



## The effects of lemon verbena (*Lippia citriodora*) verbascoside on the productive performance, plasma oxidative status, and some blood metabolites in suckling lambs

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**ABSTRACT.** The effects of two doses of a verbascoside-based dietary supplement on performance, some blood parameters, and plasma oxidative status in Lacaune suckling lambs were investigated. Twenty-four lambs were divided into three groups of eight animals each and fed *per os* a dietary supplement with a plant extract from *Lippia citriodora* containing a phenylpropanoid glycoside, verbascoside: the low-verbascoside (LVB) group received 2.5 mg per animal per day of natural extract, the high-verbascoside (HVB) group received 5.0 mg per animal per day, the control group (CON) did not receive dietary supplementation. The body weight, average daily gain, milk consumption and milk conversion index were determined for the experimental and control animals and blood samples were taken from all lambs. Dietary supplementation with verbascoside in both the LVB and HVB groups resulted in improved average daily gain ( $P < 0.01$ ), milk consumption ( $p < 0.05$ ), and serum lipid profile; the groups showed a decrease in triglycerides ( $p < 0.05$ ) and an increase in high density lipoprotein cholesterol levels ( $p < 0.05$ ). Plasma oxidative status in lambs supplemented with verbascoside showed an improvement in homeostatic stability that induced a significant decrease in reactive oxygen metabolites ( $P < 0.01$ ) and thiobarbituric acid-reactive substances ( $p < 0.05$ ) concentrations and a marked increase in serum concentrations of vitamin E ( $P < 0.01$ ) and vitamin A ( $p < 0.05$ ).

### Introduction

Oxidative stress is a particular condition of an organism that is caused by an accentuation, in the pro-oxidant sense, of the dynamic balance between oxido-reduction processes occurring in the cell with the production of radical species (Sies, 1991). Many substances present in plants are able to neutralize

the action of free radicals or to interrupt the chain reactions leading to their formation, thus inhibiting the propagation of cellular damage.

Amongst natural active compounds that have demonstrated antioxidant properties, verbascoside, a phenylpropanoid glycoside, is present in numerous medicinal plants and is called pseudo-ginseng by the populations of Northeast China.

Many studies have shown that verbascoside has anti-inflammatory (Deepak and Handa, 2000; Marzocco et al., 2007; Esposito et al., 2010), antibacterial (Ellis, 1983), antiviral (Kernan et al., 1998), immunomodulatory (Akbay et al., 2002) and antioxidant (Corino et al., 2007a,b; Rossi et al., 2009; Palazzo et al., 2011) effects by providing a scavenger effect on reactive oxygen species (Gao et al., 1999).

In our previous research (Casamassima et al., 2011, 2012; Palazzo et al., 2011) on the brown hair rabbit, and dairy sheep using verbascoside as a dietary feed, a general improvement of blood lipid profiles, plasmatic oxidative status, in particular, significant decreases of reactive oxygen metabolites (ROMs) and thiobarbituric acid reactive substances (TBARS), and an increase of endogenous vitamin A and E reserves were noted. Also, a marked increase in the milk yield of ewes was demonstrated.

The antioxidative properties of plant extracts, which can also stimulate the appetite and positively affect the secretion of endogenous digestive enzymes and influence blood constituents, are particularly important for organisms (Lee et al., 1995; Grassmann et al., 2000; Yoshioka et al., 2000; Perucka and Materka, 2001).

The administration of dietary verbascoside-based feed in suckling lambs could be useful in minimizing some types of stress on digestive organs by protecting the gastrointestinal tract from colitis (Esposito et al., 2010).

The objective of this study was to assess the effects of two doses of a verbascoside-based dietary supplement on some blood parameters, plasma oxidative status, and productive performance of suckling lambs.

## Material and methods

### Animals and diets

All experimental procedures involving animals were in accordance with European Community guidelines and approved by the Italian Ministry of Health.

Lacaune male lambs ( $n = 24$ ; age 1–3 days; born from single lambings, initial body weight  $5.08 \pm 0.46$  kg) were weighed at the beginning of the trial, divided by body weight (BW), and randomly assigned to individual treatments ( $n = 8$  per treatment). Throughout the trial lasting 42 days, the body health condition of the animals was good and the lambs received exclusively maternal milk. The ewes, intensively reared and multiparous, were

fed on 1.8 kg of permanent meadow polyphyte hay: crude protein (CP) – 8.4%, neutral detergent fibre (NDF) – 64.6%, acid detergent fibre (ADF) – 48.0%, acid detergent lignin (ADL) – 6.7%, ash – 10.2% dry matter (DM), and 700 g of a concentrate mixture (G.I.Ma., Perugia, Italy) with the following chemical composition: CP – 18.9%, crude fibre – 11.5%, ether extract – 2.9%, NDF – 13.8%, ADF – 7.3%, ADL – 1.5%, ash – 2.5% DM.

The treatments differed in terms of the dose of administered verbascoside: 0, 2.5, or 5 mg per animal per day (control group – CON, low-level verbascoside group – LVB, and high-level verbascoside group – HVB). An extract from *Lippia citriodora* leaves standardized to 20% (w/w) of verbascoside (VB; Figure 1), was used (Mazzon et al., 2009). The antioxidant supplement was prepared on an industrial scale by a standardized procedure that includes ultrasonic extraction with 60% aqueous ethyl alcohol followed by spray-drying with maltodextrins as an excipient. Verbascoside is present in an amount over 80% (w/w) of total phenylpropanoids, together with a minor mixture (<10%) of other caffeic acid derivatives (from the certificate of analysis provided by the manufacturer, I.R.B. s.r.l., Altavilla Vicentina, Vicenza, Italy). This extract, in aqueous solution, was administered daily *per os* according to the experimental dosage at 9 a.m. by means of a dispenser equipped with an appropriate dispensing device (Pfizer Animal Health, Latina, Italy).

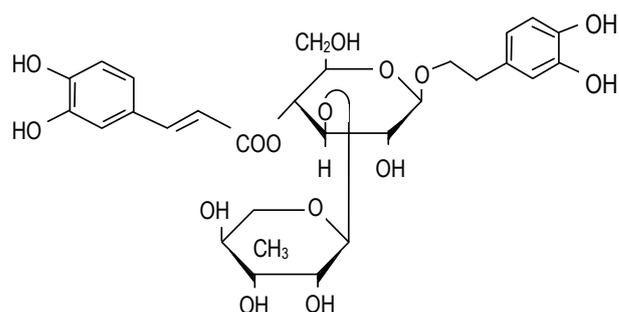


Figure 1. Chemical structure of verbascoside

During the trial, the lambs underwent the following experimental assessment: BW at 0, 14, 28, 42 days, and average daily gain (ADG) in the periods of 0–14 d, 14–28 d and 28–42 d; after a twelve-hour fast, blood samples were collected into a vacutainer from the external jugular vein on days 0, 21 and 42; daily milk consumption, using the system of double weighing, on days 0, 14, 28, and 42 was determined. On the control day, the lambs were kept away from their mothers for 24 h, except for two one-hour suckling periods at 8 a.m. and 8 p.m.

The lambs were weighed before and after each suckling period.

Daily milk consumption (between days 0–14, 14–28 and 28–42) was reported as an average of the values between two consecutive control days. Daily milk consumption was used to calculate the milk conversion index in each interval period.

### Blood analysis

Blood samples were stored in three vials: the first used a separating gel to produce serum, the second used lithium heparin to produce plasma, the third used EDTA for the haemochromocytometric test. Blood was centrifuged for 15 min at 2000 g and at room temperature and the serum was immediately tested for the following: triglycerides, total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), albumin, urea, creatinine and bilirubin using an automatic clinical chemistry analyzer, model ARCO (Biotecnica Instruments S.p.A., Italy). The concentration of ROMs in plasma was determined using a colorimetric method, as proposed by Diacron (Grosseto, Italy) and a specific commercial kit, absorbance was measured in a spectrophotometer at a wavelength of 505 nm (Cesarone et al., 1999). The results are expressed in arbitrary units called 'Carratelli units' (Carr U) where 1 CARR corresponds to  $0.024 \text{ mmol} \cdot \text{l}^{-1}$  of  $\text{H}_2\text{O}_2$ .

Plasma TBARS were determined according to Esterbauer and Zollern (1989). Briefly, a standard curve was generated using 1,1,3,3-tetramethoxypropane (Sigma Aldrich, St. Louis). Trichloroacetic acid (10% v/v) was added to the plasma samples to precipitate proteins. The resulting mixture was incubated for 15 min on ice. After centrifugation at 2200 rpm at 4°C for 15 min, 0.67% thiobarbituric acid was added to the supernatant. The mixture was incubated in a water bath at 90°C for 10 min, after which the absorbance was read at 532 nm in a spectrophotometer. The results were expressed as  $\mu\text{mol}$  of thiobarbituric acid per litre of plasma.

Vitamins A and E were extracted from plasma samples with chloroform (Zhao et al., 2004) and analysed on an HPLC system (Kontron Instruments, Italy) consisting of an autosampler (HPLC Autosampler 360) with a 20  $\mu\text{l}$  loop, a high pressure mixing pump, and a 5  $\mu\text{m}$ , 250  $\times$  4.60 mm C18 column (Phenomenex, Torrance, CA, USA). The mobile phase was nitrile acid and methanol (75:25 v/v) at a flow rate of  $1.0 \text{ ml} \cdot \text{min}^{-1}$ . A fluorimeter detector (SFM) and computer with Kroma System

2000 software were used. The concentrations of vitamins A and E were determined using an internal standard and the elution time of pure standards.

Whole blood was tested for haematological parameters, including red blood cells (RBC), white blood cells (WBC), haemoglobin (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and platelets (PLT) using an automatic blood cell counter SEAC (Radim Company, Italy).

### Statistical analysis

Analysis of variance was performed on all of the variables after the normality of the frequency distribution was assessed by the GLM procedure for repeated measurements of the SPSS statistical package (2010). Analysis included between-subjects main effect (D) of dietary supplementation (CON, LVB, and HVB), within-subjects main effect of sampling time (T), and the interaction of dietary supplementation  $\times$  sampling time (D  $\times$  T). An individual animal was the experimental unit. Data are presented as means and standard errors of groups. Differences were tested by the Scheffé test and considered significant for at least  $p < 0.05$ .

## Results

### Productive performance

In Table 1, the average values of the BW, ADG, milk consumption and milk conversion index of lambs are shown. The final BW (42 d) of the HVB group lambs was higher ( $p < 0.05$ ) than in the CON group. During the whole experimental period, the ADG values were higher ( $P < 0.01$ ) in the LVB and HVB groups than in the CON group.

In particular, the values of the weight gains in the CON group were lower ( $p < 0.05$ ) than those observed in the HVB group during the first trial period (0–14 d), and the differences also involved the LVB group in the second period (28–42 d). The ADG of lambs was significantly ( $p < 0.05$ ) affected by the effect of time only in the LVB and HVB groups. Average daily milk consumption, calculated over the entire trial, was higher ( $p < 0.05$ ) in the HVB group compared with the CON group, becoming significantly ( $p < 0.05$ ) higher in the last trial period (28–42 d).

The milk conversion index was not affected by the experimental dietary supplementation.

**Table 1.** Body weight, daily gains, milk consumptions and milk conversion index in suckling lambs

Item	Experimental group <sup>†</sup>			SEM	P-value <sup>‡</sup>		
	CON	LVB	HVB		D	T	D x T
Lambs, no.	8	8	8				
Body weight, kg							
0 d	5.11	4.90	5.24	0.09	0.187		
14 d	7.75	7.78	8.38	0.14	0.232		
28 d	10.70	10.73	11.47	0.17	0.428		
42 d	13.42 <sup>1</sup>	13.98	14.96 <sup>2</sup>	0.22	0.008	0.007	0.011
Average daily gain, g · d <sup>-1</sup>							
0–14 d	188.6 <sup>1</sup>	205.8 <sup>a</sup>	224.3 <sup>2a</sup>	5.6	0.027		
14–28 d	210.8	210.6	220.7	7.7	0.285		
28–42 d	194.2 <sup>1</sup>	232.1 <sup>2b</sup>	249.3 <sup>2b</sup>	8.5	0.018		
whole trial (0–42 d)	197.6 <sup>1</sup>	216.6 <sup>2</sup>	234.1 <sup>2</sup>	4.1	0.001	0.021	0.258
Milk consumption, g · d <sup>-1</sup>							
0–14 d	944.2	958.5	1054.9	22.9			
14–28 d	979.1	948.2	997.3	29.1			
28–42 d	934.3 <sup>1</sup>	1023.1	1121.4 <sup>2</sup>	34.1			
whole trial (0–42 d)	952.6 <sup>1</sup>	976.6	1057.9 <sup>2</sup>	15.8	0.032	0.523	0.456
Milk conversion index kg of milk · kg <sup>-1</sup> weight gain							
0–14 d	4.98	4.66	4.69	0.17			
14–28 d	4.64	4.50	4.52	0.13			
28–42 d	4.81	4.41	4.48	0.05			
whole trial (0–42 d)	4.81	4.52	4.55	0.10	0.275	0.227	0.111

<sup>†</sup> CON – control diet; LVB – 2.5 mg verbascoside per animal per day<sup>-1</sup>; HVB – 5.0 mg verbascoside per animal per day<sup>-1</sup>; <sup>‡</sup> D – fixed effect of dietary supplementation; T – fixed effect of time; D x T – interaction dietary supplementation x time; <sup>1,2</sup> within a row, means without a common superscript differ ( $p < 0.05$ ); <sup>a,b</sup> within a column, means without a common superscript differ ( $p < 0.05$ )

**Table 2.** Blood parameters of lipid profile in suckling lambs

Item	Experimental group <sup>†</sup>			SEM	P-value <sup>‡</sup>		
	CON	LVB	HVB		D	T	D x T
Lambs, no.	8	8	8				
Triglycerides, mmol · l <sup>-1</sup>							
0 d	0.24	0.25 <sup>a</sup>	0.25 <sup>a</sup>	0.01			
21 d	0.25 <sup>1</sup>	0.24 <sup>1a</sup>	0.20 <sup>2b</sup>	0.01			
42 d	0.26 <sup>1</sup>	0.22 <sup>2b</sup>	0.17 <sup>3c</sup>	0.01	0.035	0.025	0.001
Total cholesterol, mmol · l <sup>-1</sup>							
0 d	1.91	1.93	1.93 <sup>a</sup>	0.02			
21 d	1.89	1.87	1.88	0.03			
42 d	1.92	1.86	1.85 <sup>b</sup>	0.02	0.313	0.041	0.107
HDL cholesterol, mmol · l <sup>-1</sup>							
0 d	0.78	0.79 <sup>a</sup>	0.78 <sup>a</sup>	0.01			
21 d	0.76 <sup>1</sup>	0.86 <sup>2b</sup>	0.87 <sup>2b</sup>	0.01			
42 d	0.77 <sup>1</sup>	0.91 <sup>2b</sup>	0.92 <sup>2b</sup>	0.07	0.031	0.008	0.039
LDL cholesterol, mmol · l <sup>-1</sup>							
0 d	1.02	1.02 <sup>a</sup>	1.04 <sup>a</sup>	0.09			
21 d	1.01	0.90	0.92	0.03			
42 d	1.03 <sup>1</sup>	0.86 <sup>2b</sup>	0.85 <sup>2b</sup>	0.03	0.192	0.030	0.213

<sup>†</sup> CON – control diet; LVB – 2.5 mg verbascoside per animal per day<sup>-1</sup>; HVB – 5.0 mg verbascoside per animal per day<sup>-1</sup>; <sup>‡</sup> D – fixed effect of dietary supplementation; T – fixed effect of time; D x T – interaction dietary supplementation x time; <sup>1,2,3</sup> within a row, means without a common superscript differ ( $p < 0.05$ ); <sup>a,b,c</sup> within a column, means without a common superscript differ ( $p < 0.05$ )

### Blood parameters

In Table 2, the values of the blood metabolites related to the lipid profile of lambs are reported. Serum concentrations of triglycerides and HDL-C were affected by the dietary treatment with VB ( $p < 0.05$ ).

Triglycerides were lower in the HVB group than in the CON group at 21 and 42 d. In contrast, the LVB group showed a reduction in triglycerides compared with the CON group only on day 42. Moreover, on day 42, the levels of triglycerides in the LVB group were significantly higher than in the HVB group. A time effect ( $p < 0.05$ ) on serum triglycerides was observed in lambs given dietary supplementation, with lower values at the end of the trial in comparison with the initial values. The CON group did not show appreciable variations as the trial progressed. Triglycerides showed a significant interaction between treatment with verbascoside and duration of administration ( $P < 0.01$ ) leading, during the trial, to sensitive changes in blood parameters as the duration of administration increased.

Total cholesterol was not statistically affected by the dietary supplementation. In the HVB group, a time effect was observed with a significant ( $p < 0.05$ ) decrease in total cholesterol (0 d vs 42 d).

Supplementation with verbascoside significantly ( $p < 0.05$ ) increased HDL-C. In both the LVB and HVB groups, the values of HDL cholesterol increased ( $P < 0.01$ ) from the beginning to the end of the trial. The CON group did not show appreciable variations during the trial. Also, a significant ( $p < 0.05$ ) relationship between verbascoside treatment and duration of supplementation was observed.

LDL-C was not significantly affected by dietary supplementation, although in the LVB and HVB groups, its values drastically decreased ( $p < 0.05$ ) from 0 to 42 d, while the CON group did not show any variation.

In Table 3, the values of the tested haemochromocytometric parameters are reported: RBC, WBC, HGB, HCT, MCV, MCH, MCHC and PLT, as well as the serum concentrations of glucose, total proteins, albumin, urea, creatinine, magnesium, chlorine, sodium, potassium and bilirubin. Data in Table 3, except bilirubin, were synthesized by reporting only the values obtained from the average of the samples taken on days 0, 21 and 42 because the experimental treatment did not produce any statistical differences. On day 42, the blood levels of bilirubin were lower in the HVB group than in the CON group. Also, the experimental HVB group showed a significant reduction ( $p < 0.05$ ) in values from the beginning to the end of the test. In contrast,

no significant differences were found in the CON group in the same period of time.

The administration of VB significantly affected the plasma markers of oxidative status (Table 4).

The dietary treatment reduced the values of ROMs ( $P < 0.01$ ), which was already observed at mid-trial (21 d) and was confirmed on 42 d (223.87 and 162.97 vs 421.86  $U \cdot Carr^{-1}$ , respectively). Regarding the variability of ROMs during the trial, we found an increase ( $P < 0.01$ ) in the values in the CON group compared with a decrease in the HVB group, whereas the values remained unchanged in the LVB group.

The dietary supplementation with VB led to a reduction ( $p < 0.05$ ) in TBARS. At the end of the trial, the LVB and HVB groups showed lower values of TBARS than the CON group. A significant time effect was found; during the trial TBARS values decreased ( $p < 0.05$ ) in the LVB and HVB groups, respectively, but remained unchanged in the CON group.

The plasma levels of vitamin E were significantly affected ( $P < 0.01$ ) by the experimental treatment. In the HVB group, vitamin E was higher ( $P < 0.01$ ) than in the CON and LVB groups after 21 days of the trial. The values registered on day 42 confirmed the higher levels of vitamin E in the HVB group than in the LVB and CON groups (12.837 vs 8.007 and 1.439  $\mu mol \cdot l^{-1}$ , respectively) and a significant increase in those levels between CON and LVB was found. Moreover, in the LVB and HVB groups, a time effect on the values of vitamin E was shown, with a significant increase ( $P < 0.01$ ) from the beginning to the end of the trial, while in the CON group it remained unchanged over the same period of time.

The plasma concentration of vitamin A was affected ( $p < 0.05$ ) by the dietary supplementation. In particular, at the end of the trial (42 d), the HVB group showed a higher concentration of vitamin A than the CON and LVB groups ( $p < 0.05$ ). Furthermore, a significant time effect ( $p < 0.05$ ) was observed for vitamin A in the HVB group, with an increase of the values from the beginning to the end of the trial. No statistical differences were recorded in the CON and LVB groups during the same period. All of the parameters in Table 4, except TBARS values, showed a significant interaction between treatment with verbascoside and duration of administration ( $p < 0.05$ ), indicating that during the trial, sensitive changes occurred in blood parameters as the duration of administration increased.

**Table 3.** Some blood parameters in suckling lambs\*

Item	Experimental group <sup>†</sup>			SEM	P-value <sup>‡</sup>		
	CON	LVB	HVB		D	T	D x T
Lambs, no.	8	8	8				
RBC, x 10 <sup>12</sup> · l <sup>-1</sup>	8.79	8.89	8.90	0.14	0.184	0.191	0.167
WBC, x 10 <sup>9</sup> · l <sup>-1</sup>	8.90	8.67	8.69	0.09	0.210	0.813	0.808
HGB, g · l <sup>-1</sup>	106.2	102.4	107.0	1.24	0.478	0.775	0.170
HCT, %	29.49	28.96	29.32	0.85	0.185	0.112	0.895
MCV, µm <sup>3</sup>	33.50	33.27	33.54	0.39	0.278	0.785	0.169
MCH, pg	11.11	11.54	11.59	0.18	0.204	0.406	0.636
MCHC, g · dl <sup>-1</sup>	39.61	40.99	40.90	1.26	0.685	0.531	0.433
PLT, x 10 <sup>9</sup> · l <sup>-1</sup>	319.50	302.05	346.33	31.82	0.208	0.167	0.275
Albumin, g · l <sup>-1</sup>	22.20	21.90	22.50	0.21	0.063	0.654	0.710
Urea, mmol · l <sup>-1</sup>	5.46	5.58	5.49	0.05	0.329	0.099	0.074
Creatinine, µmol · l <sup>-1</sup>	69.84	68.20	68.07	0.44	0.351	0.215	0.343
Bilirubin, µmol · l <sup>-1</sup>							
0 d	6.39	6.48	6.53 <sup>a</sup>	0.11			
21 d	6.50	6.21	6.11	0.22			
42 d	6.57 <sup>1</sup>	5.83	5.70 <sup>2b</sup>	0.26	0.101	0.035	0.078

\* all data reported, excluding those related to bilirubin, were obtained from average of the samples taken at 0 d, 21 d and 42 d; <sup>†</sup> CON – control diet; LVB – 2.5 mg verbascoide per animal per day<sup>-1</sup>; HVB – 5.0 mg verbascoide per animal per day<sup>-1</sup>; <sup>‡</sup> D – fixed effect of dietary supplementation; T – fixed effect of time; D x T – interaction dietary supplementation x time; <sup>1,2</sup> within a row, means without a common superscript differ ( $p < 0.05$ ); <sup>a,b</sup> within a column, means without a common superscript differ ( $p < 0.05$ ); RBC – red blood cells; WBC – white blood cells; HGB – haemoglobin; HCT – haematocrit; MCV – mean corpuscular volume; MCH – mean corpuscular haemoglobin; MCHC – mean corpuscular haemoglobin concentration; PLT – platelets

**Table 4.** Plasma oxidative status in suckling lambs

Item	Experimental group <sup>†</sup>			SEM	P-value <sup>‡</sup>		
	CON	LVB	HVB		D	T	D x T
Lambs, no.	8	8	8				
ROMs, U · Carr <sup>-1</sup>							
0 d	214.38 <sup>a</sup>	215.61	212.21 <sup>a</sup>	6.37			
21 d	362.80 <sup>1b</sup>	224.12 <sup>2</sup>	220.44 <sup>2a</sup>	14.80			
42 d	421.86 <sup>1b</sup>	223.87 <sup>2</sup>	162.97 <sup>3b</sup>	25.11	0.006	0.005	0.017
TBARS, µmol · l <sup>-1</sup>							
0 d	0.242	0.300 <sup>a</sup>	0.250 <sup>a</sup>	0.020			
21 d	0.230	0.157 <sup>b</sup>	0.218	0.027			
42 d	0.233 <sup>1</sup>	0.136 <sup>2b</sup>	0.110 <sup>2b</sup>	0.013	0.022	0.032	0.182
Vitamin E, µmol · l <sup>-1</sup>							
0 d	0.726	1.092 <sup>a</sup>	0.778 <sup>a</sup>	0.110			
21 d	0.524 <sup>1</sup>	0.801 <sup>1a</sup>	6.575 <sup>2b</sup>	0.835			
42 d	1.439 <sup>1</sup>	8.007 <sup>2b</sup>	12.837 <sup>3c</sup>	1.257	0.003	0.031	0.047
Vitamin A, µmol · l <sup>-1</sup>							
0 d	0.057	0.058	0.058 <sup>a</sup>	0.003			
21 d	0.061	0.070	0.085 <sup>b</sup>	0.007			
42 d	0.061 <sup>1</sup>	0.073 <sup>1</sup>	0.132 <sup>2c</sup>	0.011	0.008	0.042	0.033

<sup>†</sup> CON – control diet; LVB – 2.5 mg verbascoide per animal per day<sup>-1</sup>; HVB – 5.0 mg verbascoide per animal per day<sup>-1</sup>; <sup>‡</sup> D – fixed effect of dietary supplementation; T – fixed effect of time; D x T – interaction dietary supplementation x time; <sup>1,2,3</sup> within a row, means without a common superscript differ ( $p < 0.05$ ); <sup>a,b,c</sup> within a column, means without a common superscript differ ( $p < 0.05$ )

## Discussion

### Productive performance

The improvement of weight gain in LVB and HVB suckling lambs could be ascribed to the significant improvements of the blood lipid profile characterized by reduction of ROMs and TBARS values, and an increase in vitamin A and E levels. This could have had a positive effect on the lambs' wellbeing and growth due to a stronger suckling stimulus and increased milk consumption.

Bampidis et al. (2005) observed improvements in the growth of experimental lambs fed a garlic-based feed supplement, due to the reduction of undesirable bacterial microflora, with improvement of nutrient absorption.

In an experiment carried out on fattening lambs that consumed mixed feed with a blend of essential oil compounds having antioxidant activity, Ozdogan et al. (2011) showed a slight improvement in BW and ADG, which could likely be due to the modification of rumen volatile fatty acid production by increasing the acetate-to-propionate ratio, inhibiting deamination, and the direct inhibition of methane production (Busquet et al., 2006; Castillejos et al., 2007).

Corino et al. (2007b) conducted a similar study on piglets in post-weaning; piglets were fed with a concentrate that was supplemented with two levels of VB, and the authors found considerable growth improvement in the subjects that were fed the highest level of VB. The beneficial effect of phenols and polyphenols can be ascribed, according to some authors (Giannenas et al., 2003; Arcila-Lozano et al., 2004; Bozin et al., 2006), to the antimicrobial and antifungal activity inherent in phenolic compounds. Other authors (Esposito et al., 2010; Rossi et al., 2011) have attributed that effect to the anti-inflammatory and immunomodulating activity of phenol compounds.

### Blood parameters

All of the values of the blood metabolites registered in this study were in the normal ranges for this species (Casamassima et al., 1997; Bornez et al., 2009).

The administration of VB (LVB and HVB groups) produced a general improvement in the lipid profile in lambs; this finding was highlighted by a significant decrease in triglycerides and an increase in HDL-C.

The dietary effect on the increase of plasma HDL-C concentration may be ascribed to the effect

of such phenylpropanoid glycosides as verbascoside (Carrera-Quintanar et al., 2010) acting as natural ligands of nuclear hormone receptors, peroxisome-proliferator-activated receptors (PPARs) that are involved in lipid and sugar metabolism (Goto et al., 2010; Norata et al., 2003), determining the activation of PPAR $\alpha$  receptors with the effect of modulating, on the hepatic level, the expression of key proteins involved in the metabolism of high-density lipoproteins such as both apolipoprotein A-I (Apo A-I) and apolipoprotein A-II (Apo A-II). Triglycerides would also be involved in the same mechanism of activation of PPAR $\alpha$ ; thus, this activation would induce the expression of lipoprotein lipase in peripheral tissues and induce an increase in lipolysis that would translate into a decrease in circulating triglycerides and very-low-density lipoproteins.

Our results agree with those of other authors on different species. Improvement of lipid profiles was observed in our earlier work on Italian hares (*Lepus corsicanus*), rabbits, and dairy ewes intensively reared and fed diets supplemented with water-soluble extracts of Verbenaceae (*Lippia* spp.) leaves (Casamassima et al., 2011, 2012; Palazzo et al., 2011). In particular, a decrease of total cholesterol and triglycerides and an increase of HDL-C was found. In piglets, Corino et al. (2007a) observed that dietary supplementation with VB significantly reduced serum LDL-C levels with lower values in high-verbascoside-fed piglets.

The lower bilirubin content observed in the HVB at the end of the trial than in the CON and LVB groups may be due to the antioxidant activity of polyphenols, which could inhibit the biochemical mechanisms responsible for the formation of bilirubin. In rabbits that ingested increasing (0, 15 and 30  $\mu\text{g} \cdot \text{kg}^{-1}$  BW) doses of aflatoxin B1 every day, Yousef et al. (2003) observed a decrease in bilirubin due to the use of antioxidant substances, such as ascorbic acid, in their diet.

In the present study, dietary supplementation with VB resulted in an improvement in the plasma oxidative status as observed in our earlier works (Casamassima et al., 2011, 2012; Palazzo et al., 2011). This result might be attributed to the ability of polyphenols to neutralize free-radical production at the stage of propagation of the chain reaction (Funes et al., 2009) or to inhibit the activity of pro-oxidant enzymes responsible for their production, thus protecting both LDL lipids and the  $\alpha$ -tocopherol of LDL from the attack of radicals (Cos et al., 2002), and promoting regeneration of the radical forms of  $\alpha$ -tocopherol (Zhou et al., 2005).

In our studies carried out to assess the physiological effects of dietary supplements with antioxidant activity (Casamassima et al., 2011, 2012; Palazzo et al., 2011), a significant decrease of plasma levels of ROMs and TBARS was observed. Also Li et al. (1999) reported similar results in a study on skeletal muscles of rats that were subjected to effort. Corino et al. (2007b) fed weaned piglets with a concentrate supplemented with VB and a significant decrease in the ROMs concentration in the plasma was found. In rabbits, Liu et al. (2003) observed a decrease in TBARS. In animals fed a verbascoside-based dietary supplement, the increase of plasma vitamins A and E could be due to the ability of phenylpropanoid glycoside to improve and protect the endogenous antioxidant system by better control of oxidative metabolism and the induction of antioxidant activity enzymes (Liao and Yin, 2000). Similarly to what we observed in the present study, the increase in plasma levels of vitamins A and E, apart from being stimulated by the use of antioxidants, was also affected by the supplementation of these vitamins in the diet (Corino et al., 1999; Oriani et al., 2001).

## Conclusions

Dietary supplementation with verbascoside (VB) at the highest dose produced a significant improvement in average daily gain and milk consumption. No influence was observed, however, on the milk conversion index. The administration of VB showed a significant improvement of the blood lipid profile, highlighted by a decrease in triglycerides and an increase in high-density lipoprotein cholesterol in the low-verbascoside (LVB) and high-verbascoside (HVB) lambs. Even if the groups demonstrated a decrease in values, total cholesterol and low-density lipoprotein cholesterol (LDL-C) did not differentiate among the compared groups, although the LVB and HVB groups showed a significant decrease in LDL-C at the end of the trial.

The supplementation of VB improved the plasma oxidative status in suckling lambs by affecting homeostatic stability that determined a significant decrease in the concentrations of reactive oxygen metabolites and thiobarbituric acid reactive substances and a marked increase in serum concentrations of vitamins E and A. The obtained results show that the use of the phenylpropanoid glycoside, verbascoside, led to an improvement in some production and blood parameters in suckling lambs.

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