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Recommended Citation

Wang, J. H., Wu, C. C., & Feng, J. (2011). Effect of dietary antibacterial peptide and zinc-methionine on performance and serum biochemical parameters in piglets. *Czech Journal of Animal Science*, 56(1), 30-36.

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Effect of dietary antibacterial peptide and zinc-methionine on performance and serum biochemical parameters in piglets

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ABSTRACT: The objective of this study was to evaluate the effect of dietary supplementation of antibacterial peptide and zinc methionine on performance and some serum biochemical parameters of weaned piglets. Rongchang male piglets (28 days of age, initial weight 8.4 ± 0.65 kg) were used. All piglets were randomly allotted to four diets including the control group, the antibacterial peptide (AP) group containing 10 mg antibacterial peptide/kg diet, the zinc methionine (Zn-Met) group with 1 200 mg zinc methionine/kg diet (equal to 200 mg Zn^{2+} /kg diet), the zinc methionine/antibacterial peptide (Zn/AP) group containing 10 mg antibacterial peptide and 1 200 mg zinc methionine/kg diet, respectively, in a 4-week feeding experiment. Each of these groups consisted of six replications with 8 pigs per replication. Average daily gain (ADG) and average daily feed intake (ADFI) both improved in the antibacterial peptide group ($P < 0.05$, $P < 0.05$), zinc methionine group ($P < 0.05$, $P < 0.05$) and zinc methionine/antibacterial peptide group ($P < 0.01$, $P < 0.05$), as compared with the control. Dietary zinc methionine also significantly reduced the diarrhoea ratio of piglets ($P < 0.05$). The serum immunoglobulin G (IgG) and superoxide dismutase (SOD) were increased ($P < 0.05$) and total cholesterol (TC) decreased ($P < 0.05$) in the antibacterial peptide group and zinc-methionine group, the high density lipoprotein (HDL) increased ($P < 0.05$) in the zinc methionine group, as compared with the control. The results indicated that antibacterial peptide and zinc methionine were effective in improving growth performance, enhancing immune function, blood vessel function and antioxidant enzyme activity of piglets.

Keywords: antimicrobial peptide; zinc amino acid; growth performance; serum indexes; weaned piglets

Antibacterial peptide, or antimicrobial peptide, is a series of short chain peptides usually composed of dozens of amino acid residues. It is a kind of new animal feed additives. The assets of these peptides in clinical application include their potential for broad-spectrum activity, rapid bactericidal activity and low propensity for resistance development (Alexandra et al., 2006). There is an antibacterial activity in some synthesized cyclic peptides and their intermediates (Timothy et al., 2008). Some bacterial peptides also exhibited a cytotoxic activity against cancer cells (Qiang et al., 2004; Hoskin and

Ramamoorthy, 2008). Numerous antibacterial peptides are composed of large amino acid sequences which can adopt an α -helical linear structure or a circular structure organized in a β -sheet. The α -helical conformation of active peptides is often essential with regard to their mechanism of action toward the microorganisms to arrest the growth of both Gram-positive and Gram-negative bacteria (Nicolas and Mor, 1995; Sun and Zou, 2008). Synthetic antibacterial peptide was found to have a potent antibacterial activity against both Gram-negative and Gram-positive bacteria, and to per-

Supported by The National Key Technology R and D Program of P.R. China (No. 2007BAD51B05).

meabilize the inner membrane of *E. coli* (Storici et al., 1994).

Zinc is an important element for the life of animals. It is a very important component of numerous enzymes *in vivo*. It influences the metabolism of vitamin A, enhances the immunization state and improves the performance (Zhang et al., 2005; Wu, 2007). On the one hand, the mechanism for growth promotion may be due to its positive influence on the gut mucosa (Marianne et al., 1998). On the other hand, the growth effect of zinc was proved also in pigs not affected by diarrhoea (Broom et al., 2006). The application of zinc in the raising of livestock and poultry has been widely reported (Caine et al., 2009). Dietary supplementation of 2 500 ppm ZnO for up to two weeks after weaning appears to be potentially beneficial in the prevention of postweaning diarrhoea in pigs (Marianne et al., 1998). Zinc oxide reduced anaerobic microorganisms and tended to increase the intestinal IgA concentration (Broom et al., 2006). Organic zinc inner complex or zinc amino acid exerted a better effect than inorganic zinc (Revy et al., 2004). Zinc methionine is an important source of zinc amino acid additives.

Antibiotics play an important role in cultivation and control of diseases of livestock and poultry, but their wide application also caused a lot of problems, such as the enhancement of tolerance to bacteria, antibiotic residues, environmental pollution and so on (Howard, 2004). Some studies were reported to try to find some additives with similar effects like those of antibiotics in weaned piglets, and these additives could not have the above-mentioned bad effects of antibiotics. “Rongchang” pig is an important local population of China while these pigs are often weaned at 28 days of age. After 5–6 weeks of feeding period, when they are 60–70 days of age, the male piglets are usually used as roast suckling pigs. The main purpose of the present study was to evaluate the effect of different additives of dietary antibacterial peptide and zinc methionine on growth performance and serum parameters of Rongchang weaned piglets, and provide support for the processing industry of Rongchang piglets.

MATERIAL AND METHODS

Animals, diets and experimental design

This experiment was approved by the Institutional Animal Care and Use Committee at Chongqing

Academy of Animal Sciences. 192 male Rongchang piglets, 28 days of age, were randomly assigned to four groups. Each of these groups consisted of six replications with 8 pigs per replication. The piglets were weaned at 28 days after birth. The control group received the basal diet, the other three groups received the same basal diet supplemented with 10 mg antibacterial peptide/kg diet (AP group), 1 200 zinc methionine/kg diet (equal to 200 mg Zn²⁺/kg diet, Zn-Met group), 10 mg antibacterial peptide and 1 200 mg zinc methionine/kg diet (Zn/AP group), respectively. Zinc methionine was obtained from Shanghai Luyuan Fine Chemical Plant (Shanghai, China). Purity of zinc methionine was ≥ 98.0 (concentration of Zn ≥ 17.0 , the molar ratio of zinc methionine is 1:2). Antibacterial peptide (obtained from the intestine of Rongchang pig, the activity is 3 000 IU/g) was provided by Chongqing Academy of Animal Sciences (Chongqing, China). Diets were formulated to meet or exceed requirements for all nutrients (Table 1). The treatment diets were prepared by the following processes. Initially, the calculated amounts of additives were carefully premixed with small portions of maize starch, and these premixes were then finally mixed into the feed previously supplemented with the recommended levels of all other nutrients. The feeding trial lasted for 28 days after 7 days of adaptation period. All piglets were housed in stainless steel pens with concrete floor and the size was 300 cm \times 200 cm. All piglets were given *ad libitum* access to feed and water. Average daily gain (ADG), average daily feed intake (ADFI), feed/gain (F/G) ratio and diarrhoea ratios were measured.

Diarrhoea ratios = (number of piglets with diarrhoea every day \times days of occurring diarrhoea) per (total experimental pig number \times experimental days)

Sampling procedure

After the feeding trial, blood samples from six pigs of each group (one pig per pen) were collected at approximately 8:00 a.m. for the determination of serum parameters. 5 ml blood samples were collected from the anterior vena cava of piglets, and allowed to clot at 37°C for half an hour and centrifugalized at 1 000 \times g at 4°C for 10 min, then the serum was collected and stored in Eppendorf tubes at –20°C for use.

Table 1. Composition of experimental diets

Ingredients	(g/kg)	Analyzed content	(g/kg)
Maize (85 g CP/kg)	630	dry matter	950
Soybean meal (440 g CP/kg)	200	crude protein	157.1
Wheat middlings (155 g CP/kg)	100	digestible energy (MJ/kg)	13.03
Silkworm chrysalis	40	total phosphorus	6.5
Dicalcium phosphate	12	calcium	7.3
Sodium chloride	1.5	lysine	7.9
L-Lysine HCl	2	methionine	5.3
DL-Methionine	4		
Calcium bicarbonate	10		
Vitamin premix*	0.5		

*vitamin premix provided (per kg diet): vitamin A 2 200 IU; vitamin D₃ 220 IU; vitamin E 16 mg; vitamin K₃ 0.50 mg; vitamin B₁ 1.50 mg; vitamin B₂ 4.00 mg; vitamin B₆ 2.0 mg; vitamin B₁₂ 0.020 mg; niacin 20 mg; D-pantothenic acid 12.00 mg; biotin 0.08 mg; folic acid 0.30 mg; Cu 200 mg; Fe 120 mg; Mn 50 mg; Co 2.0 mg; Se 0.3 mg; I 0.45 mg

Determination of serum biochemical parameters

Assay kits for superoxide dismutase (SOD) and immunoglobulin G (IgG) were obtained from Adlitteram Diagnostic Laboratories (ADL). SOD activity or IgG concentration was determined using the method of enzyme linked immunosorbent assay (ELISA), standards and unknown corresponding samples were binding to another defined SOD or IgG with enzyme horseradish peroxidase (HRP), respectively, after enzymatic reaction, the intensity of the colour was measured at 450 nm, the measured absorbance is directly proportional to the concen-

tration of SOD or IgG. Serum total protein (TP), urea nitrogen (BUN), glucose (GLU), glutamic pyruvic transaminase (GPT), glutamic oxalacetic transaminase (GOT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), total cholesterol (TC), triglyceride (TG) and high density lipoprotein (HDL) were determined using an Automatic Biochemistry Radiometer (Au640, Olympus).

Statistical analysis

All the statistics were analyzed using one-way ANOVA (SPSS 13.0). Duncan's multiple range test

Table 2. The effects of different dietary additives on growth performance and diarrhoea index of piglets

Item	Control	AP	Zn-Met	Zn/AP	SEM
Initial weight (kg)	8.5	8.0	8.7	8.4	0.11
End weight (kg)	22.8	23.9	24.0	25.1	0.37
Average daily gain (g)	514 ^{Aa}	568 ^{ABb}	546 ^{ABb}	596 ^{Bab}	23.9
Average daily feed intake (g)	920 ^a	966 ^b	956 ^b	942 ^b	17.5
Feed intake/gain	1.79 ^a	1.70 ^{ab}	1.75 ^{ab}	1.58 ^b	0.04
Diarrhoea ratios	4.8 ^{Aa}	4.7 ^{ABa}	1.2 ^{Bc}	3.0 ^{ABb}	0.35

Different lower case letters represent $P < 0.05$, different capital letters represent $P < 0.01$

AP = antibacterial peptide; Zn-Met = zinc methionine; Zn/AP = antibacterial peptide and zinc methionine; control = basic diet
SEM = standard error of the mean

Table 3. The effects of different dietary additives on some serum parameters of piglets

Item	Control	AP	Zn-Met	Zn/AP	SEM
TP (g/l)	79.15 ^a	73.77 ^b	78.03 ^a	77.67 ^a	0.66
BUN (mmol/l)	2.08 ^a	2.11 ^a	1.91 ^b	2.16 ^a	0.03
GLU (mmol/l)	5.18 ^a	5.22 ^a	5.19 ^a	5.15 ^a	0.13
IgG (mg/ml)	40.93 ^{Aa}	62.36 ^B	47.12 ^b	47.36 ^b	0.45
TC (mmol/l)	1.80 ^a	1.63 ^b	1.70 ^b	1.81 ^a	0.02
TG (mmol/l)	0.33 ^a	0.30 ^a	0.36 ^a	0.32 ^a	0.03
HDL (mmol/l)	0.69 ^a	0.79 ^a	0.91 ^b	0.88 ^b	0.05
GPT (U/l)	21 ^a	21 ^a	23 ^a	24 ^a	0.75
GOT (U/l)	70 ^a	68 ^a	67 ^a	68 ^a	1.01
ALP (U/l)	168 ^a	161 ^a	154 ^a	166 ^a	2.71
LDH (U/l)	660 ^a	648 ^a	668 ^a	637 ^a	7.42
SOD (µg/ml)	14.45 ^A	28.60 ^B	28.64 ^B	25.99 ^B	1.26

Different lower case letters represent $P < 0.05$, different capital letters represent $P < 0.01$

AP = antibacterial peptide; Zn-Met = zinc methionine; Zn/AP = antibacterial peptide and zinc methionine; control = basic diet; TP = total protein; BUN = urea nitrogen; GLU = glucose; IgG = immunoglobulin G; TC = total cholesterol; TG = total triglyceride; HDL = high density lipoprotein; GPT = glutamic pyruvic transaminase; GOT = glutamic oxalacetic transaminase; ALP = alkaline phosphatase; LDH = lactate dehydrogenase; SOD = superoxide dismutase
SEM = standard error of the mean

was used to compare differences among the treatment groups. The P -value of less than 0.05 or 0.01 was taken to indicate statistical significance.

RESULTS

Effect on performance and diarrhoea ratios of piglets

In Table 2, as compared with the control, dietary supplementation of AP or Zn-Met improved ADG and ADFI ($P < 0.05$), the addition of Zn-Met reduced the diarrhoea ratios ($P < 0.01$). The joint use of AP and Zn-Met did not show any synergistic effects on growth performance or diarrhoea ratios.

Effect on TP, BUN, GLU, IgG, TC, TG and HDL in the serum of piglets

Serum TG and GLU did not show any significant differences among any groups ($P > 0.05$). As

compared with the control, AP reduced TP, TC ($P < 0.05$) and increased IgG ($P < 0.01$), Zn-Met reduced BUN, TC ($P < 0.05$) and increased IgG, HDL ($P < 0.05$). (Table 3)

Some enzymes GPT, GOT, ALP, LDH and SOD activity level

Serum GPT, GOT, ALP and LDH did not show any significant differences among any groups ($P > 0.05$). As compared with the control, the addition of AP or Zn-Met increased serum SOD ($P < 0.01$). The Zn per AP group did not show any synergistic effects on levels of these serum enzymes. (Table 3)

DISCUSSION

Effect on performance and diarrhoea ratios of piglets

The effect of dietary antibacterial peptide and Zn on growth performance was reported previ-

ously. Dietary supplementation of zinc improved growth rate, feed conversion rate and diarrhoea of piglets (Wu, 2007). Weaned piglets offered antibacterial peptide exhibited a linear improvement in feed per gain with the increasing antibacterial peptide inclusion rate (Jin et al., 2008). Dietary supplementation of antibacterial peptide decreased the diarrhoea ratio and improved ADG (Wen et al., 2001; Yu et al., 2008). In our study, AP or Zn-Met could improve ADG and ADFI, which may be associated with their biological functions. The addition of Zn-Met may elevate the sensitivity of the calculus gustatorius and enzymatic activity of zinc enzymes in the saliva. Thus, it increased the feed intake and promoted the growth of piglets. The dietary supplement of Zn-Met also significantly reduced the diarrhoea ratio of piglets and ensured the health of pigs; the joint use of AP and Zn-Met was not reported previously. The current results indicated that the joint use of Zn-Met and AP was not synergetic in improving the growth performance.

Effect of different dietary additives on TP, BUN, GLU and IgG of piglets

Liver is the main site to synthesize plasma protein, and its protein metabolism is extremely vigorous. All the serum proteins are synthesized in liver, so liver is the primary source of serum proteins. The serum TP content may reflect the hepatic protein metabolic status in response to dietary treatments in weaned piglets. Serum urea nitrogen (BUN) is the main dead end product of protein metabolism. Changes in serum BUN concentration can reflect the whole body status of amino acid metabolism and utilization in animals (Eggum, 1970). In our study, serum TP concentration of AP group decreased, which may be associated with the effect of AP on protein synthesis. In addition, serum BUN concentration of Zn-Met group decreased, it would suggest the effect of Zn-Met on the enhancement of protein synthesis or the reduction of protein metabolism in animals (Brown and Cline, 1974).

The GLU has a wide array of sources because starch, flesh internal muscle glycogen, lactose in milk and maltose can be changed into glucose. Most of them are stored in liver and muscle. Weaned piglets offered the zinc diets exhibited no significant influence on the GLU concentration (Wu, 2007); it

was identical with our result. The current results indicated that the supplementation of AP or Zn-Met could not affect the GLU concentration, serum GLU concentration remains stable, the piglets grew under common state.

IgG was synthesized in spleen and lymphoid node, it existed mainly in the serum and tissue fluid and it is the most important basement for immune reaction. Its content reflects the immune state of the organism. In the present study, the supplementation of AP or Zn-Met increased IgG, which may suggest an enhancement of the immune function of weaned piglets. Consistent with this result, Ahn et al. (1998) found out that the supplementation of 200 mg/kg Zn increased ($P < 0.05$) the serum IgG concentration of piglets; the supplementation of Zn-Met increased the IgG concentration of weaned piglets (Zhang et al., 2005). In opposite, Cheng et al. (1998) reported that the supplementation of Zn did not affect the immune response of piglets. After the trial, the IgG concentration of AP group was obviously higher than that of Zn-Met and Zn/AP group. On the one hand, the IgG concentration of AP group of Rongchang piglets was in a normal range, the effect of AP on an increase in IgG concentration was higher than that of Zn-Met; on the other hand, the increase may be related to procedural errors, because the samples of AP group showed slight haemolysis.

Effect of different dietary additives on TC, TG and HDL in the serum of piglets

TC includes free cholesterol and cholesterol ester, serum TC concentration reflects the lipometabolic status. TG was mainly synthesized in liver and adipose tissue, and was stored in adipose tissue. Most tissues can use the breakdown product of triglyceride to provide energy. Dietary supplementation of coconut oil increased serum triglyceride concentrations and serum total cholesterol concentration (Allan et al., 2001). Liver is the main organ for the synthesis and storage of TC. In this study, serum TC concentration decreased in AP group and Zn-Met group, but the TG concentration remained stable. So the results may be associated with the lipometabolic status and they suggest that AP or Zn-Met could enhance the storage of cholesterol in liver and other related organs.

HDL is mainly synthesized in liver and small intestine, it cleans up cholesterol existing in rhagiocrine

cells and ensures the stabilization of cholesterol, so HDL has the anti-atherosclerosis (AS) function and prevents the occurrence of atherosclerosis. In the present study, the supplementation of Zn-Met increased serum HDL concentration and reduced serum TC concentration. It may suggest that Zn-Met induced an enhancement of HDL synthesis which may be mediated by the absorption of Zn-Met in the small intestine. In addition, the enhancement of HDL synthesis could clean up the redundant serum TC and ensure the health of blood vessels of piglets.

Effect on GPT, GOT, ALP, LDH and SOD level

GPT and GOT reside in hepatic cells mainly; if the cell death occurs, the content of GPT and GOT may increase. The content of serum ALP and LDH is also related to the health status of hepatic cells. An increase in serum ALP or LDH often reflects some diseases in the liver or gallbladder. Wang et al. (2003) found out that the addition of zinc enriched the serum ALP and LDH activity. But in our study, serum GPT, GOT, ALP and LDH of all groups had no significance, so their concentrations may have reached a stable level, and it may indicate that the addition of AP or Zn-Met in feeds was safe with respect to the growth of piglets, the addition did not have any adverse effect on hepatic cells and other organs.

SOD is an important antioxidase widely existing in animals. SOD converts superoxide to H_2O_2 , and other antioxidases, like glutathione peroxidase (GPx) and catalase (CAT), modulate the conversion of H_2O_2 to H_2O . It is likely that the increases in the activity of SOD, GPx and CAT are the main factors of a decrease in lipid peroxidation. Because of its special physiological activity, SOD is the chief substance to clean up free radicals. Zinc is the absolutely necessary ingredient of SOD. Supplementation of zinc increased the serum SOD activity (Wu, 2007). Supplementation of bovine lactoferrin (bLF) enriched serum and *longissimus muscle* copper-zinc-superoxide dismutase (CuZnSOD) (Wang et al., 2008). In accordance with their results, our study indicated that the supplementation of AP or Zn-Met could increase the serum SOD activity. The addition of AP or Zn-Met could induce the mRNA expression and promoted the synthesis of SOD (Wang et al., 2008).

CONCLUSION

The supplementation of AP or Zn-Met can improve growth performance (ADG and ADFI), increase the IgG and SOD concentration, and decrease the TC level. Dietary Zn-Met also significantly reduced the diarrhoea ratio of piglets and ensured the health of pigs. This has enlarged the application of AP or Zn-Met as feed additives. At present, antibacterial peptide is mainly purified by gel chromatography from animals or insects. So its wide use is economically restricted. It is urgent to develop new industrial procedures to raise the production of antibacterial peptide on a large scale for further utilization in the near future.

Acknowledgement

Thanks for the help of Chongqing Academy of Animal Sciences (CAAS, Chongqing, P.R. China).

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Received: 2009–12–03

Accepted after corrections: 2010–05–16

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