in vitamin E in the highest verbascoside dose group. The experimental treatment, however, did not statistically influence most of the productive parameters except for growth rate, which was significantly better in the two experimental groups. From the present research it clearly implies that both dosages of dietary verbascoside supplement had a positive role on the growing leverets, highlighted by an improvement in the haematological profile and better growth of the animals, which was probably due to the better animal welfare expressed over time by the increased feed intake capacity.

REFERENCES

Aliyu R., Gatsing D., Jaryum K.H. (2007): The effects of *Boswellia dalzielii* (Burseraceae) aqueous bark extract on rat liver function. Asian Journal of Biochemistry, 2, 359–363.

Botsoglou N.A., Florou-Paner P., Christaki E., Giannenas I., Spais A.B. (2004): Performance of rabbits and oxidative stability of muscle tissues as affected by dietary supplementation with oregano essential oil. Archives of Animal Nutrition, 58, 209–218.

Bray Y., Champely S., Soyez D. (2002): Age determination in leverets of European hare *Lepus europaeus* based on body measurements. Wildlife Biology, 8, 31–39.

Bursill C., Roach P. (2007): A green tea catechin extract upregulates the hepatic low-density lipoprotein receptor in rats. Lipids, 42, 621–627.

Casamassima D., Palazzo M., Presutti T., Colella G.E. (2009): Productive performances, plasmatic oxidative status and some blood parameters in suckling lambs supplemented with verbascoside. Italian Journal of Animal Science, 8 (Suppl. 2), 668.

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 and plasma oxidative status in White New Zealand rabbits. In: Proc. 9th Internat. Scientific Conference on Animal Physiology. Castle Mojmirovce, Slovak Republic, 12.

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, 1–8.

Castiglione G., Castellini C., Dal Bosco A. (1996): Reproductive performances in hares reared in strict captivity. Rivista Coniglicoltura, 11, 37–40. (in Italian)

Cesarone M.R., Belcaro G., Caratelli M. (1999): A simple test to monitor oxidative stress. International Angiology, 18, 127–130.

Corino C., Cannata S., Musella M., Pastorelli G., Rossi R. (2007a): Influence of verbascoside on lipid profile in weaned piglets. Annales of Nutrition and Metabolism, 51, 270.

Corino C., Rossi R., Musella M., Cannata S., Pastorelli G. (2007b): Growth performance and oxidative status in piglets supplemented with verbascoside and teupolioside. Italian Journal of Animal Science, 6, 292–294.

Cos P., Rajan P., Vedernikova I., Calomme M., Pieters L., Vlietinck A.J., Augustyns K., Haemers A., Vanden Berghe D. (2002): *In vitro* antioxidant profile of phenolic acid derivatives. Free Radical Research, 36, 711–716.

El-Manylawi M.A., Ali H.F.M. (2009): Gas chromatography-mass spectroscopy analysis and evaluate cumin seeds and their essential oil as growth promoters of New Zealand white rabbits. International Journal of Agricultural Research, 4, 107–115.

Erdelyi M., Matics Zs., Gerencsér Zs., Princz Z., Szendrö Zs., Mézes M. (2008): Study of the effect of rosemary (*Rosmarinus officinalis*) and garlic (*Allium sativum*) essential oils on the performance of rabbit. In: Proc. 9th World Rabbit Congress. Verona, Italy, 649–654.

Esterbauer H., Zollner H. (1989): Methods for determination of aldehydic lipid peroxidation products. Free Radical Biology and Medicine, 7, 197–203.

Friedman M., Henika P.R., Mandrell R.E. (2002): Bactericidal activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella enterica*. Journal of Food Protection, 65, 1545–1560.

Funes L., Fernández-Arroyo S., Laporta O., Pons A., Roche E., Segura-Carretero A., Fernández-Gutiérrez A., Micol V. (2009): Correlation between plasma antioxidant capacity and verbascoside levels in rats after oral administration of lemon verbena extract. Food Chemistry, 14, 589–598.

Hacklander K., Arnold W., Ruf T. (2002): Postnatal development and thermoregulation in the precocial European hare (*Lepus europaeus*). Journal of Comparative Physiology A, 172, 183–190.

Ibrahim Sh.A., El-Ghamry A.A., El-Mallah G.M. (2000): Effect of some plants of *Labiatae* family as feed additives on growth and metabolic changes of rabbits. Egyptian Journal of Rabbit Science, 10, 105–120.

Ibrahim Sh.A., El-Fiky A.A., Abou-El-Ella A.A. (2002): Sage and yarrow as natural bio-stimulators and detoxicants against lead in growing rabbits diets. In: Proc. 3rd Internat. Conference on Rabbit Production in Hot Climates. Hurghada, Egypt, 541–555.

Liu M., Li J., Guo H., Lee K., Qin L., Chan K. (2003): The effects of verbascoside on plasma lipid peroxidation level and erythrocyte membrane fluidity during immobilization in rabbits: a time course study. Life Sciences, 73, 883–892.

Merez A.Z., El-Harairy M.A., Salama M.M.M. (2011): Effect of using black seed on growth performance and economical efficiency of rabbits. Mansoura University Journal of Animal and Poultry Production, 2, 13–21.

Norata G.D., Pellegatta F., Catapano A.L. (2003): "Peroxisome proliferators actived receptors" and cardiovascular diseases. Italian Heart Journal, 4, 8–18. (in Italian)

Omer H.A.A., Elallawy H.M.H., AbdEl-Samee L.D., Maghraby N. (2010): Productive performance of rabbit fed diets containing lemongrass or active dried yeast. Journal of Agriculture and Environmental Science, 7, 179–187.

Paci G., Bagliacca M., Mozzicafreddo S., Lisi E. (2000): Artificial milking in leverets (*Lepus europaeus* Pallas) born in cages and in pens. Annali della Facoltà di Medicina Veterinaria di Pisa, 52-1999, 209–219. (in Italian)

Palanivel M.G., Rajkapoor B., Senthil Kumar R., Einstein J.W., Kumar E.P., Rupesh Kumar M., Kavitha K., Pradeep Kumar M., Jayakar B. (2008): Hepatoprotective and antioxidant effect of *Pisonia aculeata* L. against CCl4-induced hepatic damage in rats. Scientia Pharmaceutica, 76, 203–215.

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

Pepin D. (1979): Body weight of hares in the Paris Basin (France). In: Myers K., Mac Innes C.D. (eds): Proc. World Lagomorph Conference. Guelph, Canada, 229–238.

Rababah T.M., Hettiarachchy N.S., Horax R. (2004): Total phenolic and antioxidant activities of fenugreek, green

tea, black tea, grape seed, ginger, rosemary, gotu kola, and ginkgo extracts, vitamin E, and *tert*-butylhydroquinone. Journal of Agricultural and Food Chemistry, 52, 5183–5186.

Spagnesi M. (1972): Short observations on breeding and growing period of European leverets (*Lepus europaeus*) born in captivity. Ricerche di Biologia della Selvaggina, 53, 1–29. (in Italian)

van Raalte D.H., Li M., Pritchard P.H., Wasan K.M. (2004): Peroxisome proliferator-activated receptor (PPAR)-α: a pharmacological target with a promising future. Pharmaceutical Research, 21, 1531–1538.

Yousef M.I., Kamel K.I., Esmail A.M., Baghdadi H.H. (2004): Antioxidant activities and lipid lowering effects of isoflavone in male rabbits. Food and Chemical Toxicology, 42, 1497–1503.

Zambon A., Gervois P., Pauletto P., Fruchart J.Ch., Staels B. (2006): Modulation of hepatic inflammatory risk markers of cardiovascular diseases by PPAR-α activators. Clinical and experimental evidence. Arteriosclerosis, Thrombosis, and Vascular Biology, 26, 977–986.

Zhao B., Tham S.-Y., Lu J., Lai M.H., Lee L.K.H., Moochhala S.M. (2004): Simultaneous determination of vitamins C, E and β-carotene in human plasma by high-performance liquid chromatography with photodiode-array detection. Journal of Pharmacy and Pharmaceutical Sciences, 7, 200–204.

Zhou B., Wu L.M., Yang L., Liu Z.L. (2005): Evidence for α-tocopherol regeneration reaction of green tea polyphenols in SDS micelles. Free Radical Biology and Medicine, 38, 78–84.

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