

# Effectiveness of *Lactobacillus acidophilus* 2B4 as Biocontrol to Prevent *Salmonella enteritidis* Infection on Laying Hens

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## Abstract

Laying hen is raised and selected to produce eggs. Poultry could be infected by several kinds of *Salmonella enterica* such as *S. enteritidis* as a specific bacterium which is carried by chickens. *Salmonella enteritidis* can depress the hen weight by dehydration and contaminate the egg which is very dangerous for human health. Regarding this negative effect, the preventive treatment to eliminate *Salmonella enteritidis* contamination in eggs becomes a major concern. This research aimed to determine the optimum dosage and frequency applied of probiotic *L.acidophilus* to avoid contamination of *Salmonella enteritidis* in ovary and egg of laying hens. The result showed that probiotic treatment could increase feed consumption, egg production, however, in the other hand decreased the feed conversion. Among other, probiotic treatment was effective to reduce the population of *Salmonella enteritidis* in ovary and egg of laying hens. The optimum dosage of *L.acidophilus* probiotic was within population of 10<sup>9</sup> cfu/ml and frequency of twice a day was the most effective as biocontrol.

Key words: *L. acidophilus*, laying hen, probiotic, *Salmonella enteritidis*

## Introduction

Food security is a major issue in national development program. Food is a basic requirement for the fulfillment of - human rights for everyone. Food safety issues are major concern in public health policy. Foodborne disease and food contamination incidents occurred in various countries, not only in developing countries where sanitation and hygiene conditions are generally poor, but also in developed countries. One of the emerging pathogen is *Salmonella enteritidis* transovarian contamination of grade A eggs. These eggs are contaminated since the start of its formation in the body because its parent is infected by *S.enteritidis* in ovaries (Gantois *et al.*, 2009).

Biosecurity via biocontrol is needed to apply in layer chicken farms as preventive effort.

*Lactobacillus acidophilus* 2B4 has been proven as probiotic (Arief, 2011). This strain could inhibit the growth of *S. enteritidis* isolated from the ovary of laying hen by in vitro analysis (Ulupi *et al.*, 2009). Application of probiotic *L. acidophilus* 2B4 as a biocontrol agent for the prevention of contamination of eggs by *S. enteritidis* transovarially is very necessary, especially in laying hens. The aim of this research was to find out the dosage and frequency of probiotic *L. acidophilus* 2B4 treatment to prevent contamination of *S. enteritidis* in ovarium and egg of layer hen

## Materials and Methods

Sixty layer hens were divided into 4 Treatments. Treatment 1 (R1) was laying hens without oral administration of *L. acidophilus* 2B4 and *S. enteritidis* (as control). R2 was laying hens without *L. acidophilus* 2B4, but were infected by *S. enteritidis*. R3 was laying hens administered of *L. acidophilus* 2B4 once per day (dosage  $1 \times 10^9$  cfu/ml) and were infected by *S. enteritidis*. R4 was laying hens with oral administration twice per day (dosage  $2 \times 10^9$  cfu/ml) and were infected by *S. enteritidis*. *L. acidophilus* 2B4 was given via drinking water for 20 days, and *S. enteritidis* was given by oral administration for 5 days (day 5-day 9). Each treatment consisted of 15 laying hens. A total of 3 hens of each treatment were slaughtered for parameters evaluation. in day 5, 10, 15 and 20.

Parameters observed were performances of layer hens and Salmonella contamination on ovarium and eggs. Analysis of Salmonella contamination was done by qualitative analysis according to BAM (2007). The samples were incubated on selective media on Lysine Desoxycholate xylose media (XLD) Agar, Hectoen Eteric Agar (HEA), and Bismuth sulfite agar (BSA). These three selective media were incubated at  $35 \pm 2$  °C for  $24 \pm 2$  hours. After incubation typically appearance was observed whether there was a growing colony. The analysis followed the biochemical tests using triple sugar early Iron (TSI) and Lysine Iron Agar (LIA) in italics. Typical colonies that grew on the three specific medias XLD Agar, HE agar and BS. Each loop was inoculated using a sterile needle on TSI agar and LIA agar.

The experimental was completely random designed with 3 replications. Data were analyzed using ANOVA and then further subjected to Tukey test (Steel and Torrie, 1995) if there were any differences.

## Results and Discussion

### *Performance of layer hens*

Performance of layer hens were affected by probiotic administration as described in Table 1.

Table 1. Performance of layer hens

Performance	R1	R2	R3	R4
Feed consumption (g/hen/day)	111.81 <sup>a</sup>	112.80 <sup>a</sup>	118.29 <sup>b</sup>	118.54 <sup>b</sup>
Egg production (%)	67.64 <sup>a</sup>	70.31 <sup>a</sup>	75.91 <sup>b</sup>	81.97 <sup>c</sup>
Egg weight (g/egg)	59.43	58.89	59.57	59.58
Feed conversion	3.34 <sup>a</sup>	3.48 <sup>a</sup>	3.36 <sup>a</sup>	2.72 <sup>b</sup>
Mortality	0	0	0	0
Haugh Unit (HU)	96.40 <sup>(AA)</sup>	94.00 <sup>(AA)</sup>	94.59 <sup>(AA)</sup>	95.30 <sup>(AA)</sup>

Different superscript in the same line means significantly different ( $P < 0.05$ ), except HU

Probiotic treatment affected the amount of feed intake. R4 treatment indicated more efficient than other treatments. There was a difference between R2 and R4 treatments. Egg production of R4 was highest than the others. This was presumably because use of feed consumed by chickens R2 to improve declined condition due to the administration of *Salmonella enteritidis*. While at R2 and R4 the improved function of the body condition has been taken over by the probiotic *L. acidophilus* 2B4. R4 was better than R3. It meant that probiotic given twice per day was more effective to enhance performance than R2.

Based on the weight of eggs, there were not significant differences in all treatments. This finding was consistent with the Indonesian National Standard (1995) which states the criteria and the weight of eggs for egg consumption are extra large (more than 60 grams), large (55-60 g), moderate (51-59 grams), small (45-50 grams), and extra small (less than 46 grams). Viscosity of the albumin can be characterized by higher albumin (Sirait, 1986). The high albumin is used to determine the value of Haugh Units of eggs. The higher Haugh Unit value indicates the higher quality of the eggs (Sudaryani, 2000). Haugh Unit values of this treatment were more than 90 for each treatment, therefore, Haugh Unit value of each treatment was categorized into AA (USDA, 1964). *Salmonella* contamination on ovarium and egg of layer hens was described in Table 2.

At day-5, addition of probiotic *L. acidophilus* 2B4 could decrease *Salmonella* in layer hens (R3 and R4). After infection of *S. enteritidis* by oral administration at day 5-9, *Salmonella* could also be decreased by addition of *L. acidophilus* 2B4 twice per day. R4 was better than R2 and R3. *S. enteritidis* is invasion bacterium that invades from gastrointestinal tract to blood and reach ovarium. After *Salmonella* invasion, addition of probiotic *L. acidophilus* 2B4 twice per day (R4) could reduce *Salmonella* on ovarium at day-20. It was better than addition of probiotic only once per day (R3). Administration of probiotics enhances intestinal antibodies to alien antigens, and reduce colonization of pathogens in gastrointestinal tract (Haghighi *et al.* 2006).

Table 2. *Salmonella* infection on ovarium and egg

Treatments	<i>Salmonella</i> infection on ovarium (%)			
	day-5	day-10	day-15	day-20
R1	66.67	66.67	66.67	66.67
R2	66.67	66.67	100	100
R3	33.33	33.3	100	100
R4	0	0	100	33.3
Treatments	<i>Salmonella</i> infection on eggs (%)			
	day-5	day-10	day-15	day-20
R1	33.33	33.33	66.67	66.67
R2	33.33	33.33	100	66.67
R3	33.33	33.33	66.67	0
R4	0	33.33	33.33	0

Administration of probiotic *L.acidophilus* 2B4 could reduce *Salmonella* contamination on egg after infection *S. enteritidis* on day-10. Addition of *L. acidophilus* 2B4 twice per day (R4) was better than once per day (R3). There was correlation between *Salmonella* contamination on ovarium and egg. That proved that *Salmonella* invasion could be spread by vertical contamination from ovarium to egg.

## Conclusions

Administration of probiotic *L. acidophilus* 2B4 could increase feed consumption and egg production of layer hens which was contaminated by *S. enteritidis*. Administration of *L. acidophilus* 2B4 twice per day was more effective than once per day for 20 days treatments to reduce feed conversion. *L. acidophilus* 2B4 also could reduce *Salmonella* contamination on egg and ovarium. It proved that *L. acidophilus* 2B4 was effective used as biocontrol to prevent *Salmonella enteritidis* infection on laying hens

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