Research Article

Immunostimulant Effect of Red Ginger (*Zingiber officinale* Roscoe) in Broiler Vaccinated and Challenged with Newcastle Disease Virus

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Abstract | Ethanolic extract and essential oil of red ginger have been reported to harbor an antiviral effect on Newcastle disease virus (NDV) infection *in ovo*. This study was aimed to evaluate the activity of red ginger powder, ethanolic extract, and essential oil as immunostimulant agent during NDV challenge *in vivo*. Chickens were divided into seven groups of different treatment regimens. Group I - IV were vaccinated NDV while group V-VII were unvaccinated. Each group was treated with either red ginger powder (group I and V), ethanolic extract (group II and VI), essential oil (group III and VII). Group IV served as the untreated control. The analyses were done by measuring the NDV antibody titers using hemagglutination inhibition test and histological studies to observe the toxic effect of red ginger to the chicken livers and kidneys. Antibody titers in group I, II, III and IV were determined as HI (log)2 average and were recorded 3.81, 2.91, 3.13 and 0.95, while the geometric mean titers (GMT) were 13.9, 8.6, 7.5 and 1.9 respectively. The unvaccinated groups (V, VI, VII) exhibited 0 and 1 as HI (log)2 and GMT respectively. There was a significant difference between the measured antibody titer of group I and other groups (p<0.05). No histopathological change was found in the livers and kidney suggesting there was no toxic effect of red ginger administration to the chickens. Red ginger powder could provide the highest immunostimulant effect on NDV vaccinated chicken with no toxic effect to the chickens.

Keywords | Antibody response, Immunostimulant, Newcastle disease, Red ginger, Broiler

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INTRODUCTION

Red ginger (*Zingiber officinale*) is an herbal medicine source with a broad range of therapeutic effects. The chemical constituents of red ginger root include 6-gingerol limonene, 1,8-cineole, 6-shogaol, 8-shogaol, 6-gingerdione, arginine, acetic acid, ascorbic acid, α-linoleic acid, β-sitosterol, caprylic acid, capsaicin acid, starch, fat, protein, curcumin, and a little amount of resin fiber (Zhao and Yu, 2006).

Red ginger extract has been identified to have pharmacological effects such as anti-inflammatory, analgesic, gastrointestinal regulating agent, antioxidant, and antimicrobial properties (Qorbanpour et al., 2018). The addition of ginger aqueous extract was reported to increase performance and general health status of broiler chickens (Oleforuh et al., 2015). Water extract of red ginger combined with zinc were found to enhance testicular function including steroidogenesis and spermatogenesis in male rats (Sutyarso et al., 2016).

Furthermore, ginger extract may also increase activity of

natural killer (NK) cells during clearance of virus-infected cells (Tejasari and Zakaria, 2010). Gingerol from ginger extract was reported to inhibit replication of human immunodeficiency virus type 1 in human T cell lymphocyte cell culture (MT-4 cells) with inhibitory concentration of 1.95 – 2.50 μM (Lee et al., 2008).

Newcastle disease (ND) is an acute and contagious poultry disease, especially in chickens, which affects the gastrointestinal, reproductive, neurological, and respiratory systems (Miller and Koch, 2013). There are 5 recognized forms of ND, ranging from most severe to least, viscerotropic velogenic ND (VVND), neurotropic velogenic ND (VNND), mesogenic ND (MND), lentogenic ND (LND) and asymptomatic enteric ND (AEND). neurotropic velogenic ND and mesogenic ND is characterized by mostly neurological and respiratory symptoms without gastrointestinal involvement with manifestations such as opisthotonos, tremors, head twisting, and paralysis with the difference being MND having a lower mortality rate and is associated more with drop in egg production and mild to moderate respiratory illness, while LND and AEND rarely shows clinical signs (Bello et al., 2018).

ND is considered one of the most economically important viral diseases in the poultry industry as it can cause huge economic losses to the commercial poultry farmers globally (Ashraf and Shah, 2014). In the last major outbreak of ND in the USA, around 4 million birds died causing approximately a loss of 162 million US dollars (Rehan et al, 2019). In a study in Bangladesh by Khatun et al (2018), it is estimated that the country incurred economic loss BDT $2.44x10^{10}$ (US$ 288.49 million) per annum. The disease is caused by Newcastle Disease Virus (NDV), a virus member of the family *Paramyxoviridae*, genus *Paramyxovirus*. The virion is pleomorphic in shape with a diameter about 100 - 150 nm. The nucleocapsid is a symmetrical helix surrounded by an envelope derived from the host cell membrane (Miller and Koch, 2013).

NDV has a high genetic and antigenic diversity (Ogali et al., 2020). Recently, an increasing number of NDV genotype variation has been observed, perhaps due to the use of vaccination (Dimitrov et al., 2017). Nevertheless, it would be beneficial to seek for other alternatives to prevent NDV infection in poultry.

In this study, the beneficial effect of red ginger in promoting immune response was assessed during NDV infection in chickens. The toxic effect of red ginger was also observed employing a histopathological examination of treated chicken kidneys and livers.

MATERIALS AND METHODS

Ethical approval

The procedure involving animals were approved by the Ethical Clearance Committee of Faculty of Veterinary Medicine, Universitas Gadjah Mada, Indonesia.

Materials

A total of 154-day-old broiler chicks strain Chubb were used in this study. Red ginger powder, ethanolic extract, and essential oil were made at the Faculty of Pharmacy, University of Gadjah Mada, Indonesia. ND vaccine was obtained from PT Medion (Bandung, Indonesia). The amount of NDV used to infect the chickens was determined by calculation of lethal dose 50 (LD_{50}) done at the Department of Microbiology, Faculty of Veterinary Medicine, University of Gadjah Mada, Indonesia.

Treatment of chickens

Chickens were divided into seven groups (n = 22 per group). As shown in Table 1, group I and V were given 1% red ginger powder in their feed, group II and VI were treated with 1% red ginger ethanolic extract in their drinking water, group III and VII were treated with red ginger essential oil in their drinking water, and group IV was used as the control group therefore it was not treated with any extracts. All the chickens in group I-IV were vaccinated against NDV, while chickens in group V-VII were not vaccinated. ND vaccination was done when the chickens were seven days old using one dose of vaccine via eye drop. Red gingers were given when the chickens were 13-35 days old. Challenge test was done by infecting the chickens (21 days old) with NDV. The LD_{50} amount of NDV determined before using an intratracheal infection at a dose of 0.1 ml virus suspension. Successful NDV infection were determined from the observation of ND clinical signs. The chickens were kept until they reached 35 days old, and the serum was collected for antibody titration. Subsequently, the chickens were euthanized and submitted for necropsy.

Histological Examination

The histological examination of kidney and liver of chickens were performed by paraffin method and visualized by Hematoxylin-eosin staining at the Department of Pathology, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Indonesia.

Hemagglutination inhibition (HI) test

This test was done to observe the humoral immune response to NDV infection. Chicken sera collected from all groups were used in the test. The HI test was conducted in accordance with procedures described by OIE (2008). Briefly, chicken sera were inactivated by heating at 56°C for 30 minutes (min). The HI test performed in microplate

Table 1: Details of treatment plan

Group	Treatment (chicken age)			
	ND Vaccination (7 days old)	Red ginger administration (13-35 days old)	Challenge test (21 days old)	Necropsy, pathological changes examination (35 days old)
I	+	1% Red ginger powder in feed	+	+
II	+	1% Red ginger ethanolic extract in drinking water	+	+
III	+	1% Red ginger essential oil in drinking water	+	+
IV	+	Not treated	+	+
V	-	1% Red ginger powder in feed	+	+
VI	-	1% Red ginger ethanolic extract in drinking water	+	+
VII	-	1% Red ginger essential oil in drinking water	+	+

Note: ND: Newcastle disease

(+): performed, (-): not performed

Table 2: Antibody titers against Newcastle disease virus determined using HI test

Group	Treatment	Average of HI titer (log2)	Geometric mean titer (GMT)
I	Vaccine + 1% red ginger powder	3.81	13.9
II	Vaccine + 1% red ginger ethanolic extract	3.13	8.6
III	Vaccine + 1% red ginger essential oil	2.91	7.5
IV	Vaccine only	0.95	1.9
V	1% red ginger powder, no vaccine	0	1
VI	1% red ginger ethanolic extract, no vaccine	0	1
VII	1% red ginger essential oil, no vaccine	0	1

Table 3: Challenge test of chickens treated with red ginger

Group	Treatment	Chicken alive	%
I	Vaccine + 1% red ginger powder	7/7	100
II	Vaccine + 1% red ginger ethanolic extract	7/7	100
III	Vaccine + 1% red ginger essential oil	7/7	100
IV	Vaccine only	7/7	100
V	1% red ginger powder, no vaccine	0/7	0
VI	1% red ginger ethanolic extract, no vaccine	2/7	28
VII	1% red ginger essential oil, no vaccine	0/7	0

96 well U bottom. Thereafter, 25 µl serum two-fold diluted until the 10th wells. The 1st well was used as serum control, the 11th well was used as virus control, and the 12th well was used as red blood cells control. The 2nd to 11th wells were added with 25 µl NDV antigen (4 HA unit) and then incubated at room temperature (RT) for 30 min followed by addition of 0.5% chicken erythrocytes into each well. The antibody titers were read following a 40 min of incubation at RT or after the red blood cells controls have settled at the bottom of the microplates. HI titers were determined as the reverse of highest serum dilution which produces complete hemagglutination inhibition. The titers were then calculated as HI (log)2 and geometric mean titer (GMT) according to Brugh table by Villegas (2008).

Statistical analysis

The data obtained in this study were analyzed statistically using Analysis of variance (ANOVA). The limit of significance was considered as $p<0.05$.

RESULTS

The determined antibody titer from each group is shown in Table 2. Group I (ND vaccinated and treated with 1% of red ginger powder) showed the highest titer of HI NDV antibody (log2) of 3.81 (GMT = 13.9). Group II (ND vaccinated and treated with red ginger ethanolic extract added to the drinking water), showed average HI titer (log2) of 3.13 (GMT = 8.6). Group III (ND vaccinated and given red ginger essential oil in drinking water) had an average HI titer of 2.91 (GMT = 7.5). Group IV (the control group which was vaccinated but not treated with red ginger) showed average HI titer (log2) of 0.95 (GMT = 1.9). Interestingly, the average HI titer of the unvaccinated groups and treated with red ginger extracts (group V, VI, VII) was 0. Following the statistical analysis using ANOVA, there was a significant difference ($p<0.05$) between red ginger powder treatment (group I) with the other groups (II-VII). Chickens treated with 1% red ginger powder in their feed and vaccinated with ND showed the highest HI ND antibody titer compared with other groups.

Furthermore, the challenge test showed that the groups of vaccinated chickens had better protection against NDV infection. The chickens from these groups were alive, either in red ginger treated groups (I and III) and untreated groups (IV). However, all chickens in the unvaccinated group died following the NDV challenge test even though they were treated with red ginger extracts (group V-VII), except group VI, with only 28% alive (Table 3). This suggested a marked difference of protection during the challenge test in vaccinated and unvaccinated chickens.

While the administration of 1% red ginger powder in this study is the most effective in increasing antibody titers against NDV, it did not cause any histopathological changes in the kidneys (Figure 1). However, unbound and pale cytoplasmic vacuoles (hydropic degeneration) were found in the livers as shown in Figure 2.

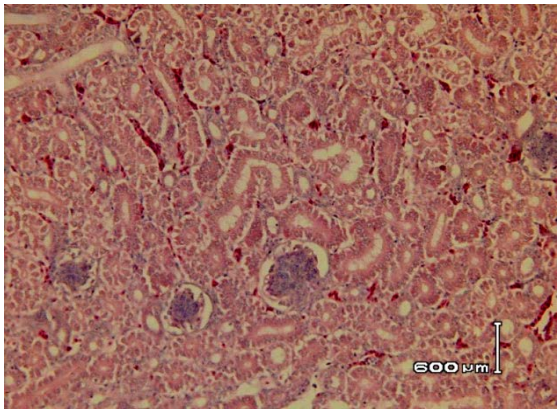

Figure 1: Histopathological examination of the kidney from a chicken treated with red ginger powder for 20 days. No pathological changes found in the glomeruli, tubules, and epithelial cells. Hematoxylin-Eosin staining at 200x magnification.

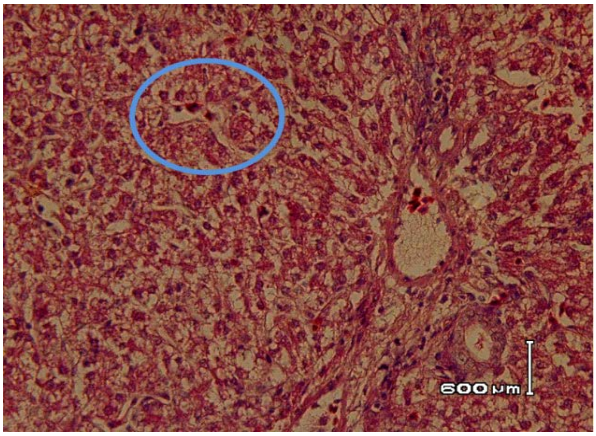

Figure 2: Histopathological examination of the liver from a chicken treated with red ginger powder for 20 days. The hepatocytes appeared swollen with pale vacuoles were found in the cytoplasm (depicted inside the blue circle). Hematoxylin-eosin stain at 200x magnification.

DISCUSSION

In this study, red ginger powder supplemented in the chicken feed was found to be more effective in strengthening the immune response in ND vaccinated chickens compared with the ethanolic extract and essential oil which were given via drinking water. Azhir et al. (2012) report that ginger powder 10g/kg in diet enhances humoral antibody titer. It is likely because the ethanol extract and essential oil of red ginger is more volatile, and the concentration of active substance is reduced. Therefore, this condition subsequently lowered the immunostimulant effect of the extracts. Aqueous extract of red ginger increased their performance, boosted their immunity as well as improved their general well-being (Oleforuh et al., 2015). Those effects are possibly due to the medicinal properties contained in ginger which is believed to improve digestion, kill parasites and their eggs, and enhancement of antibacterial and anti-inflammatory factors (Oleforuh et al., 2015). In addition, red ginger has been known as a strong antioxidant, which is considered a safe herbal medicine with a few insignificant side effects (Ali et al., 2008; Kim et al., 2007). The addition of ginger powder at a dose of 10 g per kg body weight of chicken has been reported to improve the immune response to NDV B_1 vaccine in 35- day old chicken. Taghdisi and Hejazi (2019) also reported that 1g/kg ginger in the diet improved the function of the humoral immune system. As ginger contains antioxidants, it gives protection to fatty acids of the humoral immune system and prevents the formation of free radicals. It can be concluded that ginger could have antiviral properties against the Newcastle virus by enhancing the efficiency of the immune system.

No toxic lesion was found in the kidneys following the histopathological examination. On the other hand, cytoplasmic vacuoles which are the characteristics of hydropic degeneration were found in the liver section as seen in Figure 2. This suggested a possible damage on cells that are reversible (McGavin and Zachary, 2007); however, it may not be caused by the administration of the red ginger extract. Previous study by Rong et al. (2009) reported that administration of ginger at 2000 mg/kg of body weight for 35 days in mice did not cause changes in the kidneys and liver. In addition, administration of 1% red ginger powder, 1% red ginger ethanol extract or 1% red ginger essential oil did not cause any histopathological changes in the chicken livers and kidneys.

Following the challenge test, chickens in group I-III, which were vaccinated and treated with 1% red ginger extracts, and in group IV, which were only vaccinated without any treatment with red ginger extract, all survived. Strikingly, chickens that were not vaccinated but were given ginger extracts (group V-VII), all died following the challenge test. This suggested that the administration of red ginger alone was not sufficient to promote protection against NDV infection and it must be accompanied by ND vaccination. Red ginger extracts had merely an immunostimulant effect which was not suitable for the prevention of specific diseases. Vaccines are still the most recommended agent for the prevention of specific viral infection. Protection against NDV is usually achieved using vaccine generated with low virulent NDV strains. Vaccination induced an immunity derived from neutralizing antibodies against the viral haemagglutinin and fusion glycoprotein which are responsible for virus attachment and spread. Innate cell-mediated immunity also contributes to the timed contribution of cell-mediated immune response to decrease the disease transmission potential (Kapczynski et al., 2013).

CONCLUSION

We conclude that the administration of 1% red ginger powder supplemented in chicken feed may increase the chicken immunity against NDV compared with the ethanolic extract and essential oil supplementation in drinking water. No toxicological signs were found in the chicken livers and kidneys. However, administration of red ginger powder, ethanolic extract, or essential oil did not harbor a stand-alone protective effect to NDV infection without vaccination in chicken.

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CONFLICT OF INTEREST

No conflict of interest associated with this work.

NOVELTY STATEMENT

The research origin differ from others.

AUTHORS CONTRIBUTION

Tri Untari: Prepare red gingger, prepare the chicken, Vaccination. Sitarina Widyarini: patologist analyse. Michael Haryadi Wibowo: serologis analyse. Marla Anggita: Compilation and Report the result and discuss

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