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Effect of antimicrobial peptides supplementation on commercial pig production efficiency

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Abtract

The study was conducted to evaluate of antibacterial peptides (AMPs) extracted from *Citrobacter braakii* to replace antibiotics in pig production. The work consisted of 2 main experiments: Experiment 1: Evaluation of the inhibitory ability of AMPs against *E. coli* ATCC 25922 and three *E. coli* strains isolated from diarrhea pig feces by the method of minimum inhibitory concentration (MIC). Experiment 2 was carried out on 500 commercial pigs from 70 days to 130 days old, pigs were randomly divided into 2 treatments: experimental (with the addition of 0.5 g AMPs/kg of feed and without antibiotics in feed) and control (add antibiotics in feed). Experimental results show that: At experiment 1, the MIC for *E. coli* ATCC 25922 was 25 µg/ml, for 3 strains of *E. coli* in the field, the MIC were 12,5 µg/ml. At experiment 2, the diarrhea rate of pigs in the control group was 30% and the experimental group was 20% ($P<0.05$); weight of pigs in the control and experimental groups was 100 and 101 kg, respectively ($P>0.05$); feed efficiency was 2.15 and 2.14 kg feed/kg weight gain, respectively ($P>0.05$). However, the average cost of veterinary medicine per pig in the experimental groups was 27,000 VND less than that of the control groups.

Keywords: antibacterial peptides, *Citrobacter braakii,* diarrhea, *E. coli*, feed efficiency.

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INTRODUCTION

Antibiotics have been used in the swine industry for more than 50 years to improve growth and prevent infectious diseases. However, overuse of antibiotics has created antibiotic-resistant strains of bacteria and created products containing antibiotic residues that affect human health (Diez, 2007). As a result, a global trend has emerged towards restriction of the inclusion of antibiotics in swine diets as a routine means of growth promotion. In response, a considerable amount of research has been focused on the development of alternatives to antibiotics to maintain swine performance and health (Xiao et al., 2015).

Antimicrobial peptides (AMPs) are one of the most widely researched alternatives to conventional antibiotics. AMPs are potent, broad spectrum

antibiotics which have been demonstrated to kill gram-negative and gram-positive bacteria, mycobacteria, viruses, fungi and even transformed or cancerous cells while having no effect on the cells of treated animals (Reddy et al., 2004). In recent years, studies on AMPs and their applications have become one of the hot spots in the areas of agricultural science, biology, medicine, and physiology as well as having potential applications in medicine and the food industry (Xiao et al., 2015).

Supplementation with various antimicrobial peptides has been reported to have positive effects on performance, nutrient digestibility, the intestinal microflora, intestinal morphology and immune function in pigs (Wang et al., 2011; Yoon et al., 2014; Tang et al., 2009). Yoon et al. (2014) tested AMPs to replace the antibiotic avilamycin in weaned piglets and the results after 28 days showed that pigs fed diets supplemented with AMPs improved performance, nutrient digestibility, gut morphology and reduced microbiome pathogenic bacteria. Experimental results on pigs showed that AMPs increased weight at slaughter by 13.3% (Xiao et al. 2015).

So far, in Vietnam, there has not been any research on the use of AMPs for livestock in general and pig production in particular. This study was conducted with the objective: to evaluate the ability of AMPs to inhibit bacteria to some virulent *E. coli* strains isolated from diarrhea pig feces and the effect of AMPs supplementation on the efficiency of commercial pig production

MATERIALS AND METHODS

Site and duration

The study was conducted from June to October 2022 at the Microbiology Laboratory, Faculty of Animal Science and Veterinary Medicine, Tay Nguyen University and a pig farm in Gia Lai province.

Materials

AMPs extracted from *Citrobacter braakii; E. coli* ATCC 25922*;* Three virulent *E. coli* strains isolated from diarrhea pig feces (*E. coli* strain 1, *E. coli* strain 2, and *E. coli* strain 3); Commercial pigs from 70 to 130 days old.

Research content and methods

Evaluation of the inhibitory ability of AMPs against *E. coli* ATCC 25922 and three *E. coli* strains isolated from diarrhea pig feces

Bacterial culture

The test bacteria were first inoculated on LB plates and cultured at 35 to 37°C for 16-24 hours. A single colony on the plate was picked and inoculated into 50 ml of sterile LB liquid medium, and cultured on a shaker (rotation speed of 200 rpm) at a culture temperature of 37 ℃ for 10-12 hours. The cultured bacterial solution was diluted to a physiological saline solution of 0.090-0.10 (OD600) for use.

Preparation of test solution

Prepare 800ml of LB liquid medium. Weigh 5.120 grams of AMPs sample and dissolve it in LB medium (ultrasound until completely dissolved) to bring the volume to 100 ml. Transfer the solution after constant volume to a centrifuge tube and centrifuge at a speed of 5000-10000 rpm for 5 minutes. The centrifuged supernatant is transferred to a 250ml Erlenmeyer flask (beaker), number 1. Take another 12 Erlenmeyer

flasks/250ml (beakers), add 50ml LB liquid culture medium to each bottle, numbered 2, 3, …13 in sequence. Take 10 ml of each bottle and insert it into test tube and mark. Plug the prepared test tube, sterilize it at 121°C for 15 minutes, and cool to room temperature.

Inoculant

Inoculate 1ml of diluted bacterial solution (1% inoculation volume) into each test tube, cover the stopper, and culture by slanting and shaking.

Determination of results

Temperature 37℃, rotation speed 170 rpm, cultured 4-5 hours, first observe the positive control should have bacterial growth, the negative control should have no bacterial growth, and then observe the test sample, visible bacterial growth is turbid, no bacterial growth is clear, and the lowest concentration of clear test tube is judged as minimum inhibitory concentrations (MIC).

Each bacterial strain was replicated 3 times.

Evaluation of the effect of AMPs supplementation on the efficiency of commercial pig production

Experimental design

Experimental pigs were raised in a closed barns with ventilation fans; The temperature in the barn is maintained at 23-25^{o}C. Total 500 pigs are divided equally into 10 cages (50 m^2), each cages has 50 pigs, each cages chooses 5 animals whose weight is close to the average of the herd to number the ears. Randomly divide 10 cages into 2 treatments: Control (Pigs ate the farm's basic diet supplemented with antibiotics), and Experiment (Pigs ate the farm's basic diet supplemented with AMPs). The experimental period is 60 days. The experimental scheme is shown in Table 1.

Table 1 Experimental diagram

	Control	**Experiment**
Number of pigs (head)	250	250
Number of cages	10	10
Feed	Farm's basic diet	Farm's basic diet
Colistin 10%	200g / ton of feed	0
AMPs	0	500g / ton of feed

Note: All breeding conditions during the experiment were the same

Sampling and measurements

Diarrhea rate: Daily monitoring of pig faeces status to identify diarrheal syndrome in pigs. Pigs have diarrhea when stools are thin, without mold.

Monitor growth: Pigs were weighed at the beginning of the experiment, after 1 month and at the end of the experiment (Weigh only pigs with ear numbers). Weigh each individual in the morning before feeding with a clock scale.

Feed conversion ratio (FCR): Record the amount of feed for each stage in each barn. FCR of each barn is calculated as follows:

$$FCR = \frac{\text{Total amount of feed of 1 barn in the period}}{(\text{Average weight of pigs at the end of the period} - \text{Average weight of pigs at the beginning of the period}) \times 50}$$

Estimating economic efficiency: Comparing the cost difference to

raise a pig between treatments, the costs incurred include: feed, drugs for disease prevention and treatment, experimental products added to the feed. Other costs such as: breeding stock, electricity, water, care workers are the same.

Statistical analysis

Experimental data were analyzed by Minitab 16.2 software (Minitab, 2010). Compare mean values using Student's test (T-test), compare proportions using Chi-square (χ^2).

RESULTS

Evaluation of the inhibitory ability of AMPs against *E. coli* ATCC 25922 and three *E. coli* strains isolated from diarrhea pig feces

The results in Table 2 show. After 3 experiments, the minimum inhibitory concentration (MIC) for *E. coli* ATCC 25922 was higher than that of three strains of *E. coli* isolated from diarrhea pig feces.

Table 2 Minimum inhibitory concentrations of AMPs against various strains (Unit: µg/ml)

Strain	Time 1	Time 2	Time 3
E.coli ATCC 25922	25.0	25.0	25.0
E.coli strain 1	12.5	12.5	12.5
E.coli strain 2	12.5	12.5	12.5
E.coli strain 3	12.5	12.5	12.5

Evaluation of the effect of AMPs supplementation on the efficiency of commercial pig production

Diarrhea syndrome

The experimental results showed that there was a significant difference in the incidence of diarrhea syndrome between the two treatments ($P<0.05$). Pigs raised with diets supplemented with AMPs significantly reduce the incidence of diarrhea syndrome (Table 3).

Table 3 The incidence of diarrhea syndrome

Treatments	n	Number of pigs with diarrhea	Diarrhea rate (%)
Control	250	125	50.00
Experiment	250	76	30.40

Growth of pigs

At the time of experiment, the difference in weight of pigs in the treatments was not statistically significant ($P>0.05$; Table 4). Thus, using AMPs to substitute antibiotics in feed does not affect the growth of pigs.

Table 4 Effects of AMPs supplementation on pig growth (kg)

Time	Control ($\bar{X}$ ± SD)	Experiment ($\bar{X}$ ± SD)	P–value

Beginning of the experiment	30.50 ± 3.84	31.25 ± 1.41	0.36
After 30 days of experiment	54.67 ± 5.49	54.54 ± 2.71	0.91
The end of the experiment	87.00 ± 6.90	87.75 ± 2.52	0.62

Feed conversion ratio (FCR)

Supplementing with AMPs so that antibiotic substitution in the feed does not affect the feed conversion ratio of pigs (P>0.05; Table 5). In this study, the FCR throughout the experiment was relatively low, ranging from 2.14 to 2.15 kg feed/kg weight gain.

Table 5 Effect of AMPs supplementation on FCR (kg feed/kg weight gain)

Period	**Control ($\bar{X}$ ± SD)**	**Experiment ($\bar{X}$ ± SD)**	**P–value**
From beginning to 30 days of experiment	1.59 ± 0.06	1.53 ± 0.10	0.36
From 30 to 60 days of experiment	2.32 ± 0.10	2.20 ± 0.20	0.91
From the beginning to the end of the experiment	2.15 ± 0.14	2.14 ± 0.09	0.62

Estimating economic efficiency

In this experiment the costs of feed, breeding stock, electricity, water and care workers were the same. The cost difference is mainly antibiotics, AMPs and veterinary medicine. The results in Table 6 show that when adding AMPs to the feed, the cost is reduced by about 27,000 VND/head compared to adding antibiotics to the feed.

Table 6 Cost difference between the two treatments

Item cost	**Control**	**Experiment**
Vaccine	88,000	88,000
Veterinary medicine	107,000	73,000
Colistin 10%	18,000	-
AMPs	-	25,000
Total	**213,000**	**186,000**
Difference	**27,000**	

DISCUSSION

The antibacterial activity of AMPs has been demonstrated by many studies (Diez-Gonzalez, 2007; Xiao et al., 2015; Cheung-Lee et al., 2019). Cheung-Lee et al. (2019) detected that Citrocin (a peptide of the bacterium Citrobacter braakii) has the ability to inhibit *E.coli, Salmonella, Citrobacter, Pseudomonas*. However, its strongest activity was against enterohemorrhagic *E. coli* (EHEC) O157:H7 TUV93–0 with an MIC of 16 µm.

Studies by Cutler et al. (2007) and Tang et al. (2009) showed that AMPs supplementation reduced the incidence of diarrhea in weaned piglets through antibacterial effects, modulating immune function, and improving Fe absorption.

Tang et al. (2012) and Yoon et al. (2014) also showed that AMPs supplementation to piglets significantly increased growth performance. Yoon et al. (2014) found that pigs diets supplemented with AMPs showed an increase in the apparent total tract digestibility of dry matter, crude protein and gross energy.

So, previous studies have shown that AMPs have activity against bacterial species including *E. coli*, and adding AMPs to the feed rations increases growth and feed efficiency for piglets. The results of our study showed that AMPs were active against *E. coli* ATCC 25922 and three strains of *E. coli* isolated from diarrhea pigs with MIC from 12.5 to 25.0 µg/ml. Adding AMPs to the diet reduced the incidence of diarrhea, but the effect on weight gain and feed conversion ratio was not significant. The reason for this may be that in this experiment we conducted on pigs, which were relatively large, so the digestive system was fully developed, so the addition of AMPs did not affect the growth process and the feed conversion ratio

CONCLUSIONS

AMPs extracted from Citrobacter braakii were able to inhibit some virulent E.coli strains isolated from diarrheal swine feces, with MICs ranging from 12.5 to 25 µg/ml. Adding AMPs to the feed did not affect the growth performance and feed conversion ratio of pigs at 70 to 130 days of age, but reduced the rate of diarrhea, thereby saving the cost of veterinary drugs to treat the disease.

AUTHOR CONTRIBUTIONS

Nguyen Duc Dien; Conceptualization and design the experiment, investigation, supervision, editing and finalization

Nguyen Van Thai, Le Van Khoa; Investigation, methodology, formal analysis, manuscript preparation

CONFLICT OF INTEREST

We have no conflict of interest.

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