Rabbit dietary supplementation with pale purple coneflower (Echinacea pallida). 1. Effects on the reproductive performance and immune parameters of does

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Effects of dietary supplementation with pale purple coneflower (Echinacea pallida) on reproductive performance and immunity of rabbit does and on productive results of their kits

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Running head: Pale purple coneflower in rabbit nutrition

Abstract

Echinacea pallida (EPAL), also known as pale purple coneflower, is an herbaceous flowering plant with immune-enhancement and antioxidative properties. EPAL effect was studied on rabbit does' reproductive performance, serum biochemistry and haematological parameters as well as on their kits growth performance. One hundred 21-weeks-old Grimaud rabbit does were randomly assigned to two groups. One group was fed a basal diet supplemented with 3 g EPAL /kg diet (*Echinacea* group, E) while the other was fed the basal diet without the supplementation (Control group, C). Reproductive performance of does was not affected by the treatment (*P*>0.05). Haematological parameters of pregnant rabbits showed that any interaction between gestational day and treatment was observed except for neutrophils cells (*P*=0.033). The control group was significant higher than the treatment group for basophils cells (0.55 and 0.29 %, respectively; *P*=0.049). Gestational day significantly affected most haematological parameters (*P*<0.05). No significant effect of gestational day or treatment was observed on blood serum chemistry. Regarding the immune parameters, no significant differences were observed between groups; while a significant effect of gestational day was observed for lysozymes (6.02 *vs* 7.99 *vs* 1.91; for 0, 14 and 28 days respectively; *P*=0.014). Eighty weaned kits (40 born from C does and 40 born from E does) were randomly assigned to four groups of 20 animals each fed a growing commercial diet supplemented with or without 3 g EPAL /kg diet. The following experimental groups were formed: CC (rabbits fed the C diet and born from the C does), CE (rabbits fed the E diet and born from the C does), EC (rabbits fed the C diet and born from the E does) and EE (rabbits fed the E diet and born from the E does). Dietary EPAL treatment did not significantly (*P*>0.05) affect the growth performance of weaned rabbits. In conclusion, a lack of effect of EPAL was reported. Indeed, its dietary supplementation did negatively influence the reproductive and haematological parameters of does nor the growing performance of fattening rabbits.

Keywords: pale purple coneflower, Echinacea pallida, rabbit does, haematology, fattening rabbits.

Implications

In recent years, after the ban on the use of antibiotics as growth promoters, phyto-additives have been proposed to improve rabbit health and reduce post-weaning mortality. The present study describes the effects of dietary supplementation with *Echinacea pallida* (known to possess immune-enhancement and antioxidative properties) on rabbit does reproductive performance and immunity and on their kits productive results. The EPAL dietary supplementation did not influence the reproductive and haematological parameters of rabbit does nor did promote the growth performance of their kits.

Introduction

Animal health is a critical issue in animal production strongly affecting the income generated from husbandry activity. Moreover, since the European Union has banned the use of antibiotics as feed additives, many researches in the animal nutrition area have been focused on gauging alternative feeding strategies preventing digestive diseases while enabling the achievement of a satisfactory growth performance. Given the advance in modern biotechnology, the application of naturally-occurring antimicrobial and antioxidant compounds has been preferably employed in animal nutrition due to its potential health benefits on the host physiology (Chrastinová *et al*., 2010). The immunomodulatory and anti-oxidative properties of officinal plants are well known, as well as their ability to promote positive outcomes on animal health and performance (Böhmer *et al*., 2009; Arafa *et al*., 2010).

Echinacea is a genus of herbaceous flowering plants belonging to the Asteraceae botanical family. It presents high levels of production and economic importance in the United States of America, Canada and European countries. The use of a mixture of *Echinacea purpurea*, *Echinacea angustifolia* and *Echinacea pallida* (EPAL) has been reported to have immune-enhancement properties and benefits, such as the prevention and treatment of upper respiratory tract infections (Barnes *et al*., 2005). Active components from *Echinacea* extracts (mainly alkylamides, polysaccharides and proteoglycans) have been shown to exert immunomodulatory, anti-inflammatory and anti-viral activities (Barnes *et al*., 2005). Extracts of EPAL have been proposed as phyto-immunostimulating agents and their activities are mainly directed towards the innate immune system. Most studies performed on the immunotropic properties of EPAL were related to its effect on nonspecific immunity (activation of macrophage functions, phagocytosis of granulocytes, NK cells cytotoxicity), while other studies have investigated the adaptive immune modulation of EPAL (Egger *et al*., 2008).
Improvement of immunity parameters and productive performance has been reported in various livestock species (poultry, quails and rabbits) fed diets supplemented with *Echinacea* spp. (Maass *et al*., 2005; Ahmed *et al*., 2008; Böhmer *et al*., 2009; Arafa *et al*., 2010; Nasir and Grashorn, 2010; Sahin *et al*., 2012). Nevertheless, scarce and conflicting evidence is available concerning the use of *Echinacea* spp. products in rabbit does during pregnancy. Based on this evidence, the aim of this study was to evaluate the effects of EPAL dietary supplementation on reproductive performance, blood parameters and immune indices in rabbit does as well as on the productive performance of their kits.

Material and methods

Animals, housing, diets and management of rabbit does

One hundred nulliparous does (14 week old) of a strain of Grimaud rabbits, obtained from Grimaud Italy, were housed individually in a closed rabbitry, with flat-deck wire net cages (40×50 cm^2, including nest boxes: 41×26 cm^2), and under a constant photo-period of 16 h of light per day. The rabbitry temperature was kept within 18°-22°C. A relative humidity of 60-75% was maintained by a forced ventilation system.

The does were randomly assigned to two groups (50 does per group). The first group was fed *ad libitum* a commercial pelleted diet (control diet, C) while the second one was fed the same diet supplemented with 3 g of EPAL powder /kg diet (*Echinacea* diet, E).

The doe rabbit diets were provided by the Ferrero S.p.A. feed manufacturer (Farigliano CN, Italy). Dry ground EPAL roots, obtained from Biotrade Snc® (Via Pacinotti, 21, Mirandola, Italy), was included in the treated diets during the raw material mixing process. The feeding program consisted of a diet provided from insemination to 21 days after parturition and another diet provided from 21 days after parturition to kits weaning. The diets contained the following ingredients in decreasing order: alfalfa meal, sunflower meal, barley, wheat bran, dried beet pulp, maize germ, roasted soybean meal, cane molasses, soybean oil, calcium carbonate, sodium chloride. The diets were analyzed for dry matter (DM, AOAC 925.40), crude protein by total nitrogen contents (AOAC 984.13), ether extract (AOAC 945.16), crude fiber (AOAC 962.09) and ash by ignition to 550°C (AOAC 923.03) according to the Association of Official Analytical Chemists (AOAC, 2000). NDF, ADF and ADL were determined according to Van Soest et al. (1991). Starch was determined by means of Ewer's polarimetric method (European Economic Community, 1972). The chemical composition of the different diets was reported in Table 1. Water was available *ad libitum* from nipple drinkers. The diets were completely exempt from medication (antibiotics or coccidiostat). All animals were reared under the same environmental and management conditions during the whole experimental period. Rabbit does were first artificially inseminated at 21 weeks of age (mean body weight: 3712 ± 176g). Then, artificial insemination was applied at 18 days post-partum (49 day reproductive rhythm and single batch system). Cross-fostering was applied within the experimental groups with a maximum of 8, 9 and 10 kits per litter at first, second and following kindling, respectively. The kits were freely nursed by their doe and weaned at 35 days of age.

Does performance

Data of the first five consecutive reproductive cycles were evaluated. Body weight of does at first and final kindling, does mortality and reproductive performance variables were studied. The following variables were calculated on the basis of IRRG's recommendations (International Rabbit Reproduction Group, 2005): total born; born alive; stillborn; litter size at 21 and 35 days of age; litter weight at 21 and 35 days of age; individual body weight of kits at 21 and 35 days of age; Kindling rate (%) = number of kindled does per number of inseminated does ×100; Prolificacy = number of born kits per number of does kindled; Numerical productivity at birth = number of born alive per inseminated doe; Overall productivity at birth = weight of born alive per inseminated doe; Perinatal mortality (%) = number of stillborn kits per number of total born × 100; mortality between 0-21 and 0-35 days of age.

Haematological, serum biochemistry and serum electrophoresis of rabbit does

Blood samples were collected from 8 rabbits per group at different time points during the second gestation. Considering the day of artificial insemination as starting day (T0), blood samples were collected at: day 0, day 14 and day 28, respectively. The samples were collected from the lateral saphenous vein with a heparinized syringe to prevent the blood clot. At each sampling time point, one ml of blood was collected into sterile tubes containing ethylenediaminetetraacetic acid -2K (SB-41: Sysmex Corporation) for the evaluation of haematological parameters. Meanwhile, serum obtained by collecting four ml blood samples in a sterile serum plain tube, after incubation at room temperature (22°C) for two hours and centrifugation at 2500 g for 10 minutes, was used for serum biochemistry and serum electrophoresis. Serum was stored at -80° C until analysis. Full blood count was performed using an automated laser cell counter calibrated for rabbits (MS4-S Hematology Analyzer, Melet Schloesing, Osny - France) to assess the following parameters: red blood cells (RBC, M/mm^3), haemoglobin (Hb, g/dl), haematocrit (HCT, %), mean corpuscular volume (MCV, fl), mean corpuscular haemoglobin (MCH, pg), mean corpuscular haemoglobin concentration (MCHC, g/dl), red cell distribution width (RDW, %), platelets (PLT, m/mm^3), relative volume of thrombocytes (PCT, %), mean platelet volume (MPV, fl), platelet distribution width (PDW, %), white blood cell count (WBC, m/mm^3), lymphocytes (LYM, %), monocytes (MON, %), neutrophils (NEUT,%), eosinophils (Eos, %), basophils (Bas, %). For the serum blood chemistry, the concentrations of total protein (TP, g/dl), glutamate oxaloacetate transaminase (GOT, UI/L), blood urea nitrogen (BUN, mg/dl), albumin (g/dl), urea (mg/dl) and cholesterol (mg/dl) were measured using an automated system photometer (Screen Master Touch, Hospitex Diagnostics, Sesto Fiorentino, FI, Italy).

For immune indices, the serum electrophoretic patterns were obtained using a semi-automated agarose gel electrophoresis system (Sebia Hydrasys, EVRY, France) to determine serum protein. Serum lysozyme was measured with a lysoplate assay, carried out in a moist incubator at 37°C for 18 min. The method is based on the lyses of *Micrococcus lysodeikticus* in 1% agarose. The diameter of the lysed zones was measured with a ruler and compared with the lysed zones of a standard lysozyme preparation (Sigma Aldrich, Milan, Italy). The value was expressed as µg/ml (Osserman and Lawlor, 1996). The haemolytic complement assay was carried out in microtitre plates. The complement titre is the reciprocal of the serum dilution causing 50% lysis of red blood cells of rams. Its concentration was expressed as $CH_{50\%}$ (Moscati *et al*., 2008).

Performance of fattening rabbits

At the second parturition, forty weaned kits were randomly chosen from both C and E does. Rabbits were allocated into individual wire cages (0.41 m long × 0.30 m wide × 0.28 m high) and randomly assigned to four equal-size experimental groups (n=20). Two groups of rabbits were fed a growing commercial basal diet (C) while the remaining two groups were fed the same diet supplemented with 3 g of EPAL powder / kg diet (E). According to the maternal diet, the following experimental groups were formed: CC group (rabbits fed the C diet and born from the C does), CE group (rabbits fed the E diet and born from the C does), EC (rabbits fed the C diet and born from the E does) and EE group (rabbits fed the E diet and born from the E does). The chemical composition of the different diets is reported in Table 2. The diets were completely exempt from medication (antibiotics or coccidiostat). Feed and water were provided *ad libitum*. During the whole trial, temperature was maintained at 22±2°C and a 16L: 8D photoperiod was applied. Health status was monitored daily from weaning to 77 days of age.

Rabbits were weighed at 35, 49 and 77 day of age and the following performance parameters were calculated: daily feed intake, daily weight gain and feed conversion ratio at different periods of age.

Chromatographic identification of Echinacea ingredients

Chemicals

Echinacoside (purity 98%), chlorogenic acid (purity ≥ 95%), HPLC-MS and analytical grade solvents were purchased from Sigma-Aldrich (Milan, Italy).

Extraction procedure

500 mg of dry ground EPAL roots, were sonicated for 10 min with 10 ml of a mixture of MeOH/H2O (70/30) three times. The resulting total extract (30 ml) was filtered and analyzed by UHPLC-PDA-MS/MS system.

HPLC Analysis

EPAL extract analyses were carried out on a Shimadzu Nexera X2 system equipped with a photodiode detector SPD-M20A in series to a triple quadrupole Shimadzu LCMS-8040 system provided with electrospray ionization (ESI) source (Shimadzu, Dusseldorf Germany). An Ascentis® Express C18 column (150 mm x 2.1 mm i.d., 2.7 μm particle size), (Supelco, Bellefonte, PA) was used (operated at 30°C). The mobile phase consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B), at a flow rate of 0.4 ml min^{-1}. Polyphenols elution was achieved using the following linear gradient: starting condition, 95% A, 5% B; 3 min, from 5 to 15% B; 17 min, from 15 to 100% B; 5 min and 100% B for 2 min. The injection volume was 5 μl. UV spectra were acquired in the 210-450 nm wavelength range. The identification of the components was based on the co-injection of pure standards and on their UV spectra and mass spectral information in both positive and negative ionization mode (respectively, ESI+ and ESI-).

Quantification of Echinacoside: A standard stock solution (1mg/ml) of Echinacoside was prepared in methanol and stored at -18°C. Suitable dilutions of the standard stock solution in methanol/water (1/10) were prepared to obtain final concentrations from 10 to 100 mg/ml. Calibration curve was built by analysing the resulting standard dilutions three times by HPLC-PDA.

Statistical analysis

Statistical analyses were performed using SPSS software package (IBM SPSS, 2012). Data concerning the reproductive parameters from the first to the fifth reproductive cycles were combined and analyzed in a single dataset. Statistical analyses for significant differences in reproductive performance between the control and Echinacea groups were performed using a Student's t-test. Mortality, kindling rate and prolificacy were analyzed using Chi-square test. The effect of dietary treatments on blood indices and immune parameters across three gestational periods (day 0, day 14, day 28) was statistically analyzed with a mixed between-within subjects model (GLM Repeated Measures). Performance of the fattening rabbits was analyzed using a one-way ANOVA with group as fixed factor. Duncan’s New Multiple Range test was used for post-hoc comparisons. The significance was declared at $P<0.05$.

Results

HPLC profile of EPAL

The HPLC profiles of EPAL root extract are shown in Figure 1. The analysis identified the presence of caftaric acid, cichoric acid, chlorogenic acid and Echinacoside which specifically characterized EPAL species (Hu and Kitts, 2000; Speroni *et al*., 2002; Barrett, 2003). Chromatographic analysis was reported to find 0.37 % Echinacoside. Echinacoside was found to be the main caffeic acid derivative in EPAL extract, responsible for the immunostimulatory action of Echinacea extracts (Hu and Kitts, 2000; Pellati *et al*., 2005; Dalby-Brown *et al*., 2005). Echinacoside has been studied for its antioxidant, anti-inflammatory and cicatrizing activities (Speroni *et al*., 2002). However, a purified phytochemical does not imitate the immunological effects of whole plant extracts. It appears that the immunopharmacological activities of Echinacea depend on a combination of several active compounds (Randolph *et al*., 2003).

Reproductive performance

Reproductive performances of the first five reproductive cycles are reported in Table 3. There were no significant differences between groups for any of the studied parameters. Numerical and overall productivities calculated during the five cycles were: born alive, 1438 and 1471 kits; number of kits at day 35, 1229 and 1260 for control and E groups, respectively.

Haematological findings

The haematological parameters of pregnant rabbits are reported in Table 4. The results indicated a significant (P<0.05) effect of treatment and gestational day on some haematological parameters. The control group was significant higher than the treatment group for basophils cells (0.55 and 0.29 %, respectively; P=0.049).

Gestational day significantly affected RBC, Hb, HCT, MCV, MCH, MCHC, RDW, MPV, PDW, WBC, LYM, NEUT and Eos (*P*<0.05). For any studied variables, no significant interaction between treatment and gestational period was reported except for NEUT (*P*=0.033).

No significant effect of gestational day or treatment was observed on blood serum chemistry. Regarding the immune parameters, no significant differences were observed between groups; while a significant effect of gestational day was observed for lysozymes (*P*=0.014). The higher concentration of lysozymes was observed in day 14 of gestation in comparison with days 0 (+32.7%) and 28 (+318.3%) (6.02 *vs* 7.99 *vs* 1.91; for 0, 14 and 28 days respectively).

Fattening rabbit performance

The results of fattening rabbits performance are illustrated in Table 5. For all studied variables, no statistically significant differences were reported amongst the experimental groups (*P*>0.05). In addition, regarding the health status, no illness and death were observed during the fattening period.

Discussion

Reproductive performance

Body weight of does at kindling, kindling rate, litter size at birth, at days 21st and 35th of age, and the mortality of kits did not differ between the two groups. This indicates that *Echinacea* supplements in does' diets did not exert a promoting effect on reproductive function when administered at 3 g EPAL/kg of diet. Our results differ from those obtained in mice by Barcz *et al*. (2007) who found that two *Echinacea* drugs (*Esberitox* and *Echinapur*) lowered the number of embryos in one litter, even if the results were on the edge of statistical significance. During murine pregnancy, *Echinacea purpurea* reduced the number of viable foetus (Chow *et al*., 2006). A prospective study suggested that the use of *Echinacea* in pregnancy during organogenesis is not associated with an increased risk of major malformations (Gallo *et al*., 2000). Further theoretical evidence via an expert panel on botanical medicine reported that oral consumption of *Echinacea* in recommended doses appeared safe and effective to use during pregnancy (Perri *et al*., 2006).

Haematological findings

Blood parameters in rabbits are used as an aid for the clinical diagnosis of metabolic, infectious and parasitic diseases and to assess animal condition. A variety of factors can affect animal haematological and biochemical parameters, including breed, gender, diet, age, reproductive status and seasonal variations (Ozegbe, 2001; Wells *et al*., 1999). The haematological and biochemical parameters of this study were within normal ranges for rabbit species (Archetti *et al*., 2008; Özkan *et al*., 2012). The application of Echinacea extract should booster immunological reactivity and should contribute to improve health status (Böhmer *et al*., 2009). In the present trial, EPAL had no influence the heamatological and health status of rabbit does. The change in blood coagulation-related parameters during the later stage of gestation is a common physiological response for the protection against excessive haemorrhage or for the preservation of the homeostasis at parturition (Mizoguchi *et al.,* 2010). In our study, the modulation of RBC and HCT may be related to physiological anemia resulting from haemodilution (Ozegbe, 2001). Watery supplementation with Echinacea purpurea extract induced higher results of Hb, PCV and RBC in growing rabbits (Ahmed *et al*., 2008). Likewise, a study by Chow *et al.* (2006) found an increase in

RBC in pregnant mice when fed *Echinacea purpurea*. In addition, the increment of erythropoietin level (glycoprotein hormone which controls erythropoiesis) has been reported in *Echinacea purpurea*-treated men. This should support the RBC increment deriving from the supply of phyto-additives (Whitehead *et al*., 2007). On the other hand, Maass *et al*. (2005) did not find any significant difference for these parameters in sows, piglets and grower/finisher pigs that received dried *Echinacea purpurea* herb as feed additive in their diets. Differences concerning plant species tested (EPAL *vs* *Echinacea purpurea*), preparation methods (raw material *vs* extraction), physiological status (pregnant *vs* non-pregnant) and species (rabbit *vs* swine, mice and human beings) could explain these contrasting results. An author showed that WBC parameters increased during the whole period of gestation in pregnant women (Cincotta et al., 1995), in rabbit does (Haneda *et al*., 2010) and also in rats (DeRijk *et al*., 2002). Cundell *et al*. (2003) found a significant increase of lymphocytes after one week in rats fed with dried Echinacea preparations. A higher proliferation rate of spleen lymphocytes in EPAL supplemented mice has been reported in an in vitro study, but the haematology indices were not influenced (Zhai *et al*., 2007). The increase in WBC generally is a good indicator of immunity efficiency increase (Wieslaw et al., 2006). In our study, the effect of EPAL was observed only for Bas. According to other studies, this effect may be related to its phytochemically active constituents of EPAL (Hu and Kitts, 2000; Pellati *et al*., 2005; Dalby-Brown *et al*., 2005).

With respect to blood serum chemistry, no significant difference was observed in total protein. In contrast, Wells *et al*. (1999) reported a decrease in total protein and albumin in pregnant rabbits and this is thought to reflect the increased blood volume.

Innate immunity has an important role to prevent the infection as first-line defence and also contributes antigen-presenting cells that activate the adaptive immune response, which is specific and powerful (Tizard, 2013). Dietary supplementation with *Echinacea* can stimulate the innate immunity by increasing cytokine production (Hwang *et al*., 2004) and phagocyte-stimulation (Böhmer *et al*., 2009). Lysozymes and the complement system are interesting indicators to study the innate immune function. In our experiment, only lysozyme results showed a time related change. It must be highlighted that our work was performed in a standard environment without infection, stress or other factors influencing immune responses. Therefore, the experimentation in normal conditions may hardly result in a significant effect on immunity despite the supplementation with an immunomodulating agent.

Fattening rabbit performance

Growth performance of Echinacea supplemented groups did not showed significant differences. Our results differ from Arafa *et al*. (2010) who found, in a similar study using *Echinacea purpurea* at 130 mg/kg body weight, a significant decrease in mortality rate and an increase of live weight in 6-week-old growing rabbits fed E diets ($P<0.05$). Usually, dietary herb supplementation leads to an improvement of the flavour, which accounts for an increase of feed ingestion and better performance (Franz *et al*., 2010; Christaki *et al*., 2012). Ahmed *et al*. (2008) highlighted a significant improvement of final body weight, daily weight gain and feed conversion ratio in growing rabbits to which were orally given in liquid 7.5 mg of *Echinacea purpurea* extracts/kg body weight and day. However, the outcomes of above reported references are not fully comparable with our trial due to some dissimilarities in experimental plans concerning: tested *Echinacea* species, concentration of the supplement, administration route (oral by liquid mixture), supplement preparation (extraction) and supplemented periods in doe's diet

Generally, mixtures of *Echinacea purpurea*, *Echinacea angustifolia* and EPAL are used in human medicine and animal production. To this regard, positive outcomes on productive performance were reported in rabbits with *Echinacea purpurea* addition (Ahmed *et al*., 2008; Arafa *et al*., 2010), whereas studies conducted with other livestock species did not find any improvement (Hermann *et al*., 2003; Maass *et al*., 2005; Böhmer *et al*., 2009; Sahin *et al*., 2012).

In conclusion, there is no evidence that diets supplemented with EPAL cause any beneficial effects in normal management condition. Nonetheless, further studies are suggested in order to evaluate the effect of *Echinacea pallida* on animal performance and to study the relation between its active components and physiological functions.

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Table 1 *Rabbit does diets composition*

	Does diet (from artificial insemination to 21 days after parturition)		Does diet (from 21 days after parturition to 35 days after parturition)	
	Control	Treatment	Control	Treatment
Chemical composition[1]				
Dry matter (DM)	89.3	90.2	89.9	89.9
Crude protein (% DM)	18.7	18.8	17.5	17.2
Ether extract (% DM)	2.6	2.9	4.5	4.6
NDF (% DM)	35.0	33.7	32.4	32.2
ADF (% DM)	22.4	22.2	17.5	17.9
ADL (% DM)	5.5	5.7	5.4	5.4
Ash (% MS)	9.5	9.5	7.5	7.9
Starch (% DM)	26.2	27.2	17	17.4
Echinacea pallida (g/kg)	0	3	0	3
Minerals and vitamins[2]				
Calcium (% DM)	0.9	0.9	1	1
Lysine (% DM)	0.8	0.8	0.7	0.7
Phosphorus (% DM)	0.5	0.5	0.4	0.4
Methionine (% DM)	0.3	0.3	0.4	0.4
Sodium (% DM)	0.3	0.3	0.3	0.3
Vitamin A (UI/kg)	12.5	12.5	12.5	12.5
Vitamin D3	1.2	1.2	1.2	1.2
Vitamin E	100	100	100	100
Ferrous carbonate (mg/kg)	662	662	704	704
Manganese oxide (mg/kg)	195	195	209	209
Zinc oxide (mg/kg)	186	186	186	186
Copper sulfate (mg/kg)	98	98	98	98
Potassium iodide (mg/kg)	2.4	2.4	2.5	2.5
Sodium selenite (mg/kg)	0.6	0.6	0.6	0.6

[1]The experimental diets were analyzed by the laboratory of the Department of Agricultural, Forest and Food Sciences, Turin, Italy. [2]These data were provided by the Ferrero Mangimi S.p.A, (Farigliano CN, Italy), which formulated and prepared the experimental diets.

Table 2 *Growing rabbits diets composition*

	Diets[2]	
	Control	Treatment
Chemical composition[1]		
Dry matter (DM)	89.8	89.8
Crude protein (% DM)	17.1	17.3
Ether extract (% DM)	3	3
NDF (% DM)	39.4	39.6
ADF (% DM)	23.7	24
ADL (% DM)	6.6	6.6
Ash (% DM)	9.7	10.4
Starch (% DM)	12	12.3
Echinacea pallida (g/kg)	0	3
Minerals and vitamins[2]		
Calcium (% DM)	1	1
Lysine (% DM)	0.7	0.7
Methionin (% DM)	0.4	0.4
Phosphorus (% DM)	0.4	0.4
Sodium (% DM)	0.3	0.3
Vitamin A (UI/kg)	12.5	12.5
Vitamin D3	1.2	1.2
Vitamin E	100	100
Ferrous carbonate (mg/kg)	662	662
Manganese oxide (mg/kg)	195	195
Zinc oxide (mg/kg)	186	186
Copper sulfate (mg/kg)	98	98
Potassium iodide (mg/kg)	2.5	2.5
Sodium selenite (mg/kg)	0.57	0.57

[1]The experimental diets were analyzed by the laboratory of the Department of Agricultural, Forest and Food Sciences, Turin, Italy. [2]These data were provided by the Ferrero Mangimi S.p.A, (Farigliano CN, Italy), which formulated and prepared the experimental diets.

Table 3 *Effects of pale purple coneflower (Echinacea pallida) dietary supplementation on reproductive performance of rabbit does*

	Control group	*Echinacea* group	Standard error of mean difference	*P*-value
No. of does at first kindling	50	50	-	-
No. of does at fifth kindling	37	38	-	-
Mortality of does (%)	26	24	-	0.817[1]
Body weight (LW), g				
at first kindling	3868	3869	-	0.982
at fifth kindling	4782	4770	-	0.929
No. of kindled does/artificial insemination	148 / 221	151 / 221	-	-
Kindling rate,%	67	68	-	0.760[1]
Prolificacy	8.78	8.88	-	0.852[1]
Total born	10.5	10.5	0.36	0.978[2]
Born alive	9.72	9.74	0.37	0.945[2]
Stillborn	0.78	0.76	0.18	0.907[2]
Litter size				
at 21d	8.36	8.42	0.25	0.816[2]
at 35d	8.30	8.34	0.26	0.877[2]
Litter weight (g)				
at 21d	2750	2747	101.22	0.981[2]
at 35d	7023	7038	229.82	0.946[2]
Individual body weight (g)				
at 21d	329	326	3.80	0.495[2]
at 35d	846	844	4.05	0.585[2]
Perinatal mortality (%)	7.40	7.25	-	0.868[1]
Mortality (%)				
0-21d	14	13.6	-	0.788[1]
21-35d	0.65	0.86	-	0.528[1]

[1]: parameter analyzed by Chi-square test; [2]: parameter analyzed by Student’s t-test

Table 4 *Effects of pale purple coneflower (Echinacea pallida) dietary supplementation on blood and immune parameters of pregnant rabbit does (n=8 per group)*

	Treatment		Gestational day			Within subjects effects			Between subjects effects	
	Control group	*Echinacea* group	0	14	28	Gestational day *P*-value	Gestational day × Treatment *P*-value	Root Mean Square Error	Treatment *P*-value	Root Mean Square Error
No. of animals	8	8	8	8	8					
Haematology										
RBC (M/mm^3)	5.80	5.50	5.38	5.96	5.62	0.025	0.963	0.422	0.145	0.265
Hb (g/dl)	11.99	11.40	10.96	12.27	11.86	0.013	0.992	0.885	0.274	1.912
HCT (%)	38.02	36.18	35.65	39.46	36.19	0.014	0.907	86.827	0.271	18.164
MCV (fl)	65.58	65.87	66.27	66.32	64.59	0.003	0.383	33.808	0.870	22.463
MCH (pg)	20.61	20.70	20.29	20.57	21.10	0.048	0.763	0.677	0.894	2.870
MCHC (g/dl)	31.53	31.46	30.69	31.06	32.74	<0.001	0.553	0.932	0.770	0.439
RDW (%)	10.81	11.65	10.14	11.53	12.03	<0.001	0.339	0.702	0.343	5.216
PLT (m/mm^3)	137.07	168.00	146.20	154.50	156.90	0.897	0.441	53.741	0.223	64.048
PCT (%)	0.09	0.11	0.09	0.10	0.11	0.432	0.304	0.032	0.158	0.045
MPV (fl)	6.74	6.83	6.51	6.55	7.29	<0.001	0.380	0.253	0.620	0.460
PDW (%)	6.77	6.78	6.66	6.29	7.38	0.009	0.729	0.692	0.974	0.538
WBC (m/mm^3)	9.59	9.38	11.14	11.11	6.22	<0.001	0.507	2.070	0.829	2.588
LYM (%)	14.97	14.57	15.41	12.56	16.34	0.011	0.803	2.541	0.850	5.688
MON (%)	6.53	5.91	6.62	5.49	6.54	0.052	0.663	1.055	0.533	2.606
NEUT (%)	76.87	78.17	76.87	80.49	75.21	0.009	0.033	3.396	0.657	7.717
Eos (%)	1.08	1.04	0.56	0.99	1.63	<0.001	0.626	0.475	0.851	0.565
Bas (%)	0.55	0.29	0.54	0.44	0.28	0.092	0.671	0.249	0.049	0.300
Blood serum chemistry										
BUN (mg/dl)	20.87	16.82	14.95	15.34	26.25	0.183	0.471	9.305	0.413	8.127
GOT (UI/L)	29.06	32.22	26.79	35.58	29.55	0.395	0.790	14.315	0.401	9.763
Total Protein (g/dl)	4.60	4.34	4.48	4.22	4.72	0.325	0.641	0.711	0.296	0.643
Albumin (g/dl)	2.91	2.91	2.68	2.87	3.18	0.109	0.191	0.494	0.971	0.391
Urea (mg/dl)	29.28	36.10	32.09	32.92	33.06	0.973	0.143	6.929	0.319	10.851
Cholesterol (mg/dl)	48.66	39.41	33.85	63.21	35.04	0.352	0.219	49,781	0.658	55.169

Immune parameters										
Lysozymes (µg/ml)	5.64	4.98	6.02	7.99	1.91	0.014	0.590	4.122	0.862	10.067
Complement	36.72	29.44	34.31	34.06	30.87	0.826	0.267	12.959	0.174	12.438
Alfa1 (g/dl)	0.14	0.16	0.20	0.09	0.18	0.234	0.117	0.155	0.746	0.182
Alfa 2 (g/dl)	0.28	0.19	0.32	0.19	0.19	0.176	0.304	0.170	0.164	0,158
Beta 1 (g/dl)	0.28	0.28	0.33	0.28	0.23	0.175	0.198	0.114	0.828	0.100
Beta 2 (g/dl)	0.39	0.38	0.38	0.36	0.42	0.557	0.687	0.118	0.801	0.134
Gamma (g/dl)	0.60	0.42	0.56	0.43	0.54	0.697	0.515	0.355	0.115	0.281

RBC: Red Blood Cells; Hb: Haemoglobin concentration; HCT: Haematocrit; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Haemoglobin; MCHC: Mean Corpuscular Haemoglobin Concentration; RDW: Red cell distribution width; PLT: Platelets; PCT: Relative volume of thrombocytes; MPV: Mean Platelet Volume; PDW: Platelet distribution width; WBC: White Blood Cells; LYM: Lymphocytes; MON: Monocytes; NEUT: Neutrophils; Eos: Eosinophils; Bas: Basophils; BUN: blood urea nitrogen; GOT: glutamate oxaloacetate transaminase.

Table 5 *Effect of pre and postnatal dietary supplementation with pale purple coneflower (Echinacea pallida) on growth performance of fattening* rabbits (n=20 per group)

	Groups				RSD	*P*-value
	CC	CE	EC	EE		
Live weight (g)						
At 35 day	885	889	889	882	53.8	0.976
At 49 day	1713	1711	1745	1717	79.7	0.513
At 77 day	3031	2998	3107	3041	160	0.190
Growth performance in 35-49 days						
Daily feed intake (g per day)	134	138	140	139	10.6	0.323
Daily weight gain (g per day)	59.2	58.7	61.2	59.6	3.58	0.160
Feed conversion ratio	2.28	2.36	2.29	2.35	0.15	0.200
Growth performance in 49-77 days						
Daily feed intake (g per day)	176	178	181	181	10.8	0.478
Daily weight gain (g per day)	45.4	44.4	46.9	45.7	4.05	0.254
Feed conversion ratio	3.87	4.03	3.88	3.98	0.30	0.282

Growth performance in 35-77 days						
Daily feed intake (g per day)	162	165	168	168	11.5	0.368
Daily weight gain (g per day)	49.9	49.0	51.6	50.2	3.11	0.082
Feed conversion ratio	3.25	3.37	3.26	3.34	0.19	0.122

CC: rabbits fed the C diet and born from the C does, CE: rabbits fed the E diet and born from the C does, EC: rabbits fed the C diet and born from the E does, EE: rabbits fed the E diet and born from the E does.

Figure 1

LC-PDA profile at 325 nm of Echinacea pallida (Nutt.) Nutt root extract at 325 nm.