Influence of *Lippia citriodora* verbascoside on growth performance, antioxidant status, and serum immunoglobulins content in piglets

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ABSTRACT: Phytogenic feed additives are used in animal feeding to improve livestock performance. The aim of this study was to determine whether a dietary plant extract from *Lippia citriodora*, standardized for verbascoside, can modify various immunological, oxidative, and biochemical serum parameters in weaned piglets. A total of 144 piglets, half female and half barrows (7.99 ± 1.40 kg BW), were allocated to three dietary treatments with six replicates per treatment (pens of 8 piglets each). Piglets were supplemented with the following levels of plant extract standardized for verbascoside: 0 (CON = control group), 5 (LV = low verbascoside group), or 10 (HV = high verbascoside group) mg/kg of diet for 56 days. Body weight (BW) and feed consumption were recorded at days 0 and 56 to determine the average daily gain and gain : feed. Twelve piglets from each treatment were randomly selected, and blood was collected by anterior vena cava puncture on days 0, 14, and 56 for glucose, triglycerides, cholesterol, urea, and oxidative status, and on days 0 and 56 for IgG and IgA concentrations. The HV group grew more ($P < 0.05$) than the CON and LV groups. No significant differences were observed for any of the biochemical parameters between the groups; urea, high density lipoprotein cholesterol, total cholesterol, and low density lipoprotein cholesterol increased significantly over time. Reactive oxygen metabolites (ROM) showed significant time, time × treatment, and treatment effects ($P < 0.001$). Both serum Igs increased ($P < 0.005$, $P < 0.001$ for IgG and IgA respectively) over time in all groups; treatment ($P < 0.05$), and time × treatment ($P = 0.056$) effects were found for serum IgA concentration. The *Lippia citriodora* verbascoside positively influenced antioxidant status and IgA content with a tendential effect on growth performance.

Keywords: verbascoside; immunoglobulin; reactive oxygen metabolite; piglet

In January 2006 the use of antibiotics as growth promoters was prohibited in the European Union, largely due to concerns regarding bacterial resistance to antibiotics and consumer food safety. The development of alternative additives and strategies to replace the role of antibiotic feed additives in pig production is therefore of major concern. Particular interest is now being paid to phytogenic feed additives (PFAs). PFAs are natural substances that contain low-molecular-weight reactive oxygen species-scavenging substances, which include polyphenols. Verbascoside is a natural phenethylalcohol glycoside that occurs in many plants belonging to Bignoniaceae, Buddlejaceae, Gesneriaceae, Labiatae, Oleaceae, Orobanchaceae, Scrophulariaceae, and Verbenaceae. Verbascoside contains a rhamnose unit bound to glucose, where the glucose acts as a bridge and belongs to the extensive family of phenylpropanoids.

Verbascoside exhibits a number of biological activities including anti-oxidative, anti-bacterial, and anti-tumor actions as recently reported by Santoro et al. (2008). Recently, Rossi et al. (2009a) found that verbascoside has a greater antioxidant power compared to other phenolic compounds and compared to trolox, a standard water soluble

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antioxidant analogue of vitamin E. It showed an antioxidant activity equal to 4.22 EAR (mg equivalent trolox/g).

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). In particular this species scaveneges the hydroxyl radical in a concentration-dependent manner. It is well known that *Lippia* contains phenolic compounds, including iridoids, flavonoids, phenolic acids, and phenylpropanoids with interesting antioxidant properties.

Phenylpropanoids, particularly verbascoside, are the most abundant compounds in *Lippia* extracts (Funes et al., 2009; Quintanar et al., 2010).

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*.

The effects of nutrition on oxidative stress in pigs have been widely studied, since the ability of pigs to neutralize reactive oxygen species (ROS) plays a key role in their welfare and performance (Lauridsen et al., 1999; Brambilla et al., 2001; 2002). Oxidative stress occurs when the antioxidant system is overwhelmed by the production of ROS which can lead to increased prevalence of infectious disease via an impaired immune cell function. On the basis of recent studies (Quintanar et al., 2010; Bergman 2011) we also believe that due to its antioxidant activity, verbascoside could contribute to maintaining an effective immune response.

The aim of this study was to assess whether dietary supplementation of piglets with a plant extract standardized for verbascoside exerted any effect on growth performance, oxidative, immunological, and biochemical blood status in the post-weaning period.

MATERIAL AND METHODS

Animal and dietary treatments

A total of 144 Dalland piglets, with an average age of 24 days (7.99 ± 0.85 kg BW) were allotted randomly to each treatment with weight, gender, and ancestry equalized among treatments. The treatments were applied to the three tested groups, each with six replicates, with eight pigs per pen, half barrows and half females.

Three dietary treatments were given to the pigs with differing levels of plant extract standardized for verbascoside: 0 (CON = control group), 5 (LV = low verbascoside group), and 10 (HV = high verbascoside group) mg/kg of diet for 56 days.

A commercial extract from *Lippia citriodora* was used, which was devoid of essential oils, and standardized in 20% (w/w) of verbascoside (I.R.B. s.r.l., Altavilla Vicentina, Vicenza, Italy).

To avoid oxidation in the complete feed, the supplement was microencapsulated within a protective matrix of hydrogenated vegetable lipids using spray-cooling (Sintal Zootecnica, Isola Vicentina, Vicenza, Italy).

The pigs were housed in an environmentally-controlled nursery room and given *ad libitum* access to water and a pelleted diet (Table 1) formulated to meet or exceed the nutritional requirements of a weaned piglet (NRC, 1998). Pen pig weight and feed consumption were recorded at days 0 and 56 to determine the average daily gain (ADG), and gain : feed (G : F). Mortality was recorded daily throughout the trial.

All procedures involving animals were carried out in accordance with European Community guidelines (No. 86/609/CEE) and approved by the Italian Ministry of Health (L. No. 116/92).

Collection of serum samples

Blood samples were obtained from 12 piglets (2 per pen, 1 male and 1 female) per treatment by puncture of the anterior vena cava in 5 ml tubes containing a gel and clot activator (Becton and Dickinson, Milano, Italy) at days 0, 14, and 56 of the trial.

Samples were centrifuged (1000 × *g*, 4°C, 15 min) within 30 min of collection, and the serum was stored in the dark at −35°C pending analysis.

Blood analyses

Glucose, total cholesterol, high-density lipoprotein cholesterol (HDL-C), triglycerides, and urea were determined by enzymatic spectrophotometric assay (Alfa Wasserman, Milano, Italy). The con-

Table 1. Ingredients and chemical analyses of the diets (g/kg)

Live weight range	8–15 kg	15–30 kg
Ingredients		
Steam-rolled corn	280	180
Corn yellow	150	200
Barley	150	200
Wheat middings	80	80
Dried whey	50	20
Soy protein concentrate, 64% CP	40	44
Soybean meal, 48% CP	60	80
Fish meal, 70% CP	28	–
Rice protein meal, 65% CP	24	20
Dextrose	25	10
Wheat bran	30	80
Soy oil	30	30
Experimental supplement[1]	1	1
Vitamin-mineral premix[2]	34	34
Dicalcium phosphate	10	14
L-Lysine·HCl	5	4
Preservative[3]	3	3
Calculated chemical composition[4]		
Crude protein	206.1	195.4
Ether extract	83.3	75.6
Crude fiber	30.9	39.9
Ash	61.3	65.9
Lysine	13.1	11.9
Methionine + cysteine	7.9	7.2
Threonine	8.5	7.7
Tryptophan	2.6	2.4

[1]quantities of plant extract standardized for verbascoside provided per 1 kg of complete diet: 0 (only maltodextrins), 5, and 10 mg for control (CON), low verbascoside (LV), and high verbascoside (HV) groups respectively
[2]provided per 1 kg of complete diet: Ca 2.8 g, P 0.14 g, Na 1.33 g, vit. A 16 000 IU, vit. D3 2000 IU, vit. E 175 IU, vit. K (menadione sodium bisulfite) 3.8 mg, vit. B_1 4.9 mg, vit. B_2 9.8 mg, calcium D-pantothenate 40 mg, niacin 57.8 mg, vit. B12 0.09 mg, vit. B6 7.7 mg, folic acid 3.4 mg, biotin 0.33 mg, choline chloride 1000 mg, Zn (ZnO) 85 mg, Cu ($CuSO_4$) 85 mg, Mn (MnO) 108 mg, Fe ($FeCO_3$) 470 mg, I (KI) 3.85 mg, Co ($CoSO_4$) 1.4 mg, Se (as Na_2SeO_3) 490 μg. Premix containing calcium formiate, *Saccharomices cerevisiae*, sodium chloride, barley, butyric acid, DL-tryptophan, DL-methionine, L-treonine
[3]composition per 1 kg of complete feed: formic acid 0.3 g, lactic acid 1.1 g, colloidal silica carrier 1.6 g
[4]calculation based on INRA (2004)

centration of low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald equation according to Johnson et al. (1997).

Serum IgG and IgA concentrations were measured with an ELISA kit (Bethyl Laboratories, Inc., Montgomery, USA) following the manufacturer's instructions.

Serum concentrations of reactive oxygen metabolites (ROMs) were determined using a commercial kit (DIACRON Srl., Grosseto, Italy). The d-ROM test is a spectrophotometric test based on the capacity of transition metal ions to generate *in vitro* alkoxyl and peroxyl radicals in the presence of hydroperoxides. A chromogenic reagent (*N,N*-diethylparaphenylen-diamine) is then added to this solution. This chromogen compound is oxidized by hydroperoxyl and alkoxyl radicals, and then transformed into a pink to red coloured complex that is directly related to the hydroperoxide levels of the sample. The measurements from the kit cannot be considered specific as reactive oxygen species (ROS); however, the kit mostly estimates alkoxyl and peroxyl radicals. The results are expressed in arbitrary units called Carr Units where 1 Carr Unit corresponds to 0.024 mmol/l of H_2O_2.

Statistical analysis

Performance data were analyzed using the ANOVA procedure with treatment as the main effect. For the blood parameter data, gender and time effects were also evaluated by performing a repeated measurements analysis. Dietary treatment, time, gender, time × treatment, and gender × treatment interactions were included in the model. No effect of gender was noted, and no treatment × gender interaction was observed for any blood variables; these data are thus not presented. Treatment effects were deemed significant at $P < 0.05$, and a trend was noted when $P < 0.10$. A contingency table with the corresponding chi-squared statistics was used to analyze piglet mortality. All analyses were performed with SPSS software (SPSS Inc., Chicago, USA).

RESULTS

Growth performance

At the beginning of the trial, the piglets had an average body weight of 7.99 kg. After 56 days of

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Table 2. Growth performance of piglets fed control diet (CON) or diets supplemented with a plant extract standardized for verbascoside, 5 mg/kg (LV) or 10 mg/kg (HV)

	Diet			P
	CON	LV	HV	
Initial BW (kg)	7.98 ± 0.31	7.77 ± 0.38	8.22 ± 0.39	ns
End weight (kg)	29.60 ± 0.70	29.63 ± 0.95	32.07 ± 0.82	0.087
ADG (g)	386[a] ± 41	389[a] ± 39	425[b] ± 43	0.017
G : F (kg /kg)	0.494 ± 0.05	0.508 ± 0.05	0.523 ± 0.06	ns
ADFI (kg/day)	0.781 ± 0.07	0.766 ± 0.07	0.812 ± 0.08	ns

LV = low verbascoside, HV = high verbascoside, BW = body weight, ADG = average daily gain, G = gain, F = feed, ADFI = average daily feed intake, ns = nonsignificant
[a,b]means within rows with different superscript letters differ significantly ($P < 0.05$)
n = 6 (6 pens with 8 pigs each)

the experimental diet, the HV group showed a tendentially higher final weight ($P = 0.087$) and a greater ($P < 0.05$) ADG compared to the CON and LV groups. Throughout the entire experimental period, the G : F of the group fed with the highest concentration of verbascoside was numerically higher but not statistically significant than the CON and LV groups (Table 2). Mortality was low (2.08%) and not affected by diet ($P = 0.22$).

Blood analyses

No significant treatment effect was found for glucose, urea, HDL-C, total cholesterol, triglyc-erides, and HDL-C serum concentrations. Only LDL-C showed a trend effect for treatment ($P = 0.054$). Urea, HDL-C, total cholesterol, and LDL-C serum concentrations varied significantly with time (Table 3). No interaction time × dietary treatment was found for the serum parameters.

Oxidative status, measured as ROMs, shows time, time × treatment, and treatment effects (Figure 1). The average value was within the range of 30 to 40 mg H_2O_2/100 ml. In the CON group the ROMs increased as the piglet grew (time effect), showing a significantly higher value of the HV and LV groups at day 56.

Serum IgG and IgA increased significantly over time; the final IgG average concentrations (data not
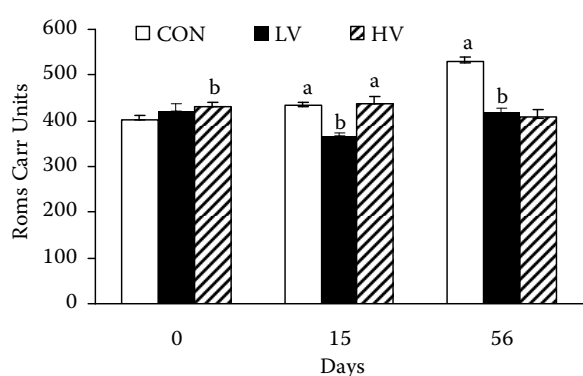

Figure 1. Reactive oxygen metabolite (ROMs in Carr Units) levels in serum of piglets fed a control diet (CON) or a diet supplemented with a plant extract standardized for verbascoside, 5 mg/kg (LV) or 10 mg/kg (HV)

Time, time × dietary treatment, treatment effect: $P < 0.001$
LV = low verbascoside, HV = high verbascoside
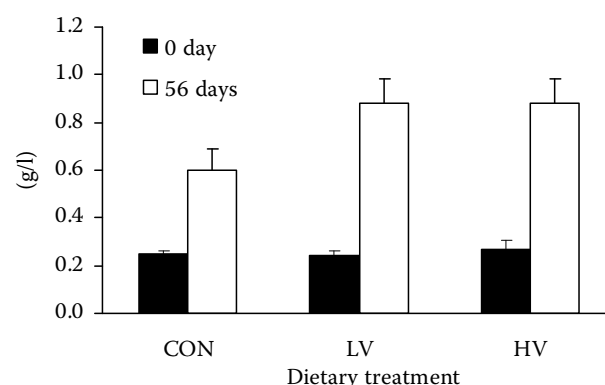[a,b]means within column with different superscript letters differ significantly

Figure 2. Effect of control diet (CON) or diets supplemented with a plant extract standardized for verbascoside, 5 mg/kg (LV) or 10 mg/kg (HV) on IgA serum concentration

Time ($P < 0.001$), time × dietary treatment ($P < 0.10$), and treatment effect ($P < 0.05$)
LV = low verbascoside, HV = high verbascoside

Table 3. Serum biochemical parameters (mmol/l) of piglets fed a control diet (CON) or diets supplemented with a plant extract standardized for verbascoside, 5 mg/kg (LV) or 10 mg/kg (HV)

	Dietary treatment			Probability		
	CON	LV	HV	average[1]	time	dietary treatment
Glucose						
Day 0	4.97 ± 0.18	4.91 ± 0.05	4.85 ± 0.29	4.91		
Day 14	4.79 ± 0.17	5.14 ± 0.72	5.12 ± 0.24	5.01		
Day 56	4.25 ± 0.13	4.79 ± 0.29	4.44 ±0.15	4.49	ns	ns
Urea						
Day 0	4.62 ± 0.92	4.03 ± 0.93	4.83 ± 0.64	4.49[a]		
Day 14	2.86 ± 0.53	2.56 ± 0.35	2.85 ± 0.53	2.76[b]		
Day 56	8.20 ± 0.96	6.82 ± 1.29	7.80 ± 0.58	7.61[c]	**	ns
HDL-C						
Day 0	0.85 ± 0.04	0.90 ± 0.03	0.88 ± 0.05	0.87[a]		
Day 14	0.92 ± 0.05	0.94 ± 0.06	1.00 ± 0.05	0.96[b]		
Day 56	1.12 ± 0.04	1.06 ± 0.02	1.06 ± 0.03	1.08[c]	**	ns
Total cholesterol						
Day 0	2.56 ± 0.04	2.74 ± 0.07	2.54 ± 0.07	2.61[a]		
Day 14	2.44 ± 0.07	2.51 ± 0.10	2.57 ± 0.14	2.50[a]		
Day 56	3.14 ± 0.09	3.07 ± 0.03	2.82 ± 0.09	3.01[b]	**	ns
LDL-C						
Day 0	1.41 ± 0.06	1.53 ± 0.09	1.33 ± 0.09	1.42[a]		
Day 14	1.19 ± 0.10	1.28 ± 0.13	1.21 ± 0.15	1.22[b]		
Day 56	1.74 ± 0.06	1.73 ± 0.05	1.43 ± 0.11	1.63[c]	*	+
Triglycerides						
Day 0	0.65 ± 0.06	0.64 ± 0.09	0.69 ± 0.05	0.66		
Day 14	0.68 ± 0.06	0.59 ± 0.07	0.76 ± 0.03	0.67		
Day 56	0.55 ± 0.10	0.59 ± 0.08	0.64 ± 0.01	0.59	ns	ns

CON = control group, LW = low verbascoside, HV = high verbascoside, HDL-C = high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol
ns = not significant ($P > 0.10$), + = $P < 0.10$, *$P < 0.01$; **$P < 0.001$
[1]average of 0, 14, 56 days (n = 36 for main effects)
results are presented as mean ± SE, n = 12
[a,b,c]means with different letters in the column differ for the P indicated

shown) were 8.53, 10.51, and 9.97 g/l in the CON, LV, and HV groups, respectively ($P < 0.10$).

The IgA concentration of the LV and HV groups at day 56 showed values increased by 46.66% with respect to the control group (Figure 2) showing a significant treatment effect ($P < 0.05$) and a trend for time and treatment interaction ($P < 0.10$).

DISCUSSION

Growth performances

The weaning transition is a complex period when piglets have to adapt rapidly to major changes in their nutrition and environment. Weaning at an

early age (21–28 days), as in intensive production systems in Europe, probably exacerbates the level of general stress in these immature animals.

Phytogenic feed additives as growth promoters are a subject of debate. Phytogenic feed additives have been shown to positively influence daily feed intake, daily weight gain, and feed utilization in growing pigs, and improved growth performance in pigs (Baumann et al., 2003).

Gertenbach and Bilkei (2001) reported that giving feed supplemented with 60 g carvacrol and 55 g thymol per ton (1000 ppm Oregpig) during the postweaning period significantly improves the weight gain and health of pigs.

Wang et al. (2008), on the other hand, found no significant differences in the performance of piglets from sows fed with PFA from 7 days prior to farrowing until the end of lactation, probably due to the short duration of the supplementation. Our results on performance agree with Asghar et al. (1991), who reported a daily gain improvement in post-weaning piglets fed with a natural antioxidant and vitamin E. Other botanicals (herbs and/or spices) administered as feed additives have shown a similar improvement in feed conversion (Maass et al., 2005). An improvement of 4.5 and 5.2% in the feed conversion ratio and in the average daily gain due to phytogenic compounds has been observed in trials conducted at Kansas State University and in Denmark as reported by Steiner (2009).

Optimizing the feed conversion ratio is crucial for efficiency in swine production; the improvement found in this study is of even more importance in the time of rising prices for feed ingredients. Our results suggest that the plant extract used has the potential to boost performance and is likely to improve gut health probably by enhancing the antioxidant activity in the small-intestinal mucosa. Funes et al. (2009) utilized an extract from lemon verbena standardised in 25% verbascoside (w/w) and seven major phenolic compounds in the extract were identified: luteolin-7-diglucuronide, apigenin-7-diglucuronide (Clerodendrin), chrysoeriol-7-diglucuronide, verbascoside, isoverbascoside, Eukovoside, Martynoside. Their study revealed a stronger antioxidant activity than exhibit other antioxidant extracts such as olive leaf and tea catechins under the same conditions. This may be related to the stronger capacity shown by this extract in the lipophilic TEAC assay, which may also explain the capability of lemon verbena extract (and therefore verbascoside) to scavenge

free radicals in a specific environment, such as biological membranes. Our findings would seem to indicate that well-selected phytogenics can be successfully used to improve growth performance in post-weaning piglets.

Blood analyses

Little information is available on the effects of phenylpropanoid glycosides (PPGs) on serum biochemical parameters. An international study with a large sample size has been conducted on humans (Covas et al., 2006) but not, to our knowledge, on animals.

In this study, participants were randomly assigned to three sequences of a daily administration of 25 ml of olive oil. The olive oil had low (2.7 mg/kg of olive oil), medium (164 mg/kg), or high (366 mg/kg) phenolic contents. Each intervention period lasted three weeks and was preceded by a two-week washout period. Results showed that the polyphenol content of an olive oil can benefit HDL cholesterol levels and oxidative damage. All interventions increased HDL cholesterol levels, decreased the total cholesterol/HDL cholesterol ratio and triglyceride levels, and improved the reduced glutathione/oxidized glutathione ratio. The results obtained by Covas et al. provided evidence to recommend the use of polyphenol-rich olive oil as a source of fat to achieve additional benefits against cardiovascular risk factors.

On day 0, the blood biochemical profiles were similar for all the pigs regardless of dietary treatment and agreed with the values reported in the literature for suckling piglets of the same age (de Rodas et al., 1995).

During the experiment biochemical parameters between groups did not differ. The significant increase in serum urea values at day 56 in our study could be explained by a greater feed intake by the piglets and, to some extent, by a dietary protein that was not optimally balanced.

Serum LDL-C was reduced by 17% HV at day 56. The lowest increment between basal time and day 56 of LDL-C found for the treated group vs. the CON group, may contribute to reducing the risk of cardiovascular disease, if confirmed in humans. A significant LDL-C reduction of 30% was obtained by Lin et al. (1998) in rats fed a diet containing 2.5% green tea in a long-term supplementation period (63 weeks). Ali et al. (2007) noted a marked de-

crease in total cholesterol, LDL cholesterol, trigly-cerides, and total lipids in hens that received feed supplemented with thyme plants due to the carvac-rolo as also found by Lee et al. (2003). Some authors (Norata et al., 2003; Bursill and Roach, 2007) have suggested that polyphenols activate the peroxisome proliferator-activated receptor (PPARa), thus modulating the expression of key proteins involved in the metabolism of HDL in the liver.

There is a considerable amount of literature on the role of oxidative stress in the development of chronic pathological conditions and ageing in general. As a result, it has been suggested that oxidative damage and reactive oxygen play a role in the initiation or progression of numerous disorders, including atherosclerosis and cancer (Ames et al., 1995; Hoeschen, 1997). In farm animals, oxidative stress may be involved in several pathological conditions, including conditions that are relevant for animal production and the general welfare of the individuals. Thus, common diseases in pigs such as pneumonia (Lauritzen et al., 2003a, b) and sepsis (Basu and Eriksson, 1998; 2001) have been shown to involve an altered redox balance.

Antioxidants provide a potentially important and cheap alternative treatment to diseases related to oxidative stress, although its use remains controversial. Susceptibility to oxidative stress is a function of the overall balance between those factors that exert oxidative stress and those that exhibit antioxidant capabilities. Thus, methods for quantifying oxidative stress mostly include direct or indirect measures of oxidants and antioxidants. The test system used in our study has already been applied in livestock animals such as cattle (Bernabucci et al., 2005), pigs (Brambilla et al., 2002), and rabbits (Oriani et al., 2001). However ontogenetic data are lacking. ROMs are defined as oxygen-centered free radicals and their metabolites (Powell, 1991). We determined the concentration of ROMs in serum, mostly using levels of alkoxyl and peroxyl radicals that originate from hydroperoxides in the presence of ferryl ions. ROMs are generated during normal metabolism and, particularly, during the activation of lipid metabolisms involving both the phospholipid and arachidonic acid pathways in the cell membranes (O'Flaherty et al., 1985). The average of the ROM values corresponds with the results reported by Brambilla et al. (2002). ROMs can have reactions which, if uncontrolled, can impair animal performance (Kanner et al., 1987). Sauerwein et al. (2007) found the highest ROM values in the first

week after weaning which is associated with decreasing of growth rates. In our study the highest concentrations of ROMs were detected at day 56 post-weaning in the control animals, whereas the animals in the HV and LV groups had by 23 and 21% lower values, respectively. This result is in agreement with the lowest ADG found in the control group. Similar results were obtained by Cannata et al. (2010) on pigs fed the same plant extract used in this trial, from weaning to 150 days. The lowest values of total blood antiradical activity were registered at 60 days post weaning thus confirming the weakness of this physiological period (Cannata et al., 2010). On the basis of their overall trial, the treated groups showed higher ($P = 0.09$) values for the same blood parameter. The effect of weaning on total antiradical activity has also been observed in the untreated pigs (Rossi et al., 2009b). In addition, in order to prevent the adverse effects of stress on welfare and health, measuring oxidative stress in pigs weighing around 40 kg is critical. Moreover the Mulberry heart disease, which is caused by severe lipid peroxidation, is found most commonly in 2–4-month-old animals, thus confirming this period is critical in pigs (Brambilla et al., 2002).

The lowest values in the treated groups showed that antioxidants can be up-regulated and mobilized to neutralize excessive ROS formation. As reported by Liao et al. (1999), ROS scavenging along antioxidative activities of PPGs, such as verbascoside or martynoside, were dependent on the number of phenol hydroxyl groups at conjugating positions in PPG. The higher is the number of these groups, the stronger are their antioxidant activities; verbascoside presents four phenol hydroxyl groups and therefore strong antioxidant activities, as highlighted in the treated groups. Liu et al. (2003) found a lower value of thiobarbituric acid-reactive substances in the plasma of rabbits fed 0.8 mg verbascoside per kg (dissolved in normal saline) twice a day. Previous studies reported decreased lipidic peroxidation in the muscles of rats (Li et al., 1997), and high scavenging of superoxide anions and superoxide radical hydroxyl (Gao et al., 1999). Superoxide indirectly initiates lipid peroxidation, because the superoxide anion can function as a precursor of singlet oxygen and hydroxyl radicals. With our kit we found that hydroxyl radicals can abstract hydrogen atoms from the membrane lipid and this results in a lipid peroxide reaction that generates peroxyl radicals.

Recent trends and developments in the area of animal nutrition have been characterized by the

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increasing interest in the potential impact of plants, herbs, and spices on the immune function of animals (Gallois et al., 2009). Empirical evidence suggests that plant extracts may boost the immune system, thus preventing disease in production animals (Wenk, 2003). It has also been noted that a diet that includes vitamin E and C and selenium in doses exceeding the physiological requirements produces a pronounced immunostimulating effect (Sheffy and Shultz, 1979; Hayak et al., 1989). In this study, changes in IgA and IgG concentrations over time in all groups of pigs most likely reflect the active synthesis of antibodies by the pig's own immune system.

In the post-weaning period, the immunoregulatory and immunoprotective components of maternal milk are both removed (Bailey et al., 2005), and from day 56, the Ig contents reflect *de novo* synthesis by piglets (Kanitz et al., 2004).

To the best of our knowledge, no data are available regarding the potential effects of verbascoside on IgA secretion. In contrast, there are several reports demonstrating the stimulatory effects of other substances such as mannan oligosaccharides (MOS) (Davis et al., 2002), and CLA (Corino et al., 2009) on IgA in pig species. Although the mechanism(s) whereby dietary additives enhance the immune response has not been resolved, possibilities include the activation of toll-like receptors, luminal captation by dendritic cells or the stimulation of epithelial cells and the release of proinflammatory cytokines.

Cheled-Shoval et al. (2011) proposed the direct binding of MOS to transmembranal receptors on the small intestine of chickens and the consequent induction of a response. The receptors include host transmembrane molecules, potentially including the toll-like receptors (TLR) (Harris et al., 2006), mannose receptors (Netea et al., 2006), the galectin family receptors (Almkvist and Karlsson, 2004), and the transmembrane mucins (MUC1 and MUC4) (Carraway et al., 2003). In our study the plant extract supplementation induced an increase in the total seric IgA.

Immunity provides us with the specific mechanisms to resist particular pathogen or foreign substances, by involving the production of antibodies or the activation of T cells (Vinardell and Mitjans, 2008). There are two different clones of T cells: T-helper cells can be classified as Th1 (producing interleukins IL-2, IL-3, and interferon-gamma (IFN-γ)) and Th2 (producing IL-4, IL-5, IL-6, and IL-10). Some of these cytokines are able to activate B cells and mediate proliferation, switching, and differentiation of these cells to become committed to secrete IgA (Shanahan, 1994). B cells

proliferate and mature into IgA plasma cells in response to certain signaling by cytokines produced by T cells and macrophages. Plasma cells produce polymeric IgA which is then secreted across the epithelial cell into the lumen (Pestka, 1993). The IgA that is secreted by B cells in the *lamina propria* can be released into the intestine following the bowel content or to the general circulation, thus raising serum IgA concentrations.

The time-related increase in IgA serum observed in this study is in accordance with previous research (Klobasa et al., 1981). IgA may have a role in mucosal defense, and may be able to neutralize intracellular pathogens such as viruses during their passage through the lining epithelial cells of mucous membranes en route to the secretions (Larsen et al., 2000). They agglutinate antigens, neutralise viruses and bacterial toxins, and prevent the adhesion of enteropathogenic bacteria to mucosal epithelial cells. The increase in blood IgA may be due to an increase in intestinal IgA synthesis, as Vaerman et al. (1997) demonstrated that roughly 30% of the total plasma IgA originated daily from local intestinal synthesis.

This effect is beneficial because the increase in IgA in the intestine prevents local infection and allergen absorption (He et al., 2005), thus representing an immune barrier against the adhesion of pathogens to the intestinal mucosa. This is achieved by binding either to the surface of bacteria and trapping them in the mucous layer of the intestinal epithelia, or to bacterial surface molecules that mediate adhesion to epithelial cells (Rodrigues et al., 2000). In conclusion, we believe that the results presented in this paper should encourage the scientific community to continue investigations of PFAs as alternatives to antibiotics. Antioxidant status measured as ROMs reflects the physiological responsiveness of animals to the feeding plant extract standardized for verbascoside.

In addition, the results regarding the modulating IgA synthesis suggest that *Lippia citriodora* could be useful in conditions associated with local or systemic inflammation, such as the weaning-period which is characterized by intestinal inflammation.

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