Epidermal Growth Factor Ameliorates Essential Trace Element Absorption in The Gastrointestinal Tract by Regulating The Expression of Microelement Transport-Relative Genes in Lipopolysaccharide Challenged Early Weaning Piglets

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Research

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Abstract

Background: Epidermal growth factor (EGF) plays an important role in nutrient utilization. A 14-days trial was conducted to investigate how EGF attenuates the effect of lipopolysaccharide (LPS) on the growth performance and the nutrient utilization of early-weaned pigs.

Methods: A total of 48 early weaned piglets were randomly distributed among 4 groups, which included the control, EGF, LPS and EGF + LPS groups. Each group had 6 replicates and each replicate consisted of 2 piglets. The experiment lasted for 14 d.

Results: The results showed that LPS exposure significantly decreased the average daily gain (ADG) and significantly increased the feed conversion ratio (FCR) of the weaned pigs compared with the groups without LPS treatment ($P<0.05$), while dietary EGF could significantly increase the average daily feed intake (ADFI) compared with the diet without EGF supplementation ($P<0.05$). The LPS treatment significantly decreased the apparent digestibility of crude fat compared with the groups without LPS treatment ($P<0.05$). The LPS treatment resulted in a significantly increased concentration of Cu, Fe, Zn and Mn in the stomach, jejunum and ileum chyme, and feces compared with the groups without LPS treatment ($P<0.05$), while the EGF treatment significantly decreased these indicators compared with the diet without EGF supplementation ($P<0.05$). In the gastrointestinal tract, the LPS treatment significantly decreased the expression levels of the zrt-irt-like protein 4 (Zip4), zrt-irt-like protein 7 (Zip7), copper transport protein 1 (Ctr1), antioxidant 1 (Atox1), copper chaperone for superoxide dismutase (CCS), cytochrome c oxidase copper chaperone 17 (Cox17), copper-transporting P-type 7A (ATP7A), copper-transporting P-type 7B (ATP7B), divalent metal transporter 1 (DMT1), cytochrome b (CYTB), and transferrin (Tf) genes compared with the groups without LPS treatment ($P<0.05$), while the EGF treatment significantly increased the expression levels of the Zip4, Ctr1, Atox1, CCS, Cox17, ATP7A, ATP7B, DMT1, CYTB, and hephaestin (Hp) genes compared with the diet without EGF supplementation ($P<0.05$).

Conclusion: Dietary EGF could attenuate the effect of LPS exposure on the essential trace element absorption by changing the expression levels of microelement transport-relative genes of early-weaned pigs.

Background

Essential trace elements are the indispensable nutrition for animals, and especially the Cu, Fe, Zn, and Mn are required for the normal growth, development, and many physiological functions in animals [1–4]. Cu is a part of Cu-transporting P-type ATPase and Cu/Zn superoxide dismutase [5]. Fe as the part of hemoglobin and myoglobin plays important role in deliver the oxygen, it also plays a vital role in the host immunity [6]. Zn takes part in the growth, oxidation resistance and immunity [7]. Mn as the part of phosphoenolpyruvate carboxykinase takes part in the gluconeogenesis, and it is related to the neuronal health [8]. However, the absorption of essential trace element is lower in the body.

Pig (*Sus scrofa*) is one of the most raised animals in the world. Piglets are weaned early to increase the reproductive performance of the sow and to reduce pathogen transmission [9]. However, because of rapid and dramatic change of the living environment and expose to the bacteria [10], early weaning piglets are easy to suffer from stress, which reduce the growth performance and feed intake [11], and decrease the digestibility through digestive disorders [12]. The reduction of nutrient absorption leads to the resources wasting and the environment pollution which limits the sustainable development of animal husbandry. Meanwhile, the replacement of milk with a less digestible diet will also lead to maldigestion during the period of weaning when the digestive system of piglet is immature [13]. The absorption of nutrition is closely related to the intestinal health; however, early weaning stress increase the intestinal permeability of piglets which has negative effect on the nutrient utilization [14]. Lipopolysaccharide (LPS) is the primary component of gram-negative bacteria outer cell walls [15], and it can induce severe bacterial diarrhea, apoptosis [16], inflammatory responses [17], intestinal barrier damage [18], and then inhibits the growth and decrease the nutrient absorption of the animal [19]. Due to its good repeatability, the LPS stress mode is widely used in the study of animal stress.

Many of growth factors exist in the milk, such as insulin, nerve growth factor (NGF), and epidermal growth factor (EGF) which can improve the intestinal development of piglet and thus improve their growth performance [20]. Early weaning prevents the supply of those growth factors from milk to piglets. Interestingly, EGF is one of the most abundant growth factors in the milk [21, 22], which indicates its irreplaceable role for young mammals. EGF was first isolated by Dr. Cohen from the mouse (*Mus musculus*) submaxillary gland in 1962 [23]. It is a polypeptide comprising 53 amino acids [24]. It is found in many body fluids such as the milk, blood, saliva, and intestinal fluid [25], and it plays important roles in the regulation of cell growth, proliferation, apoptosis and tumorigenesis [26–28]. Previous studies showed that EGF could improve the growth performance of broiler chicks (*Gallus gallus*) [27] and rats (*Rattus norvegicus*) [29]. Dietary EGF can augment the intestinal length and villus height by activating the phosphatidylinositol-3-kinases/protein-serine-threonine kinase (PI3K/AKT) and RAS/mitogen-activated protein kinase (RAS/MAPK) signaling pathways [30, 31]. Meanwhile, EGF can also promote the proliferation of goblet cells [13] and increase the activity of digestive enzymes in the intestine [32]. However, the effect of EGF on growth performance and nutrient utilization in the early-weaning pigs is unclear. In this experiment, a model of LPS stress was established to examine how EGF attenuates the effect of LPS on the growth performance and nutrient utilization (especially the absorption of Cu, Fe, Zn, and Mn) in the early-weaning pigs.

Methods

Experimental animals and diets

A total of 48 Duroc × Landrace × Large White early weaned piglets (castrated male pigs, average initial weight was 7.84 ± 0.30 kg), aged 25 d, were randomly distributed among 4 groups. Each group had 6 replicates and each replicate consisted of 2 piglets. The basal diet (control group) met the nutrient requirements of pigs according to NRC 2012 (Table 1). The Piglets in the EGF and EGF + LPS groups were fed the basal diet supplemented with 2 $mg \cdot kg^{-1}$ EGF (Peprotech, Rocky Hill, USA). The concentration of essential microelements in these two kinds of diets are shown in Table 2. There are no significantly differences of Cu, Fe, Zn, and Mn concentration between these two kinds of diets ($P<0.05$). Piglets in the LPS (dietary the basal diet) and EGF + LPS groups

were intraperitoneally injected with the 100 μg·kg^{-1} body weight LPS (Sigma-Aldrich, Saint Louis, USA) at 7 d and 15 d. Meanwhile, the control and EGF groups were injected with the corresponding volume physiological saline (Nanjing Jiancheng Biotechnical Institute, Nanjing, China).

The experiment lasted for 14 d and the pigs had ad libitum access to feed and water during this period. The environmental conditions were suitable for pigs, and the temperature kept at 20°C. The pigs were weighed in the morning at 1 d and 15 d, and feed intake was recorded every day. The following variables were calculated: Initial body weight (IBW, g) = W_i/N_0; Final body weight (FBW, g) = W_f/N_t; Average daily feed intake (ADFI, g) = F_t/D_t; Average daily gain (ADG, g) = $(W_f - W_i)/D_t$; Feed conversion ratio (FCR, g·g^{-1}) = $F_t/(W_f - W_i)$. Where W_i and W_f were the total initial and final pig weight, respectively; N_0 and N_t were the initial and final number of pigs; and F_t and D_t were the total feed intake and total experimental days.

Sample collection

Feces were collected from 11 d to 14 d during the trial and were stored at −20°C. At the end of the experiment, all pigs were slaughtered 4 h after the final injection of LPS. Before slaughter, the weaned piglets were euthanized with Zoletil (Virbac, Beijing, China) at 15 mg·kg^{-1} body weight. The chyme samples from the stomach, jejunum and ileum of each replicate were collected and immediately frozen at −20°C. The mucosa of stomach, duodenum, jejunum and ileum samples of each replicate were collected and immediately frozen at −80°C for Q-RT-PCR analysis.

Nutrient digestibility and essential microelements concentration

The diet and the feces and chyme retrieved from the stomach, jejunum and ileum were dried at 105°C. Then, they were ground into a fine powder and passed through a 40 μm mesh. Gross energy, crude protein, crude fat, crude fiber, and P were tested according to the methods of the Association of Official Analytical Chemists International, 2007. The digestibility of nutrients was calculated as follows: digestibility (%) = 100 − $(I_d/I_s) \times (N_s/N_d) \times 100$, where I_d and I_s were the content of the indicator in the diet and the sample, respectively, and N_s and N_d were the content of the nutrient in the sample and the diet, respectively.

Samples of diet, feces and chyme were digested in concentrated nitric acid and perchloric acid (the addition ratio was 4:1) to dissolve the Cu, Fe, Zn and Mn (GBT 23942-2009), and concentration were analyzed by electron coupled plasma atomic emission spectrum (Ke Jie Instrument Limited Company, Nanjing, China).

Quantitative real-time PCR (Q-RT-PCR) analysis

The relative expression levels of zrt-irt-like protein 4 (Zip4), zrt-irt-like protein 7 (Zip7), zinc transporter 1 (ZnT1), zinc transporter 4 (ZnT4), copper transport protein 1 (Ctr1), cytochrome c oxidase copper chaperone 17 (Cox17), antioxidant 1 (Atox1), copper-transporting P-type 7A (ATP7A), copper-transporting P-type 7B (ATP7B), copper chaperone for superoxide dismutase (CCS), divalent metal transporter 1 (DMT1), cytochrome b (CYTB), hephaestin (Hp), and transferrin (Tf) in the mucosa of the stomach, duodenum, jejunum and ileum which from 6 pigs per treatment were detected by Q-RT-PCR. The primers (Sangon Biotech, Shanghai, China) used are listed in Table 3. The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was chosen as the reference gene for sample normalization. Total RNA from the intestinal tissue was extracted using the TRIzol reagent (Invitrogen, Carlsbad, CA). The integrity of each RNA sample was estimated by 1% agarose gel electrophoresis (Sangon Biotech, Shanghai, China). The cDNA was synthesized using a SMART cDNA Synthesis Kit (Clontech Laboratories, Palo Alto, CA) by following the manufacturer's protocol. Q-RT-PCR reactions were carried out in a BIO-RAD CFX96 touch Q-PCR system (Applied Biosystems, Foster City, USA) in 20 μL volumes that contained the following components: 10 μL of SYBR Green Mix (Takara, Changsha, China), 2 μL cDNA (1000 ng·μL^{-1}), 0.4 μL of each primer (10 mM) and 7.2 μL dH2O, followed by 40 cycles of 95°C for 30 s, 55°C or 58°C for 30 s, and 72°C for 30 s. Finally, a melt curve analysis was used to detect the single product (temperature from 65°C to 95°C). All samples were tested in triplicate. The $2^{-\Delta\Delta CT}$ method was used to analyze the relative expression level. The standard curve was obtained by using 5-fold serial dilutions of cDNA (in triplicate), and the amplification efficiency was calculated by using the following equation: E = 10*(-1/slope)-1, and the amplification efficiencies of all primes ranged from 0.90 to 1.10.

Statistical analysis

The date about the concentration of essential microelements in the diets were subjected to independent-samples T test. The other data were subjected to two-way ANOVA to obtain the statistical differences among the groups with EGF and LPS treatment as independent variables. When a significant interaction was observed, data were subjected to one-way ANOVA, and differences were tested by Duncan's multiple-range test (SPSS 22.0). The level of significance was set at $P < 0.05$. The results are presented as the mean values with their standard deviation (means ± SD).

Results

Growth performance

Compared with the diet without EGF supplementation, supplied with EGF in the diet increased the ADFI ($P < 0.05$). The IBW, FBW, ADG, and FCR were not affected by EGF supplementation in the diet ($P > 0.05$). Injected with LPS decreased the ADG and increased the FCR compared with the treatment without LPS injection ($P < 0.05$). The IBW, FBW, and ADFI were not affected by whether injected with LPS ($P > 0.05$). The present study revealed a significantly interaction between EGF and LPS treatment regarding the ADFI ($P < 0.05$), while there was no significant interaction regarding other growth performance indexes ($P > 0.05$). The lowest ADFI was observed in the control group, which was significantly lower than the other groups ($P < 0.05$), while there were no significant differences among the EGF, LPS and EGF + LPS groups ($P > 0.05$). Besides, there were no significant differences among all groups for the IBW, FBW, ADG and FCR ($P > 0.05$) (Table 4).

Nutrient apparent digestibility

The results of apparent nutrient digestibility are shown in Table 5. Injected with LPS decreased the apparent digestibility of the crude fat compared with the treatment without LPS injection ($P < 0.05$). The apparent digestibility of crude protein, crude fiber, gross energy, and P were not affected by whether injected with LPS ($P > 0.05$). All the indexes of apparent digestibility were not affected by EGF treatment in the diet ($P > 0.05$). There were no interactions between EGF and LPS treatment regarding all the indexes of apparent digestibility ($P > 0.05$). The lowest apparent digestibility of crude fat was observed in the LPS group, which was significantly differed from the other groups ($P < 0.05$), while there were no significant differences among the control, EGF and EGF + LPS groups ($P > 0.05$). Besides, there were no significant differences among all groups for the apparent digestibility of crude protein, crude fiber, gross energy, and P ($P > 0.05$).

Concentration of essential microelements in the gastrointestinal tract chyme and feces

The essential microelement concentration in the gastrointestinal tract chyme and feces are shown in Table 6. Compared with the diet without EGF supplementation, supplied with EGF in the diet decreased the Cu concentration in the jejunum and ileum chyme, and feces ($P < 0.05$). The Cu concentration in the stomach chyme was not affected by EGF supplementation in the diet ($P > 0.05$). Injected with LPS decreased the Cu concentration in the ileum chyme and increased the Cu concentration in the jejunum chyme compared with the treatment without LPS injection ($P < 0.05$). The Cu concentration in the stomach chyme and feces were not affected by whether injected with LPS ($P > 0.05$). The present study revealed a significantly interaction between the EGF and LPS treatment regarding the Cu concentration in the stomach, jejunum and ileum chyme ($P < 0.05$), and there was no interaction in the feces ($P > 0.05$). The LPS group had a significantly greater Cu concentration compared with the other groups in the jejunum chyme ($P < 0.05$), while there were no significant differences among the control, EGF, and EGF + LPS groups ($P > 0.05$). The control and LPS groups had significantly greater Cu concentration compared with the EGF and EGF + LPS groups in the ileum chyme ($P < 0.05$), and EGF group had a significantly greater Cu concentration compared with the EGF + LPS group ($P < 0.05$), while there was no significant difference between the control and LPS groups ($P > 0.05$). There were no significant differences among all groups for the Cu concentration in the stomach chyme and feces ($P > 0.05$).

Compared with the diet without EGF supplementation, supplied with EGF in the diet decreased the Fe concentration in the ileum chyme, and feces ($P < 0.05$). The Fe concentration in the stomach and jejunum chyme were not affected by EGF supplementation ($P > 0.05$). Injected with LPS decreased the Fe concentration in the ileum chyme and increased the Fe concentration in the jejunum chyme and feces compared with the treatment without LPS injection ($P < 0.05$). The Fe concentration in the stomach chyme was not affected by whether injected with LPS ($P > 0.05$). The present study revealed a significantly interaction between EGF and LPS treatment regarding the Fe concentration in the feces ($P < 0.05$), and there were no interactions in the stomach, jejunum and ileum chyme ($P > 0.05$). In the jejunum chyme, the LPS group had a significantly greater Fe concentration compared with the control and EGF groups ($P < 0.05$), and the EGF + LPS group had a significantly greater Fe concentration compared with the control group ($P < 0.05$), while there were no significant differences between the control and EGF groups, or the LPS and EGF + LPS groups, or the EGF and EGF + LPS groups ($P > 0.05$). In the ileum chyme, there were no significant differences between the control and LPS groups for Fe concentration ($P > 0.05$), but these two groups had significantly greater Fe concentration compared with the EGF and EGF + LPS groups ($P < 0.05$), and the EGF group had a significantly greater Fe concentration compared with the EGF + LPS group ($P < 0.05$). In the feces, the LPS group had a significantly greater Fe concentration compared with the other groups ($P < 0.05$), and the control group had a significantly greater Fe concentration compared with the EGF and EGF + LPS groups ($P < 0.05$), while there were no significant differences between the EGF and EGF + LPS groups ($P > 0.05$). There were no significant differences among all groups for the Fe concentration in the stomach chyme ($P > 0.05$).

Compared with the diet without EGF supplementation, supplied with EGF in the diet decreased the Zn concentration in the jejunum and ileum chyme, and feces ($P < 0.05$). The Zn concentration in the stomach chyme was not affected by EGF supplementation ($P > 0.05$). Injected with LPS increased the Zn concentration in the jejunum and ileum chyme, and feces compared with the treatment without LPS injection ($P < 0.05$). The Zn concentration in the stomach chyme was not affected by whether injected with LPS ($P > 0.05$). The present study revealed significantly interaction between EGF and LPS treatment regarding the Zn concentration in the stomach and ileum chyme, and feces ($P < 0.05$), and there was no significant interaction in the jejunum chyme ($P > 0.05$). A significantly greater and lowest Zn concentration was respectively observed in the LPS and EGF groups in the ileum chyme, which significantly differed from the other groups ($P< 0.05$), and the control group had a significantly greater Zn concentration compared with the EGF + LPS group ($P < 0.05$). In the feces, the LPS group had a significantly greater Zn concentration compared with the other groups ($P < 0.05$), and the control group had a significantly greater Zn concentration compared with the EGF group ($P < 0.05$), while there was no significant difference between the control and EGF + LPS groups, or the EGF and EGF + LPS groups ($P > 0.05$).

Compared with the diet without EGF supplementation, supplied with EGF in the diet decreased the Mn concentration in the jejunum and ileum chyme, and feces ($P < 0.05$). The Mn concentration in the stomach chyme was not affected by EGF supplementation ($P > 0.05$). Injected with LPS increased the Mn concentration in the stomach chyme, and feces compared with the treatment without LPS injection ($P < 0.05$). The Mn concentration in the jejunum and ileum chyme were not affected by whether injected with LPS ($P > 0.05$). There were no interactions between EGF and LPS treatment regarding the Mn concentration in the stomach, jejunum and ileum chyme, and feces ($P > 0.05$). In the stomach chyme, the LPS and EGF + LPS groups had significantly greater Mn concentration compared with the other groups ($P < 0.05$), while there were no significant differences between the control and EGF groups, or the LPS and EGF + LPS groups ($P > 0.05$). In the jejunum chyme, the control and LPS groups had significantly greater Mn concentration compared with the other groups ($P < 0.05$), while there were no significant differences between the control and LPS groups, or the EGF and EGF + LPS groups ($P > 0.05$). For the ileum chyme, the control and LPS groups had significantly greater Mn concentration compared with the other groups ($P < 0.05$), and EGF + LPS group had a significantly greater Mn concentration compared with the EGF group ($P < 0.05$), while there was no significant difference between the control and LPS groups ($P > 0.05$). In the feces, the LPS group had a significantly greater Mn concentration compared with the other groups ($P < 0.05$), and the control group had a significantly greater Mn concentration compared with the EGF and EGF + LPS groups ($P < 0.05$), while there was no significant difference between the EGF and EGF + LPS groups ($P > 0.05$).

Expression of essential microelement transport-relative genes in the mucosa of the gastrointestinal tract

The expression levels of Zn transport-related genes in the mucosa from the gastrointestinal tract are shown in Fig. 1A-D and Table 7. Compared with the diet without EGF supplementation, supplied with EGF in the diet significantly increased the expression level of Zip4 in the stomach, jejunum and ileum, and significantly increased the expression level of ZnT1 in the stomach ($P < 0.05$). Injected with LPS significantly decreased the expression levels of Zip4 and Zip7 in the stomach and ileum, and significantly increased the expression level of ZnT1 in the stomach compared with the treatment without LPS injection ($P < 0.05$). The present study revealed significantly interaction between EGF and LPS treatment regarding the expression level of Zip4 in the stomach, and the expression level of Zip7 in the stomach, jejunum and ileum ($P < 0.05$) (Table 7).

The EGF and control groups had significantly greater and lowest expression levels of Zip4 in the stomach compared with the other groups, respectively ($P < 0.05$), and the EGF + LPS group had a significantly greater expression level compared with the control and LPS groups ($P < 0.05$). The EGF and EGF + LPS groups had significantly greater expression levels of Zip4 in the jejunum compared with the LPS group ($P < 0.05$), whereas there were no significant differences among the control, EGF and EGF + LPS groups or between the control and LPS groups ($P > 0.05$). The EGF group had a significantly greater expression level of Zip4 in the ileum compared with the other groups ($P < 0.05$), while the control and EGF + LPS groups had significantly greater expression levels compared with the LPS groups ($P < 0.05$), whereas there was no significant difference between the control and EGF + LPS groups ($P > 0.05$) (Fig. 1A). The control group had a significantly greater expression level of Zip7 in the stomach compared with the LPS and EGF + LPS groups ($P < 0.05$), and the EGF group had a significantly greater expression level compared with the LPS group ($P < 0.05$), while there was no significant difference between the control and EGF groups, or between the EGF and EGF + LPS groups, or between the LPS and EGF + LPS groups ($P > 0.05$). The LPS group had a significantly greater expression level of Zip7 in the jejunum compared with the EGF + LPS group ($P < 0.05$), while there were no significant differences among the control, EGF and LPS groups, or among the control, EGF and EGF + LPS groups ($P > 0.05$). The control group had a significantly greater expression level of Zip7 in the ileum compared with the other groups ($P < 0.05$), and the EGF group had a significantly greater expression level compared with the LPS group ($P < 0.05$), while there was no significant difference between the EGF and EGF + LPS groups, or between the LPS and EGF + LPS groups ($P > 0.05$) (Fig. 1B). The greatest expression level of ZnT1 in the stomach was observed in the EGF + LPS group, which significantly differed from the other groups ($P < 0.05$), and the EGF group had a significantly greater expression level compared with the control and LPS groups ($P < 0.05$), while there was no significant difference between the control and LPS groups ($P > 0.05$). The ZnT4 expression level was not obviously changed in any of the samples ($P > 0.05$) (Fig. 1C-D).

The expression levels of the Cu transport-related genes in the mucosa from the gastrointestinal tract are shown in Fig. 1E-J and Table 7. Compared with the diet without EGF supplementation, supplied with EGF in the diet significantly increased the expression levels of Ctrl and CCS in the stomach and jejunum, and significantly increased the expression level of Cox17 in the stomach and duodenum, and significantly increased the expression level of Atox1 in the stomach, jejunum and ileum, and significantly increased the expression levels of ATP7A and ATP7B in the stomach, duodenum and jejunum ($P < 0.05$). Compared with the treatment without LPS injection, injected with LPS significantly decreased the expression level of Ctrl in the jejunum, and significantly decreased the expression level of Cox17 in the duodenum and ileum, and significantly decreased the expression level of Atox1 in the stomach and jejunum, and significantly decreased the expression level of ATP7A in the duodenum, and significantly decreased the expression level of ATP7B in the stomach, duodenum and ileum, and significantly decreased the expression level of CCS in the duodenum, jejunum and ileum ($P < 0.05$). The present study revealed significantly interaction between EGF and LPS treatment regarding the expression levels of Cox17 and ATP7B in the stomach and duodenum, and the expression level of Atox1 in the stomach, duodenum and ileum, and the expression level of ATP7A in the stomach, and the expression level of CCS in the jejunum ($P < 0.05$) (Table 7).

The LPS group had a significantly lowest expression level of Ctrl in the jejunum compared with the other groups ($P < 0.05$), while there were no significant differences among the control, EGF and EGF + LPS groups ($P > 0.05$). (Fig. 1E). In the stomach, the EGF group had a significantly greater expression level of Cox17 compared with the other groups ($P < 0.05$), and the EGF + LPS group had a significantly greater expression level compared with the control and LPS groups ($P < 0.05$), but there were no significant differences among the control and LPS groups ($P > 0.05$). In the duodenum, the LPS group had a significantly lowest expression level of Cox17 compared with the other groups ($P < 0.05$), whereas there were no significant differences among the control, EGF and EGF + LPS groups ($P > 0.05$). In the ileum, the control and EGF groups had significantly greater expression levels of Cox17 compared with the other groups ($P < 0.05$), but there was no significant difference between the control and EGF groups, or between the LPS and EGF + LPS groups ($P > 0.05$) (Fig. 1F). The EGF group had a significantly greater expression level of Atox1 in the stomach compared with the other groups ($P < 0.05$), and the control group had a significantly greater expression level compared with the LPS and EGF + LPS groups ($P < 0.05$), while there was no significant difference between the LPS and EGF + LPS groups ($P > 0.05$). The EGF group had a significantly greater expression level of Atox1 in the jejunum compared with the other groups ($P < 0.05$), while the EGF and EGF + LPS groups had significantly greater expression levels compared with the LPS group ($P < 0.05$), but there was no significant difference between the control and EGF + LPS groups ($P > 0.05$). The EGF and EGF + LPS groups had significantly greater expression levels of Atox1 in the ileum compared with the other groups ($P < 0.05$), and the control group had a significantly greater expression level compared with the LPS group ($P < 0.05$), while there was no significant difference between the EGF and EGF + LPS groups ($P > 0.05$) (Fig. 1G). The EGF and EGF + LPS groups had significantly greater expression levels of ATP7A in the stomach compared with the other groups ($P < 0.05$), and the control group had a significantly greater expression level compared with the LPS group ($P < 0.05$), while there were no significant differences between the EGF and EGF + LPS groups ($P > 0.05$). The control and EGF groups had significantly greater expression levels of ATP7A in the duodenum compared with the LPS group ($P < 0.05$), while there were no significant differences among the control, EGF and EGF + LPS groups, or between the LPS and EGF + LPS groups ($P > 0.05$). The EGF and EGF + LPS groups had significantly greater expression levels of ATP7A in jejunum compared with the other groups ($P < 0.05$), while there was no significant difference between the EGF and EGF + LPS groups, or between the control and LPS groups ($P > 0.05$) (Fig. 1H). In the stomach, the EGF group had a significantly greater expression level of ATP7B compared with the other groups ($P < 0.05$), and the LPS group had a significantly greater expression level compared with the control and EGF + LPS groups ($P < 0.05$), while there was no significant difference between the control and EGF + LPS groups ($P > 0.05$). In the duodenum, the EGF group had a significantly greater expression level of ATP7B compared with the other groups ($P < 0.05$), while there were no significant differences among the control, LPS and EGF + LPS groups ($P > 0.05$). In the jejunum, the EGF and EGF + LPS groups had significantly greater expression levels of ATP7B compared with the control and LPS groups ($P < 0.05$), while there

was no significant difference between the control and LPS groups, or between the EGF and EGF + LPS groups ($P > 0.05$). In the ileum, the greatest expression level of ATP7B was observed in the control and EGF groups, which significantly differed from the other groups ($P < 0.05$), but there was no significant difference between the control and EGF groups, or between the LPS and EGF + LPS groups ($P > 0.05$) (Fig. 1I). The greatest expression level of CCS in the stomach and jejunum was observed in the EGF group, which significantly differed from the other groups ($P < 0.05$), and the EGF + LPS group had a significantly greater expression level compared with control and LPS groups ($P < 0.05$), but there were no significant differences among the control and LPS groups ($P > 0.05$). In the ileum, the greatest expression level of CCS was observed in the control and EGF groups, which significantly differed from the other groups ($P < 0.05$), but there was no significant difference between the control and EGF groups, or between the LPS and EGF + LPS groups ($P > 0.05$) (Fig. 1J).

The expression level of the DMT1 gene, which is revolved in the transport of Mn, Fe, Zn, and Cu in the mucosa of the gastrointestinal tract, is shown in Fig. 1K and Table 7. Compared with the diet without EGF supplementation, supplied with EGF in the diet increased the expression level of DMT1 in the stomach, duodenum and jejunum ($P < 0.05$). Injected with LPS decreased the expression level of DMT1 in the duodenum, jejunum and ileum compared with the treatment without LPS injection ($P < 0.05$). The present study revealed significantly interaction between EGF and LPS treatment regarding the expression level of DMT1 in the duodenum and ileum ($P < 0.05$) (Table 7).

In the stomach, the EGF and EGF + LPS groups had significantly greater expression levels of DMT1 compared with the control and LPS groups ($P < 0.05$), while there was no significant difference between the control and LPS groups or the EGF and EGF + LPS groups ($P > 0.05$). In the duodenum, the greatest expression level of DMT1 was observed in the EGF group, which significantly differed from the other groups ($P < 0.05$), and the control group had a significantly greater expression level of DMT1 compared with the LPS and EGF + LPS groups ($P < 0.05$), while there was no significant difference between the LPS and EGF + LPS groups ($P > 0.05$). In the jejunum, the EGF group had a significantly greater expression level of DMT1 compared with the other groups ($P < 0.05$), and the control group had a significantly greater expression level compared with the LPS group ($P < 0.05$), but there was no significant difference between the control and EGF + LPS groups, or between the LPS and EGF + LPS groups ($P > 0.05$). In the ileum, the LPS group had a significantly lowest expression level of CYTB compared with the other groups ($P < 0.05$), but there were no significant differences among the control, EGF, and EGF + LPS groups ($P > 0.05$) (Fig. 1K).

The expression levels of Fe transport-related genes in the mucosa of the gastrointestinal tract are shown in the Fig. 1L-N and the Table 7. Compared with the diet without EGF supplementation, supplied with EGF in the diet significantly increased the expression level of CYTB in the stomach, duodenum and ileum, and significantly increased the expression level of Hp in the stomach, jejunum and ileum, and significantly increased the expression level of Tf in the stomach ($P < 0.05$). Injected with LPS significantly decreased the expression levels of CYTB in the jejunum, and significantly decreased the expression level of Tf in the stomach, duodenum and jejunum compared with the treatment without LPS injection ($P < 0.05$). The present study revealed significantly interaction between EGF and LPS treatment regarding the expression level of CYTB in the stomach and duodenum, and the expression level of Hp in the ileum ($P < 0.05$) (Table 7).

In the stomach, the EGF group had a significantly greater expression level of CYTB compared with the other groups ($P < 0.05$), and the LPS and EGF + LPS groups had significantly greater expression levels compared with the control group ($P < 0.05$), but there was no significant difference between the LPS and EGF + LPS groups ($P > 0.05$). The greatest expression level of CYTB in the duodenum was observed in the EGF + LPS group, which significantly differed from the control and LPS groups ($P < 0.05$), but there were no significant differences among the control, EGF and LPS groups, or between the EGF and EGF + LPS groups ($P > 0.05$). In the jejunum, the control and EGF groups had significantly greater expression levels of CYTB compared with the other groups ($P < 0.05$), but there was no significant difference between the control and EGF groups, or the LPS and EGF + LPS groups ($P > 0.05$) (Fig. 1L). In the stomach, the greatest expression level of Hp was observed in the EGF + LPS group, which significantly differed from the other groups ($P < 0.05$), and the EGF group had a significantly greater expression level compared with the control and LPS groups ($P < 0.05$), while there was no significant difference between the control and LPS groups ($P > 0.05$). In the jejunum, the EGF and EGF + LPS groups had significantly greater expression levels of Hp compared with the control group ($P < 0.05$), whereas there were no significant differences between the control and LPS groups, or among the EGF, LPS and EGF + LPS groups ($P > 0.05$). In the ileum, the EGF + LPS group had a significantly greater expression level of Hp compared with the control and LPS groups ($P < 0.05$), and the control and EGF groups had significantly greater expression levels compared with the LPS group ($P < 0.05$), whereas there was no significant difference between the control and EGF groups, or between the EGF and EGF + LPS groups ($P > 0.05$). (Fig. 1M). In the stomach, the EGF group had a significantly greater expression level of Tf compared with the other groups ($P < 0.05$), and the EGF + LPS group had a significantly greater expression level compared with the control and LPS groups ($P < 0.05$), while there was no significant difference between the control and LPS groups ($P > 0.05$). In the duodenum, the control and EGF groups had significantly greater expression levels of Tf compared with the other groups ($P < 0.05$), while there was no significant difference between the control and EGF groups, or between the LPS and EGF + LPS groups ($P > 0.05$). In the jejunum, the EGF group had a significantly greater expression level of Tf compared with the EGF + LPS group ($P < 0.05$), while there were no significant differences among the control, EGF and LPS groups, or among the control, LPS and EGF + LPS groups ($P > 0.05$) (Fig. 1N).

Discussion

Recently, the application of EGF has received increasing amounts of attention due to its positive impacts on animals. Previous studies had shown that dietary LPS could significantly decrease the ADG and ADFI of weaned piglets [33]. Our results indicated that injected with LPS significantly decreased the ADG and significantly increased the FCR of weaned piglets, which was in agreement with previous studies. Previous studies had also shown that dietary EGF could increase bodyweight of early-weaned mice [34]. Our results also suggested that dietary EGF could increase the FBW of early-weaned piglets, however, the change did not reach statistical significance. Changes of growth performance induced by EGF and LPS may be related to the changes of nutrient absorption.

Nutrient absorption is closely related to intestinal health. Complete gastrointestinal development of the piglet is very important in swine farming, and it is also a big problem, especially in the early-weaned stage [30]. Dietary EGF can improve villus heights and intestinal length in the jejunal and duodenal of weaned piglets [30]. It can also decrease the lamina propria width and upregulate the expression level of interleukin-13 (IL-13) in the jejunal of weaned piglets, which

indicates that EGF can minimize the inflammation index of the intestine [13]. A previous study showed that dietary 400 μg/kg EGF had no significantly influence on the apparent digestibility of crude protein, gross energy or P [35]. Our results also indicated that EGF and LPS treatment had no significantly influence on the apparent digestibility of crude protein, crude fiber, gross energy and P, which is in agreement with the previous studies. Our results indicated that injected with LPS significantly decreased the apparent digestibility of crude fat which may be related to the changes of ADG and FCR.

Indispensable microelements such as Cu, Fe, Zn and Mn take part in the regulation of the body physiological functions, such as participating in the redox cycling [1-4], oxygen transport, DNA biosynthesis [36], cellular signal recognition [37, 38], and nutrient metabolism [39, 40]. A greater concentration of these compounds in the gastrointestinal tract chyme and feces means a lower absorption level. Our results showed that intraperitoneal injection with LPS significantly increased the concentration of Cu, Fe, Zn and Mn in the stomach, jejunum and ileum chyme, and faces, while these indexes were significantly decreased by EGF treatment. The LPS treatment decreased the absorption of essential microelement possibly by significantly decreasing the expression levels of the Zip4, Zip7, Ctr1, Atox1, CCS, Cox17, ATP7A, ATP7B, DMT1, CYTB, and Tf genes, while the EGF treatment increase the essential microelement absorption by significantly increasing the expression levels of the Zip4, Ctr1, Atox1, CCS, Cox17, ATP7A, ATP7B, DMT1, CYTB, and Hp genes.

Conclusion

In conclusion, the present findings suggested that injection with LPS decreased the growth performance, and essential microelement absorption of the early-weaned pigs. Dietary EGF could eliminate the adverse impact of LPS treatment. EGF and LPS treatment influence the absorption of trace element through changing the expression levels of microelement transport-relative genes in the mucosa of gastrointestinal tract.

Abbreviations

EGF: epidermal growth factor; LPS: lipopolysaccharide; ADFI: average daily feed intake; Zip4: zrt-irt-like protein 4; Zip7: zrt-irt-like protein 7; ZnT1: zinc transporter 1; ZnT4: zinc transporter 4; Ctr1: copper transport protein1; Cox17: cytochrome c oxidase copper chaperone; Atox1: antioxidant 1; ATP7A: copper-transporting P-type 7A; ATP7B: copper-transporting P-type 7B; CCS: copper chaperone for superoxide dismutase; DMT1: divalent metal transporter 1; CYTB: cytochrome b; Hp: hephaestin; Tf: transferrin; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; NGF: nerve growth factor; PI3K/AKT: phosphatidylinositol-3-kinases/protein-serine-threonine kinase; RAS/MAPK: RAS/mitogen-activated protein kinase; IBW: initial body weight; FBW: final body weight; ADG: average daily weight gain; FCR: feed conversion ratio; Q-RT-PCR: quantitative real-time PCR; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; IL-13: interleukin-13; SGLT1: sodium-glucose linked transporter 1.

Declarations

Acknowledgments

Not applicable.

Authors' contributions

JJX designed the research and wrote the original draft. LX analyzed and interpreted the data. BL, KMA and LYZ performed experiments. YJH participated in the design of the animal experiments. RJF edited the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

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

Ethics approval and consent to participate

All experiments involving animals were supported by the Animal Care Committee of Hunan Agricultural University (Changsha, Hunan Province, China) and were conducted according to the Chinese guidelines for animal welfare.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1 Composition of the basal diets and nutrition level (dry matter)

Item	Content
Ingredient, %	
Corn	63.70
Squeezed soybean meal	16.00
Expanded soybean	8.00
Fish meal	4.50
Whey powder	2.00
Glucose	2.00
Limestone	0.78
$CaHPO_4$	1.30
Lys	0.35
Met	0.07
Thr	0.06
NaCl	0.24
Premix	1.00
Total	100
Nutrient level, %	
DE ($MJ \cdot kg^{-1}$)	14.22
Crude protein	19.59
Lys	1.56
Met + Cys	0.88
Ca	0.86
Available P	0.45
Total P	0.61

The premix provided per kilogram of complete feed: vitamin A, 10,000 IU; vitamin D_3, 1,500 IU; vitamin E, 60 mg; vitamin K_3, 3 mg; vitamin B_1, vitamin $B_{12,}$ 0.024 mg, 1.8 mg; riboflavin, 6 mg; folic acid, 0.3 mg; biotin, 4.5 mg; nicotinic acid, 24 mg; D-pantothenic acid, 15 mg; choline, 1,000 mg; Zn, 100 mg; Fe, 120 mg; Cu, 150 mg; I, 0.3 mg; Se, 0.3 mg. Content of crude protein, total P, and Ca were measured value, and others were calculated value.

Table 2 The concentration of essential microelements in two kinds of diets (dry matter, ug·g^{-1})

Items	basal diet	B+EGF	P
Cu	79.40±5.95	63.04±4.93	0.060
Fe	453.23±11.87	452.85±9.86	0.981
Zn	365.20±12.01	341.54±6.65	0.207
Mn	112.24±9.70	91.13±6.62	0.102

B+EGF the basal diet supplemented with 2 mg·kg^{-1} EGF.

Table 3 The primers for quantitative real-time PCR

Gene	Primer Sequence (5′ to 3′)	Accession number	Size (bp)
Zip4	F: TGGCTGTGTATGGGCTGTCT	JF346412.1	141
	R: ACTGGCTGAGCTGGTCCTG		
Zip7	F: TCCAGGCATCAAGCAAGA	NM_001131045.1	171
	R: CCACCCGAAGCAAAACT		
ZnT1	F: AACCGACCAGGAGGAGAC	FJ374262.1	244
	R: TACTACAATCACGGAACCCA		
ZnT4	F: GTGGACCCCTGTGACAACTG	EU835903.1	277
	R: CTGACAAGACCTCTAAGCGATG		
Ctr1	F: CCTATGACCTTCTACTTTGGCTT	AF320815.2	137
	R: CGGGCTATCTTGAGTCCTTC		
Cox17	F: CTGAATCGCAGGAGAAGAAG	NM_001348525.1	121
	R: TGGGCCTCAATTAGGTGTC		
Atox1	F: CTCTAACGCAGTCACTCGGG	NM_001167641.2	79
	R: CAGACCTTCTTGTTGGGCA		
ATP7A	F: CAGGAGTAGGTGCTCAAAATG	AB271958.1	107
	R: ATGGGTAATGGTTCCAGTCTT		
ATP7B	F: GAACCCCAAGGCTCATCAC	AB271959.1	228
	R: CCGAGGAACTGGACAAAGG		
CCS	F: CGATGAGGGAGAAGACGACC	AY573056.1	98
	R: AGCGAGCGATGATGCCA		
DMT1	F: TCTTATGAGCATTGCCTACCT	EU647217.1	199
	R: AACCTTGGGATACTGACGG		
CYTB	F: TACTTATGGGAGTGACCGAGA	AM268434.1	137
	R: GACTATCCAAAAAATGAGAGCC		
Hp	F: ATCTCCCACCATAACCTCACC	NM_214000.2	157
	R: CCACCTCCTGTTTCTTTCCC		
Tf	F: AAACAGTGGTGGGAAAATAGAG	NM_001244653.1	119
	R: CCGCAATGTAGATGTAGCCT		
GAPDH	F: ATTCCACGGCACAGTCAA	AF017079.1	214
	R: AGCAGAAGGGGCAGAGAT		

Table 4 EGF attenuates the effect of LPS on growth performance of early-weaning piglets

Items	IBW, kg	FBW, kg	ADFI, g	ADG, g	FCR, g·g-1
Treatment					
Control	7.92±0.44	10.52±0.54	264.54±38.94^{a}	198.93±11.62	1.64±0.27
EGF	7.84±0.31	10.67±0.55	350.00±23.89^{b}	202.50±28.03	1.61±0.12
LPS	7.65±0.17	9.43±1.00	319.34±11.54^{b}	158.57±24.19	2.08±0.33
EGF+LPS	7.65±0.49	10.42±0.09	327.64±9.29^{b}	167.86±5.05	1.95±0.00
Main effect					
L E					
-	7.88±0.36	10.61±0.51	321.51±50.93	201.31±22.40^{B}	1.63±0.19^{A}
+	7.65±0.34	9.76±0.93	322.11±10.75	162.29±18.02^{A}	2.03±0.24^{B}
-	7.78±0.34	9.90±0.97	301.07±34.41^{A}	174.71±28.55	1.86±0.36
+	7.74±0.39	10.59±0.45	342.55±22.20^{B}	190.95±28.22	1.75±0.21
Source of variation	*P*-value				
L	0.247	0.134	0.254	0.036	0.030
E	0.844	0.196	0.007	0.670	0.602
E×L	0.834	0.336	0.019	0.849	0.733

L LPS addition, *E* EGF addition, *L×E* interaction between LPS and EGF, *IBW* initial body weight, *FBW* final body weight, *ADFI* average daily feed intake, *ADG* average daily gain, *FCR* feed conversion ratio. Values in the same row with the same superscript (case sensitive) or absence of a superscript were not significantly different ($P > 0.05$).

Table 5 EGF attenuates the effect of LPS on apparent nutrient digestibility (dry matter)

Items	Crude protein	Crude fat	Crude fiber	Gross energy	P
Treatment					
Control	83.56±1.31	60.11±4.50^{b}	49.49±8.05	83.89±2.21	64.68±2.18
EGF	79.40±3.12	60.17±1.31^{b}	44.00±0.98	82.17±0.96	58.31±4.80
LPS	74.93±13.88	48.36±6.61^{a}	51.30±9.19	80.67±9.10	58.70±5.53
EGF+LPS	79.53±6.77	58.28±1.82^{b}	47.04±4.76	82.55±4.65	58.91±8.72
Main effect					
L E					
-	80.78±3.29	60.14±3.07^{B}	46.36±5.54	83.03±1.79	60.86±4.99
+	76.46±11.42	52.33±7.23^{A}	49.60±7.30	81.29±7.41	58.77±5.79
-	77.81±11.66	55.07±8.01	50.40±7.79	82.05±6.78	60.70±5.37
+	79.44±3.87	59.54±1.62	45.01±2.75	82.32±2.43	58.55±5.53
Source of variation	*P*-value				
L	0.466	0.020	0.540	0.697	0.469
E	0.969	0.068	0.233	0.982	0.410
E×L	0.452	0.071	0.876	0.624	0.381

L LPS addition, *E* EGF addition, *L×E* interaction between LPS and EGF. Values in the same row with the same superscript (case sensitive) or absence of a superscript were not significantly different ($P > 0.05$).

Table 6 EGF attenuates the effect of LPS on the concentration of essential microelements in the gastrointestinal tract chyme and feces (dry matter, ug·g^{-1})

Items	Treatment				Main effect		
					L		E
	Control	EGF	LPS	EGF+LPS	-	+	-
Cu							
Stomach	46.72±2.86	49.06±0.84	49.65±0.01	45.40±2.34	48.06±2.16	47.10±2.86	47.89±2.58
Jejunum	57.59±1.12^{a}	59.91±1.27^{a}	91.36±12.11^{b}	56.69±5.59^{a}	58.75±1.66^{A}	68.24±19.20^{B}	71.10±19.48^{B}
Ileum	183.85±2.12^{c}	119.72±15.04^{b}	179.97±7.27^{c}	57.42±4.05^{a}	145.37±36.71^{B}	103.38±63.62^{A}	181.52±5.66^{B}
Feces	431.71±109.42	329.25±40.49	511.53±10.99	339.75±21.12	373.16±88.38	425.64±100.13	463.64±89.04^{B}
Fe							
Stomach	317.02±16.65	328.78±14.11	327.66±9.74	312.51±24.31	321.72±15.16	320.08±17.47	321.28±14.01
Jejunum	330.67±31.98^{a}	360.77±8.19^{ab}	405.73±5.23^{c}	391.30±4.95^{bc}	348.73±23.68^{A}	398.52±9.31^{B}	368.20±47.20
Ileum	1333.46±9.67^{c}	919.54±31.52^{b}	1256.44±195.13^{c}	675.66±18.65^{a}	1057.52±215.18^{B}	966.05±353.89^{A}	1294.95±121.24^{B}
Feces	2321.65±199.76^{b}	1797.26±58.30^{a}	2929.12±190.06^{c}	1824.33±68.56^{a}	2059.45±315.94^{A}	2376.72±618.46^{B}	2625.38±375.65^{B}
Zn							
Stomach	166.62±14.99	156.81±2.28	159.87±6.30	177.43±6.86	161.01±10.25	168.65±11.28	163.25±10.93
Jejunum	266.60±6.71	214.69±2.87	290.97±61.83	266.62±23.75	240.64±28.80^{A}	274.73±35.51^{B}	276.34±34.01^{B}
Ileum	412.50±18.42^{c}	138.89±22.06^{a}	894.09±39.17^{d}	281.11±5.34^{b}	275.70±150.96^{A}	587.60±336.67^{B}	653.30±265.19^{B}
Feces	1447.58±5.37^{b}	1199.16±129.92^{a}	1972.72±144.57^{c}	1332.86±92.05^{ab}	1298.53±164.19^{A}	1607.09±358.03^{B}	1762.67±305.27^{B}
Mn							
Stomach	38.94±0.26^{a}	38.49±0.58^{a}	45.11±0.99^{b}	43.96±1.50^{b}	38.72±0.45^{A}	44.53±1.30^{B}	42.64±3.45
Jejunum	179.48±9.18^{b}	127.30±13.47^{a}	190.53±3.20^{b}	135.17±3.74^{a}	153.39±31.56	162.85±32.08	185.00±8.49^{B}
Ileum	275.11±11.49^{c}	224.85±10.58^{a}	283.15±3.73^{c}	247.99±1.77^{b}	249.98±30.39	265.57±20.44	279.13±8.38^{B}
Feces	508.09±13.69^{b}	389.36±18.20^{a}	590.53±27.57^{c}	414.04±2.65^{a}	436.85±66.64^{A}	502.28±103.14^{B}	549.31±50.81^{B}

L LPS addition, *E* EGF addition, *L*×*E* interaction between LPS and EGF. Values in the same row with the same superscript (case sensitive) or absence of a superscript were not significantly different ($P > 0.05$).

Table 7 Summary of two-way ANOVA between the effect of EGF and LPS on the expression levels of microelement transport-relative genes

Items	Source of variation	F-value				*P*-value			
		Stomach	Duodenum	Jejunum	Ileum	Stomach	Duodenum	Jejunum	Ileum
Zip4	L	6.270	0.835	1.464	54.521	0.046	0.403	0.261	0.000
	E	179.885	0.636	18.005	52.197	0.000	0.461	0.003	0.000
	E×L	51.828	0.167	1.011	5.712	0.000	0.699	0.344	0.054
Zip7	L	26.586	4.474	0.244	34.323	0.002	0.079	0.647	0.002
	E	0.003	3.807	3.937	0.750	0.961	0.099	0.118	0.426
	E×L	10.986	0.280	7.757	8.053	0.016	0.616	0.050	0.036
ZnT1	L	12.940	*0.718*	0.408	0.953	0.023	0.425	0.558	0.374
	E	63.936	*2.533*	3.477	3.356	0.001	0.156	0.136	0.126
	E×L	0.726	*1.718*	4.689	0.517	0.442	0.231	0.096	0.504
ZnT4	L	0.007	0.077	1.251	0.057	0.936	0.791	0.326	0.823
	E	1.002	3.961	0.333	4.304	0.363	0.094	0.595	0.107
	E×L	0.011	0.602	0.051	3.473	0.919	0.467	0.833	0.136
Ctr1	L	0.323	0.892	17.512	2.179	0.594	0.381	0.006	0.183
	E	7.242	2.235	7.465	0.158	0.043	0.186	0.034	0.702
	E×L	0.003	0.310	1.489	0.094	0.960	0.598	0.268	0.768
Cox17	L	4.957	30.481	0.000	43.154	0.077	0.003	0.118	0.003
	E	146.232	9.225	0.000	0.176	0.000	0.029	0.158	0.696
	E×L	21.456	8.216	0.819	0.580	0.006	0.035	0.421	0.489
Atox1	L	130.033	1.862	43.032	0.267	0.000	0.215	0.000	0.632
	E	51.703	0.222	54.651	320.924	0.001	0.652	0.000	0.000
	E×L	20.320	5.975	0.057	18.811	0.006	0.044	0.819	0.012
ATP7A	L	1.207	12.704	0.238	0.000	0.322	0.009	0.638	0.988
	E	468.415	6.148	28.471	0.389	0.000	0.042	0.001	0.556
	E×L	13.023	0.710	1.304	0.017	0.015	0.427	0.286	0.902
ATP7B	L	10.866	14.156	0.124	95.946	0.022	0.006	0.739	0.000
	E	23.530	45.783	25.666	0.393	0.005	0.000	0.004	0.551
	E×L	77.283	11.400	0.105	1.783	0.000	0.010	0.759	0.224
CCS	L	6.672	12.236	46.840	44.292	0.061	0.017	0.001	0.001
	E	47.602	1.283	441.862	0.001	0.002	0.309	0.000	0.982
	E×L	3.513	0.644	13.717	0.720	0.134	0.459	0.014	0.429
DMT1	L	0.236	365.855	36.553	24.411	0.647	0.000	0.004	0.003
	E	74.810	104.849	9.928	4.842	0.000	0.000	0.034	0.070
	E×L	0.011	83.206	2.734	31.408	0.919	0.000	0.174	0.001
CYTB	L	0.452	0.387	28.722	0.157	0.531	0.553	0.006	0.706
	E	19.555	9.032	0.143	11.793	0.007	0.020	0.725	0.014
	E×L	19.454	6.172	0.818	0.107	0.007	0.042	0.417	0.755
Hp	L	4.939	0.229	3.077	0.722	0.090	0.650	0.154	0.443
	E	95.816	0.899	14.727	40.043	0.001	0.380	0.018	0.003
	E×L	3.914	0.104	2.193	21.389	0.119	0.758	0.213	0.010
Tf	L	17.312	42.426	13.502	1.750	0.014	0.001	0.014	0.243
	E	128.333	5.368	0.002	2.209	0.000	0.060	0.968	0.197
	E×L	5.574	1.288	2.565	2.185	0.078	0.300	0.170	0.199

L LPS addition, *E* EGF addition, *L*×*E* interaction between LPS and EGF.

Figures

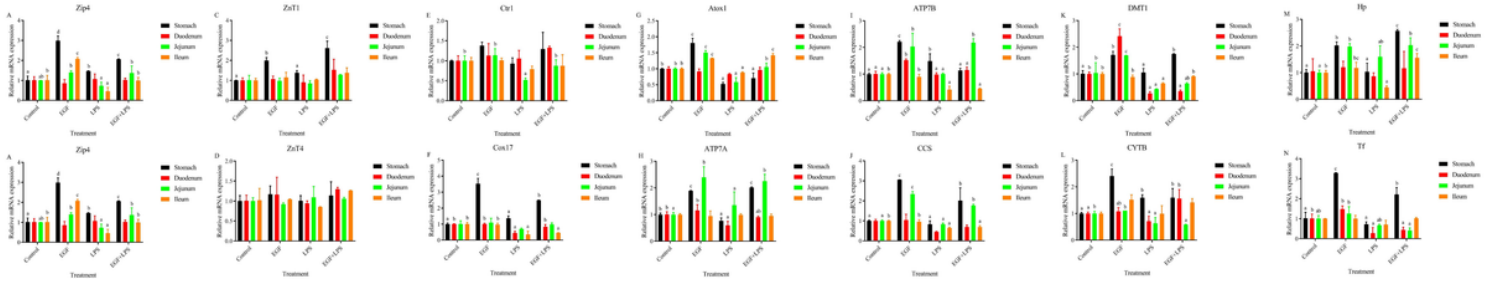

Figure 1

EGF attenuates the effect of LPS on the expression levels of microelement transport-relative genes. (A) zrt-irt-like protein 4 (Zip4) (B) zrt-irt-like protein 7 (Zip7) (C) zinc transporter 1 (ZnT1) (D) zinc transporter 4 (ZnT4) (E) copper transport protein1 (Ctr1) (F) cytochrome c oxidase copper chaperone (Cox17) (G) antioxidant 1 (Atox1) (H) copper-transporting P-type 7A (ATP7A) (I) copper-transporting P-type 7B (ATP7B) (J) copper chaperone for superoxide dismutase (CCS) (K) divalent metal transporter 1 (DMT1) (L) cytochrome b (CYTB) (M) hephaestin (Hp) (N) transferrin (Tf). Vertical bars in the same pattern with the same superscript or the absence of superscripts were not significantly different ($P > 0.05$).