Effects of dietary licorice extract on serum Biochemical index, tissues antioxidant capacity and immunity function of weaned piglets

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Research

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Abstract

Background: This study investigated the effects of dietary licorice extract (LE) on antioxidant capacity and immunity in weaned piglets. A total of 96 DLY (Duroc × Landrace × Yorkshire) weaned piglets were randomly assigned to four treatments. The control group were fed a corn–soybean meal-based diet (basal diet, BD), and three LE level groups were fed on BD supplied with 50□150 and 250 mg/kg LE. The trial lasted 5 weeks. At day 35, six piglets per treatment were killed and blood, liver, spleen, and thymus were collected.

Results: The result showed that: 1)Dietary LE increased ($P < 0.05$) activity of the alkaline phosphatase (ALP) and reduced ($P < 0.05$) the activity of glutamic oxalacetic transaminase (AST), 50 mg / kg LE reduced ($P < 0.05$) total cholesterol (TC) and high density lipoprotein cholesterol (HDL-C) in serum. 2) The addition of 150 and 250 mg / kg LE increased ($P < 0.05$) glutathion peroxidase (GSH-Px) activity in liver and spleen, increased ($P < 0.05$) the total antioxidant capacity (T-AOC) in serum and spleen . 50 mg / kg LE increased ($P < 0.05$) the total superoxide dismutase (T-SOD) activity in serum. Three doses of LE reduced ($P < 0.05$) serum malondialdehyde content (MDA). 3) 150 mg / kg LE increased ($P < 0.05$) serum IgG level. 4) Dietary LE down-regulated ($P < 0.05$) the mRNA levels of 7 immune-related genes (*IL-6*, *IL-8*, *IL-10*, *IL-1β*, *TNF-α*, *MCP-1*, *ICAM-1*) in the thymus; 50 mg / kg LE and 150 mg / kg LE down-regulated ($P < 0.05$) the mRNA levels of *TNF-α*, while 250 mg / kg LE up-regulated the mRNA levels of 2 inflammatory genes (*IL-1β*, and *ICAM-1*) in the spleen; three levels of LE down-regulated ($P < 0.05$ the mRNA levels of 3 inflammatory genes (*IL-6*, *TNF-α*, *ICAM-1*) in the liver.

Conclusions: In summary, LE supplementation regulates the activity of serum biochemical enzyme, improves the antioxidant capacity and immune function of in serum, liver, spleen and thymus, those improvement may contributes to the promotion of growth performance of weaned piglets. In general, 150 mg / kg LE exhibits better effect.

Introduction

Piglets are often subjected to nutritional, physiological and social pressures during weaning, leading to significant changes in immunology [1], reduced the production of antibodies and depressed the function of cellular immunity [2]. Following the global ban on application of antimicrobial growth promoters in animal feed, the investigation for potential antibiotic alternatives that could alleviate the weaning stress and promote health of piglets become a big challenge.

Weaned piglets will faced many challenge, including oxidant stress and immune stress. Oxidant stress is the result of the combined action of a large number of biological and environmental factors and stress, which is a common phenomenon in animals [3]. Under normal physiological conditions, the generation and scavenging of free radicals are in a balanced state. If the balance is disturbed, which could lead to oxidant stress [4]. It has been reported that many chronic diseases are associated with excessive production of reactive oxygen species (ROS) [5]. Antioxidant enzymes including glutathione peroxidase

and superoxide dismutase, neutralize toxic oxygen products, thereby normalizing the balance of the homeostasis of the body [4]. The malondialdehyde (MDA) is the main product of lipid peroxidation in animals, which indirectly reflect the damage level of cells or tissue *in vivo* [6].

The immune function is one of defense mechanisms against infection in the animal body. Immune function is closely related to inflammation, which is caused by the activation of immune cells in local tissues. The inflammatory response can be regulated by the expression of cytokines, such as IL-1β, IL-6, and TNF-α [7]. However, inflammation with the excessive production of inflammatory cytokines depresses the growth performance of animal [8]. Decrease in the release or expression of pro-inflammatory mediators indicates alleviating inflammatory responses in animals. Therefore, enhancement of body antioxidant capacity and immunity function will help the weaned piglets to copy with weaning stress.

Licorice has been frequently used as a herbal medicine to suppress peroxidation and inflammation. Licorice extracts (LE) are enrich of bioactive compounds such as flavonoids and glycyrrhizin, which have anti-oxidization [9], immune-regulatory [10] and anti-inflammatory [11] properties. Studies have shown that compounds from Licorice have anti-oxidant properties. Chalcone A and chalcone B inhibit lipid peroxidation of rat liver microsomes, and dose-dependently inhibit lipopolysaccharide-induced macrophage reactive oxygen production [9]. Licorice chalcone inhibit mitochondrial lipid peroxidation [12], and has a strong inhibitory effect on the production of superoxide anions and scavenging activity on free radicals, thus protect red blood cells from oxidant hemolysis and effectively protect biological systems free from all kinds of oxidant stress [13]. Furthermore, triterpenoids of licorice increase catalase and glutathione peroxidase activities in rats [14].

Previous research indicates that *Glycyrrhiza uralensis* reduced the release of NO and PGE2, and reduced the mRNA levels of *TNF-α*, *IL-6*, *COX-2*, *IL-1β* in LPS-treated macrophages [11]. In a mouse inflammation model, LE relieves skin swelling, reduces the mRNA expression of pro-inflammatory cytokines *TNF-α*, *IL-1β* and *IL-6*, and inhibits the mRNA expression of *INOS* and *COX-2* [15]. Hence, the biological activities of LE render it a potential alternative for in-feed antibiotics.

We hypothesized that dietary LE supplementation could enhance the antioxidant capacity and immune function of weaned piglets fed on feeds free of antibiotics. Therefore, the study aimed to assess the beneficial effects of dietary LE supplementation on weaning piglets, and biochemical indicators, immunoglobulin, antioxidant capacity and mRNA expression of inflammation-related genes in serum or tissues were investigated.

Materials And Methods

Animals and diets

Ninety-six DLY piglets with initial average body weight of 8.05 ± 0.23 kg) were blocked and randomly assigned to one of four experimental diets based on the initial body weight. Each dietary treatment was replicated with six pens of four pigs per replicate. The four treatments included a control group and three

levels of dietary licorice extract groups that consisted of a basal diet supplemented with 50, 150 or 250 mg/kg licorice extract. The basal diet (S1 Table) was formulated to meet requirements of piglet accroding to the NRC (2012), and diet were provided in a powder form. The licorice extract was supplied by Kaimeijia Biotechnology Company(Xinjiang, China)and contained flavonoid（60%）and glycyrrhizin（10%). The pigs received feed and water on *ad libitum* basis throughout the trial. The trial lasted for five weeks. The room temperature was maintained at 28 ± 1 °C throughout the trial.

Sample collection and preparation

At 35 d, pigs were fasted for eight hours and then six pigs per group with average body weight were selected and sacrificed to collect blood, liver, spleen and thymus samples. Liver, spleen and thymus tissue weight were measureed and divided into aliquots using the surgical scissors. The tissue samples were snap-frozen in liquid nitrogen, and stored at -80 ^{o}C until use. Serum samples were prepared by centrifugation of the whole blood (sodium ethylenediaminetetraacetic acid (EDTA) as anticoagulant, 2000 × g for 15 min, 5804R Centrifuge, F45-30-11 rotor, Eppendorf) and stored at -20 ^{o}C.

Biochemical assays

Serum glutamic oxalacetic transaminase (AST), glutamic pyruvic transaminase (ALT), total protein (TP), albumin (ALB), alkaline phosphatase (ALP), glutamyltranspeptidase (GGT), glucose (GLU), lactate dehydrogenase (LDH), total cholesterol (TC), triglyceride (TG) and high density lipoprotein (HDL), low density lipoprotein cholesterin(HDL), urea nitrogen (BUN)were determined using an Automatic Biochemistry Radiometer (Au720, Olympus).

Serum immunoglobulin analyses

Serum immunoglobulin IgA, IgG and IgM were measured using ELISA assay kits (Meimian, Bioengineering, Jiangsu, China). All samples were analyzed in duplicate. Standard, control and samples were added to wells previously coated with monoclonal antibodies specific to each immunoglobulin. After incubation at 37℃ for 30 minutes, the unbound compounds were washed away and enzyme-linked polyclonal antibodies for each immunoglobulin were added. After incubation at 37℃ for 30 minutes, unbound antibody enzymes were removed by washing. Then, the substrate solution was added to the hole and the color was produced in proportion to the binding amount of immunoglobulin in the initial step. With the addition of the stop fluid, the color development terminates. Color intensity was measured at 450 nm. The concentration is calculated according to the standard curve.

Antioxidant capability

Total antioxidant capability (T-AOC), malondialdehyde (MDA), activity of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) in serum, liver and spleen were measured using corresponding assay kits (Jiancheng Bioengineering, Nanjing, China). Concentration of protein was determined with bicinchoninic acid (BCA) method using a commercial BCA protein assay kit (Jiancheng Bioengineering, Nanjing, China). The optical density (OD) values were measured with an ultraviolet-visible

spectrophotometer (Model 680, Bio-rad, Hercules, CA, USA). For each measurement, the compared samples were run on the same plate to eliminate potential errors origin from different plates.

Q-PCR analyses of mRNA abundance

Total RNA of liver, spleen and thymus samples was extracted by using Trizol (Invitrogen, USA) method and cDNA was synthesized using the PrimeScript RT reagent kit (Takara, China). Real-time quantitative PCR (qPCR) was performed on QuantStudio 6 Flex system (Applied Biosystems, USA) using SYBR Premix Ex Taq™ II kit (No. RR820A, TaKaRa, China). The primers (S2 Table) for 9 inflammation-related genes, 2 reference genes: β-actin (*ACTB*) and glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*), were designed using Primer Express 3.0 (Applied Biosystems, Foster City, CA). The relative mRNA abundance was quantified as previously described using $2^{-\Delta\Delta Ct}$ method [16],For each of the target gene in a given tissue, all samples were run on the same 384-well plate (Applied Biosystems, Foster City, CA) and the average of each duplicate value expressed as the number of copies was used for statistical analysis.

Statistical analysis

Statistical analysis was performed using SPSS for Windows (Version 22.0, Chicago, IL, USA). Difference between groups was evaluated by analysis of variance (ANOVA). Data are presented as means ± standard error and *P* values of less than 0.05 were considered statistically significant.

Results

Effect of LE supplementation on organ index of weaned piglets

The effects of LE supplementation on the organ index of weaned piglets are shown in Table 1. LE supplementation moderately increased the liver index and piglets receiving the 250 mg / kg LE had a higher ($P < 0.05$) liver index than that of the control group. Dietary supplementation with 50 mg / kg LE lowered ($P < 0.05$) the thymus index of piglets, while there were no difference ($P > 0.05$) in the 150 and the 250 mg / kg LE groups compared with the control group. Dietary LE supplementation had no effects ($P > 0.05$) on spleen index and kidney index of piglets .

Table 1
Effect of LE on organ index of weaned piglets.

Item	0 mg/kg LE	50 mg/kg LE	150 mg/kg LE	250 mg/kg LE	*P*-value
liver	24.92 ± 0.92^{a}	27.19 ± 0.74^{ab}	26.21 ± 1.20^{a}	29.96 ± 1.20^{b}	0.017
spleen	2.15 ± 0.16	2.00 ± 0.17	2.10 ± 0.13	2.22 ± 0.29	0.776
thymus	0.97 ± 0.09^{b}	0.72 ± 0.07^{a}	0.83 ± 0.07^{ab}	0.92 ± 0.04^{ab}	0.105
kidney	5.47 ± 0.35	5.20 ± 0.35	4.97 ± 0.23	5.50 ± 0.16	0.514

Data are presented as means ± SE, n = 6. $^{a, b}$ Mean values within a row with different superscript letters were significantly different ($P < 0.05$).

Effect of LE supplementation on serum biochemistry index of weaned piglets

The effects of LE supplementation on piglet serum biochemistry index are presented in Table 2. Dietary LE supplementation increased ($P < 0.05$) activity of the alkaline phosphatase (ALP), and reduced ($P < 0.05$) the activity of glutamic oxalacetic transaminase (AST) in serum of the piglets. Compared with the control group, 50 mg / kg LE supplementation reduced ($P < 0.05$) serum total cholesterol (TC) and high density lipoprotein cholesterol (HDL-C) content, and 150 and 250 mg/kg LE had a trend to reduced ($P > 0.05$) serum TC and HDL-C. The addition of LE at various concentrations had no effects ($P > 0.05$) on serum alanine aminotransferase (ALT) activity, low-density lipoprotein cholesterol content (LDL-C), lactate dehydrogenase (LDH), total protein, globulin, albumin, glutamyl transpeptidase, glucose, urea nitrogen (BUN), and triglyceride (TG) .

Table 2
Effect of LE on serum biochemical parameters of weaned piglets.

Item	0 mg/kg LE	50 mg/kg LE	150 mg/kg LE	250 mg/kg LE	*P*-value
ALT (U/L)	29.00 ± 4.02	33.80 ± 1.71	32.00 ± 2.20	29.00 ± 1.95	0.578
AST (U/L)	57.50 ± 5.95^{b}	38.20 ± 1.98^{a}	38.83 ± 2.01^{a}	41.50 ± 1.80^{a}	0.001
TP (g/L)	55.83 ± 0.82	54.77 ± 1.54	54.93 ± 0.70	55.25 ± 0.67	0.921
ALB (g/L)	26.50 ± 0.29	25.72 ± 1.04	26.52 ± 0.66	25.30 ± 1.23	0.799
GLOB (g/L)	30.37 ± 1.39	29.67 ± 2.04	28.42 ± 1.17	28.37 ± 2.00	0.874
ALP (U/L)	115.2 ± 10.36^{a}	184.4 ± 13.33^{b}	154.8 ± 11.74^{b}	156 ± 13.40^{b}	0.009
GGT (U/L)	33.67 ± 3.55	33.80 ± 4.34	34.67 ± 4.06	30.00 ± 3.42	0.823
GLU (mmol/L)	4.56 ± 0.27	4.26 ± 0.30	4.65 ± 0.14	4.08 ± 0.41	0.531
TC (mmol/L)	2.29 ± 0.07^{b}	1.97 ± 0.07^{a}	2.13 ± 0.07^{ab}	2.06 ± 0.08^{ab}	0.042
TG (mmol/L)	0.40 ± 0.03	0.38 ± 0.02	0.40 ± 0.00	0.40 ± 0.07	0.980
HDL-C (mmol/L)	0.68 ± 0.03^{b}	0.56 ± 0.03^{a}	0.65 ± 0.04^{b}	0.62 ± 0.02^{ab}	0.017
LDL-C (mmol/L)	0.85 ± 0.03	0.91 ± 0.03	0.93 ± 0.03	0.90 ± 0.05	0.611
LDH (U/L)	313.36 ± 31.85	300.66 ± 22.92	271.52 ± 10.91	304.53 ± 32.80	0.612
BUN (mmol/L)	1.30 ± 0.10	1.24 ± 0.05	1.18 ± 0.04	1.36 ± 0.04	0.727

Data are presented as means ± SE, n = 6. $^{a, b}$ Mean values within a row with different superscript letters were significantly different (P < 0.05).

Effect of LE on antioxidant capacity of serum, liver and spleen in weaned piglets

We investigated the effect of LE supplementation on antioxidant measurements in serum,liver and spleen of weaned piglets (Table 3). Compared with the control group, 150 mg/kg and 250 mg/kg LE supplementation increased ($P < 0.05$) serum total antioxidant capacity (T-AOC). Three levels of dietary LE reduced ($P < 0.05$) serum malondialdehyde (MDA) content. 50 mg/kg LE increased ($P < 0.05$) enhanced serum total superoxide dismutase (T-SOD). Dietary LE had no limited ($P > 0.05$) effects on the activity of serum GSH-PX, although 150 mg/kg LE group had a highest activity. In liver, Dietary LE exhibited limited effects ($P > 0.05$) on T-AOC, MDA, T-SOD and GSH-PX, except that 250 mg/kg LE increased ($P < 0.05$) activity of GSH-PX when compared with the control. In spleen, 150 mg/kg and 250 mg/kg LE supplementation increased ($P < 0.05$) T-AOC, and 150 mg/kg LE increased ($P < 0.05$) activity of GSH-PX compared with the control. Dietary did not affected ($P > 0.05$) spleen MDA and T-SOD.

Table 3
Effect of LE on antioxidant capacity of serum, liver and spleen in weaned piglets.

Item	0 mg/kg LE	50 mg/kg LE	150 mg/kg LE	250 mg/kg LE	*P*-value
serum					
T-AOC (U/ml)	1.10 ± 0.13^{a}	1.21 ± 0.11^{ab}	1.83 ± 0.18^{c}	1.57 ± 0.13^{bc}	0.007
MDA (nmol/ml)	2.98 ± 0.33^{b}	2.38 ± 0.11^{a}	2.41 ± 0.15^{a}	2.33 ± 0.17^{a}	0.106
T-SOD (U/ml)	119.46 ± 5.62^{a}	135.36 ± 2.12^{b}	124.67 ± 1.20^{a}	124.38 ± 4.54^{a}	0.015
GSH-P_X (U/ml)	764.74 ± 44.29	682.13 ± 29.66	808.10 ± 43.75	742.52 ± 8.33	0.284
Liver					
T-AOC (U/mgport)	0.69 ± 0.04	0.77 ± 0.04	0.80 ± 0.07	0.81 ± 0.03	0.384
MDA (nmol/mgport)	0.83 ± 0.07	0.77 ± 0.07	0.63 ± 0.09	0.78 ± 0.09	0.356
T-SOD (nmol/mgport)	470.81 ± 19.35	478.43 ± 24.44	470.55 ± 13.89	497.46 ± 19.02	0.724
GSH-P_X (nmol/mgport)	585.26 ± 14.68^{a}	603.47 ± 26.30^{ab}	604.07 ± 22.80^{ab}	676.23 ± 34.42^{b}	0.089
Spleen					
T-AOC (U/mgport)	0.76 ± 0.08^{a}	0.81 ± 0.10^{a}	1.44 ± 0.15^{b}	1.29 ± 0.13^{b}	0.001
MDA (nmol/mgport)	1.08 ± 0.15	1.07 ± 0.17	0.99 ± 0.13	0.79 ± 0.06	0.400
T-SOD (nmol/mgport)	7.57 ± 0.45	7.86 ± 0.37	7.62 ± 0.51	7.09 ± 0.24	0.376
GSH-P_X (nmol/mgport)	542.22 ± 29.68^{a}	544.27 ± 17.58^{a}	599.62 ± 8.49^{b}	559.00 ± 12.95^{ab}	0.080

Data are presented as means ± SE, n = 6. $^{a, b}$ Mean values within a row with different superscript letters were significantly different ($P < 0.05$).

Effect of LE supplementation on serum immunoglobulin levels

We investigated serum immunoglobulin levels (Table 4). Relative to the control group, the addition of 150 mg/kg LE increased ($P < 0.05$) the serum IgG content of piglets. LE addition had no effect ($P > 0.05$)

on serum IgA, but had a tendency to increase ($P > 0.05$) the serum IgM, and piglets supplied with 150 mg/kg LE had the highest serum IgM levels.

Table 4
Effect of LE on piglet serum immunoglobulin levels of weaned piglets (μg/ml).

Item	0 mg/kg LE	50 mg/kg LE	150 mg/kg LE	250 mg/kg LE	*P*-value
IgA	39.95 ± 2.65	38.41 ± 3.24	38.34 ± 3.45	41.26 ± 3.44	0.603
IgG	423.17 ± 13.96^{a}	446.54 ± 22.79^{a}	514.70 ± 18.44^{b}	485.60 ± 23.82^{ab}	0.043
IgM	35.55 ± 2.14	40.22 ± 3.90	41.23 ± 3.88	38.19 ± 3.37	0.657

Data are presented as means ± SE, n = 6. $^{a, b}$ Mean values within a row with different superscript letters were significantly different ($P < 0.05$).

Effect of LE supplementation on mRNA of inflammation-related genes in liver, spleen and thymus

The mRNA expression of 9 inflammation-related genes (*ICAM-1*, *IL-1β*, *IL-2*, *IL-6*, *IL-8*, *IL-10*, *MCP-1*, *TNF-α* and *INOS*) in liver were determined (Fig. 1). Compared with the control group, dietary LE down-regulated ($P < 0.05$) the mRNA levels of 3 inflammatory genes (*IL-6*, *TNF-α*, *ICAM-1*) in the liver. Dietary LE also affected expression of inflammation-related genes in spleen (Fig. 2). Relative to the control group, 50 mg / kg LE and 150 mg / kg LE down-regulated ($P < 0.05$) the mRNA levels of *TNF-α*, while 250 mg / kg LE up-regulated ($P < 0.05$) the mRNA levels of 3 inflammatory genes (*IL-1β*, *MCP-1*, *ICAM-1*) in the spleen. It seems that dietary LE exhibited great impact on expression of expression of inflammation-related genes in thymus (Fig. 3) and moderately down-regulated ($P < 0.05$) the mRNA levels of 7 inflammation-related genes (*IL-6*, *IL-8*, *IL-10*, *IL-1β*, *TNF-α*, *MCP-1* and *ICAM-1*). In general LE supplementation suppresses the expression of inflammation-related genes in liver, spleen and thymus, thus enhanced the immunity function of weaned piglets and 150 mg /kg dietary LE exhibited the optimal effect.

Discussion

Weaning stress decrease growth performance and damage the immune function of piglets [1, 2, 17]. Supplementation of plant extracts improve growth performance, alleviate oxidant stress and improve immune function, thereby reduce the negative effects of weaning stress on piglets[18, 19]. LE exhibits antioxidant function [12–14] and immunomodulatory function on RAW264.7 cells and mice [11, 20–22], thus is a potential potential alternatives protection against early weaned stress in piglets.

Serum biochemical indicators reflects the metabolism and deposition of substances in the animal body. We investigated dietary LE supplementation on serum biochemical indicators of piglets. LE supplement reduced AST level in the serum of piglets (Table 2). Transaminase plays an important role in the process of amino acid metabolism, and AST acts as a indicator intracellular enzyme relative to liver function and be used as the index of liver damage [23]. Reduced serum AST level indicated improvement of the liver

function of weaned piglets. Studies have revealed that licorice extract or its active ingredient glycyrrhizic acid have hepatoprotective function, and licorice extract inhibited the increase of serum AST and ALT in a liver injury rat model induced by carbon tetrachloride [24]. Glycyrrhetinic acid, a decomposition product of glycyrrhizic acid, improves the liver index of piglets and promoted liver development [25],and prevents the toxic accumulation of bile acids, and protect the liver from cholic acid [24], protects liver cells from oxidant damage induced by tert-butyl hydroperoxide and prevents cell death by inhibiting the reduction of intracellular glutathione and reactive oxygen species and depolarizing mitochondrial membrane [26]. Therefore, it not strange that LE increased the liver index of weaned piglets (Table 1), indicating a protective effect of LE on the liver function [27]. The activity of alkaline phosphatase (AKP) in serum reflects the metabolic efficiency of lipids. AKP is an isozyme with genetic markers. Animal performance are affected by AKP activity and the daily body weight gain is positively correlated with AKP activity [28]. In present study LE increased the serum AKP activity (Table 2), which is consistent with improvement of the growth performance of weaned piglets. Serum cholesterol reflects the absorption and metabolism of lipids by the animal body. 50 mg/kg LE reduced TC and HDL-C in serum (Table 2), similar results are found that supplementation of LE through drinking water reduces the serum glucose, low density lipoprotein cholesterol (L-DLC) and total cholesterol in broilers [29]. This decreases of serum TC and HDL-C may be related to the inhibition of lipid peroxidation by licorice, the active ingredient of licorice inhibits the formation of lipid peroxides, and in the subsequent liver clearance process increases the rate of conversion of cholesterol into bile acids, thereby reducing cholesterol and increased liver bile acid content [30]. LE had no effect on the spleen index and kidney index of weaned piglets (Table 1). Studies shown that licorice extract mainly affects liver and intestine of weaned piglets [20, 31].

Weaning stress results in oxidant stress, which induces a variety of diseases. We explored the effect of dietary LE on the antioxidant capacities in serum, liver and spleen. Dietary LE supplementation moderately increased activity of GSH-Px, SOD and T-AOC, and decreased MDA level in those tissues (Table 3). The antioxidant activity of LE may be attributed to its chemical components, including glycyrrhizin flavonoids, glycyrrhizin and polysaccharides [32, 33], which have multiple phenolic hydroxyl groups and benzene ring structures. Chalcone C attenuates the inflammation induced by lipopolysaccharide and interferon by reducing the expression of inducible NO and regulating the activity of antioxidant enzymes such as glutathione peroxidase [34] Glycyrrhizin inhibit mitochondrial lipid peroxidation and scavenges free radicals [12], Chalcone has a strong inhibitory effect on the production of superoxide anions, and also has a strong scavenging activity on free radicals[14]. The mechanism of antioxidant capacity of flavonoids compounds include inhibiting the generation of ROS by affect their conformation and inhibiting enzyme activity involved in their production, scavenging of ROS, or up-regulation or protection of antioxidant defense [35, 36]. Furthermore, flavonoids obviously inhibit the formation of malondialdehyde (MDA) and has a strong scavenging effect on hydroxyl radical and superoxide anion radical [33]. The LE used in present study contains 60% flavonoids, therefore supplementation of dietary LE enhanced the antioxidant capacity of weaned piglets.

To investigate whether LE exhibits immune-regulatory and anti-inflammatory properties in weaned piglets, we measured expression of 9 inflammation-related genes (*ICAM-1*, *IL-1β*, *IL-2*, *IL-6*, *IL-8*, *IL-10*, *MCP-1*, *TNF-*

α and *INOS*) in liver, spleen and thymus. LE down-regulated the mRNA levels of 3 inflammatory genes (*IL-6*, *TNF-α*, *ICAM-1*) in liver(Fig. 1), *TNF-α* in spleen (Fig. 2) and 7 genes (*IL-6*, *IL-8*, *IL-10*, *IL-1β*, *TNF-α*, *MCP-1* and *ICAM-1*) in thymus (Fig. 3). IL-6 and TNF-α were important mediator of inflammatory response, which were commonly used as markers for systemic pro-inflammatory cytokine activation [37], and TNF-a, IL-1β and IL-6 are also important inducers of acute phase proteins [38]. IL-2 is known to be associated with cellular immunity and play important roles in animal immune function [39]. IL-8 plays a key role in the recruitment and activation of neutrophils during inflammation [40]. The immune response can be regulated by the expression of INOS, ICAM-1 and MCP-1[41, 42], and ICAM-1 is an adhesion molecule that promotes the firm adhesion of leukocytes to endothelial cells [43], ICAM-1 and MCP-1 have a low basal expression in epithelial cells but is up-regulated in response to a variety of inflammatory mediators [44]. The down-regulated of those inflammation-related genes in liver,spleen and thymus indicated enhancement of immunity function of the weaned piglets by LE. Similar results are found that licorice extract reduces the secretion and mRNA levels of TNF-α, IL-6 and IL-1β in macrophages, and inhibits skin swelling and the expression of INOS and COX-2 in a mouse inflammation model [45]. Ural Glycyrrhiza reduces the release of NO and PGE2 in LPS-treated macrophages, and down-regulates mRNA levels of IL-6, TNF-α, IL-1β and COX-2 [11]. Chalcone A (LCA), the active ingredients of flavonoids in licorice extract, inhibits the release of cytokines in mouse [46]. IL-10 is an anti-inflammatory cytokine associated with humoral immunity [47]. The down-regulation on expression of *IL-10* in thymus by LE (Fig. 3), may indicated that piglets were in a health condition and not need to expression more IL-10 to maintain the immune homeostasis (Fig. 3).

We further investigated the serum immunoglobulin levels of piglets. LE increased the serum IgG and had a tendency to increase the serum IgM (Table 4). IgG is accounting for about 80% of the total serum immunoglobulin, and play roles in resisting the invasion of viral infections [48]. The increase of serum IgG indicated enhancement of the serum immunity of weaned piglets by dietary LE. The active ingredients in licorice extract, such as glycyrrhizin flavonoids and glycyrrhizic acid, increase the phagocytic function of phagocytic cells and regulate the number and function of lymphocytes [49], thus prevent some inflammatory mediators by regulating immune-related signaling pathways or enzyme activities produced to activate macrophages, indirectly inhibit platelet aggregation [30], inflammatory cytokine secretion [30, 50] and neutrophil adhesion [43, 51], thus regulate the inflammatory response and improve immune function of piglets.

Conclusions

In summary, dietary supplementation with licorice extract regulates serum biochemistry enzyme activity, promotes liver metabolic function, improves the antioxidant capacity, inhibites the expression of inflammation-related genes and enhances the serum IgG content, thus promotes the health status and improves growth performance of weaned piglets.

Declarations

Acknowledgement

None.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

Author contributions were as follows. H. Z. and T. Y. designed the research; T. Y. and J. T. conducted the experiments; J.T.,G. J., G. L., X. C., G. T., J. C., B.K. and H. Z. collected sample and analyzed the data; H. Z. and T. Y. wrote the paper; and H. Z. had primary responsibility for the final content. All authors read and approved the final manuscript.

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Ethics approval and consent to participate

The experiment followed the actual law of animal protection and was approved by the Animal Care and Use Committee of the Sichuan Agricultural University (Ethic Approval Code: SCAUAC201308-1).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Abbreviations

LE: licorice extract; T-AOC: Total antioxidant capability; MDA: malondialdehyde; SOD: superoxide dismutase; GSH-Px: glutathione peroxidase; ELISA: Enzyme linked immunosorbent assay; IgA: Immune globulin A; TNF-α: Tumor necrosis factor α; IL: Interleukin; INOS: Inducible nitric oxide synthase; MCP-1: Monocyte chemoattractant protein

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Figures

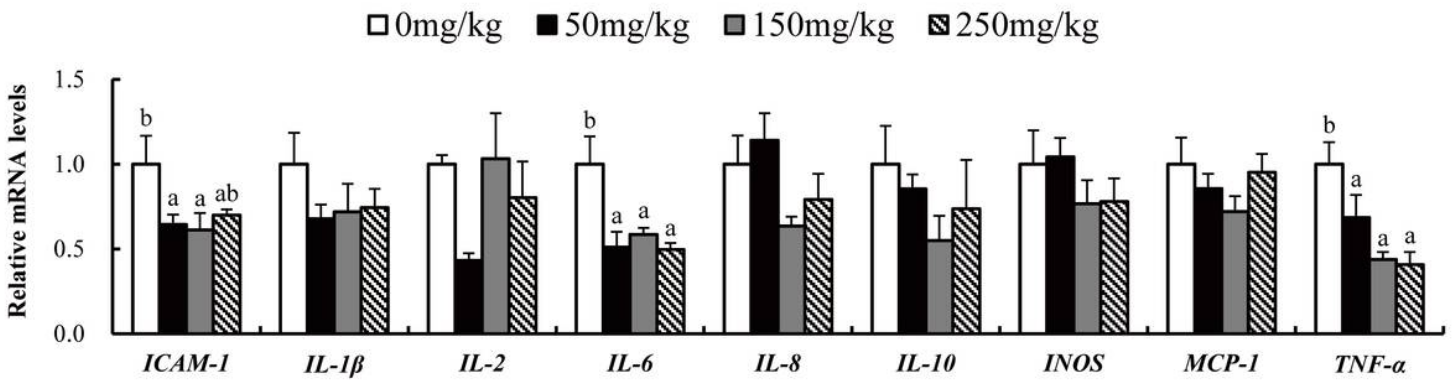

Figure 1

Effect of LE on mRNA levels of liver inflammation-related genes in weaned Piglets. Data are presented as means ± SE (n = 6). a, b Mean values with unlike letters were significantly different (P < 0.05).

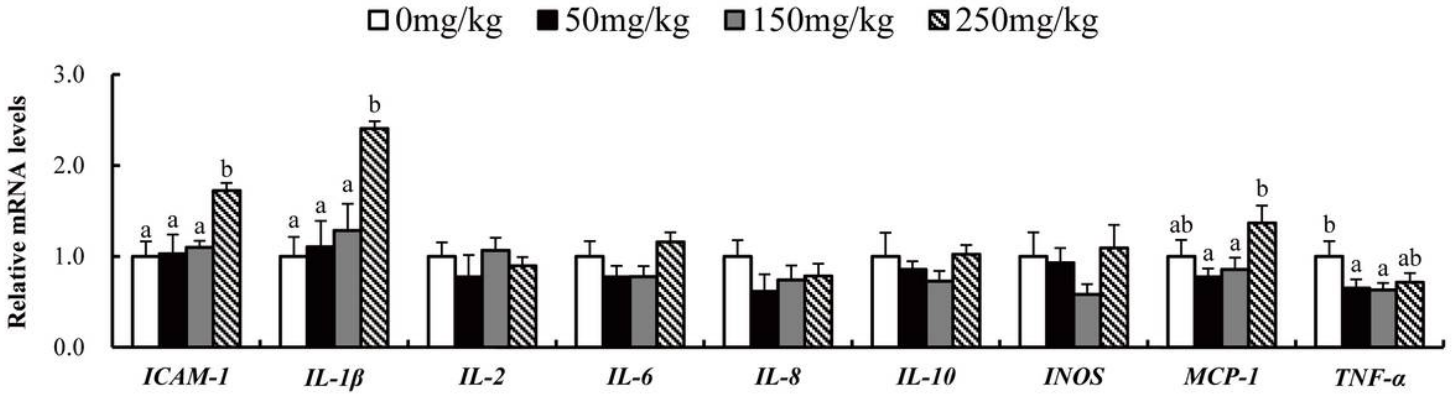

Figure 2

Effect of LE on mRNA levels of spleen inflammation-related genes in weaned Piglets. Data are presented as means ± SE (n = 6). a, b Mean values with unlike letters were significantly different (P < 0.05).

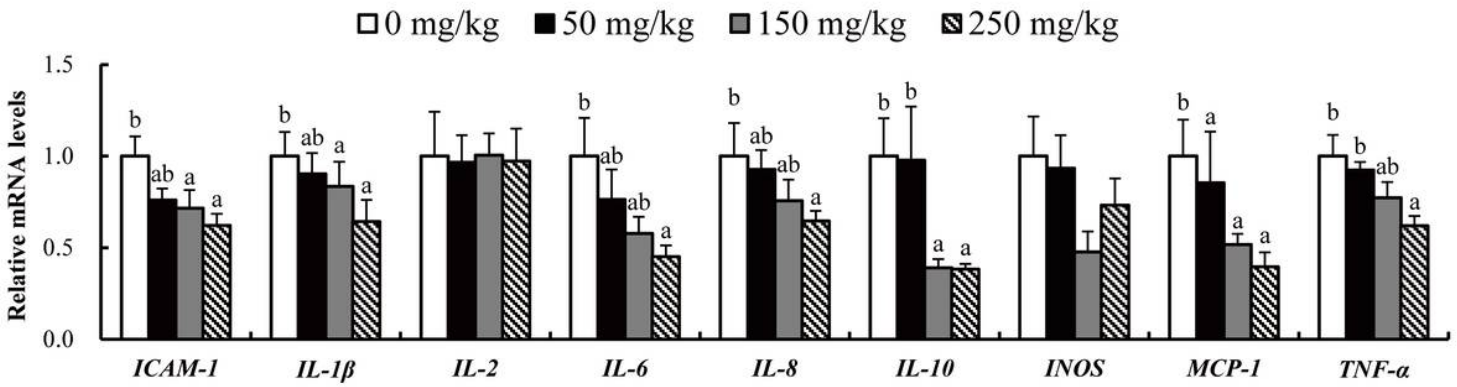

Figure 3

Effect of LE on mRNA levels of thymus inflammation-related genes in weaned piglets. Data are presented as means ± SE (n = 6). a, b Mean values with unlike letters were significantly different (P < 0.05).

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