Organic Acid and Inhibition of Complete Silage Ration on the Growth of *Salmonella enteritidis*

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ABSTRACT: Giving feed by silage technology is expected to replace the benefits of antibiotics in poultry feed. The purpose of this study was to determine organic acids content and the ability of silage ration complete in *Salmonella enteritidis* inhibiting in vitro. Silage complete ration composed of some feedstuffs with the crude protein content of 21% and an additional 1 ml inoculum per 1 liter of water was added until 45% water content. Inoculum contained a total BAL 5 x 10^8 cfu/ml. An aerobic fermentation process was done by 1 kg of complete feed compacted into plastic and put into silos, stored according to treatment. Organic acids content were: lactic acid, propionic acid, butyric acid analyzed using HPLC with a wavelength of 215 nm. Completely randomized design was apllied in this experiment with 4 treatments stored (7, 14, 21, and 28 days) each repeated four times, the data was tested using SPSS, differences between treatments were tested by Duncant. The average results showed that lactic acid very significantly higher ($P < 0.01$) on each day of observation: 7 days = 305.64 ppm; 14 days = 876.52 ppm; 21 days = 1666.79 ppm; 28 days = 4038.70 ppm. Propionic acid content on 7 and 14 days were not significantly differently: 164.1 ppm and 158.9 ppm, while on 21 days and 28 days storaged very significantly higher ($P < 0.01$): 244.2 ppm and 325.9 ppm. However, butyric acid content below the detection limits. Inhibition activity analyzed using agar well diffusion method. Silage ration complete with 100% concentration was capable for forming a clear zone with the average area 7,61 mm against 10^6 cfu/ml of *Salmonella enteritidis*. In conclusion silage ration complete with 21% protein had a number of organic acids that could inhibit pathogenic bacteria so that poultry feed was safe and free from antibiotics.

Keywords: Organic acid, Inhibition, Silage, *Salmonella enteritidis*

INTRODUCTION

Healthy feed produce healthy livestock. Nowadays healthy poultry feed comes with antibiotics. Eventhough beside the positive impact gained there are the negative effects of antibiotics are also obtained in the form of residues in animal products. Several studies of alternative antibiotics have been written by Sanchez *et al.* (2015) describe another things that is safe and does not leave a residue, which phytobiotic derived from plants, spices and herbs. Research on 'organic acids and inhibition of complete silage ration to the growth of *Salmonella enteritidis* ' in addition aimed at improving the performance of the livestock also as healthy fodder and able to act as an antibiotic.

Silage is made because of abundant feed ingredients, on the other hand of making silage is also intended to maintain nutritional feed ingredients. Silage also contains lactic acid bacteria are able to produce antimicrobial substances. Silage is also able to improve performance such as that provided by the probiotic effect (McDonald *et al*, 1991). Complete ration silage has a pH that is acidic and increase the content of total acid (Allaily *et al*., 2011).

MATERIALS AND METHODS

Materials

Feed ingredients were used for the preparation of complete silage ration as: yellow corn, rice bran, coconut meal, soybean meal, fish meal, coconut oil, CaCO3, DCP, premix, water and liquid inoculum containing a total BAL 5 x 10^8 cfu/ml. Equipment were used plastic, silo, petri dish, biuret, laminar, scales, isolative, cool box and a pH meter.

Method

All feed ingredients were mixed according to the formula of laying ducks with 21% crude protein and 45% moisture content. Making 45% water content was refers to Allaily *et al*. (2011). Water was already containing a total BAL 5 x 10^8 cfu/ml. After all ingredients mixed with water, and then compacted for 1 Kg in each plastic. Then covered and stored in a silo in an aerobic fermented during 7, 14, 21, and 28 days (for treatment). Organic acids such as lactic acid, propionic acid, butyric acid were calculated by using HPLC (High Performance Liquid Chromatography). Clear zone formed from 100% concentration challenged of 10^6 cfu/ml *S.enteritidis* concentration.

Sample Preparation

The sample was 2 g, then was put in a 125 mL Erlenmeyer. Added water 25 ml and stirred until dissolved. Then pH was set 1-2 with HCl 3 N. Extracted with diethyleter 25 mL for 2 minutes and 2 times. Results extract basified to pH 8 with 2 N NaOH, and then evaporated down to the water phase. A layer of water left behind was diluted to 2 mL with mobile phase, filtered with millex 0.45 pM and 20 mL injected into the HPLC. Challenge test was using the well diffusion method with MHA (Muller Hilton Agar), using a standard fluid Max Farlan 10^6 cfu/ml. Complete silage ration for 100% fluids was dripped into the well contents of *S. enteritidis* 10^6 cfu/ml, then was put into an incubator and 24 hours later formed clear zone can be observed.

Standard

Standard stock of 10,000 ppm lactic acid, Standard series, respectively: Lactic acid: 312.5; 625; 1250 and 2500 ppm, propionic acid: 62.5; 125; 250 and 500 ppm, butyric acid: 62.5; 125; 250 and 500 ppm. 10 mL injected into the HPLC.

HPLC conditions

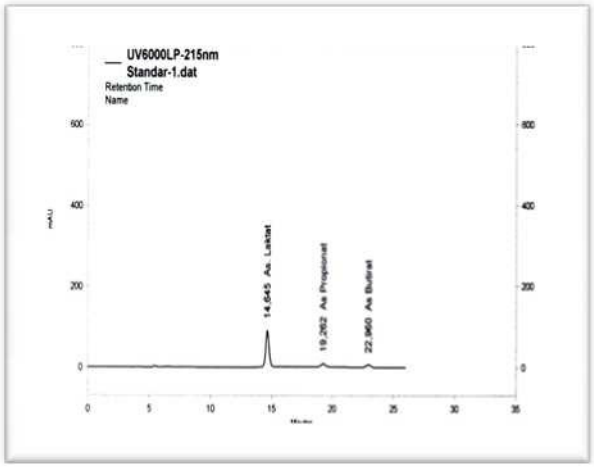
Column: Meta Carb H Plus coloum 300 x 7.8 mm, Phase Motion: H_2SO_4 125 mL in 500 mL of water, Flow: 0.6 mL / min, Gel Length: 215 nm, temperature: 800 C.

Analysis Procedures

Day observation 7.14, 21, and 28 days was the treatment, and each treatment was repeated 4 times. The design used was completely randomized design with 4 treatments and 4 replications. Results were analyzed using SPSS and tested duncan to see a noticeable difference.

RESULTS AND DISCUSSION

Complete silage ration with fermentation time of 7, 14, 21, and 28 days showed an increase significantly, lactic acid content as well as propionic acid in the treatment of 14, 21, and 28 days was increased significantly, but 7 and 14 days showed no different result. While the content of butyric acid in 7 and 14 days was below the limit of detection, treatment of 21 and 28 days were not significantly different. Pictures HPLC analysis results can be seen as follows:



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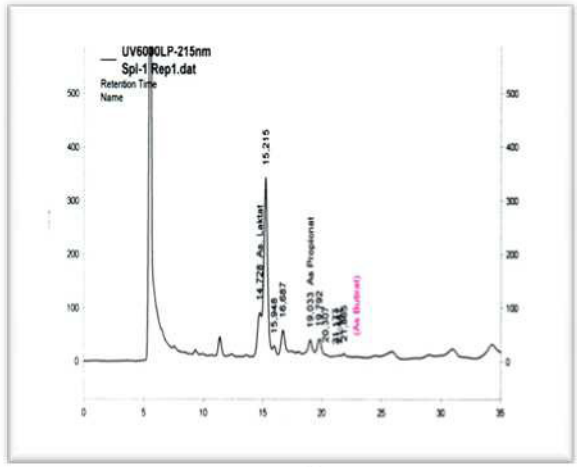

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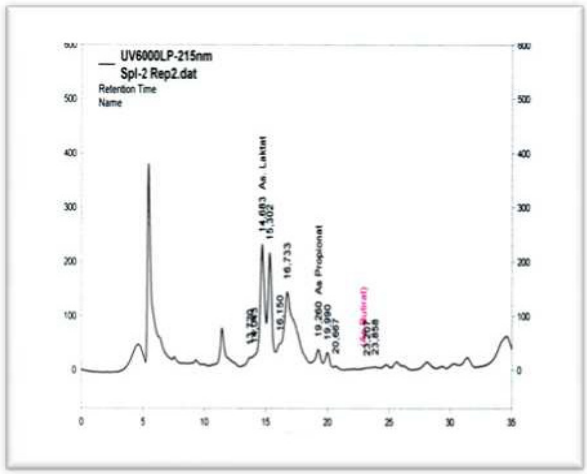

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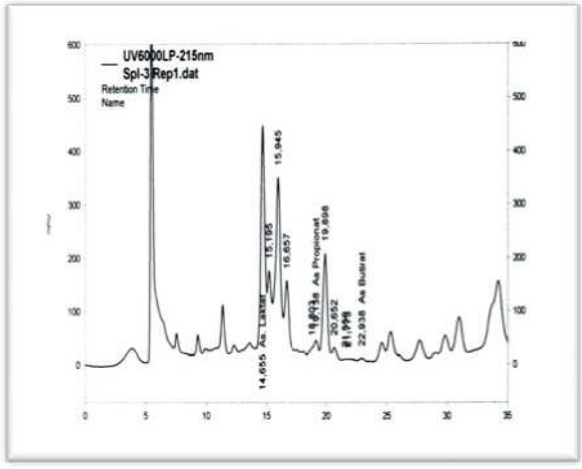

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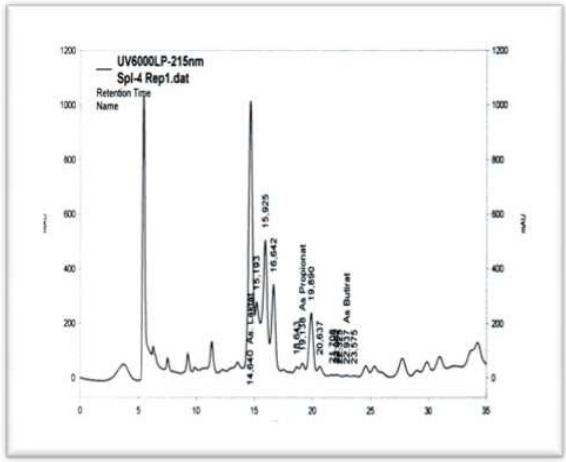

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Picture : a. Standard, b. 7 days, c. 14 days, d. 21 days, e. 28 days of fermentation time.

Table 1. Organic acid content in silage treated with fermentation time

Parameter	Fermentation Time (Day)			
	7	14	21	28
Lactic acid (ppm)	305.64±5.44a	876.52±19.95b	1666.79±20.87c	4038.7±86.48d
propionic acid (ppm)	164.05±2.92a	158.88±2.60a	244.16±3.65b	325.92±44.96c
butyric acid (ppm)	<0.62	<0.62	52.94±0.78	48.48±0.67

Source: Integrated Research and Testing Laboratory UGM (2015)

Organic acids have an antimicrobial effect (Tharmaraj and Shah 2009), which examines the acetic acid, lactic, formic, propionic, butyric, benzoic, and phenillaktat able to inhibit pathogens such as *E. coli, B.cereus, S. Aureus*. Nurjama'yah *et al.* (2014) succeed to see the antimicrobial activity of lactic acid bacteria against *E. coli, S. Thypmurium and L.monocytogenes*. Negara *et al.* (2008) wrote that the organic acid salts of a complete ration of corn silage fluids had inhibitory ability against S.typhimurium and E. coli. Fermentation products was not only produced organic acids as antimicrobials, but was able of being a factor to improve animal performance (Fasina and Thanissery, 2011; Milbradt *et al.*, 2014).

Complete silage ration at 100% concentration was able to inhibit pathogenic bacteria S. enteritidis with a concentration of 106 cfu ml. Clear zone produced with the average area of 7.61 mm. Formed clear zone can be seen in the picture below.

Picture of clear zone formed by 100% concentration of complete silage ration

Miyamoto *et al.* (2000) reported about the ability of lactic acid bacteria form a clear zone covering an area of 0.7 cm - 1.2 cm. Clear zone area formed by complete silage ration measuring 7.61 mm is almost the same as the ability of lactic acid bacteria derived from the cloaca and vagina chicken inhibited *Salmonella enteritidis*. But the clear zone formed on the Oyarzabal and Conner (1985) researched was wider at 13 -30 mm, it was probably due to Salmonella sp challenged with bacteria that have been isolated and selected.

CONCLUSIONS

Complete Silage ration with 21% protein had a number of organic acids that was able to inhibit *Salmonella enteritidis* so that a poultry feed was safe and can replace antibiotics function.

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