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Effects of Bio-probiotic Betel Bokashi on Raising Pigs and Preventing Diarrhea in Young Piglets in Central Vietnam

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Abstract: Infection of *Escherichia coli* in piglets is identified as one of major problems in pig husbandry. Data were collected from different experiments conducted at farm levels and results showed that the percentage of piglets infected with *E. coli* was very high. *E. coli* infected healthy piglets were up to 48.9% and *E. coli* infected diarrhea piglets accounted for 88.3%. Using Betel Bokashi and antibiotics in treatment brings about different results. Out of 48 infected piglets, 15 piglets were selected for three groups treatment with five each group. Results showed that 92.3% diarrhea piglets treated with Betel Bokashi recovered in 3.85 d, while 100% and 88.23% treated with antibiotics were cured in 4 d and 4.01 d, respectively. Post-treatment piglets in group 3 grew up faster than group 1 and group 2, with gains 231, 218 and 212 g, respectively.

Key words: Piglets, *Escherichia coli*, diarrhea, Betel Bokashi, health and treatment.

1. Introduction

Escherichia coli infection in piglets is identified as one of major problems in pig husbandry. The major serotypes causing the problems as usual include: O140, O147, O141, O139, O138, O117 and O115 as listed in Vietnam practices [1]. According to Loi [2], *E. coli* is permanently present in healthy pigs as well. In healthy pigs, there is *E. coli* K88. When the number of *E. coli* multiplies, which means that the environment in pig intestines has been altered, it is suitable for the development of poisonous *E. coli*. Because the strains of *E. coli* can cause diarrhea, antibiotics have long been considered the most effective way to prevent and treat the disease [3-5]. Diarrhea is a worrisome problem caused by *E. coli* and has been researched extensively to produce effective medications [6, 7].

However, recently, using conventional antibiotics to treat the disease proves to be not effective. Some antibiotics are completely ineffective due to resistance to antibiotics of the bacteria. The research on replacing antibiotics in treating diarrhea by using herbal medicines, such as "Betel Bokashi", was reported in Binh Dinh province [2, 6]. Betel Bokashi is a naturally occurring herb that inhibits bacteria with substances, such as eugenol, chavicol, estradiol and some other phenolic compounds added into the dietary composition of piglets [2, 8]. The research aimed to evaluate the impact of Betel Bokashi as treatment of diarrhea by *E. coli* and the dose used in pig farms for minimizing the health risk for reproductive sows and suckling piglets in comparison of antibiotics.

2. Materials and Methods

2.1 Experimental Design

Suckling piglets were selected from different litters at one pig farm of 50 sows. Just only 10 sows had farrow at duration of one week at each batch. These

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sows had the same feeding and housing condition. There were 54 young piglets with diarrhea from 124 young piglets born alive and raising on farm. There were 18 young piglets in each trial catch for treatment and three trials in each experiment using different doses of Betel Bokashi for treatment (Table 1). Trials were as follows:

Control group (T_1): Norflocacine was applied with the dose of 1 mL/5 kg body weight (according to the manufacturer's instructions);

T_2: Enroflocacine was given after 12 h of birth with a dose of 0.5 mL/piglet. When piglets were infected and had diarrhea, the dose of 1 mL/5 kg of body weight was added (according to the manufacturer's instructions);

T_3: Betel Bokashi was supplemented to the piglets at first week postpartum with direct dose of 3 mL/piglet by oral and 5-8 mL/d of Betel Bokashi was supplemented to piglets at the 2nd week for 3-5 consecutive days.

2.2 Sample Analysis

Fecal samples of suckling pigs having diarrhea were collected at farm and stored at freezer condition of -20 °C until analysis. All the equipment and materials as sterilized chambers, ovens, freezers and autoclaves were used. Microscopes, culture incubator, peptri plates, test tubes, alcohol lamps, triangles, measuring cups, electronic scales, glass slides, syringes and other utensils were used in all of protocols and analysis of samples.

All stool samples were transferred to the laboratory in warm cabinets at 37 °C for 24 h after that cultured in an EMB agar medium where *E. coli* produced purple iridescent colonies. All bacteria are stained grams and isolates are sprouting, appearing pink and reading the results.

2.3 Environment, Solution and Chemicals

Types of media that transport, culture, isolate and reading screen *E. coli* bacteria include common and specific environments as common jelly, EMB, blood agar, etc.. Chemicals used in the experiments are: alcohol gram dyes, gentian violet, lugol and fuchsin. Ten bacteria samples were randomly isolated from the total numbers to determine the biochemical characteristics of the bacteria. Pure monoculture was kept for the identification of some biochemical properties in the following environment: citrate, Kligler's iron agar (KIA), motility indol urease (MIU), methyl red, glucose, saccharose and lactose.

Biochemical reagents were used for sugar fermentation reaction and reading on the Petri was by microscopy. The experimental factor is Betel Bokashi to compare the differences between T_1 (Norfloxacine), T_2 (Enrofloxacine) and T_3 (Betel Bokashi) under treatment regimens.

The experimental data were processed according to the biostatistical method. All collected data was managed using Microsoft Excel 2013 software and analyzed statistically using Minitab software. Data were presented as mean ± standard error of the mean. Monitoring indicators include infection rate of *E. coli*, cure rate, disease rate and weight gain, and they were calculated by the following Eqs. (1)-(4):

$$\text{Infection rate of } E. coli\ (\%) = \frac{\text{positive samples}}{\text{isolated samples}} \times 100\% \quad (1)$$

$$\text{Cure rate } (\%) = \frac{\text{healthy piglets}}{\text{infected piglets}} \times 100\% \quad (2)$$

$$\text{Disease rate } (\%) = \frac{\text{infected piglets}}{\text{piglets born alive}} \times 100\% \quad (3)$$

$$\text{Mortality rate } (\%) = \frac{\text{dead piglets}}{\text{piglets born alive}} \times 100\% \quad (4)$$

3. Results and Discussion

3.1 Morphological and Biochemical Characteristic of E. coli Strains

Recognization via the morphological and culture characteristics of *E. coli* strains isolated in this study

Table 1 Experimental setting-up.

Criteria	Group 1	Group 2	Group 3
Piglets per batch	18	18	18
Replicates	3	3	3
Total	54	54	54

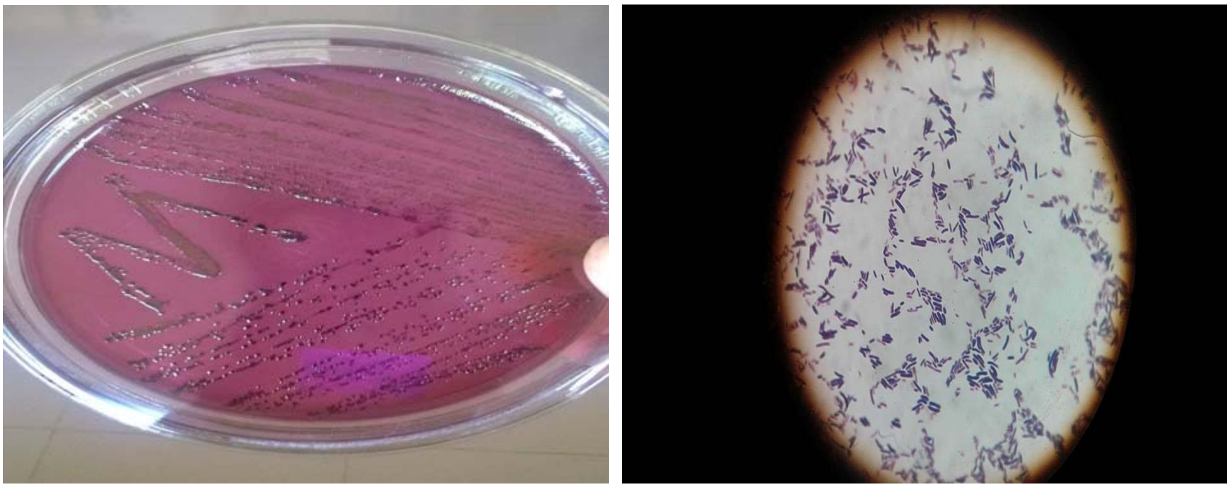

Fig. 1 ***E. coli*** **on EMB environment.**

showed common typical characteristics of *E. coli* as documented and published by other authors and reference.

After incubation at 37 °C for 18-24 h, the following results were obtained. The color of the medium did not change in citrate tubes, suggesting that the bacteria were not capable of using a negative citrate source. In the KIA environment test tube, there was the possibility of sugar fermentation. The stain at the bottom of the test tube disappeared, pushing the agar off the bottom, and no black sign at the bottom of the tube indicated that the isolated bacteria were capable of producing. In the test tube containing the MUI environment, the bacteria were observed to grow around the implants indicating that the bacteria were mobile. After 4-5 drops of Kovac's reagent was added, a red ring appeared on the testicle. It was caused by bacteria capable of forming tryptophan in the indol environment. After the reagent was added, indole combined with the paradigm ethylamino benzaldehyde contained in the reagent to form a red compound called rosindol, so that on the contact surface, there was a dark red ring. In the methyl red reaction, 2-3 d after incubation in a warm incubator, 2-3 drops of methyl red reagent were added. It was observed that the medium turned red, indicating that the bacteria had a positive methyl red response. The usual environment was peptone glucose. This reaction was used to distinguish *E. coli* from *Enterobacter aerogene*s. *E. aerogenes* fermented glucose into pyruvic acid and continued to metabolize acetyl methyl carbonyl neutral, then the pH reached at 5.4. The methyl blue changing into the red is negative reaction on medium (Fig. 1).

E. coli also fermented glucose to pyruvic acid and further converted acid to ethanol, acetic acid, H_2, CO_2, lactic acid and succinic acid, making the pH of the medium lower to 4.5 or lower. When small red methyl was added, the medium turned red, so the methyl red response was positive. In the fermentation of sugar glucose, saccharose and lactose, the environment turned from red to yellow. Because the medium contained phenol red indicator, it was red at alkaline pH and turned yellow at acidic pH. Bacterial isolates were capable of using this sugar. Fermentation of acids should lower the pH of the medium and alter the color of the indicator.

From Table 2, it was shown that all bacteria were tested by biochemical reactions with differences and isolated for different types of *E. coli*, as described in other publications [2, 9-11].

3.2 Test Results at Farm Level

According to the results collected during the experiments, it was clear that the rate of young piglets having diarrhea was very high, almost all piglets had the disease at least once. The diarrhea situation of the camp is presented in Table 3.

It was shown that the incidence of diarrhea in pigs was 48.9%. In the first treatment T_1 due to postnatal disease, the diarrhea rate was higher than the other two (T_2 and T_3). In T_3, Betel Bokashi was used to prevent piglets from diarrhea after birth, so the incidence of diarrhea in this batch was the lowest. This proves that the use of Betel Bokashi to prevent bacterial diarrhea in piglets was more effective and safe for pigs.

3.2.1 Rate of Samples Positive for *E. coli*

All samples were collected for laboratory testing to determine the prevalence of *E. coli* infection throughout the herd. After that, *E. coli* bacteria were isolated according to the guidelines of the laboratories the Department of Microbiology Infectious Diseases, Department of Animal Husbandry, University of Agriculture and Forestry, Hue University. All samples were taken at the pig farm and results of isolation are presented in Table 4.

The isolates showed that the number of samples positive for *E. coli* in young piglets was quite high at 83.33%. Thus, the rate of *E. coli* isolation was present in the piglets' manure, which matched the findings in Refs. [2, 12, 13] who reported that 100% healthy pigs' isolates have *E. coli*, while a lower percentage has other bacteria, *Salmonelle* and *Staphylococcus*. It was shown in Table 4 that in the first block because of no pre-diarrhea prevention, the number of samples positive for *E. coli* was higher than the other two. In both plots 2 and 3, *E. coli* was found to be 3.4% and 6.7%, respectively. It is found that if there is prevention of diarrhea for pigs with antibiotics or Betel bokashi, the result would be better than the control.

3.2.2 Results after Treatment

The results of treatment using antibiotics and Betel Bokashi proved to be both highly effective. Specifically in T_1 with norfloxacine, the number of piglets cured was 15/17 piglets, the cure rate was 88.23% and the mortality rate was 6.67%. In T_2 using enrofloxacine, the number of piglets cured was 14/14, the cure rate was 100% and no piglets died during treatment. In T_3 treatment using Betel Bokashi, the number of cured piglets was 12/13, the cure rate was 92.3% and the mortality rate was 3.33%. From Table 4, it can be seen that in T_2, when using enrofloxacine for diarrhea treatment, all treated pigs became healthy and none of them died. In T_1 with norflocacine and T_3

Table 2 Biochemistry results of *E. coli*.

Variables	Citrate	Glucose	Lactose	Saccharose	Air occurred	H_2S	Moving	Urea	Indol	MR
n		10	10	10	10		10		10	10
Results	-	+	+	+	+	-	+	-	+	+

+: positive; -: negative.

Table 3 Situation of diarrhea in pig farms and number of infected piglets.

Trials	Litters (*n*)	No. of piglets (*n*)	No. of piglets infected (*n*)	Diarrhea (%)
T_1	3	30	17	56.7
T_2	3	30	14	46.7
T_3	3	30	13	43.3
Total	9	90	44	48.9

Table 4 *E. coli* infection in piglets and litters.

Trials	Samples (*n*)	Infected samples (*n*)	Infected percentage/head (%)
T_1	30	26	86.7
T_2	30	25	83.3
T_3	30	24	80.0
Total	90	75	83.33

Table 5 Average percentage of healthy piglets after the treatments.

Trial	Infected rate (%)	Healthy rate (%)	No. of treatment days	*P*
T_1	56.67	88.23	4.01 ± 0.75^a	
T_2	46.67	100	4.00 ± 0.68^b	0.26
T_3	43.33	92.3	3.85 ± 0.69^c	
Total	48.89	88.74	3.97 ± 0.69	

[a-c] Means in the same column with different letters are of significant difference ($P < 0.05$).

with Betel Bokashi, the mortality rates were higher than that of T_2, but negligible. Of the three drugs used, the cure rate for all three drugs was all higher than 85%. Specifically, the cure rate with enrofloxacine was the lowest, but also reached 85.7%. Especially when using Betel Bokashi which was proved to be more effective, the recovery rate was higher at 92.3%. Duration of treatment is also a measure of effectiveness of the drugs used; the shorter the treatment time, the more effective the drug. Duration of treatment is counted from the beginning of treatment to the complete removal of the disease when no symptoms existed. Table 5 is presented to provide a specific number of treatment days for each batch.

Based on the results obtained from Table 5, it can be found that the treatment days of the three treatments varied from 3.85 d to 4 d, and there was no statistically significant difference ($P > 0.05$). The rate of disease was 56.67% in T_1, 46.67% in T_2 and 43.33% in T_3, respectively.

Table 5 also shows that the duration of treatment for diarrhea of the young piglets' diarrhea at the farm was relatively long with a median number of days of treatment up to 3.97 d. The duration of treatment of diarrhea using antibiotics or Betel Bokashi in camps were the same, 4.01 d in T_1, 4 d in T_2 and 3.85 d in T_3, respectively ($P > 0.05$). It can be concluded that the addition of Betel Bokashi for the treatment proved to be more effective than antibiotics enrofloxacine or norfloxacine.

The organs in the body of piglets in the period from birth to weaning are not yet complete. Gastrointestinal pepsin with the ability to slow the secretion is not digested, so it is easy to be infected by gastrointestinal tract. Piglets' ability to regulate body temperature is poor due to thin layers of fat under the skin. Piglets are then easily affected by factors, such as temperature and humidity. At this time, the piglets had an immune system for resistance to diseases. At first, piglets' immune system is passive, completely dependent on the amount of antibody received from the mother pigs through milk. Climate factors, weather conditions, housing conditions, techniques, care and feeding are also factors that cause bacterial diarrhea. In order to treat bacterial diarrhea in pigs, it is necessary to identify an effective antibiotic or herb that inhibits or destroys the pathogen and enhances resistance of the body. Currently, according to the study results of many authors, many types of antibiotics have become completely resistant to bacteria and no longer work in disease prevention. According to Thuy and Phu [11], *E. coli* isolated from white feces showed that 40% of *E. coli* were resistant to streptomycin, 50% resistant to sulphamid and 12% resistant to chlotetracyclin. Therefore, bio-preparations should be used to treat diarrhea instead of antibiotics so as to reduce the risk of drug resistance.

Betel Bokashi was used to treat the 13 infected piglets in T_3. On the first day using Betel Bokashi, the piglets' feces were still loose, fur was still ruffled and pigs were still moody, traveled less. However, on the second day, the pigs were more agile and the stools became more solid. On the third day, the pigs ate and drank as normal, and could concentrate better.

The piglets' feces on day 4 had clearly visible shapes, forming stools. It also depended on the ability of each piglet to absorb drugs, as observed, there were some piglets that recovered on day 4 or 5, while there were also some piglets that recovered on day 3.

The experiment was given a relative distribution of neonatal birth weight between the experimental plots. However, the weaning weight at 21 d of age and the probability of gaining grams per head per day varied between the following groups. The piglets in T_2 were supplemented with antibiotics to prevent diarrhea and the control weaning weight varied from 6.08 kg/head to 6.25 kg/head, corresponding to an average weight gain of 212-220 g/head/day. Meanwhile, in T_3, the piglets were supplemented with Betel Bokashi, average weaning weight was 6.76 kg/head and average gain of 231 g/head/day. Thus, it can be seen that in the three treatments on weaned piglets, weaning weights were similar and the weight gain per gram per day was similar. However, when supplemented with Betel Bokashi to prevent diarrhea caused by *E. coli*, piglets' weaning weight was higher than that of the other two groups. The treatment of antibiotic to piglets of T_2 and Betel Bokashi for T_3 made the differences of growing (6.08-6.25 kg/head at weaning and average weight gain of 212-218 g/head/day) as shown in Table 6. It can be found that piglets treated using antibiotics after weaning grew slower than that using Betel Bokashi. According to the study by Trung [4], the addition of 2% of *Achyranthes aspera* L. to pigs' feeds during the second month of pregnancy and during pregnancy, the resistance to diarrhea would be enhanced for young piglets and the mortality rate was reduced (9%); weaning weight was very high (8.46 kg), but weaning time was 45 d old. Based on the above results, it can be concluded that the supplementation of Betel Bokashi for suckling piglets results in a higher average weight per day gain as compared to supplementation of antibiotic in prevention and treatment of diarrheal disease in piglets caused by *E. coli* in suckling piglets.

Table 7 showed that the infected percentages between different treatments were not significantly; when piglets having an addition of Betel Bokashi showed a better than the treatment of Norflocacine (T_1), but was not better than Enroflocacine used (T_2), as according to the study of Phuong et al. [7], in which they also use freeze-dried Biotactyl for piglets. However, the rate of dead piglets are different between antibiotics applied and Betel Bokashi used ($P < 0.05$).

4. Conclusions and Recommendation

The percentage of *E. coli* positive specimens isolated from fecal samples of mother pig infected with diarrhea was 82.23%. All isolates carry the full biochemical characteristics of *E. coli*.

The prevalence of diarrhea in the camp was high at 48.9%, and the average number of days of treatment of diarrhea with Betel Bokashi was 3.85 d. Weight of pigs after 21 d of age using Betel Bookish was 6.26 kg and average weight gain was 221 g/head/day. When using betel Bookish to prevent diarrhea on piglets, there was no statistically significant difference.

Table 6 Growth of piglets on experimemt.

Trial	N	Piglets (n)	Everage weight at birth	Everage weight at weaning	*P*	ADG (g)	*P*
T_1	3	30	1.62 ± 0.11	6.08 ± 0.17^b		212 ± 10^b	
T_2	3	30	1.61 ± 0.13	6.25 ± 0.21^b	0.016	218 ± 10^b	0.004
T_3	3	30	1.62 ± 0.17	6.86 ± 0.38^a		231 ± 14^a	

$^{a\text{-}c}$ Means in the same column with different letters are of significant difference ($P < 0.05$).

Table 7 Percentage of piglets recovering health.

Trials	No. of Piglets	No of Infected piglets (n)	Infected percentage (%)	*P*	No. treatment piglets (n)	Percentage of piglets (%)	Percentage of dead piglets (%)
T_1	90	48	56.67		15	88.23	6.67
T_2	90	48	46.67	0.56	15	100	0
T_3	90	48	43.33		15	92.3	3.33
Total	90	48	48.89		39	88.74	3.33

a-c Means in the same column with different letters are of significant difference ($P < 0.05$).

Through the results of this study, it can be concluded that the use of Betel Bokashi as probiotics resulted a higher than antibiotics, and that the use of Betel Bokashi orally is more effective as compared with other applications in the traditional using, but it needs more time for labour.

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