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Morinda citrifolia L. Leaf Extract as Antibacterial *Salmonella typhimurium* to Increase Productivity of Quail (*Coturnix coturnix japonica*)

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Abstract: The objective of this study was to apply effect of *Morinda citrifolia* L. *citrifolia* L. leaf extract as antibacterial of *Salmonella typhimurium* on mortality of Day Old Quail (DOQ), egg production and Hen day, hatchability of layer quail. This research was conducted at Laboratory of microbiology and laboratory of poultry nutrition, faculty of animal science, bogor agricultural university and slamet quail farms cilangkap, sukabumi, west java, Indonesia on March-July 2012. Two hundred and forty heads of quail were randomly assigned to four dietary treatments (sixty heads of quail/ treatment). Experimental design used was Completely Randomized Design (CRD). The treatments consist of level of biscuit *Morinda citrifolia* L. *Citrifolia* L. leaf extract i.e R1 = 0%, R2 = 5%, R3 = 10%, R4 = 15%. The results indicated the treatments had significant effect ($p<0.05$) on mortality of Day Old Quail (DOQ). The average mortality of Day Old Quail (DOQ) was given extract *Morinda citrifolia* L. leaf were R1 (4.00%), R2 (1.00%), R3 (1.33%), R4 (0.67%). The average mortality of Day Old Quail (DOQ) was given 15% extract *Morinda citrifolia* L. leaf (R4) was lowest than control treatment (R1). The results of the analysis indicated that *Morinda citrifolia* L. leaf of quail drink had not significant effect ($p>0.05$) on egg production, hen day and hatchability. It was concluded that the *Morinda citrifolia* L. leaf extract 15% can reduce mortality of Day Old Quail (DOQ) and can increase its egg production, hen day and hatchability.

Key words: *Morinda citrifolia* L. *Salmonella typhimurium*, mortality, quail, productivity

INTRODUCTION

The quail is one of the types of birds that holds the fastest production cycle compared to other birds. Wilson *et al*. (1961) states that female quail starts laying eggs at the age of 35 days (average 40 days) and is in full production at the age of 50 days. In an appropriate environment quail reproduces over a long period, resulting in an average of 250 eggs per year grain. High egg production until the end of the phase can be achieved by providing good quality food as needed. Increased productivity is closely related to quail physiological conditions by pressing the percentage of mortality in laying phase.

Salmonella typhimurium is one of the pathogenic bacteria that thrive in the digestive tract of livestock. Ohl and Miller (2001) states that *Salmonella typhimurium* is one of the bacteria that often attacks birds and can contaminate the product to be harmful to humans who consume them. Livestock that infected with *Salmonella typhimurium* bacteria will exhibit symptoms such as diarrhea, so it will inhibit the growth of livestock. Livestock that infected with *Salmonella typhimurium* can spread the disease through meat, eggs and milk. To prevent the bacteria from growing, the farmers usually provide antibiotics.

Giving antibiotics may suppress the growth of pathogenic bacteria such as *Salmonella typhimurium*. Unfortunately the use of antibiotics contains a bad effect for animals since animals are resistant to these kinds of specific pathogenic microorganisms. In addition, residues of antibiotics are carried in animal products such as meat, eggs, milk and are harmful to consumers who consumes. Residues of antibiotics may be poisonous for consumers and cause health problems in the long term.

The use of traditional medicine as natural antibiotics in livestock is now further enhanced to replace the use of synthetic antibiotics. One of the natural antibiotics is *Morinda citrifolia* L. leaf extract in drinking water for quails. *Morinda citrifolia* L. leaf contains antraquinon, amino acids, glycosides, phenolic compounds and acid ursulat. The content of alkaloids, phenols, glycosides and

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anthraquinone contains an active substance i.e., antimicrobial, antibacterial and anti-inflammatory. Therefore *Morinda citrifolia* L. leaf can be used as an herbal solution to synthetic antibiotics. The purpose of giving *Morinda citrifolia* L. leaf extract in the form of this research is to enhance homogeneity and to advance absorption.

MATERIALS AND METHODS

The research was conducted at Laboratory of Microbiologi and Laboratory of Poultry Nutrition, Faculty of Animal Science, Bogor Agricultural University and Slamet Quail Farms Cilangkap, Sukabumi, West Java, Indonesia on March-July 2012. This research used 240 heads of Day Old Quail (DOQ). The quails were randomly assigned to four dietary treatments (sixty heads of quail/treatment).

Process of *Morinda citrifolia* L. leaf extract: Figure 1 showed process of *Morinda citrifolia* L. leaf extract by drying, boiling, with temperature 100°C for 30 min to get the *Morinda citrifolia* L. leaf extract and than cooling in room temperature.

Inhibition zone of *Salmonella typhimurium*: Inhibition zone test of *Salmonella typhimurium* conducted to determine the best concentration of *Morinda citrifolia* L. leaf extract against the bacteria *Salmonella typhimurium*. Bacteria cultured on agar slant that has been sterilized and then incubated for 24 h at 37°C. Bacterial cultures were taken as one loop and inoculated into test tubes containing 10 mL of liquid medium sterile sodium broth. Then incubated on a shaker water bath for 24 h.

Bacterial culture that has rejuvenated taken by 50 μL using a micro pipette and put in a sterile petri dish. Selective media sterile 15 mL was poured into a petri dish and then mixed evenly and allowed to solidify at room temperature. After a solid medium, 0.5 cm diameter hole was made using the base of a Pasteur pipette and then poured with *Morinda citrifolia* L. leaf extract 5, 10 and 15% and then incubated at 37°C for 24 h. Antibacterial power of each treatment is shown by the diameter of the clear zone around the hole. Measurement of the strength of antibiotics against bacteria use the method of Davis and Stout with the following conditions: very strong (inhibition zone 20 mm or more), strong (inhibition zone 10-20 mm), medium (5-10 mm inhibition zone) and weak (inhibition zone<5 mm) (Davis and Stout, 1971).

Process of inhibition zone of *Salmonella typhimurium*: Figure 2 showed that a diagram process of Inhibition zone of *Salmonella typhimurium* by sterilizing, incubating and inoculating to get the diameter of the clear zone of *Salmonella typhimurium*.

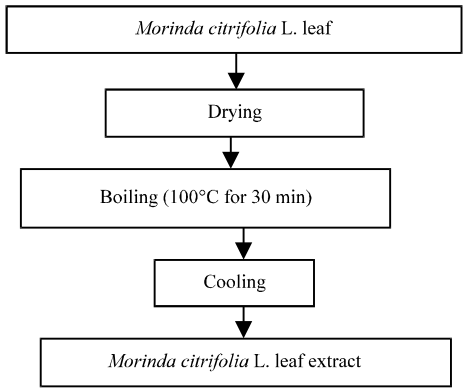

Fig. 1: Diagram process of *Morinda citrifolia* L. leaf extract

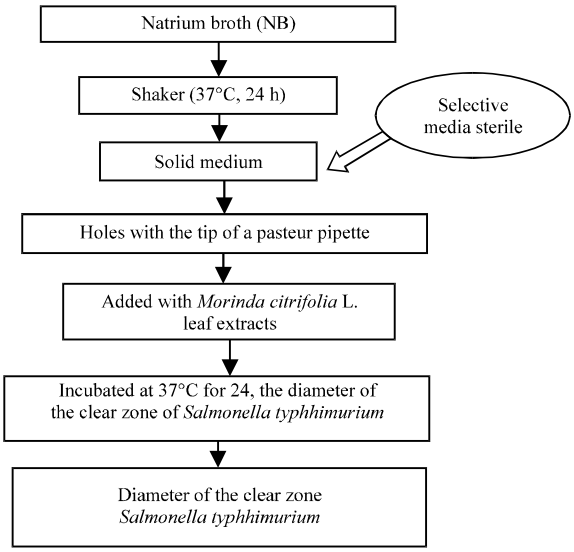

Fig. 2: Diagram process of inhibition zone of *Salmonella typhimurium*

Experimental design: The experimental design used in this research was completely randomized design with four treatments and three replications, the treatments were *Morinda citrifolia* L. extract composition i.e: R1 (quail drink was given vitamin vita chicks), R2 (quail drink was given 5% of *Morinda citrifolia* L. leaf extract), R3 (quail drink was given 10% *Morinda citrifolia* L. leaf extract) and R4 (quail drink was given 15% *Morinda citrifolia* L. leaf extract). The data was analysed using Analysis of Variance. The differences among treatments were examined with Duncan test (Steel and Torrie, 1993).

The parameters measured were mortality of DOQ and feed intake, feed conversion, hen day, egg production and hatchability of layer quail.

Mortality of day old quail: Mortality (%) is computed by number of day old quail that died divided by the total number of DOQ multiplied by 100%:

$$\text{Mortality (\%)} = \frac{\text{DOQ dead} \times 100\%}{\text{DOQ total}}$$

Egg production: Egg production (kg) is the total weight of eggs produced during the research.. eggs were weighed every day from 7 weeks-15 weeks old of quail.

Hen day (%): Hen day (%) is number of egg during research divided by number of quail during the research multiplied by 100%:

$$\text{Hen day (\%)} = \frac{\text{No. of eggs during research}}{\text{No. of quail} \times \text{number of days during research}} \times 100\%$$

Hatchability (%): Hatchability (%) is the number of eggs that can be hatched compared with the total number of eggs produced by laying quail during the study multiplied by 100%:

$$\text{Hatchability (\%)} = \frac{\text{No. of egg hatching}}{\text{Total No. of egg}} \times 100\%$$

RESULT AND DISCUSSION

The result showed that *Morinda citrifolia* L. extract had inhibition zone of *Salmonella typhimurium* (Fig. 3, 4). *Morinda citrifolia* L. extract with a concentration of 10% can inhibit the growth of *Salmonella typhimurium* bacteria with inhibition zones of 3 mm, while concentration of 15% had a inhibition zones 6 mm, meanwhile, concentration of 5% could not inhibit the growth of *Salmonella typhimurium* (Fig. 4).

Inhibition zone *Morinda citrifolia* L. extract (3-6 mm) against *Salmonella typhimurium* is smaller than the inhibition zone *Mimusops elengi* L. leaf extract (12.16 mm) (Noor, 2006). Strength of antibiotics against bacteria using Davis and Stout (1971) states that the 5-10 mm zone inhibition has medium strength. So the use of *Morinda citrifolia* L. leaf extract against *Salmonella typhimurium* has medium strength.

Flavonoids contained in the *Morinda citrifolia* L. leaf extracts act directly as an antibiotic to interfere with the function of microorganisms such as bacteria or viruses (Cushnie and Lamb, 2005). This causes the concentration of *Morinda citrifolia* L. leaf extract 10 and 15% had the inhibition of *Salmonella typhimurium*. Activity biological flavonoids compound against the bacteria *Salmonella typhimurium* do with damaging the wall of the bacterium *Salmonella typhimurium* are composed of lipids and amino acids react with the alcohol

Fig. 3(a-c): Media sterile before Inhibition zone of *Morinda citrifolia* against *Salmonella typhimurium*

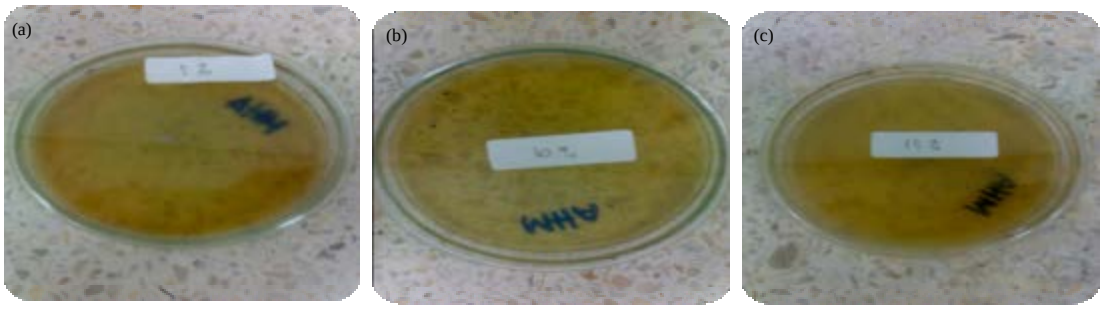

Fig. 4(a-c): Media sterile after inhibition zone of *Morinda citrifolia* against *Salmonella typhimurium*

Table 1: Phytochemical results *Morinda citrifolia* L. Leaf extract

Chemical compounds	Qualitative results
Alkaloids	+++
Saponin	++++
Tannins	-
Phenolics	+
Flavonoid	++
Triterfenoid	++++
Steroid	-
Glycosides	++++

-: Negative, +: Weak positive, ++: Positive, +++: Strong positive, ++++: Very strong positive

Table 2: Productivity of layer quail for 15 weeks

	Treatment of extract			
Variable	R1	R2	R3	R4
Mortalities	4.00±1.00^a	1.00±0^b	1.33±0.58^b	0.67±0.58^b
Egg production (kg/head)	1.24	1.28	1.44	1.54
Hen day (%)	36.02	40.18	42.69	46.48
Hatchability (%)	70	100	100	100

R1: Quail drink was given vitamin vita chicks, R2: Quail drink was given 5% of *Morinda citrifolia* L. leaf extract, R3: Quail drink was given 10% *Morinda citrifolia* L. leaf extract and R4: Quail drink was given 15% *Morinda citrifolia* L. leaf extract

groups on the flavonoid compounds that will break down the cell walls and the compound can enter the bacterial cell nucleus. Furthermore, the compounds also bacterium cell nucleus will be in contact with the DNA in the cell nucleus bacteria *Salmonella typhimurium* and by the difference between the polar lipid constituent DNA with alcohol groups on the flavonoid compounds would be the reaction that would destroy the lipid structure of DNA that bacteria *Salmonella typhimurium* bacterial cell nucleus will also be analyzed and *Salmonella typhimurium* bacteria will also undergo lysis and death (Gunawan, 2009).

Qualitative test showed that *Morinda citrifolia* L. content alkaloids, saponin, phenolic, flavonoids, triterfenoid dan glycosides (Table 1). Bioactive compounds flavonoids and alkaloids in the leaves of *Morinda citrifolia* L. extract are usable alternative antibiotics. Bioactive compounds can reduce the population of *Salmonella typhimurium* in quail and can disrupt the digestive tract. This is demonstrated by the inhibition zone owned by *Morinda citrifolia* L. leaf extract against *Salmonella typhimurium*.

Morinda citrifolia L. leaf contains anthraquinone, amino acids, glycosides, phenolic compounds and acid ursulat. The content of alkaloids, phenolic, glycosides and anthraquinone is an active substance that is antimicrobial, antibacterial and anti-inflammatory.

The results of the analysis indicated that *Morinda citrifolia* L. leaf of quail drink had significant effect ($p<0.05$) on mortality of Day Old Quail (DOQ). The average mortality of Day Old Quail (DOQ) given extract *Morinda citrifolia* L. leaf was R1 (4.00%), R2 (1.00%), R3 (1.33%), R4 (0.67%). The average mortality of Day Old Quail (DOQ) was iven 15% extract *Morinda citrifolia* L. leaf (R4) was lowest than control treatment (R1).

This showed that *Morinda citrifolia* L. leaf extract can reduce the percentage of quail mortality in starter period. Bioactive compounds from alkaloids and flavonoids contained in the *Morinda citrifolia* L. leaf extracts that act as antioxidants and antibacterial is expected to lower the percentage of mortality.

The results of the analysis indicated that *Morinda citrifolia* L. leaf of quail drink had no significant effect ($p>0.05$) on egg production. Egg production is a trait passed down by the parent (Ensminger, 1992). Besides, the production of eggs is influenced by light and the protein content of the feed (Tarasewicz *et al*., 2006), further Romanoff And Romanoff (1963) says that in order to spawn the birds must have properties (ability) laying a good, free emergency physiological disorder da mendaptkan feed and a good environment.

Quail production peak occurred at the age of five months with an average percentage of spawn 76 times. In this study, the egg production is low since the quail are still in a phase of adaptation to spawn. Hen day egg production is a unit. Hen day egg production is a percentage of the production in a certain period based on the number of quail that exist at any time in the relevant period (North and Bell, 1990). Hen day in this study was calculated at the end of the study. The results of the analysis indicated that *Morinda citrifolia* L. leaf of quail drink had no significant effect ($p>0.05$) on hen day. Table 2 showed that the treatment quail drink was given 15% *Morinda citrifolia* L. leaf extract (R4) was 46.48% higher than the average hen day in control treatment (R1).

Hen day (%) in this research is different from the results of research conducted by Subekti *et al*. (2006) 61.03-63.71% which maintained for 25 weeks. The difference is due to the different treatment given and the length of culture.

The results of the analysis indicated that *Morinda citrifolia* L. leaf of quail drink had no significant effect ($p>0.05$) on hatchability. The quail given the *Morinda citrifolia* L. leaf of quail drink has 100% hatchability while control had 70% hatchability (Table 2). This showed that *Morinda citrifolia* L. leaf extract the improve hatchability of quail.

CONCLUSION

It was concluded that the Morinda *citrifolia* L. leaf extract 15% can reduce mortality of Day Old Quail (DOQ) and can increase its egg production, hen day and hatchability.

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