

Original Research Article

Effects of Red Onion Extract (*Allium ascalonicum* L.) in Pulmonary Histopathological Lesions of Layer Chickens: A Possible Herbal Medicine Solution to Overcome Avian Influenza and Newcastle Disease Viruses

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Abstract: Layer chicken farms in Indonesia have still obstacles to increase their productivity, especially due to poultry diseases. The red onion (*Allium ascalonicum* L.) is a plant that contains flavonoid and saponin. Flavonoid is useful as anti-inflammatory and antioxidant, while saponin is useful as antivirus, anti-inflammatory, antifungal, and immunomodulator. The present study aimed to know and determine the effect of onion extracts to pulmonary histopathological lesions in layer chickens which raised in battery cage. The eighteen layer chickens at the age of 17 days were divided into three groups of six each, namely without administration of red onion extract (Group I), with administration red onion extract through drinking water (Group II) and with administration red onion extract peroral (Group III). Every ten days, six chickens were necropsied and then the lungs were processed for histopathological preparations and stained with routine hematoxylin and eosin. The results showed that the lungs of the Group I had severe congestion and diffuse hemorrhages. In Group II, lungs had moderate congestion and hemorrhages. In group III, lungs had mild congestion and hemorrhages. It is concluded, that red onion extract apparently has reduced the lungs lesions in layer chickens.

Keywords: Red onion extract, layer chickens, lungs, histopathological lesions, hematoxylin and eosin.

Novelty

The results of the present study are unique and have not been previously reported. It is likely that novel strategies using red onion extracts as a medicinal herb is advisable for overcoming viral diseases, especially avian influenza and Newcastle disease in poultry.

INTRODUCTION

Both broiler and layer chicken farms has good prospect and shown growth rapidly in Indonesia. The egg and flesh of the chicken product is nutritional food which its price achievable by people relatively. Avian influenza (AIV) and Newcastle disease virus (NDV) has been endemic in Indonesia (Sedyaningsih *et al.*, 2006; Wasito R *et al.*, 2018). In the field, the primary target organ of AIV is the lungs. The lungs had petechial and linear hemorrhages (Yunita N *et al.*, 2017; Wasito R *et al.*, 2018). It was also reported that

the diseases causing disturbances of lung function are avian influenza (AI) and Newcastle disease (ND) (Pattison M, 2008; Wasito R *et al.*, 2018). The AIV and ND resulted in edema, congestion, necrosis, hemorrhage and mononuclear cell infiltration in lung parenchyma (Mathias A, 2010). Lung is respiration organ for oxygen transport from environment to blood circulation and release dioxide carbon from blood circulation to environment. Impaired lung function resulting tissues lack of oxygen so the body's metabolism process inhibited (Cunningham JG, 2002).

Low pathogenicity *avian influenza* causes respiratory distress, feathers become matted and dull, weight loss and decrease of egg production. Morbidity and mortality rate of avian influenza are 100% and \pm 5-20% (Mutinelli F *et al.*, 2003). ND causes considerable impact on the poultry industry worldwide, with

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significant morbidity and mortality and high economic losses (OIE, 2009).

Most of layer chicken raised in battery cage. Chickens in battery cages are under high stress, which inhibit their immune response. Stress caused by restriction of chicken movement and prevention of natural behaviour such as build private nest, take dustbaths and explore their surrounding. Chicken in high-density flocks suffer respiratory disease and eye irritation from exposure to fecal dust, osteoporosis from lack of exercise and foot disorders from standing on cage wire for their entire lives. In Indonesia, red onion (*Allium ascalonicum* L.) is a primary food seasoning ingredient that beneficial to health. The red onion contains the active substance of flavonoid or quercetin (an antioxidant and anti-inflammatory) and saponins (antivirus, anti-inflammatory, antifungal and immunomodulatory) (Corea G *et al.*, 2005; Shaik YB *et al.*, 2006; Dalbagh-Bazarbachi H *et al.*, 2014). Therefore, this study mainly aims to know and determine the effect of red onion extract against layer chicken's lung histopathological lesions were raised in battery cage.

MATERIAL AND METHODS

Materials

In the present study, 18 hens Lohmann brown at 17 days of age were used. The chickens were placed in a cage-type battery made of wire with length, width and height are 120x40x37 cm. Cage is divided into three partitions with the length, width and height of each partition are 40x40x37 cm. Each partition occupied by 6 chickens. Drink water were given 250 ml /day/partition and feed provided in the form of feed crumble (small grains) RN-42 as much as 22 grams/chicken/day on days 1-10, 27 grams/chicken/day on days 11-20 and 32 grams/chicken/day on days 21-30.

Preparation Of Red Onion Extract

Two kilograms of red onions processed by maceration method becomes semi-solid form of the extract in the Pharmacy Laboratory, Faculty of Pharmacy, Gadjah Mada University. Two kilograms of onion peeled and washed and then cut into pieces and dried in an oven with temperature of 50° C so the weight of onion become \pm 0.45 kg. Red onion is dry blended to form a powder (simplicia) as much as \pm 0.44 kg. Furthermore red onion simplicia soaked in solvent ethanol 70% by volume \pm 2.25 liters for 24-48 hours so that the volume becomes \pm 2.82 liters. The result of this process is then filtered with a Buchner funnel and obtained \pm 2, 82 liters. The result evaporated with rotary evaporator at temperature of 55° C to obtain semi-solid form of extract as much as \pm 200 g.

The implementation stage

The present field study was carried out for four weeks. For the implementation of the study, 18 layer

chickens were randomly divided into three groups by the number of chicken in each group are six. The Control (Group I) is layer chicken that were not given the extract of red onion; Group II is a group of layer chicken given the red onion extract in 250 ml of water at a dose of 0.264 g extract of red onion semi solid/chicken/day in the first ten days, 0.324 g extract of red onion semi solid/chicken/day in the second ten days and 0.384 g extract of red onion semi solid/chicken/day in the last ten days; Group III is a group of layer chicken given red onion extract through feeding at a dose of 0.132 g extract red onion semi solid/chicken/day in the first ten days, 0.162 g extract of red onion semi solid/chicken/day in the second ten days and 0.192 g extract red onion semi solid/chicken/day in the last ten days. Frequency of giving red onion extract in drinking water is once a day and the frequency of giving red onion extract through feeding is twice a day.

During the treatment process, red onion extract given by using 1 ml syringe. Every ten days, two chickens in Groups I, II and III were necropsied, respectively. The lungs were collected, processed histopathologically and stained with hematoxylin-eosin at the Pathology Laboratory, Department of pathology, Faculty of Veterinary Medicine, Gadjah Mada University, Yogyakarta, Indonesia. In each treatment Group, the lungs histopathology were examined under light microscope and descriptively analyzed.

RESULT AND DISCUSSION

In the present study, histopathologic lesions of the lungs in chickens given red onion extract (*Allium ascalonicum* L.) were observed at all time points. In chickens, the lung is a primary organ for respiration and the damage of lung function can cause systemic disorders with high morbidity and mortality rates (Pattison M, 2008; Mathias A, 2010); Wasito R *et al.*, 2018). Results of the present study indicated that at 10 days, lungs of the chickens fed diets either no red onion extract (*Allium ascalonicum* L.) (Group I) or supplemented with red onion extract through the drinking water (Groups II) were severe and diffuse congestion and hemorrhages. Whereas, in chickens administered red onion extract peroral (Group III), the lungs had moderate congestion and hemorrhages. By day 20, the addition of red onion extract (*Allium ascalonicum* L.) to groups II and III caused moderate pulmonary congestion and hemorrhages. Severe and diffuse congestion and hemorrhages occurred in chickens in Group I at 20 days. At 30 days, chickens in Group I had severe and diffuse congestion and hemorrhages of the lungs (Figs 1-2), while chickens in Group II (Figs 3-4) had moderate pulmonary hemorrhages, and all chickens in Group III (Figs 5-6) had mild histopathologic lesions of the lungs at this time.

Hemorrhages is discharge of blood from the blood vessels (extravasation) due to ruptured blood

vessels. Hemorrhages and congestion that occurs in the lungs of Group I, Group II and Group III is a form of inflammatory response caused by infectious microorganisms (viruses, bacteria, fungi, protozoa) (Chen *et al.*, 2018). In the inflammation process, body released chemical compounds, such as prostaglandins, histamine, leukotrienes, bradykinin, platelet-activating factor (PAF) which resulted in dilation of blood vessels and increased blood flow, causing congestion, hemorrhages and edema on the organ infected (Vane J

and Botting R, 1987; Chaithra *et al.*, 2018). It was reported that infection of blood vessels endothelial cells and viremia are the main factors of pathogenesis during viral infection in poultry (Toffan *et al.*, 2008). In the case of chicken diseases in the field, AIV and NDV-infected chickens shows similar histopathologic lesions in the lungs (Wasito R *et al.*, 2018). The lungs are the primary target for AIV and NDV infections (Yunita N, 2017; Wasito R *et al.*, 2018).

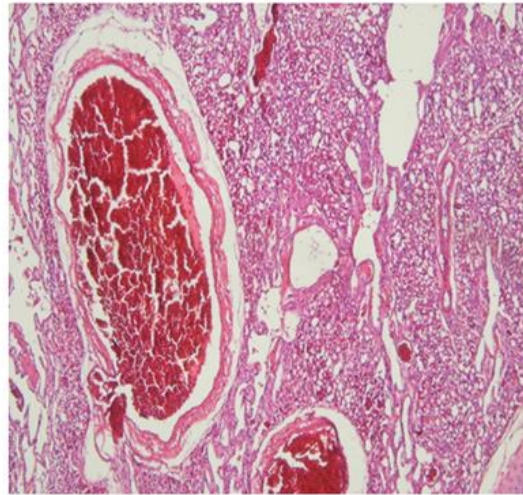


Fig.1. Histopathology lungs of the chickens fed diets no red onion extract (*Allium ascalonicum* L.) (Group I) for 30 days. Notice: severe and diffuse congestion and hemorrhages within lung parenchyme and blood vessels (Hematoxylin-eosin, 250x.).

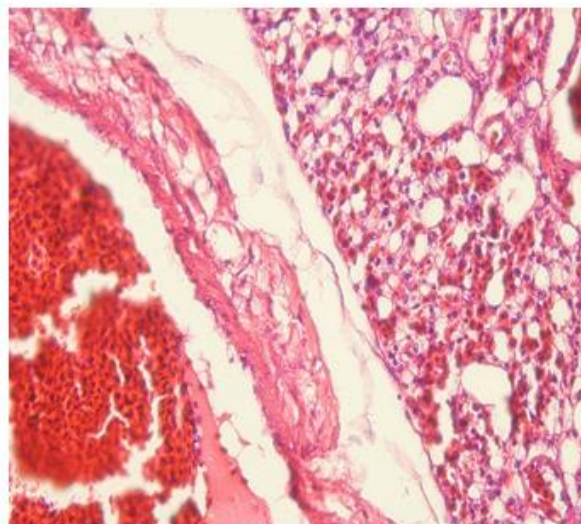


Fig.2. Histopathologic lungs of the chickens fed diets no red onion extract (*Allium ascalonicum* L.) (Group I) for 30 days. Notice: severe and diffuse congestion and hemorrhages within lung parenchyme and blood vessels (Hematoxylin-eosin, 500x.).

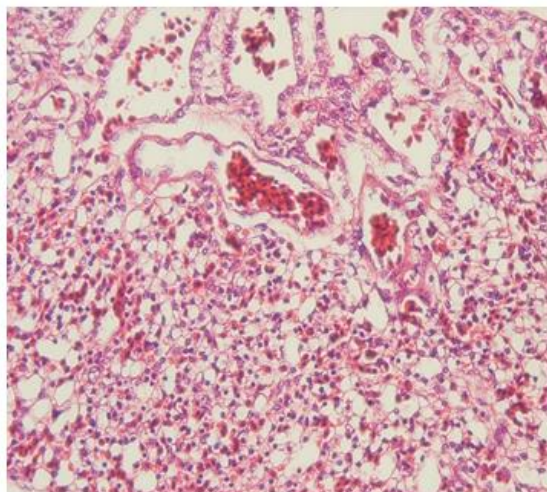


Fig. 3. Histopathologic lungs of the chickens supplemented with red onion extract (*Allium ascalonicum* L.) through the drinking water (Group II) for 30 days. Notice: moderate congestion and hemorrhages within lung parenchyme and blood vessels (Hematoxylin-eosin, 250x).

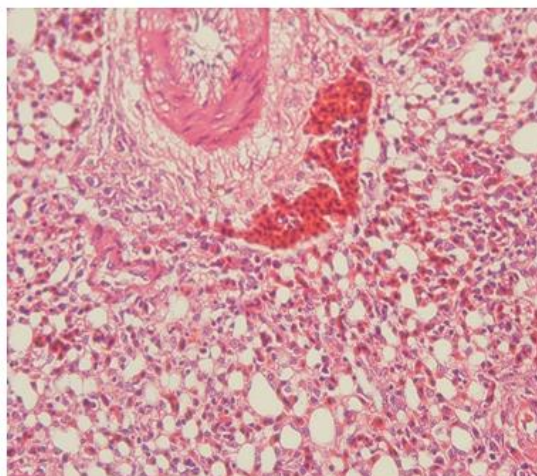


Fig.4. Histopathologic lungs of the chickens supplemented with red onion extract (*Allium ascalonicum* L.) Through the drinking water (Group II) for 30 days. Notice: moderate congestion and hemorrhages within lung parenchyme and blood vessels (Hematoxylin-eosin, 250x).

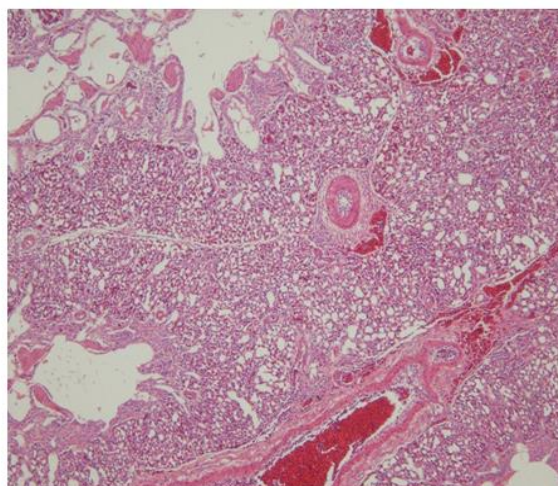


Fig.5. Histopathologic lungs of the chickens administered red onion extract (*Allium ascalonicum* L.) peroral (Group III) for 30 days. Notice: mild congestion and hemorrhages within lung parenchyme and blood vessels (Hematoxylin-eosin, 250x).

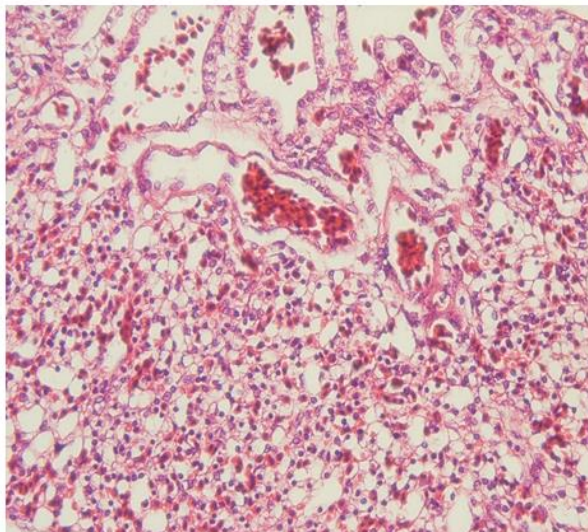


Fig.6. Histopathologic lungs of the chickens administered red onion extract (*Allium ascalonicum* L.) peroral (Group III) for 30 days. Notice: mild congestion and hemorrhages within lung parenchyma and blood vessels (Hematoxylin-eosin, 250x.).

In the lungs of Groups II and III were found hemorrhages and congestion lighter than that of Group I. The red onion containing useful compounds as an antioxidant, anti-inflammatory, antimicrobial and immunomodulatory so as to reduce or prevent lesions on the tissue, especially congestion and hemorrhages in the lungs (Kumar KPS *et al*, 2010; D'Andra G 2015). Kumar KPS *et al* (2010) proved that congestion and hemorrhages may also occur in the bronchi and lung. The antioxidant effect of red onions due to red onions contained chemical compounds, including flavonoids, saponins and S-alk(en)yl-L-cysteine (ACSO) which are capable of cleaning free radicals in the body (Vu QH *et al*, 2012). Antimicrobial activities, especially related to flavonoids, ascalin, tannin, essential oils, coumarin and the isoflavone genistein contained in the onions. Such compounds can block the development of the mycelium fungi, interruption of cell wall synthesis process, disruption cytoplasm membrane and inhibition of microorganisms DNA synthesis, so that the cells lose viability and die. In an *in vivo* study, the anti-inflammatory activity of saponin showed that saponin inhibited inflammatory mediators and affected the stability of the membrane so that the permeability of blood vessels is reduced (Motlagh HRM *et al*, 2011). Flavonoids especially quercetin is able to increase the production of interferon gamma (IFN- γ) and the proliferation of lymphocytes that have the potential to improve the body's immune system (Farhadi L, 2014). Molecule IFN- γ is a lymphokine produced by T-lymphocytes due to infection by viruses, bacteria, protozoa, rickettsiae and fungi. IFN- γ molecules work to activated and improved macrophage phagocytic function, and inhibited protein synthesis and reproduction of the virus (Chen L *et al*, 2018; Chaithra VH *et al*, 2018).

CONCLUSION

The red onion extract developed in the present study has potential value in further research study as well as in possibly commercialized herbal medicine anti viral infection, especially AIV and/or NDV in order to be able to reduce and/or eradicate morbidity and mortality rates, and also to improve the productivity in poultry.

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