Effect of dietary copper sources and concentrations on serum lysozyme concentration and protegrin-1 gene expression in weaning piglets

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Abstract

The aim of this study was to investigate the effect of dietary copper sources and concentrations on serum immune indices and the protegrin-1 (NPG1) expression level in the bone marrow of weaning piglets. A total of 80 crossbred piglets (Duroc × Landrace × Yorkshire), with the average age of 21 days and the initial body weight of 7.00±0.03 kg, were randomly assigned to five treatments for 14 days by different kinds of diet. The dietary treatments were: 1, basal diet; 2, basal diet + 20 mg/kg Cu as CuSO4; 3, basal diet + 20 mg/kg Cu as cupric citrate (CuCit); 4, basal diet + 180 mg/kg Cu as CuSO4; 5, basal diet + 180 mg/kg Cu as CuCit. The results showed that compared with basal diet, supplementation with 20 mg/kg Cu as CuCit had no significant difference on growth performance (P>0.05). The incidence of diarrhoea was reduced by 71.57% and serum lysozyme concentration was increased by 170.73% (P<0.05), but there were not significant differences on serum IgA, IgG and IgM concentration (P>0.05). The mRNA expression level of NPG1 was significantly increased by 2.32-fold (P<0.01). However, the other three trial groups showed no significant differences on the experimental results compared with the basal diet group. These results indicated that supplemental 20 mg/kg Cu as CuCit could increase serum lysozyme concentration and NPG1 mRNA expression level, and reduce the incidence of diarrhoea in weaning piglets.

Introduction

It is generally demonstrated that the digestive systems are immature in the early weaning piglets. During weaning, the digestive tract of piglets must adapt to solid feed instead of sow milk. The change in the piglets’ diet could result in disturbances of digestive function. An unhealthy digestive tract often causes the problem of post-weaning stress syndrome characterized by diarrhoea and seriously restricts the production potential of the piglets. Researches showed that the supplementation of 125 to 250 mg/kg copper (Cu) as CuSO4 could stimulate growth rate and improve feed efficiency in weaning piglets (Bunch et al., 1961; Cromwell et al., 1989). It has also been observed that such high concentration of Cu supplementation can result in its high excretion in faeces (Kornegay and Harper, 1997). Recent studies have indicated that the growth stimulatory effects of organic Cu, such as cupric citrate (CuCit) (Armstrong et al., 2004), cupric methionate (Huang et al., 2010), cupric proteinate (Veum et al., 2004) and cupric lysine (Zhou et al., 1994) was an alternative to CuSO4 in the piglets’ diet. Moreover, CuCit has been reported to improve growth performance at lower dietary concentrations than CuSO4 in broiler chickens (Ewing et al., 1998). In addition, supplementation with 125 mg/kg Cu as CuCit and 250 mg/kg Cu as CuSO4 were equally effective at stimulating growth and improving feed efficiency in weaning pigs (Armstrong et al., 2004).

Although the mode of action of growth promoting effect on Cu remains unknown, it may be attributed to the antibacterial properties. In vitro studies have shown that Cu deficiency can impair the bactericidal activity of neutrophils and macrophages (Jones and Suttle 1981; Babu and Failla, 1990). These studies provide indirect evidences for immune defense of Cu in weaning pigs. Antimicrobial peptides (AMPs) are an important and effective component of innate immune defenses, which display direct antimicrobial activity against pathogens (Zasloff, 2002). Cathelicidins are the largest family of AMPs in pigs, which include protegrins 1 (NPG1) to 5, proline-arginine-rich 39-amino acid peptide (PR-39), prophenin 1 to 2, and porcine myeloid antimicrobial peptides 23, 36, and 37 (Kosciuczuk et al., 2012). Researches have shown that cathelicidins are synthesized by bone marrow progenitor cells and cathelicidins such as, NPG1 and PR-39 are inducible (Zanetti et al., 1995; Wu et al., 2000). Moreover, NPG1 mRNA expression was significantly decreased after weaning in piglets, and supplemental lactoferrin in diet increased the mRNA expression level of NPG1 in pigs (Wang et al., 2006; Han et al., 2007). These results suggested that extrinsic modulation of porcine NPG1 expression by supplemental Cu may be possible. However, there is no available data about the regulatory effects of Cu on NPG1 expression in weaning piglets. Thus, this study aimed to investigate the effect of dietary Cu sources (CuCit vs CuSO4) and concentrations (20 mg/kg vs 180 mg/kg) on serum immune indices and the NPG1 expression level in the bone marrow of weaning piglets.

Materials and methods

Materials

In this study, CuCit and CuSO4 were provided by Sichuan Animtech Feed Co., Ltd., Chengdu, China. CuCit is a kind of organic Cu, with a purity of approximately 98.5% and the content of Cu is 34.5%.

Animal care and experimental design

All procedures were approved by the Institutional Animal Care and Use Committee of Sichuan Academy of Animal Science. All the animal experiments were done according to the guidelines for animal experiments at the National Institute of Animal Health. A total of 80 crossbred piglets (Duroc × Landrace × Yorkshire), with the average age of 21 days and the initial body weight of 7.00±0.03 kg, were randomly assigned to five groups with four replicates and four piglets (two gilts and two barrows) per pen. The piglets were raised for 14 days after 3 days adaptation. The dietary treatments were: 1, basal diet (control group); 2, basal diet + 20 mg/kg Cu as CuSO4; 3, basal diet + 20 mg/kg Cu as CuCit; 4, basal diet + 180 mg/kg Cu as CuSO4; 5, basal diet + 180 mg/kg Cu as CuCit.
Cu as CuCl; 4, basal diet + 180 mg/kg Cu as CuSO4; 5, basal diet + 180 mg/kg Cu as CuCl2. The basal diet used in the experiment on maize-soybean meal-extruded soybean basis (Table 1), was formulated to meet the requirements for National Research Council (NRC, 2012), feeding standard of swine of China in 2004 and feed description and proximate composition of China in 2013. Piglets were housed in temperature-controlled nursery rooms and grouped in elevated pens with wire flooring. The room temperature and the relative humidity were respectively maintained 25-28°C and 50-70% throughout the experiment. Piglets were fed at 8:00, 12:00, 16:00 and 20:00 every day. During the entire experimental period, the piglets were allowed ad libitum access to feed and water. The nurseries were cleaned every day and disinfected every four days.

Growth and serum
Piglets were weighed individually and feed consumption per pen was measured at the beginning and end of the experiment. Feed wastage was collected each day and taken into account in the calculation of feed consumption and feed conversion efficiency. Feed conversion efficiency was calculated by the average daily gain (ADG) and the average daily feed intake (ADFI) of the piglets. At the end of the experiment, venous blood were obtained from four barrows (one pig per pen) randomly selected from each treatment for the determination of serum lysozyme and immunoglobulin concentration. Serum was obtained by centrifugation of the blood samples at 3000 rpm for 30 min at 5°C. The serum was stored at -20°C until measured by the kits purchased from Nanjing Jian Cheng Bioengineering Institute.

Incidence of diarrhoea
Severity of diarrhoea in piglets each treatment was monitored three times per day (i.e., at morning, noon and evening) during the entire experimental period. A faecal consistency score was used to evaluate the severity, which was based on a scale from 0 to 3 (0 = normal faeces, 1 = soft faeces, 2 = mild diarrhoea, and 3 = watery diarrhoea). When the score was 2 or 3, diarrhoea was recorded once. Incidence of diarrhoea was calculated according to the following equation: incidence of diarrhoea = numbers of diarrhoea pigs / (numbers of pigs in treatment ×numbers of trial days) ×100%.

RNA extraction and cDNA synthesis
The barrows were slaughtered under general anaesthesia. The bone marrow from left femur of barrows was aseptically removed and imme-
diately frozen in liquid nitrogen. The samples were stored at -80°C until they were analysed for RNA isolation. Total RNA was isolated from the bone marrow by using the RNAiso Pure RNA Isolation Kit (TAKARA BIOTECHNOLOGY CO., LTD. Dalian, China). Purified RNA samples were reverse-transcribed by using PrimeScript RT reagent Kit with gDNA Eraser for qRT-PCR (TAKARA). The process for total RNA isolation and cDNA synthesis was completed according to the manufacturer’s instructions.

Quantitative real-time PCR
The NPG1 mRNA expression was assessed by quantitative real-time PCR (qRT-PCR). The qRT-PCR procedure was in accordance with the minimum information for publication of qRT-PCR experiments guidelines (Bustin et al., 2009). Specific primers were designed according to the porcine NPG1 (NM_001123149.1) and 18S rRNA (AY265350) gene sequences by using Primer Premier 5.0 (Premier Biosoft International, CA, USA) and listed in Table 2.

The qRT-PCR amplification was performed by using a total volume of 12.5 µL containing 1 µL cDNA (50 ng/µL), 6.25 µL 2 ×SYBR Premix Ex Taq (TaKaRa), 0.5 µL 10 pM/µL of each primer and 4.25 µL ddH2O. Reactions were amplified by using CFX96 real-time PCR detection system (Bio-Rad, Hercules, CA, USA) and quantified by using the manufacturer’s software. The thermocycling conditions comprised 3 min at 95°C and then 40 cycles with 10 s at 95°C, 30 s at 58°C, followed by a standard melting curve analysis to validate the specificity of the PCR products. All samples were amplified in triplicate from the same cDNA preparation. A 10-fold dilution series of PCR products were used to determine PCR efficiency by constructing a relative standard curve. PCR efficiencies were determined for each gene with efficiency = 100%±5%. The NPG1 gene expression level was analysed by the 2-ΔΔCT method and normalized by 18S rRNA gene (Livak and Schmittgen, 2001; Wang et al., 2012).

Statistical analysis
The obtained data were presented as means±standard errors (SD) and analysed by One-way ANOVA test and Tukey test. The incidence of diarrhoea of two groups was analysed using U-Test. All statistical analyses were performed by using SAS ver. 8.0 statistical package (SAS Institute, NC, USA). A P-value <0.05 was considered statistically significant.

| Ingredients                      | %     | Nutrient item               | Nutrient levels |
|----------------------------------|-------|-----------------------------|-----------------|
| Maize                            | 60.17 | Digestible energy, MJ/kg    | 14.23           |
| Soybean meal                     | 12.00 | Crude protein, %            | 20.33           |
| Extruded soybean                 | 15.00 | Calcium, %                  | 0.81            |
| Fish meal                        | 6.00  | Available phosphorus, %     | 0.41            |
| Wheat bran                       | 2.50  | D-lysine, %                 | 1.19            |
| Soybean oil                      | 1.50  | D-methionine, %             | 0.42            |
| L-Lysine-HCl                     | 0.30  | D-threonine, %              | 0.74            |
| DL-Methionine                    | 0.10  | D-tryptofan, %              | 0.22            |
| Threonine                        | 0.07  |                             |                 |
| Limestone                        | 0.95  |                             |                 |
| Calcium phosphate tribasic       | 0.50  |                             |                 |
| Choline chloride                 | 0.06  |                             |                 |
| Salt                             | 0.30  |                             |                 |
| Vitamin premix ⁶                   | 0.05  |                             |                 |
| Trace mineral premix ⁷           | 0.50  |                             |                 |

⁶Provided per kg of diet: vitamin A, 2200 U; vitamin D, 220 U; vitamin E, 16.00 mg; vitamin K₃, 0.50 mg; vitamin B₁, 1.50 mg; vitamin B₂, 4.00 mg; vitamin B₆, 2.00 mg; niacin, 20.00 mg; calcium pantothenic, 12.00 mg; folic acid, 0.30 mg; biotin, 0.08 mg; vitamin B₁₂, 20.00 μg.

⁷Provided per kg of diet: Fe, 105.00 mg; Cu, 10.00 mg; Mn, 4.00 mg; Zn, 110.00 mg; I, 0.14 mg; Se, 0.30 mg.

| Gene          | GenBank Accession NO. | Primer sequence (5‘–3’)                  | PCR product size, bp |
|---------------|-----------------------|-------------------------------------------|----------------------|
| protegrin-1   | NM_001123149.1        | Forward: CCCACATTTTTCCGGGGGCGCAG          | 121                  |
|               |                       | Reverse: GTGATTGGCTGCAAAATCTCTTACCC       |                      |
| 18S rRNA      | AY265350              | Forward: CTGCCITCCTTGGATGTTG             | 195                  |

Table 2. Primers used for the quantitative real-time PCR.
Results

Effects of copper on growth performance

The growth performance of weaning piglets was shown in Table 3. Compared with the control group, ADG, ADFI and the feed intake:gain (F/G) ratio did not differ when piglets receiving diets supplemented with 20 mg/kg or 180 mg/kg Cu as CuCit or CuSO₄ (P>0.05) throughout the study.

Effects of copper on incidence of diarrhoea

The incidence of diarrhoea of weaning piglets during the experimental period was shown in Figure 1. Compared with the control group, supplementation with different Cu sources and concentrations had reduced the incidence of diarrhoea. Moreover, supplementation with 20 mg/kg Cu as CuCit had significantly reduced the incidence of diarrhoea by 71.57% (P<0.05).

Effects of copper on serum lysozyme and immunoglobulin

The serum lysozyme and immunoglobulin concentration of weaning piglets was shown in Table 4. Compared with the control group, supplementation with 20 mg/kg Cu as CuCit and 180 mg/kg Cu as CuSO₄ had significantly increased serum lysozyme concentration by 170.73% (P<0.05) and 96.60% (P<0.05), respectively. No difference was observed among the trial groups of 20 mg/kg Cu as CuSO₄, 180 mg/kg Cu as CuCit and the control group (P>0.05). However, there was no significant difference between trial groups and the control group on serum IgA, IgG and IgM concentration (P>0.05).

Effects of copper on the protegrin-1 gene expression

As shown in Figure 2, compared with the control group, supplementation with 20 mg/kg Cu as CuCit had significantly increased the relative mRNA expression of NPG1 by 2.32-fold (P<0.01). However, the difference between the other three trial groups and the control group was not significant (P>0.05).

Discussion

Previous studies have demonstrated no con-

Table 3. Effects of copper on growth performance in weaning piglets.

|                        | Control group | CuSO₄ 20 mg/kg | CuCit 20 mg/kg | CuSO₄ 180 mg/kg | CuCit 180 mg/kg |
|------------------------|---------------|----------------|---------------|----------------|----------------|
| Initial weight, kg     | 7.038±0.035   | 7.016±0.016    | 7.006±0.018   | 6.976±0.027    | 7.006±0.015    |
| Final weight, kg       | 8.480±0.187   | 8.553±0.225    | 8.784±0.209   | 8.655±0.182    | 8.650±0.195    |
| ADG, kg                | 0.103±0.014   | 0.112±0.019    | 0.127±0.022   | 0.120±0.015    | 0.117±0.025    |
| ADFI, kg               | 0.210±0.015   | 0.222±0.014    | 0.215±0.019   | 0.211±0.021    |                |
| F/G, kg                | 1.893±0.092   | 1.875±0.101    | 1.748±0.080   | 1.792±0.082    | 1.803±0.100    |

CuCit, cupric citrate; ADG, average daily gain; ADFI, average daily feed intake; F/I, feed intake:gain.

Table 4. Effects of copper on serum lysozyme and immunoglobulin concentration in weaning piglets.

|                        | Control group | CuSO₄ 20 mg/kg | CuCit 20 mg/kg | CuSO₄ 180 mg/kg | CuCit 180 mg/kg |
|------------------------|---------------|----------------|---------------|----------------|----------------|
| Lysozyme, µg/dL        | 52.07±7.09c   | 54.40±3.46c    | 140.97±19.90c | 102.37±14.68b  | 63.60±7.48c    |
| IgA, g/L               | 0.002±0.003   | 0.004±0.001    | 0.005±0.001   | 0.005±0.001    | 0.004±0.003    |
| IgG, g/L               | 5.153±1.424   | 5.165±1.478    | 6.636±1.303   | 6.193±1.300    | 5.312±1.108    |
| IgM, g/L               | 0.184±0.062   | 0.201±0.014    | 0.312±0.052   | 0.304±0.070    | 0.280±0.019    |

CuCit, cupric citrate. a,b,cIn the same row, values with different small-letter superscripts indicate significant difference (P<0.05).

Figure 1. Effects of copper on incidence of diarrhoea in weaning piglets. Control, basal diet; CuSO₄-20, basal diet + 20 mg/kg Cu as CuSO₄; CuCit-20, basal diet + 20 mg/kg Cu as cupric citrate; CuSO₄-180, basal diet + 180 mg/kg Cu as CuSO₄; CuCit-180, basal diet + 180 mg/kg Cu as cupric citrate. Different superscripts with small letters are significantly different (P<0.05).

Figure 2. Effect of copper on the gene expression of protegrin-1 in weaning piglets. Control, basal diet; CuSO₄-20, basal diet + 20 mg/kg Cu as CuSO₄; CuCit-20, basal diet + 20 mg/kg Cu as cupric citrate; CuSO₄-180, basal diet + 180 mg/kg Cu as CuSO₄; CuCit-180, basal diet + 180 mg/kg Cu as cupric citrate. Each column represents the mean ±SD of 4 individual pigs. Different superscripts with capital letters are significantly different (P<0.01).
sistent effect of either CuSO₄ or CuCit on performance in weaning piglets (Armstrong et al., 2000). In the present study, compared with the control group, piglets receiving diets supplemented with 20 mg/kg or 180 mg/kg Cu as CuCit or CuSO₄ had no significant difference on growth performance during the first two weeks after weaning. Surprisingly, the incidence of diarrhea was significantly reduced when piglets receiving 20 mg/kg Cu as CuCit compared with the control group. Therefore, CuCit could be used at lower concentration in diet than CuSO₄ without adversely affecting growth in the early weaning piglets.

Copper appears to play an important role in the body that apparently relate, among others, to the maintenance of immune function (Bonham et al., 2002). Research speculated that the growth promoting effect of Cu may be due to the antibiotic-like action of Cu in the gastrointestinal tract of piglets (Bunch et al., 1961). Indeed, our study showed that piglets receiving 20 mg/kg Cu as CuCit had significantly increased the concentration of serum lysozyme when compared to other piglets in the experiment. Lysozyme is one of the protein widely existed in animal blood and plays an essential role in defence against gastrointestinal pathogens and the decrease of gastrointestinal illness in weaning piglets (Nyachoti et al., 2012). Moreover, lysozyme is more effective against gram positive bacteria than gram negative bacteria (Masschalck and Michiels, 2003). The participation of lysozyme in immune bacteriolyis may be in relation to serum immunoglobulin. Lysozyme could increase the rate of bactericidal activity of immunoglobulin with complement (Hill and Porter, 1974). This study found that supplemental 20 mg/kg Cu as CuCit could significantly increase the serum lysozyme concentration and NPG1 mRNA expression level, and reduce the incidence of diarrhea in weaning piglets. Our study also indicated that the lower dietary concentration Cu as CuCit was more effective than CuSO₄ in improving the serum immune indices and stimulating the expression of AMPs in weaning piglets. Further studies are required to confirm the immune defence of CuCit and its dietary use in order to protect the weaning piglets from post-weaning stress syndrome.

Conclusions

In conclusion, this study has shown that supplemental 20 mg/kg Cu as CuCit could increase serum lysozyme concentration and NPG1 mRNA expression level, and reduce the incidence of diarrhea in weaning piglets. Moreover, serum lysozyme is more effective against gram positive bacteria than gram negative bacteria (Masschalck and Michiels, 2003). The participation of lysozyme in immune bacteriolyis may be in relation to serum immunoglobulin. Lysozyme could increase the rate of bactericidal activity of immunoglobulin with complement (Hill and Porter, 1974). This study found that supplemental 20 mg/kg Cu as CuCit could significantly increase the serum lysozyme concentration and NPG1 mRNA expression level, and reduce the incidence of diarrhea in weaning piglets. Our study also indicated that the lower dietary concentration Cu as CuCit was more effective than CuSO₄ in improving the serum immune indices and stimulating the expression of AMPs in weaning piglets. Further studies are required to confirm the immune defence of CuCit and its dietary use in order to protect the weaning piglets from post-weaning stress syndrome.

References

Armstrong, T.A., Cook, D.R., Ward, M.M., Williams, C.M., Spears, J.W., 2004. Effect of dietary copper source (cupric citrate and cupric sulfate) and concentration on growth performance and fecal copper excretion in weaning pigs. J. Anim. Sci. 82: 1234-1240.

Bonham, T.A., Spears, J.W., van Heugten, E., Engle, T.E., Wright, C.L., 2000. Effect of copper source (cupric citrate vs cupric sulfate) and level on growth performance and copper metabolism in pigs. Asian Austral. J. Anim. 13:1134-1161.

Babu, U., Failla, M.L., 1990. Respiratory burst and candidicidal activity of peritoneal macrophages are impaired in copper-deficient rats. J. Nutr. 120:1692-1699.

Bonham, M., O’Connor, J.M., Hannigan, B.M., Strain, J.J., 2002. The immune system as a physiological indicator of marginal copper status? Brit. J. Nutr. 87:393-403.

Bunch, K., Speer, V., Hays, V., Hawbaker, J., Catron, D., 1991. Effects of copper sulfate, copper oxide and chlorotetracycline on baby pig performance. J. Anim. Sci. 20:723-726.

Bustin, S.A., Benes, V., Garson, J.A., Hellemans, J., Huggett, J., Kubista, M., Mueller, R., Nolan, T., Pfaffl, M.W., Shipley, G.L., Vandesompele, J., Wittwer, C.T., 2009. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin. Chem. 55:611-622.

Cromwell, G.L., Stahly, T.S., Moneghe H.J., 1989. Effects of source and level of copper on performance and liver copper stores in weaning pigs. J. Anim. Sci. 67: 2996-3002.

Ewing, H.P., Pesti, G.M., Bakali, R.I., Menten, J.F., 1998. Studies on the feeding of cupric sulfate pentahydrate, cupric citrate, and copper oxychloride to broiler chickens. Poultry Sci. 77:445-448.

Han, F., Wang, Y., Feng, J., Guo, J., Xu, Z., 2007. Developmental gene expression of antimicrobial peptide Protegrin-1 and effect of weaning on gene regulation of Protegrin-1 in piglets. J. Anim. Feed Sci. 16:86-95.

Hill, I.R., Porter, P., 1974. Studies of bacterial activity to Escherichia coli of porcine serum and colostral immunoglobulins and the role of lysozyme with secretory IgA. Immunology 26:1239-1250.

Huang, Y., Zhou, T., Lee, J., Jang, H., Park, J., Kim I., 2010. Effect of dietary copper sources (cupric sulfate and cupric methionate) and concentrations on performance and fecal characteristics in growing pigs. Asian Austral. J. Anim. 23:757-761.

Jones, D., Suttle, N., 1981. Some effects of copper deficiency on leucocyte function in sheep and cattle. Res. Vet. Sci. 31:151-156.

Kokryakov, V.N., Harwig, S.S., Panyutich, E.A., Shevchenko, A.A., Aleshina, G.M., Shamova, O.V., Korneva, H.A., Lehrer, R.I., 1993. Protegrins: leukocyte antimicrobial peptides that combine features of corticosteroid antagonists and tachypleins. FEBS Lett. 327:231-236.

Kornegay, E., Harper, A., 1997. Environmental nutrition: nutrient management strategies to reduce nutrient excretion of swine. Prof. Anim. Sci. 13:99-111.

Kosciuczuk, E.M., Lisowski, P., Jarczak, J., Strzalkowska, N., Jozwik, A., Horbanczuk, J., Krzyzewski, J., Zwierzchowski, L., Bagnicka, E., 2012. Cathelicidins: family of antimicrobial peptides. A review. Mol. Biol. Rep. 39:10957-10970.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25:402-408.

Masschalck, B., Michiels, C.W., 2003.
Antimicrobial properties of lysozyme in relation to foodborne vegetative bacteria. Crit. Rev. Microbiol. 29:191-214.
Nyachoti, C.M., Kiariie, E., Bhandari, S.K., Zhang, G., Krause, D.O., 2012. Weaned pig responses to Escherichia coli K88 oral challenge when receiving a lysozyme supplement. J. Anim. Sci. 90:252-260.
Steinberg, D.A., Hurst, M.A., Fujii, C.A., Kung, A.H., Ho, J.E., Cheng, F.C., Lowry, D.J., Fiddes, J.C., 1997. Protegrin-1: a broad-spectrum, rapidly microbicidal peptide with in vivo activity. Antimicrob. Agents Ch. 41:1738-1742.
Veum, T.L., Carlson, M.S., Wu, C.W., Bollinger, D.W., Ellersieck, M.R., 2004. Copper proteinate in weanling pig diets for enhancing growth performance and reducing fecal copper excretion compared with copper sulfate. J. Anim. Sci. 82:1062-1070.
Wang, J., Zhao, S.M., Song, X.L., Pan, H.B., Li, W.Z., Gao, S.Z., Chen, D.W., 2012. Low protein diet up-regulate intramuscular lipogenic gene expression and down-regulate lipolytic gene expression in growth–finishing pigs. Livest. Sci. 148:119-128.
Wang, Y., Shan, T., Xu, Z., Liu, J., Feng, J., 2006. Effect of lactoferrin on the growth performance, intestinal morphology, and expression of PR-39 and protegrin-1 genes in weaned piglets. J. Anim. Sci. 84:2636-2341.
Wu, H., Zhang, G., Minton, J.E., Ross, C.R., Blecha, F., 2000. Regulation of cathelicidin gene expression: induction by lipopolysaccharide, interleukin-6, retinoic acid, and Salmonella enterica serovar typhimurium infection. Infect. Immu. 68:5552-5558.
Zanetti, M., Gennaro, R., Romeo, D., 1995. Cathelicidins: a novel protein family with a common proregion and a variable C-terminal antimicrobial domain. FEBS Lett. 374:1-5.
Zasloff, M., 2002. Antimicrobial peptides of multicellular organisms. Nature 415:389-395.
Zhou, W., Kornegay, E.T., van Laar, H., Swinkels, J.W., Wong, E.A., Lindemann, M.D., 1994. The role of feed consumption and feed efficiency in copper-stimulated growth. J. Anim. Sci. 72:2385-2394.