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E-mail: editorijps@gmail.com

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Research Article
Effect of *Phyllanthus buxifolius* Leaf as a Feed Supplement on Liver Function and Haematological Response of Quail (*Coturnix coturnix japonica*) Challenged with Infectious Newcastle Disease Virus

[1]Wardah, [2]J. Rahmahani and [3]T. Sopandi

[1]Department of Development Economic, Faculty of Economic, 17 Agustus 1945 University, Surabaya, Indonesia
[2]Department of Veterinary Microbiology, Faculty of Veterinary Medicine, Airlangga University, Indonesia
[3]Department of Biology, Faculty of Mathematical and Natural Science, PGRI Adi Buana University, Surabaya, Indonesia

Abstract

Background and Objective: Quail breeders are continuously facing high mortality rates due to Newcastle disease virus infection. Administration of 4-6% *Phyllanthus buxifolius* leaf powder in the diet can decrease fat and cholesterol levels in egg yolk and increase the immunity of unchallenged quail against Newcastle disease virus. This study examines the effects of *P. buxifolius* leaf powder on liver function and the haematological responses of quail challenged with Newcastle disease virus. **Materials and Methods:** One day old quail were acclimatized for 14 days in collective bamboo cages. Seventy-five female quail with similar weights were transferred to individual cages and randomized into five groups, each group being fed a commercial diet containing either 0, 2, 4, 6 and 8% *P. buxifolius* leaf powder. At the age of 47 days, all quail were infected with velogenic Newcastle disease virus. Haemogglutination inhibition tests were conducted on quail at the ages of 45, 65 and 80 days. Liver function tests and white blood cell and platelet counts were evaluated on quail at the ages of 45 and 75 days. **Results:** Supplementation with *P. buxifolius* leaf powder significantly increased antibody titers in 75 and 90 days old quail, significantly decreased aspartate amino transferase and alanine amino transferase levels and decreased total leucocyte, thrombocyte, lymphocyte and monocyte counts. Leaf powders of *P. buxifolius* have a high potential to protect poultry from infection with Newcastle disease virus and reduce spread of the disease. **Conclusion:** Dietary supplementation with 4-6% *P. buxifolius* leaf powder does not cause liver damage or inflammation in quail and may protect against infection.

Key words: Liver function, haematological, *C. coturnix japonica*, *Phyllanthus buxifolius*, Newcastle disease

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Corresponding Author: Wardah, Department of Development Economic, Faculty of Economic, 17 Agustus 1945 University, Surabaya, Indonesia

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Newcastle disease (ND) and avian influenza viruses are major problems currently facing quail breeders. Often, the application of good farm management practices and use of vaccinations and hygiene programs are not enough to prevent the spread of the ND virus. Vaccination programs have been implemented using various types of vaccines and vaccine timetables; however, despite these measures, quail have received much attention as important carriers of the ND virus[1]. Quail were found to be susceptible to natural infection with a velogenic strain of ND virus[1-3]. Conventional control strategies such as surveillance, stamping out (euthanizing infected or susceptible animals), movement restriction and enforcement of biosecurity measures did not prevent the virus from spreading, particularly in developing countries[4].

Naturally, the immune system can be improved by prevention of infectious diseases and administration of immune stimulation components. Herbal remedies have also been suggested as alternatives to prevent virus spread. Medicinal plants are known to contain various polyphenols with antiviral activities[5]. *Phyllanthus buxifolius* leaf contains flavonoids, polyphenols, tannins, saponins, alkaloids, quinones and steroid triterpenoids, all of which can nourish the tissues of animals without eliciting an inflammatory response. The compounds in *P. buxifolius* leaf are considered safe for poultry consumption and have been found to lower blood cholesterol levels in broiler chickens[6]. Preliminary studies have shown that supplementation of commercial feed with 4-6% *P. buxifolius* leaf powder can decrease fat and cholesterol levels of egg yolk and increase the immunity of unchallenged quail against ND virus[7].

Although not fatal, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were significantly elevated in unvaccinated quail infected with ND virus[8], suggesting liver injury. Velogenic strains of ND virus can cause haemorrhaging in the respiratory tract and brain and necrosis of hepatocytes in unvaccinated pigeons[9].

Several investigators have reported the effects of *P. amarus*, another species of *Phyllanthus*, on liver function and blood biochemistry in various animals. The methanol extract of *P. amarus* leaves (50-800 mg kg^{-1}) can reduce AST and ALT levels in male guinea pigs[10]. Similarly, oral administration of *P. amarus* aqueous extracts can reduce AST and ALT levels in albino rats[11]. The ethanol extract of *P. amarus* at 200 and 300 mg kg^{-1} has hepato-protective and nephron-protective properties when administered to gentamicin-treated rats[12]. However, previous studies have indicated that some chromatographic fractions obtained from *P. amarus* may have potentially deleterious effects on blood biochemistry[13]. Liver functions and hematological responses play important roles in quail production performance. This study explores the possible effects of *P. buxifolius* leaf as a feed supplement on preventing the spread of ND disease in poultry. This study addresses the critical area of ND virus-associated losses in poultry productivity using medicinal plants, an approach that has not yet been widely used. Thus, this study contributes to a new theory on the treatment and prevention of ND disease.

MATERIALS AND METHODS

Preparation of *P. buxifolius*: *Phyllanthus buxifolius* was locally sourced from a farm in Sumberingin, Sanankulon, Blitar, Indonesia; air-dried for six days; oven-dried at 50-60°C for 4 h and then ground into approximately 2 mm diameter particles using a grinder mill. *Phyllanthus Buxifolius* leaf powder was added to commercial quail feed at concentrations of 0, 2, 4, 6 and 8%, after which the feed was mixed and re-crumbled. The resulting feed crumble was chemically analyzed to determine the percentage of crude protein and fats, phosphorus, acid detergent fiber (ADF), neutral detergent fiber (NDF), cellulose, hemicellulose, silica, pectin, lignin and the following metabolic compounds: flavonoids, tannins and saponins. Table 1 shows the chemical composition of the commercial basal diet supplemented with 0, 2, 4, 6 and 8% *P. buxifolius* leaf powder.

Birds, housing and feeding: One-hundred 1 day old quail (DOQ) were obtained from local breeders and acclimatized for 14 days in collective bamboo cages. Seventy-five female quail with similar weights were randomized and transferred into individual cages (130 cm^2/head). After 15 days, quail were divided into five groups, with each group being fed a diet of commercial feed mixed with either 0, 2, 4, 6 or 8% *P. buxifolius* leaf powder. All quail were reared under the same conditions: At temperature ranging from 28-33°C, with 16 h of light and 8 h of darkness and with *ad libitum* access to food and water. All quail were reared for a total of 90 days. At the age of 47 days, all quail were infected with a velogenic strain of ND virus. Haemagglutination inhibition tests were conducted on quail aged 45, 65 and 80 days. Liver function tests (AST and ALT levels) and white blood cell (leucocyte, lymphocyte and monocyte) and thrombocyte counts were performed in quail aged 45 and 75 days.

Table 1: Chemical composition of basal feed diet supplemented with powder of *P. buxifolius* dry leaf

	Composition (%) of commercial basal feed diet plus powder of *P. buxifolius* leaf				
Components	0	2	4	6	8
Crude protein	22.39	22.56	23.48	23.77	23.07
Crude fat	7.61	7.44	7.39	7.29	7.25
Carbohydrate	58.22	59.08	59.71	60.98	62.88
NDF	15.76	15.96	15.24	19.21	19.31
ADF	6.33	7.60	7.29	7.37	7.17
Hemicellulose	6.53	7.04	7.97	8.33	9.76
Cellulose	4.31	4.71	4.99	5.21	5.33
Silica	0.24	0.19	0.18	0.20	0.30
Lignin	2.97	3.23	3.80	5.88	6.81
Pectin	0.40	3.98	7.95	8.67	8.91
Total flavonoid (routine equivalent)	0.06	0.23	0.55	0.73	0.75
Tannin total (tannic acid equivalent)	1.14	1.75	2.53	2.74	2.97
Saponin	2.05	3.42	4.45	5.56	5.75

NDF: Neutral detergent fiber, ADF: Acid detergent fiber

Haemagglutination inhibition assay (HI titer): For this assay, red blood cells (RBCs) were isolated using the following method: A total of 8 mL of blood was taken from healthy quail without anticoagulant and centrifuged at 2000 rpm for 10 min. The supernatant was transferred into a tube containing 5 mL of normal saline using a sterile pipette. Tubes were then centrifuged at 2000 rpm for 10 min. This sequence was repeated three times to produce a pure serum sample containing RBCs.

Haemagglutination titers were determined as described by Alan *et al.*[14]. Prior to the assay, serum samples were heated in a water bath at a temperature of 56°C for 30 min to destroy non-specific agglutinin. Using a multichannel dispenser, 50 µL of normal saline was dispensed into wells 1-12 of a microtiter plate, along with 50 µL of serum, which was progressively diluted 2-fold for each new row of the plate and 50 µL of antigen was added to the wells. One row served as a control and did not contain serum. Finally, 50 µL of RBC suspension was added to each well. The plate was then incubated at room temperature for 15-30 min until haemagglutination was observed.

Haemagglutination inhibition assays (HI titer) were carried out as described by Hashmi[15]. In the ND virus vaccine vial at a dose of 100 added 1 mL of normal saline. Furthermore, 50 µL of normal saline and 50 µL of antigen were pipetted into wells 1-12 of a microtiter plate and homogenized, after which 50 µL of 1% RBC solution was added and the wells were mixed. The plate was incubated at room temperature and observed every 10-15 min for haemagglutination.

AST and ALT: The AST and ALT were analyzed using the EnzyChrom™ Aspartate Transaminase Assay Kit (EALT-100; BioAssay Systems, Hayward, CA, USA) and the EnzyChrom™ Aspartate Transaminase Assay Kit (EASTR-100; BioAssay Systems), respectively. Briefly, 1000 µL of reagent A (L-aspartate+NADH or L-alanine+NADH) was added to each cuvette. Centrifuged serum samples (100 µL) were then added and the mixtures were incubated at 37°C for 1 min. Reagent B (2-oxoglutarate+LDH; 250 µL) was added, the solutions were mixed and the cuvette was incubated for 1 min at room temperature. The optical density was read 340 nm after 3 min.

Total and differential leucocytes: Aseptically, 3 mL blood was taken from the vena brachiali of each quail, transferred to a tube containing EDTA and mixed. The mixture (0.5 mL) was diluted with Turk's solvent (10 mL). Turk's is a composed of a Gentian violet stain and 2% acetic acid. The blood was then shaken for 3 min until homogenous and 2-3 drops were placed onto a counting chamber and incubated for 1 min. Total leucocytes were counted (total leucocyte count; TLC) under a microscope at 100× magnification. The differential leucocyte count (DLC) was determined using swab blood stained with 10% Giemsa for 30 min. Leucocytes were counted under a microscope at 100× magnification.

Statistical analyses: The statistical analysis of data was performed by one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* analysis. In all cases, differences were considered significant at $p<0.05$.

RESULTS

HI titer: The HI titer was not detected in serum of quail that was not fed *P. buxifolius* leaf powder and not infected ND virus. The HI titer also was not detected in serum of quail at 45 days old fed with *P. buxifolius* leaf powder and infected with the ND virus. However, the HI titer was detected in serum of quail at 65 and 80 days old fed with *P. buxifolius* leaf powder and infected with ND virus.

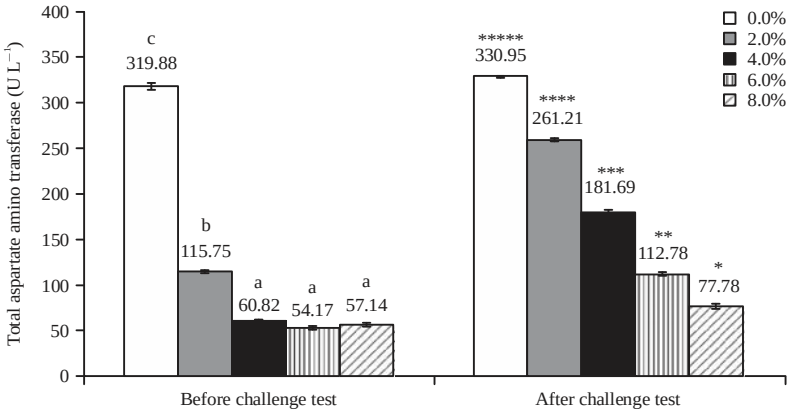

Fig. 1: Effect of *P. buxifolius* leaf powder on serum AST levels of quail before and after the viral challenge

Values and error bars represent means and standard deviations (n = 5), respectively. ANOVA was followed by Tukey's test. Each symbol, a, b and c (for data before the viral challenge) and *,**,***,**** and *****(for data after the viral challenge) indicates statistical significance compared to other symbols within the respective group

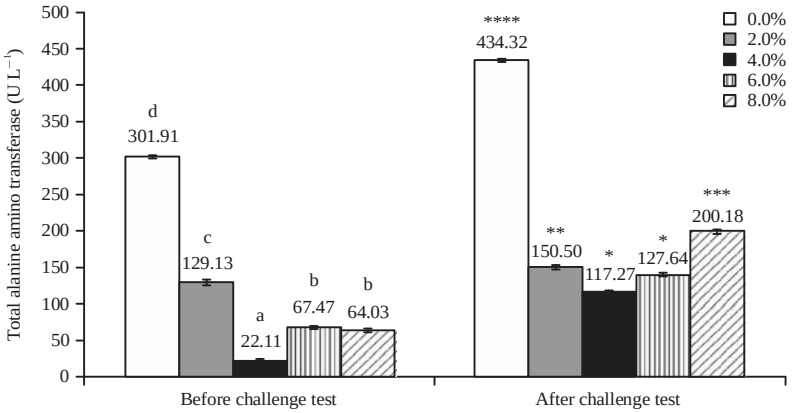

Fig. 2: Effect of *P. buxifolius* leaf powder on serum ALT levels of quail before and after the viral challenge

Values and error bars represent means and standard deviations (n = 5), respectively. ANOVA was followed by Tukey's test. Each symbol, a, b, c and d (for data before the viral challenge) and *,**,*** and ****(for data after the viral challenge) indicates statistical significance compared to other symbols within the respective group

Haemagglutination was not prevented using the serum of 45 days old quail (those not infected with ND virus) fed *P. buxifolius* leaf powder, but was prevented using serum from 65 and 80 days old quail (those infected with ND virus), as well as that from quail receiving a 1 mL IM dose of ND vaccination 1 week before the assay.

AST: Supplementation of commercial feed with *P. buxifolius* leaf powder significantly decreased serum AST levels in quail challenged with Newcastle disease virus (Fig. 1). Before the viral challenge, the serum AST levels of quail fed 4% (60.82±1.42 U L^{-1}), 6% (54.17±1.21 U L^{-1}) and 8% (57.14±14.05 U L^{-1}) *P. buxifolius* leaf powder were significantly lower than those of quail fed 0% (319.88±52.94 U L^{-1}) and 2% (115.75±8.43 U L^{-1}) *P. buxifolius* leaf powder. Moreover, the serum AST levels of quail fed 2.0% *P. buxifolius* leaf powder were significantly lower than those of quail fed 0% *P. buxifolius* leaf powder. After the viral challenge, the serum AST levels of quail fed 2% (261.21±9.85 U L^{-1}), 4% (181.69±22.06 U L^{-1}), 6% (112.78±18.03 U L^{-1}) and 8% (77.78±21.70 U L^{-1}) *P. buxifolius* leaf powder was significantly lower than those of quail fed 0% *P. buxifolius* leaf powder, with the lowest AST levels being found in the birds that were fed a diet supplemented with 8.0% *P. buxifolius* powder.

ALT: Supplementation of commercial feed with *P. buxifolius* leaf powder significantly decreased serum ALT levels in quail before and after challenged with Newcastle disease virus (Fig. 2). Before the viral challenge, the lowest serum ALT levels

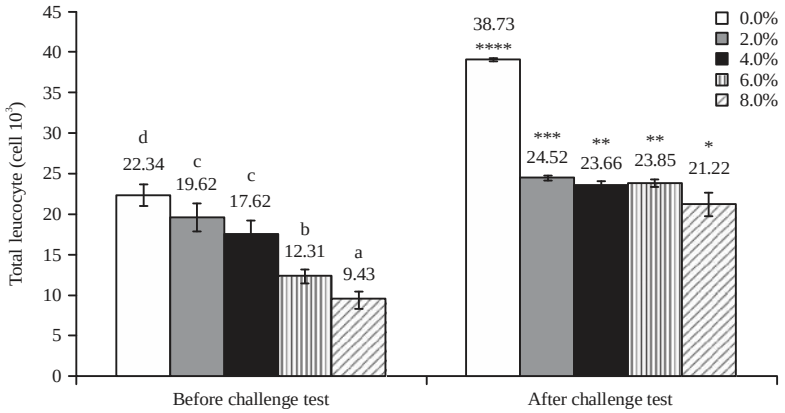

Fig. 3: Effect of *P. buxifolius* leaf powder on total leucocyte counts of quail before and after the viral challenge

Values and error bars represent means and standard deviations (n = 5), respectively. ANOVA was followed by Tukey's test. Each symbol, a, b, c and d (for data before the viral challenge) and *,**,*** and ****(for data after the viral challenge) indicates statistical significance compared to other symbols within the respective group

were detected in quail fed a diet supplemented with 4% (22.11±1.42 U L^{-1}) *P. buxifolius* leaf powder. Serum ALT levels were significantly lower in quail fed 6% (67.47±18.69 U L^{-1}) and 8% (64.03±17.32 U L^{-1}) *P. buxifolius* leaf powder compared to those of quail fed 2% (129.13±46.49 U L^{-1}) and 0% (301.91±31.98 U L^{-1}) *P. buxifolius* leaf powder. After the viral challenge, serum ALT levels were lowest in quail fed a commercial diet supplemented with 4% (117.27±8.53 U L^{-1}) *P. buxifolius* leaf powder, followed by those of quail fed 6% (127.64±12.40 U L^{-1}) *P. buxifolius* leaf powder. Quail in these two groups had serum ALT levels significantly lower than those fed 0% (434.32±195.59 U L^{-1}), 2% (150.50±26.12 U L^{-1}), or 8% (200.18±40.08 U L^{-1} *P. buxifolius* leaf powder.

Leucocytes: Supplementation of commercial feed with *P. buxifolius* leaf powder significantly decreased TLC in quail (Fig. 3) before and after the ND virus challenge. Before the challenge, the TLC of quail fed 0% (22.34±1.29×10^3 cell) and 8% (9.43±1.02×10^3 cell) *P. buxifolius* leaf powder were significantly higher than those of quail fed 2% (19.62±1.67×10^3 cell), 4% (17.62±6.56 x 10^3 cell), or 6% (12.31±6.95×10^3 cell) *P. buxifolius* leaf powder. No significant difference was observed in the TLC between quail fed 6% and those fed 8% *P. buxifolius* leaf powder. After the viral challenge, the TLC of quail fed 8% (21.22±1.45×10^3 cell) *P. buxifolius* leaf powder was significantly lower than that of those fed 2% (24.52±3.72×10^3 cell), 4% (23.66±1.68×10^3 cell) and 6% (22.22±1.80×10^3 cell) *P. buxifolius* leaf powder and those fed 2, 4, 6 and 8% *P. buxifolius* leaf powder had significantly lower TLC than those fed 0% (38.73±2.65×10^3 cell) *P. buxifolius* leaf powder. The lowest TLC was measured in quail fed a diet supplemented with 8% *P. buxifolius* leaf powder.

Thrombocytes: Supplementation of commercial feed with *P. buxifolius* leaf powder significantly decreased total thrombocyte counts in quail (Fig. 4) before and after challenged with Newcastle disease virus. Before the viral challenge, thrombocyte counts of quail fed 4% (12.60±0.41×10^6 cell) and 8% (11.40±0.05×10^6 cell) *P. buxifolius* leaf powder was significantly lower than those of quail fed 0% (29.80±3.27×10^3 cell), 2% (16.40±4.56×10^3 cell) and 6% (13.20±1.30×10^3 cell) *P. buxifolius* leaf powder; however, no significant difference in thrombocyte counts was observed between quail fed 4% and those fed 8.0% *P. buxifolius* leaf powder. After the viral challenge, thrombocyte counts in quail fed 4% (13.60±0.31×10^6 cell) and 6% (14.20±0.22×10^6 cell) *P. buxifolius* leaf powder was significantly lower than those of quail fed 0% (32.60±2.51×10^3 cell), 2% (18.20±4.02×10^3 cell) and 8% (14.60±1.34×10^3 cell) *P. buxifolius* leaf powder, with TLC of quail fed 8% *P. buxifolius* leaf powder significantly lower than those of quail fed 2 and 0% *P. buxifolius* leaf powder. The lowest thrombocyte counts were measured in quail fed a diet supplemented with 4% *P. buxifolius* leaf powder.

Lymphocytes: Supplementation of commercial feed with *P. buxifolius* leaf powder significantly decreased lymphocyte counts (Fig. 5) in quail before and after challenged with Newcastle disease virus. Before the viral challenge, lymphocyte counts of quail fed 2% *P. buxifolius* leaf powder (45.80±0.22%) were significantly lower than those of quail fed

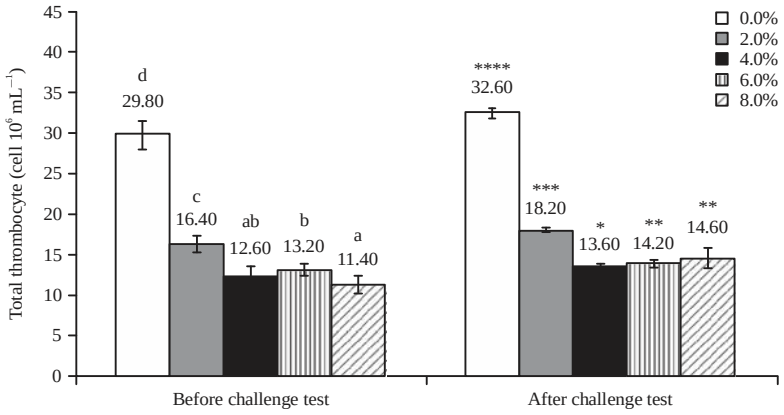

Fig. 4: Effect of *P. buxifolius* leaf powder on total thrombocyte counts of quail before and after the viral challenge

Values and error bars represent means and standard deviations (n = 5), respectively. ANOVA was followed by Tukey's test. Each symbol, a, b, c and d (for data before the viral challenge) and *,**,*** and ****(for data after the viral challenge) indicates statistical significance compared to other symbols within the respective group

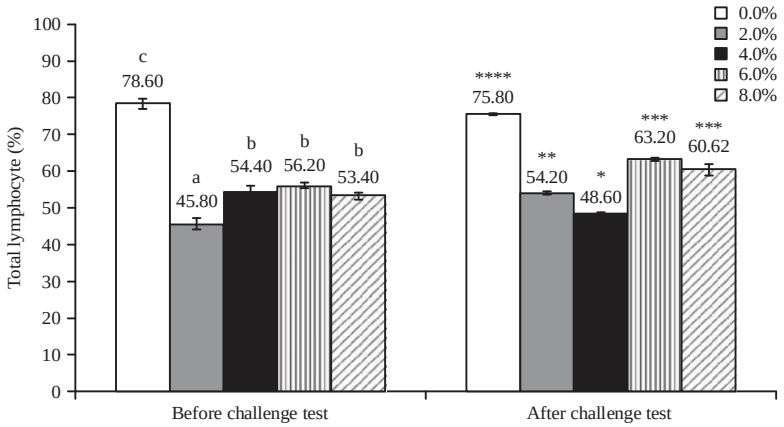

Fig. 5: Effect of *P. buxifolius* leaf powder on total lymphocyte counts of quail before and after the viral challenge

Values and error bars represent means and standard deviations (n = 5), respectively. ANOVA was followed by Tukey's test. Each symbol, a, b and c (for data before the viral challenge) and *,**,*** and ****(for data after the viral challenge) indicates statistical significance compared to other symbols within the respective group

0% (78.60±1.29%), 4% (54.40±0.41%), 6% (56.20±0.73%) and 8% (53.40±1.02%) *P. buxifolius* leaf powder; however, there was no significant difference in lymphocyte counts between quail fed 4, 6 and 8% *P. buxifolius* leaf powder. After the viral challenge, lymphocyte counts of quail fed 4% *P. buxifolius* leaf powder (48.60±0.51%) were significantly lower than those of quail fed 0% (75.80±1.66%), 2% (54.20±0.32), 6% (63.20±0.43%) and 8% (60.62±1.45%) *P. buxifolius* leaf powder. Lymphocyte counts of quail fed 6 and 8% *P. buxifolius* leaf powder was significantly higher than those of quail fed 2%, with those of quail fed 2% *P. buxifolius* leaf powder significantly lower than those of quail fed 0% *P. buxifolius* leaf powder.

Monocytes: Supplementation of commercial feed with *P. buxifolius* leaf powder significantly decreased monocyte counts of quail (Fig. 6) before and after the ND virus challenge. Before the viral challenge, monocyte counts in quail fed 6% (0.04±0.0001%) *P. buxifolius* leaf powder was significantly lower than those of quail fed 2% (0.20±0.005%), 4% (0.20±0.002%) and 8% (0.18±0.002%) *P. buxifolius* leaf powder. After the viral challenged, monocyte counts in quail fed 2% (0.30±0.002%) *P. buxifolius* leaf powder was significantly lower than those of quail fed 4% (0.60±0.002%), 6% (0.80±0.004%) and 8% (1.0±0.05%) *P. buxifolius* leaf powder. There were no significant ($p>0.05$) differences in monocyte counts in quail fed 0% (0.80±0.003%), 6 and 8% *P. buxifolius* leaf powder.

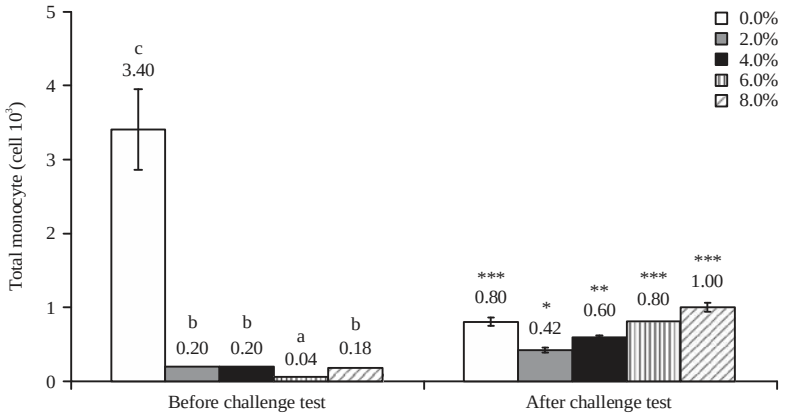

Fig. 6: Effect of *P. buxifolius* leaf powder on total leucocyte counts of quail before and after the viral challenge

Values and error bars represent means and standard deviations (n = 5), respectively. ANOVA was followed by Tukey's test. Each symbol, a, b and d (for data before the viral challenge) and *,** and ***(for data after the viral challenge) indicates statistical significance compared to other symbols within the respective group

DISCUSSION

The results of this study demonstrate that dietary administration of *P. buxifolius* leaf can modulate the humeral immune response to ND virus for up to 65 days in quail. Modulation of the immune response is thought to be due to the activity of secondary metabolite components such as alkaloids, flavonoids, tannins and saponins. Wardah and Wurlina[6] reported that *P. buxifolius* leaves contain flavonoids, polyphenols, saponins, alkaloids, quionones, steroids and triterpenoids. The results of the present study are in accordance with those of Babu and Panda[16], who reported that species of *Phyllanthus* can modulate the immune response to ND virus in chickens up to 20 weeks of age and with those of Wardah and Sopandi[7], who reported that feeding quail 4-6% *P. buxifolius* powder can improve immunity to ND virus.

ND virus contains haemagglutinin in the envelope[17], allowing the virus to agglutinate RBCs of poultry. The HI assay is used to measure antibody titers against those viruses capable of agglutinating RBCs[18], such as ND virus. Detection of an HI titer indicates binding of a virus to antibody, thus inhibiting its ability to agglutinate RBCs. The present study indicated that HI titer was detected in serum of quails that infected ND virus and fed *P. buxifolius* leaf powder at the age of 65 and 80 days. Some investigators have reported the effects of *Phyllanthus* plants against certain viruses. Venkateswaran *et al.*[19] reported that aqueous extracts of the plant *P. niruri* inhibit the endogenous DNA polymerase activity of hepatitis B virus (HBV) and bind to the hepatitis B surface antigen *in vitro*. Liu *et al.*[20] reported that flavonoids, a major compound isolated from *Phyllanthus* species, have antiviral activities and that two other compounds widely available in *Phyllanthus* extracts, tannins and ellagitannins, can inhibit the activity of the Epstein-Barr virus DNA polymerase enzyme. Saputra *et al.*[21] reported that the ability of *P. niruri* to function as immune therapy is mediated by its immunostimulatory properties. *Phyllanthus Buxifolius* has been shown to increase NK cell cytotoxicity, allowing for increased lysis of mutated cells and to facilitate the phagocytic activities of monocytes/macrophages. Malhortra and Singh[22] reported that *P. amarus* can inhibit the DNA polymerase activity of HBV, woodchuck hepatitis virus and human immunodeficiency virus (HIV-1-RT). Jung *et al.*[23] reported that *P. urinaria koreana* extracts can inhibit HBV DNA synthesis and the secretion of HBV surface antigens HBsAg and HBcAg by replicating cells harbouring HBV wild-type and lamivudine-resistant mutants, likely by inducing the expression of IFN-β, COX-2 and IL-6.

The results of this study showed that dietary administration of *P. buxifolius* leaf powder can decrease serum AST and ALT levels in quail before and after challenging the birds with ND virus. However, ALT levels after viral challenge, hit a peak low at 4% powder leaf and rise steadily with 6 and 7%. Because increases in AST and ALT levels indicate liver damage or disease, these data indicate that *Phyllanthus* leaf powder does not cause hepatotoxicity in quail. The decrease of AST and ALT levels in quail fed with *P. buxifolius* leaf powder was allegedly due to the activity of the secondary metabolite components contained in *P. buxifolius* and increased protein feed content.

A low-protein diet can result in a decrease in the activity of liver enzymes involved in the catabolism of amino acids; however, these decreases can be reversed if animals are fed a high-protein diet[24]. The AST, also known as serum glutamic oxaloacetic transaminase (SGOT), is an enzyme that is released in the blood when certain organs or tissues (particularly liver and heart) are injured. The result of the current study support several reports on the effects of *P. buxifolius* on ALT and AST levels. Adeneye *et al*.[25] reported that flavonoids, tannins and saponins found in *P. buxifolius* may be hepatoprotective in rats. Obianime and Uche[10] reported that *P. amarus* significantly decreased levels of cholesterol, AST, ALT, urea, uric acid, total protein, alkaline and acid phosphates in mice. The current study found that supplementation of 4% *P. buxifolius* leaf powder in the diet is an optimum concentration to decrease levels of both AST and ALT.

The results of this study showed that supplementation of commercial feed with *P. buxifolius* leaf powder influenced the haematological characteristics of quail both before and after a ND virus challenge. An increase in leucocytes, or white blood cells, is an indicator of infection in an organism. The results of this work showed that *P. buxifolius* leaf powder can reduce the number of leucocytes, indicating that *P. buxifolius* leaf powder does not cause infection in quail. Knowledge of bird haematology is a useful diagnostic tool in veterinary medicine, as these values can be used as physiological indicators of stress or disease[26]. Haematological values are commonly used as indicators of health in birds housed in cage systems and to detect stress caused by various factors, such as environmental, nutritional and pathological factors[27,28]. However, Montejo *et al*.[29] reported no significant effects on total white blood cell counts of mice fed *P. niruri* leaf powder for 4 weeks.

The results of this study clearly demonstrated that administration of *P. buxifolius* leaf powder decreases the number of lymphocytes in quail, although the magnitude of that change did vary with different concentrations of the herb. This decrease may be due to the inhibition of ND virus growth by *P. buxifolius*, leading to the destruction of lymphocytes or inhibition of lymphocyte proliferation. An increased number of lymphocytes suggest activation of the body's defence systems[30], resulting in increased lymphopoiesis and/or enhanced release of lymphocytes from lymph myeloid tissue[31]. Similar results have been reported by Nwankpa *et al*.[32], who demonstrated that the number of lymphocytes in Wistar albino rats infected with *Salmonellae typhi* and fed *P. amarus* extracts was significantly lower than those of rats infected with *S. typhi* but not fed *P. amarus*. *Phyllanthus Buxifolius* lignans, especially phyltetralin and phyllanthin, have demonstrated the ability to strongly inhibit lymphocyte proliferation[33]. This inhibition may be due to a failure to initiate lymphocyte transformation or interference with the exponential increase in the number of dividing cells[34]. Jantan *et al*.[35] reported that all compounds of *P. amarus* have monocyte-inhibitory activities. Papatriantafyllou[36] reported that methanol extract of *P. amarus* reduces the volume of exudate and migration of neutrophils and monocytes in male Wistar rats.

Highly pathogenic ND virus causes devastating disease in poultry. Decreasing the incidence of ND virus in poultry requires a focus on prevention and control of poultry infections. The ND virus spreads rapidly from bird to bird and is considered an airborne disease, although it may also be transmitted through body contact and contact with objects such as drinkers, feeders, feed and water. Conventional control strategies in poultry based on surveillance, stamping out, movement restriction and enforcement of biosecurity measures have not succeeded in preventing the virus spread, particularly in developing countries[4]. Vaccination may prevent the clinical disease, but cannot prevent the actual infection, which can easily spread among poultry flocks[37-39]. In the present study, dietary supplementation of *P. buxifolius* leaf powder significantly enhanced the serum antibody response to ND virus in quail without causing liver damage. Immunomodulation may play an important role in reducing morbidity and mortality of poultry due to viral infection. The use of *Phyllanthus* leaf as a feed supplement can support vaccination program for the treatment or prevention of the spread of ND virus in modern poultry production. Feed formulations containing medicinal plants can help improve immune status without disrupting poultry productivity.

CONCLUSION

Phyllanthus buxifolius leaf powder increases the antibody response of poultry infected with ND virus. Dietary supplementation of *P. buxifolius* leaf powder at the concentrations of 4-6% can increase quail antibody response and immunity without causing liver damage, infection or inflammation. The use of this medicinal plant as a commercial poultry feed additive can reduce mortality and the spread of ND virus.

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