Providing Ultraviolet Recovery on *Salmonella* sp Bacteria and Haematological Examination in Infected Salmonellosis

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Abstract. Ducks are one of the most popular livestock in Indonesian society. Maintenance of ducks is mostly done extensively, namely grazing in paddy fields or ditches. This method can cause ducks to be susceptible to disease and can spread quickly because ducks are grazed to move places. One of the diseases that attacks ducks is Salmonellosis caused by the *Salmonella* sp. bacteria. One way to kill *Salmonella* sp. bacteria is by using ultraviolet (UV) light. The purpose of this study was to find the right UV irradiation method to weaken the *Salmonella* sp. bacteria which then weakened bacteria would be made into Salmonellosis vaccine. In this study, *Salmonella* sp. bacteria were isolated directly from ducks’s cloaca by swabs. *Salmonella* sp. were given UV irradiation treatment with different times of 5 minutes, 10 minutes, 15 minutes, 20 minutes, 25 minutes, 30 minutes and 35 minutes, with the same distance of 10 cm. *Salmonella* sp which is irradiated with UV light for 25 minutes is the best, supported by the duck’s immune system increases after 2 weeks of *Salmonella* infection. This result gives opportunities to develop vaccine for Salmonelosis disease in the duck in the future.

1. Introduction
Ducks are one of the livestock known and very popular in Indonesian society. In terms of population and economy, duck animals rank second after chicken. Maintenance of ducks by breeders is mostly done extensively. This method can cause ducks to become susceptible to disease and can spread widely because ducks are grazed in place.

One of the diseases that attacks ducks is Salmonellosis caused by the *S. typhimurium* bacteria. These bacteria can suppress the immune system of poultry (immune suppression) and cause the death of broilers [1]. Young ducklings will be easily infected with bacteria and if this duck can live to adulthood it will act as a carrier or carrier and can produce eggs infected with *S.typhimurium* bacteria. These infected eggs can transmit bacteria to humans if consumed in less mature conditions, besides that the bacteria can be transferred to newly hatched ducklings.

One way to kill bacteria is by using ultraviolet (UV) light. UV light can be sourced from sunlight or from lamps that have low wavelengths and contain mercury at low pressure [2]. The use of UV light is the best choice to turn off bacteria without causing adverse effects on the environment. UV rays are effective in killing microorganisms, viruses and protozoa [3]. Radiation using ultraviolet light in *Salmonella* sp with a certain irradiation time can cause death by damage to the structure of DNA. Based on the above, it is necessary to do research about what is the right UV irradiation time to weaken the activity of *Salmonella* sp bacteria.

2. Material and Methods
This research was conducted in the Laboratory of Microbiology, Laboratory of Animal Physiology Department of Biological Science, Faculty of Mathematics and Natural Sciences, University of Udayana and Veterinary Laboratory Denpasar. This study was conducted from July 2018 to September 2018. The work procedure in this study was divided into 3 stages:

2.1. Isolation of Salmonella sp pathogens, sample test and sample treatment.

Isolation of pathogens Salmonella sp was isolated from duck cloaca taken randomly in the Mengwi Badung area. Duck cloaca is rubbed using a sterile cotton bud and then the cotton bud is inserted into a tube containing Selenit broth media. The samples were tested using selective media for Salmonella. Identification of bacteria using an identification reference book from Bergeys Manual Bacteria Determinative.

2.2. The sample treatment of Salmonella sp bacteria.

The sample treatment of Salmonella sp bacteria that had been obtained was grown on Salmonella Shigella Agar (SSA) media and incubated for 24 hours at 37 ° C. The bacterial culture was diluted using distilled water to obtain a population density of 1 x 10^8 cells / ml by standardizing it with the Mc Farland standard 1 x 108. The culture was poured as much as 1 ml into 24 sterile petri dishes. Then it was divided into control group (K) that is without UV irradiation and the treatment group with ultraviolet light exposure with 7 different time treatments namely irradiation 5 minutes (P1), 10 minutes (P2), 15 minutes (P3), 20 minutes (P4), 25 minutes (P5), 30 minutes (P6) and 35 minutes (P7) with the same distance of 10 cm. Then, 15 ml of SSA was poured, levelled and compacted and incubated at 37 ° C for 24 hours. The experiment was repeated 3 times. The next day, the colonies growing on petri dishes were calculated using a colony counter.

2.3. Treatment for administering Salmonella sp bacteria that have been irradiated.

Salmonella sp bacteria obtained from the results of the irradiation and incubation above, then taken and diluted with 2 ml of distilled water. Given to the ducklings aged 30 days orally as much as 2 ml. Each treatment consists of 3 ducks. Ducklings are kept in cages and samples are taken every week for cloaca swabs and blood samples for haematological examination. The taking of cloaca swabs and blood samples was carried out until the third week aimed at seeing the growth of the bacteria in the body of the ducks infected with bacteria and seeing the relationship between infection and haematological conditions.

3. Results and Discussion

3.1. Isolation of Salmonella sp pathogens and sample testing (Figure 1).

The types of leukocytes that play a role in the protection of invasion of foreign substances are lymphocytes. The number of lymphocytes also increased from the first week to the third week. This explains that lymphocytes also play a role in the immune defense because they are able to produce antibodies when the body is infected with microorganisms (Table 1).

The results of isolation of Salmonella bacteria in ducks showed positive containing Salmonella sp bacteria with black color shown on Salmonella Shigella Agar (SSA) media with small, round and smooth colony morphology on the surface of the colony. The results are in accordance with the research found by [4]. Black Salmonella sp colonies on SSA media isolated in chicken spleen at Sibreh Aceh Besar farm. This shows that the presence of these bacteria is indeed a carrier or carrier and can produce eggs infected with Salmonella sp bacteria.
Figure 1. Results of isolation of cloaca duck swabs

Caption: Samples of 1, 2, 3, 4 showed positive for *Salmonella* sp bacteria (black colony color on SSA media). Sample 5 shows negative results (clear SSA media)

Figure 2. Growth of *Salmonella* sp bacteria that have been irradiated

Description: K, P1, P2, P3 and P4 positively contain *Salmonella* sp bacteria. P5, P6 and P7 negatively contain *Salmonella* sp

3.2. Treatment of UV irradiation.

The treatment of UV irradiation on *Salmonella* sp bacteria showed the results in Figure 2. Some *Salmonella* species found to infect birds such as *Salmonella enteric* PT6 serovar Enteritidis isolated from chicks hatching [5]. Furthermore, a total of 34 *Salmonella* bacteria including *Salmonella enterica* subsp. Entericaserovar Senftenberg (*S. senftenberg*) was successfully isolated in chicken food and tolerant to dry [6].

The results of irradiation using Ultra Violet light showed a time of up to 20 minutes and the control of bacteria was still alive while the irradiation of 25 minutes, 30 and 35 minutes of bacteria was dead. With the results of the 20-minute irradiation, it is expected that the *Salmonella* sp bacteria can function as a vaccine. The results of Ref. [7] research showed that the manufacture of vaccines with ultraviolet radiation can produce the best antisera titre in 320 nm irradiation for 8 x irradiation every 5 minutes irradiation.
UV radiation can affect microorganisms to a significant degree. UV radiation can be used to control the microbes needed. UV radiation causes a high decrease in the number of bacteria with increased exposure to time [8].

![Figure 3](image.png)

**Figure 3.** Total bacteria in ducks infected with *Salmonella* sp. that has been weakened.

| No | Code | WBC (x10³/μL) | Lymphocyte (%) | Neutrophil (%) |
|----|------|---------------|----------------|---------------|
|    |      | Week 2        | Week 3         | Week 2        | Week 3         |
| 1  | K    | 153           | 171.2          | 58            | 68             | 27            | 20            |
| 2  | P1   | 164.8         | 181.2          | 53            | 62             | 31            | 31            |
| 3  | P2   | 153.8         | 154.3          | 60            | 75             | 21            | 17            |
| 4  | P3   | 170           | 156.6          | 55            | 80             | 28            | 13            |
| 5  | P4   | 165           | 176.6          | 56            | 61             | 16            | 22            |
| 6  | P5   | 159.2         | 163.3          | 62            | 58             | 18            | 18            |
| 7  | P6   | 150.1         | 183.4          | 61            | 66             | 16            | 6             |
| 8  | P7   | 161.4         | 178.8          | 64            | 64             | 19            | 20            |

Cultures that have been UV irradiated are then given to ducklings and the total bacteria and hematologic conditions are calculated every week. The results of total bacterial calculations showed that the total bacteria from the first week to the third week showed that the results were decreasing. This is also consistent with research from [8] which states that UV radiation can affect microorganisms to a significant degree. Thus, UV radiation can be used to control the microbes needed. UV radiation causes a high decrease in the number of viable bacteria with increased exposure to radiation. UV light will not penetrate very far into the bacteria so the exposure must last long enough for the bacteria to change, and the DNA moves closer to the surface, for UV light to destroy DNA.
Hematologic conditions indicate that packed cell volume (hematocrit) increases from the first week to the third week. The number of white blood cells (leukocytes) in most treatments shows an increase in number from the first week to the third week. This increase in white blood cells is the body's way of defending itself from bacterial infections that are inserted into the body. White blood cells play a role in the defense of the immune system from invasion of disease-causing microorganisms.

The number of neutrophils from the first week to the third week showed that there was an increase in P4 (20'), and P7 (35'), some experienced a decrease in K, P2 (10'), P3 (15'), and P6 (30'), while P1 (5') and P5 (25') the results remain. The difference in results in each treatment depends on the individual ducklings in their body defenses. Neutrophils are a phagocytic specialist on bacteria and are important in the response to inflammation and debris clearance.

Haematological conditions affected by Salmonella bacteria also occur in research conducted by Adam., C.H (2017). Haematological changes in the infected part of Salmonella gallinarum showed a significant (P <0.05) decrease in cell volume (PCV), haemoglobin concentration and red blood cell (RBC) concentrations in the acute phase of typhoid poultry which is anemia. This is suspected because modification of erythrocytes is directly related to the cytopathic effect of Salmonella gallinarum lipopolysaccharide / outer membrane of protein or indirectly by the induction of antibodies or both, to the number of bacteria in the tissue.

The results of research conducted by Ref. [9] showed that antibody titre from local isolates irradiated with UV light 10x 5 minutes were 88.76 ± 33.06 IU / mL at week 4 with the lowest antibody titre value of 11.15 ± 9.18 IU / mL in negative control.

Splenocytes from mice immunized with S. typhlieve mutants showed a significant proliferative increase and antibody titres increased at week 4 to week 8 and were not found to increase after week 8 [10]. Research by [11] resulted in an increase in the immune response achieved at 6 weeks post vaccination with recombinant SalmonellasppYA3494engineered chitosan based oral salmonella nano vaccine targeting intestinal PPs immune cells of birds, and demonstrated its ability to induce antigen specific mucosal antibody and T cell responses. Thus, our candidate oral salmonella vaccine has the potential to mitigate Salmonellosis in poultry.

4. Conclusion
Salmonella sp which is irradiated with UV light for 25 minutes is the best, supported by the duck’s immune system increases after 2 weeks of Salmonella infection. This result gives opportunities to develop vaccine for Salmonellosis disease for the duck in the future.

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Acknowledgement

On this occasion, the authors would like to thank profusely to the Institute for Research and the Community Services University of Udayana which facilitates to obtain the Competitive Research Grant funds from the Ministry of Education and Culture of the Republic of Indonesia based on the Letter of Assignment No; 3769/UN14.2.8.II/LT/2018.