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Researching the existence of a number of harmful microorganisms in biological padding for raising pigs used in livestock of Vietnam

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Abstract

The research aims to determine the fluctuation of the number of some kinds of harmful microorganisms in pig bed after 5 months using biological preparation. We also compared the inhibition harmful microorganism of biogical preparation producted by Vietnam National University of Agriculture (VNUA) with commercial preparation BALASA.No1. BALASA.No1 preparation was researched and judged in the paper "Evaluating the effect of applying in fermented technology for raising pig in small farm" by Do Quang Dai Master, 2011. So we assessed the effect of VNUA biogical preparation by comparing the microbiological criterias between experimential group (using VNUA preparation) and control group (using BALASA.no1). The results shows that fermenting pig bed by these preparation both intibited the growth of *Salmonella* and constrained the growth of *Coliform* and *E.coli*. Based on this study it could be conclude that using VNUA bio preparation to ferment pig bed can prevent the disease from *Salmonella* and *Coliform.*

Keywords: Fermented bed technology, Biological preparation, BALASA.No1, Vietnam national university of agriculture

1. Introduction

In recent years, the livestock industry of Vietnam has a strong raising in the number of cattles and poultries as well as the total quantity of livestock waste, at 85-90 milion tons (GSO of Vietnam, 2014). Along with this development, there are many methods for handling livestock waste like biogas cellar, organic farming method, livestock biosecurity [1-2]. Recently, fermented bed technology has applied in many coutries. With this technology, the useful microorganisms compete and destroy the harmful microorganisms that cause fermentation making uncomfortable smells so that all the feces and urine are construed without poison gas [3]. Famers will save money for cleaning and waste treatment, water sources and surroungding environments are not polluted, that creates "firewall" to prevent the disease thank for effective microorganisms [4]. Therefore, the research for the production of biological preparation applied ecological farming in Vietnam are necessary.

Nowaday, Vietnam National University of Agriculture (VNUA) has researched and manufactured the biological product used for bed in pig production and applied it in reality. However, the process of raising pigs on the floor using bio product for long periods can make harmful microorganisms survive and cause disease in animals or not? In this paper, to answer this question, we verified and assessed the effect of this preparation after using 5 months by researching the presence of harmful microorganisms: *Coliform, Escherichia coli and Salmonella* [5-6]. We assessed the inhibitory effect of harmful microorganisms in the barn padding of preparation producted by VNUA and a popular commercial preparation in Vietnam- BALASA.No1 based on the following criteria:

1. Reasearch the presence of *Coliform* in biological padding.
2. Reasearch the presence of *E. coli* in biological padding.
3. Research the presence of *Salmonella* in biological padding.

2. Materials and methods

2.1 Sampling

To have the samples, we conducted the experiment in a pig farm in Bach Thuong commune, Duy Tien district, Ha Nam province by following way: 18 market hogs was disposed into 6 barns; the experiment group included the barn number 1,2,3 and the control group included the barn number 4,5,6. The same conditions in all the barns are: body weight of hogs when beginning the experiment, density, type of food, food intake, feeding method, hygiene, prevention. Different condition: using biological preparation producted by VNUA for the padding of experimential group; using bio

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preparation BALASA-No.1 producted by Minh Tuan facility for the padding of control group.

The padding samples were collected from the barns in 3 times- after 1, 3 and 5 months using the preparations, at 5 points including 4 corners and the middle of the barn at the 20cm depth of the padding. Total number of collected samples is 30 samples for each time.

2.2 Quantitative determination of total number of Coliform and E.coli

We used spread plate method [7] to quantate the number of total *Coliform* and *E.coli* in the samples.

- Preparing samples: take 1g of the sample into 15ml centrifuge tubes, using pipette to absorb 10 ml sterile distilled water into each tube, then vortex the tubes.
- Samples dilution: making a 10-fold serial dilutions of sample solution into 10-1, 10-2, 10-3, 10-4,…
- Inoculating samples: Choose 3 appropriate consecutive dilutions to inoculate. Each dilution was spreaded on 2 MacConkey agar plates with 100µl of each dilution per plate. Marking the dilutions in each agar plate and cultivating in an incubator at 37˚C for 24-48 hours. After 24-48 hours, we counted the number of colonies on both agar plates of each dilution. Select the plates with 30 to 300 colonies in the plates of 2 consecutive dilutions. The *Coliform* has formed colonies with traits: small, round, slightly convex, smooth, slightly red and non-diffusible pigment [7]. For calculating the density of *E.coli*, we need to calculate the confirmation rate of *Coliform* according to following method:

Getting 5 typical colonies to streak and stab into TSI agar slant tube for evaluation *Coliform*. Then the tubes were incubated at 37˚C for 24 hours and removed to read the results. In TSI agar, *Coliform* make the bottom and the slant of agar turn pink. The confirmation rate of *Coliform* is the rate between the number of colonies positive with the test in TSI agar and the total number of colonies selected for this test.

- The positive *Coliform* colonies on agar were inoculated onto EMB agar plate. Incubated the plates at 42,5˚C for 24 hours then removed them to read the results. The colonies of *E.coli* is green metallic sheen in EMB agar. For calculating the density of *E.coli*, we need to calculate the confirmation rate of *E.coli* according to following methods:

Get 5 typical colonies (round, flat, green metallic sheen [7]) we continued to expertise by the biochemical reactions: IMViC, catalase. It is *E.coli* if the results of the biochemical will be IMViC (++--), catalase (+). The confirmation rate of *E.coli* is the rate between the number of colonies positive with the biochemical reactions of *E.coli* and the total number of colonies selected for this test.

- Calculation results: based on the number of counted colonies and the confirmation rate of *Coliform* and *E.coli*, we can calculate the density of *Coliform, E.coli* according to the following formula:

$$A = R \times \frac{N}{V.(n_1 + 0{,}1.n_2).d}$$

with:

A(CFU/ml): The number of bacteria cells (unit forming colony) in 1g or 1ml sample.

N: The total number of colonies counted on the selected plates in 2 consecutive dilutions.

V(ml): The volume of the sample solution streak into each plate.

n_1, n_2: Number of plates are selected for colony counts on 1st, 2nd dilutions.

d: dilution factor of the first dilution.

R: the confirmation rate of *E.coli* or *Coliform*

Rounding the results with retaining only 2 significant figure. Results were expressed as a decimal between 1.0 and 9.9 multiplied by 10^n (n is the appropriate exponent of 10).

2.3 Method for isolating Salmonella spp bacteria

We isolated *Salmonella spp* bacteria according to ISO 6759:2002 method.

3. Results

3.1 Determination the presence of Coliform in the padding samples

As the result shown in Figure 1: In MacConkey agar, after cultivating at 37ºC in 24h, the *Coliform* has formed colonies with traits: small, round, elevation is convex, smooth, slightly red and non-diffusible pigment.

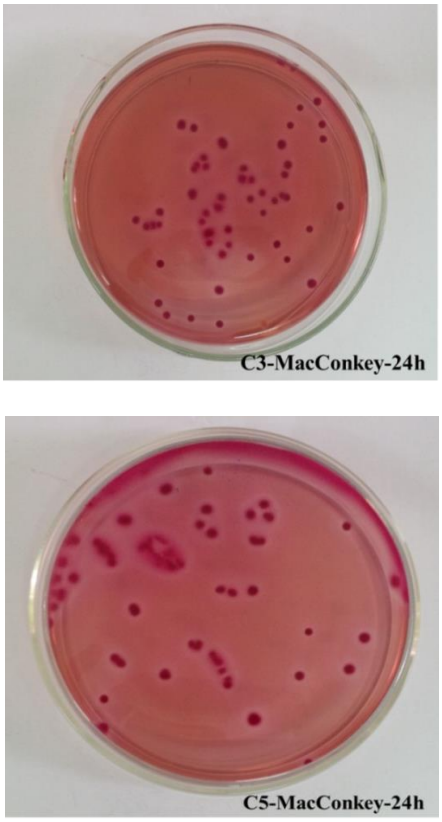

Figure 1 *Coliform* colonies on MacConkey agar

The result in Figure 2 has indicated: in TSI agar, *Coliform* has colored the agar, both the slant and the bottom has turned pink. We can calculate the confirmation rate of Coliform from the growing property of *Coliform* in TSI agar. The summary of total *Coliform* counts is calculated by that result.

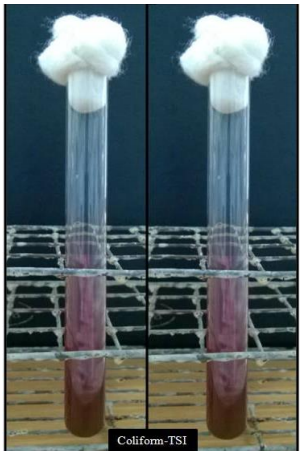

Figure 2 Results of expertising *Coliform* on TSI agar

Table 1 Total *Coliform* counts (10^3 CFU/g)

Serial	Group	Barn number	Total Coliform counts (10^3 CFU/g)		
			After 1 month ($\bar{X} \pm$ SD)	After 3 months ($\bar{X} \pm$ SD)	After 5 months ($\bar{X} \pm$ SD)
1	Exp	1	97,15 ± 3,56	93,43 ± 2,26	90,07 ± 3,05
		2	95,20 ± 2,14	95,74 ± 4,10	91,22 ± 5,53
		3	93,44 ± 4,22	90,09 ± 4,56	89,01 ± 3,13
	Average		95,34 ± 2,02	92,14 ± 4,02	89,98± 1,26
2	Control	4	95,05 ± 5,34	96,35 ± 4,24	97,45 ± 3,45
		5	97,76 ± 9,87	95,13 ± 6,83	91,50 ± 1,56
		6	96,09 ± 6,78	92,12 ± 3,74	90,14 ± 3,73
	Average		95,12 ± 1,32	93,01 ± 4,32	90,16 ± 2,45

Result from Table 1: there's slight change in the Total *Coliform* density in both the exp and the control group. Total *Coliform* density tends to slightly decrease and oscillate between 95,34.10^3 CFU/g and 88,98.10^3 CFU/g in exp group, 95,12.10^3 CFU/g and 90,16.10^3 CFU/g in control group. This proved the pig model based on biology padding without cleaning the barns for a long time, will make Total *Coliform* density decrease over time due to the activities of beneficial microorganisms. The bio preparation in both exp and control group has about the same effect on the inhibition of *Coliform* in the padding.

To explain this result, we consider the microbial components of 2 bio preparations. Both of them have *Bacillus subtilis, Streptoccocus latis* (in BALASA.No1), *Lactobacillus acidophilus* (in preparation made by VNUA), which secrete bacterioxin and enzyme creating acid, so pH environment reduce and harmful microorganisms like *Coliform*, *Salmonella*, others harmful bacterial spore was killed and contrained the growth [8-9].

3.2 Determination the presence of E.coli in the padding samples

From our result on the previous test (*Coliform* on MacConkey agar), we took the resulted colonies to EMB agar and cultivated in incubator at 42,5°C for 24 hours. We can see in Figure 3, these colonies which are taken from MacConkey agar are growing well in EMB agar.

After that, we cultivated the suspected colonies in Tryptone agar and take 5 typical *E.coli* colonies in EMB agar to expertise with bio-chem reaction IMViC [7] in order to calculate the faceal *E.coli* confirmation rate. The result is shown in Figure 4.

From the *E.coli's* bio-chem reactions, we can calculate the confirmation rate, therefore deterrmine the *E.coli* density in the padding. Result shown in Table 2.

Table 2 *E.coli* density in padding samples

Serial	Group	Barn number	E.coli density (10^3 CFU/g)		
			After 1 month ($\bar{X} \pm$ SD)	After 3 months ($\bar{X} \pm$ SD)	After 5 months ($\bar{X} \pm$ SD)
1	Exp	1	74,74 ± 3,56	73,43 ± 2,26	90,07 ± 3,05
		2	76,16 ± 2,14	75,74 ± 4,10	91,22 ± 5,53
		3	77,44 ± 4,22	72,07 ± 4,56	89,01 ± 3,13
	Average		76,27 ± 2,02	73,71 ± 4,02	89,98 ± 1,26
2	Control	4	76,04 ± 5,34	77,10 ± 4,24	77,96 ± 3,45
		5	78,21 ± 9,87	76,10 ± 6,83	73,20 ± 1,56
		6	76,87 ± 6,78	73,70 ± 3,74	72,11 ± 3,73
	Average		76,10 ± 1,32	74,41 ± 4,32	72,13 ± 2,45

As we can see, *E.coli* density changes little between months. The average *E.coli* density of oscillates between 76,27.10^3 CFU/g and 89,98.10^3 CFU/g in exp group, 76,10.10^3 CFU/g and 72,13.10^3 CFU/g in control group. This means the benefical microorganisms in 2 bio preparations both inhibited the growth of *E.coli*. However, the figure for 2 groups after 5 months using bio preparation saw that BALASA.No1 inhibited the growth of *E.coli* longer than the bio prepation made by VNUA.

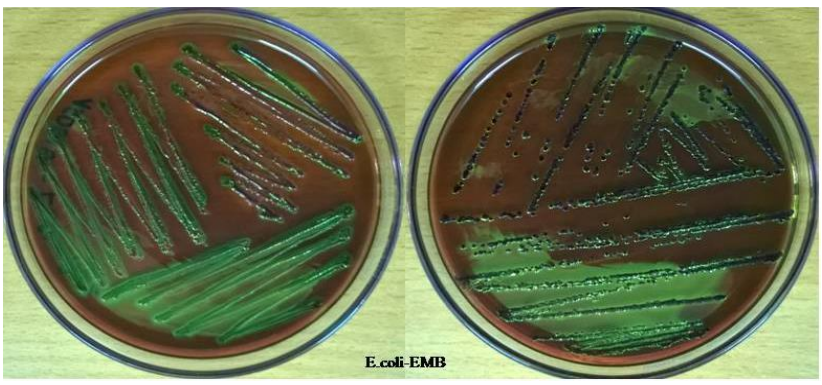

Figure 3 Results of cultivating *E.coli* on EMB agar

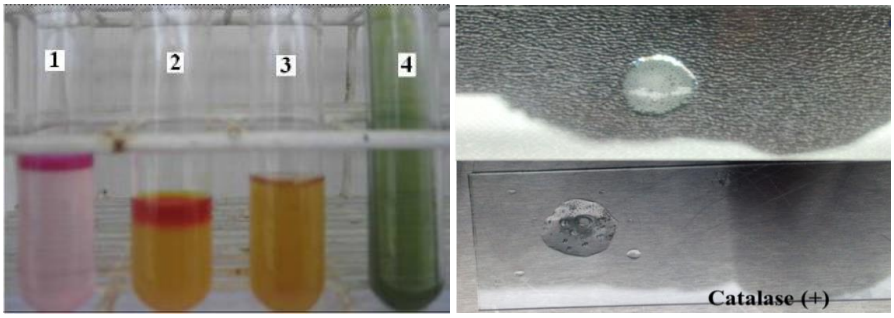

Figure 4 Results of the *E.coli's* bio-chem reactions
1: Indole (+), 2: Methyl Red (+), 3: Voges Prokauer (-), 4: Citrate (-), Catalase (+)

3.3 Determination the presence of Salmonella in the padding samples

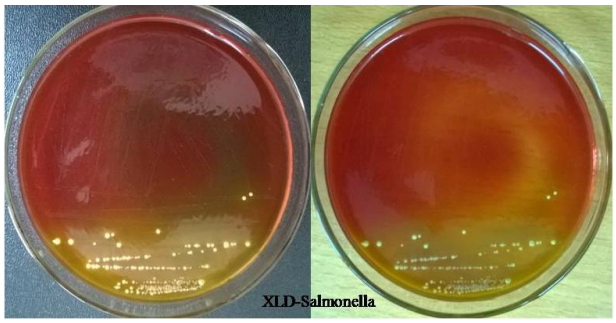

Figure 5 Results of cultivating *Salmonella* on XLD agar

We cultivated the colonies suspected with *Samonella*, which make XLD agar turn lemon yellow (Figure 5), in TSI and SS agar, incubated at 37°C in 24 hours then tested a few bio-chem reactions to confirm. From the resulted research shown in Figure 6, we can see there's no presence of *Salmonella* in barns of both exp and control group. This has proven that the bio preparation made by VNUA and the preparation BALASA No.1 both produced good results for intibited the growth of *Salmonella*. So that, raising pigs in fermented bed without cleaning the barn for a long time did not save *Salmonella*.

Following Nguyen Lan Dung, 1983 [10], *Salmonella* exists in environment with pH 6-9, while the benefit microorganism in fermented bed reduce pH environment by secreting acid lactic, acid acetic (*Lactobacillus*), resovling carbonate, protein into organic acid and acid amin (*Lactobacillus, Bacillus, Saccharomyces, Actinomyces*). Therefore, *Salmonella* cannot exist in the padding using bio preparation.

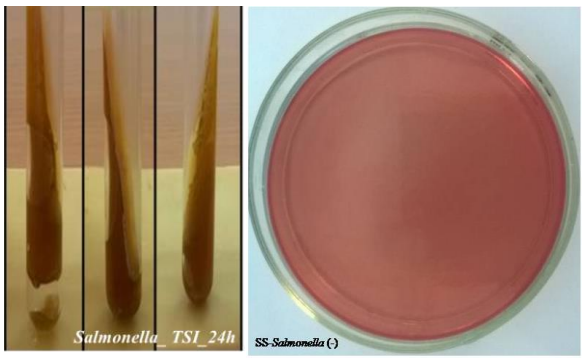

Figure 6 Results of testing *Salmonella* on TSI agar and SS agar

4. Discussion

Because of the limited time, we only evaluated methods of farming on a small scale and on market hog. The research would be practiced with other objects such as sows, piglets for all the seasons of the year.

5. Conclusions

5.1 The changes in total *Coliform* in both exp and control group is not significant. Total *Coliform* tends to decrease slightly and oscillate between 95.34.103 CFU/g and 88,98.103 CFU/g in exp group, 95.12.103 CFU/g and 90,16.103 CFU/g in control group. The bio preparation in both exp and control group has about the same effect on the inhibition of *Coliform* in the padding.

5.2 *E.coli* density changes little between months. The average *E.coli* density oscillates between 76,27.103 CFU/g and 89,98.103 CFU/g in exp group, 76,10.103 CFU/g and 72,13.103 CFU/g in control group. This means the benefical microorganisms in 2 bio preparations both inhibited the growth of *E.coli*. However, the figure for 2 groups after 5 months using bio preparation saw that BALASA.No1 inhibited the growth of E.coli longer than the bio prepation made by VNUA.

5.3 Both experimental groups and the control group did not have *Salmonella* bacteria in the padding.

5.4 The results of this analysis show that the bio preparation made by VNUA and the preparation BALASA No.1 both intibited the growth of *Salmonella* and constrained the growth of *Coliform* and *E.coli*. Therefore, the process of raising pigs on the floor using bio product for a long time can prevent diseases in animal. However, ability of preparation BALASA No.1 to constrain the growth of *E.coli* is better than preparation of VNUA when using for a long time.

6. Acknowledgements

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