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Research Article

Egg Cholesterol and Immunity of Quail (*Coturnix coturnix japonica*) Diet *Phillanthus buxifolius* Leaves as Feed Supplement

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Abstract

Phyllantus buxifolius is an herbal feed supplement that can increase immunity and reduce cholesterol. A study has been conducted to investigate the effect diet of *P. buxifolius* leaves powder as feed supplement on mechanism of cholesterol reduction and increase immunity of quail. Seventy five quail at 30 day old randomly divided into 5 groups and each group fed leaves powder of *P. buxifolius* 0.0, 2.0, 4.0, 6.0 and 8.0% kg^{-1} in commercial feed for 75 days, respectively. Powder of *P. buxifolius* leaves significantly ($p<0.05$) decreased fat, cholesterol and low density lipoprotein yolk egg quail but significantly increased High Density Lipoprotein (HDL) at measurement 45, 60 and 75 days old. Fed of 4.0 and 6.0% leaves of *P. buxifolius* powder significantly ($p<0.05$) increased the expression of interleukin-β and lymphocytes but significantly ($p<0.05$) decreased nitric oxide synthase (iNOS), leukocytes and monocytes even undetectable levels. Leaf powder of *P. buxifolius* potential as feed additive for decreasing fat and cholesterol yolk egg and increasing immunity of quail at 4-6% commercial feed. The use of this medicinal plant as a commercial feed supplementation can reduce quail mortality due to viral infection and may increase the interest of the market for the consumption of quail eggs.

Key words: *Phillanthus buxifolius*, egg, cholesterol, immunity, quail

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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

The market of quail eggs in Indonesia is increasing, being offered mainly as a side dish in restaurants. The content of vitamins A, B1 and B2 in quail egg is twice larger, meanwhile, choline, iron and potassium are five times larger than chicken eggs. Protein content in quail eggs also higher than protein content in chicken eggs (Jenkins *et al*., 2001). Quail eggs present, in average, 13.1% protein, 1.1% minerals and 11.2% lipids (Panda and Singh, 1990) and calcium, phosphorus, iron, vitamin A and energy contents per 100 g are 59, 220, 3.8 mg, 300 IU and 158 kcal, respectively (Fernendez *et al*., 2011). However, quail egg have high cholesterol content (364 mg g^{-1}) compared cholesterol chicken eggs (50 mg g^{-1}). High cholesterol content in quail eggs not favored mainly by people who suffer from cardiovascular diseases, disorders of cholesterol, high blood pressure and obesity. High cholesterol in eggs is suspected because of continuous use synthetic supplements that stimulate growth but the residue is difficult and can cause health problems to consumers, can increase feed consumption and egg production so that the target maximum production achieved.

In addition, the high mortality of quail due to the disease primarily ND and avian influenza viruses is a major problem faced quail breeders in Indonesia. Viral infection of the quail also received attention because considered it as important carriers for ND virus (Lima *et al*., 2004). Quails were found to acquire the natural infection with a velogenic strain of ND virus (Czirjak *et al*., 2007; Lima *et al*., 2004; Sa'idu *et al*., 2004). Conventional control strategies in poultry based on surveillance, stamping out, movement restriction and enforcement of biosecurity measures did not prevent the virus spreading, particularly in developing countries (Abdelwhab and Hafez, 2012).

Herbal remedies, including Traditional Chinese Medicine (TCM) have also been suggested as alternatives. Various polyphenols are present and the antiviral activities have been attributed to medicinal plant (Hudson, 2009). *Phyllanthus buxifolius* is a medicinal plant and has been widely used to treat various diseases by Indonesian people and this plant leaves known contain flavonoid, polyphenol, saponin, alkaloids, quionones, steroids and triterpenoids (Wardah and Wurlina, 2007).

Provision of natural herb that have potential antilipidemic effect. The use of natural feed supplements that have the potential to lower cholesterol, are easily available at low cost is expected to lowering cholesterol of egg and increase the immune of poultry. Natural antioxidants have been to offer vast array of health effects including lowering the cholesterol level (Nurulhuda *et al*., 2012).

Phyllanthus genus contains many medicinal secondary metabolites (Zhang *et al*., 2000). Phyllanthus known have functional and activity of immunocompetent cells, immunotherapy and potentially as antihiperlipidemik and antikolesterolemik in blood (Adeneye *et al*., 2006; Obianime and Uche, 2008; Umbare *et al*., 2009). *Phyllantus buxifolius* (family: Euphorbiaceae) is medicinal plant and has been widely used to treat various types of diseases by Indonesian people. *Phyllanthus buxifolius* leaves contain the flavonoids, polyphenols, tannins, saponins, alkaloids, quinones and steroid triterpenoids (Sopandi, 2005; Wardah and Wurlina, 2007), which can nourish the liver and tissues of animals, do not cause infection and inflammation (inflammatory) that is safe for consumption poultry as well as lowering blood cholesterol levels in broiler chickens (Wardah and Wurlina, 2007). This study aimed to examine the effect *P. buxifolius* leaves powder in feed on increase immunity in blood and reduce yolk egg cholesterol of *C. coturnix japonica*.

MATERIALS AND METHODS

Preparation of *P. buxifolius*: Locally farm-sourced *P. buxifolius* from Sumberingin, Sanankulon, Blitar, Indonesia, were air-dried for 6 days, oven-dried at 50-60°C for 4 h and then grounded to approximately 2 mm diameter particles using a mill. Powder of *P. buxifolius* leaves were added to commercial feed quail (0.0, 2.0, 4.0, 6.0 and 8.0%), mixed and re-crumbling. The crumble of feed after adding powder *P. buxifolius* leaves were chemicals analysis to determination of crude protein and fat, phosphorus, Acid Detergent Fiber (ADF), Neutral Detergent Fiber (NDF), cellulose, hemicellulose, silica, pectin and lignin and metabolic compound: Flavonoids, tannins and saponins.

Experimental design: One hundred Day Old Quail (DOQ) obtained from locally breeder were acclimatized for 2 weeks in collective bamboos cages and then selected 75 quails (female) which have same weight relatively, randomized and transferred to individual cages (130 cm^2 $head^{-1}$). At the age of 30 days, quail were divided into 5 group and each group diet commercial feed mixed 0, 2, 4, 6 and 8% leaves powder *P. buxifolius*. All quail reared at same condition, at temperature of 23-25°C, the lighting regime consisted of 16 h of light and 8 h of darkness and *ad libitum* feeding and drinking and all quail reared for 75 days.

Data collection: Cholesterol, LDL and HDL serum observed at 15 days before the quail treated. At the age of 45, 60 and 75 days, fat content in yolk egg was analyzed by Soxhlet

extraction method, yolk egg cholesterol, Low Density Lipoprotein (LDL) and High Density Lipoprotein (HDL) in yolk egg were analyzed by Liebermann Burchard methods, respectively. Meanwhile, observation of the immune response covering Total Leukocytes Count (TLC), the Differential Leukocytes Count (DLC) included monocyte and lymphocyte, the amount of iNOS and the expression of interleukin 1β (ELISA methods) were done at the age of 45 and 75 days.

Statistical analysis: All data were analyzed using split plot base on a Completely Randomized Design (CRD) with five replications. Tukey's honestly significant difference multiple comparison tests were used to segregate significantly different treatments using SPSS 17 software.

RESULTS

Fat yolk egg: The present study (Fig. 1) showed fed of commercial feed with supplementation *P. buxifolius* powder significantly ($p<0.05$) decreased fat yolk egg at the age of quail 45, 60 and 75 days. Fat content of yolk egg at the age of quails 45 days which dieted commercial feed with supplementation 8.0% *P. buxifolius* powder (1.67±0.20%) significantly ($p<0.05$) lower than fat content of yolk egg quail, which dieted commercial feed with supplemented 6.0% (2.34±0.07%), 4.0% (3.02±0.14%), 2.0% (3.13±0.09%) and 0.0% (3.65±0.21%) *P. buxifolius* powder. Fat content of yolk egg quail which diet commercial feed with supplemented 6.0% *P. buxifolius* powder significantly ($p<0.05$) lower than fat content of yolk egg 4.0, 2.0 and 0.0% *P. buxifolius* powder. No significant ($p>0.05$) were observed fat content of yolk egg quail diet commercial feed with supplemented 4.0 and 2.0% *P. buxifolius* powder but the both of supplemented (4.0 and 2.0%) *P. buxifolius* powder significantly ($p<0.05$) lower than fat content of yolk egg quail dieted commercial feed with supplemented 0.0% *P. buxifolius* powder.

At the age of quails 60 days, no significant ($p>0.05$) different were observed fat content of yolk egg with supplemented 8.0% *P. buxifolius* powder to commercial feed (1.42±0.04%) and fat content of yolk egg with supplemented 6.0% (1.45±0.12%) but fat content both supplemented *P. buxifolius* powder to commercial feed significantly ($p<0.05$) lower than fat content of yolk egg with supplemented 4.0% (1.93±0.07%), 2.0% (3.11±0.04%) and 0.0% (5.26±0.07%) *P. buxifolius* powder to commercial feed. Fat content of yolk egg with supplemented 4.0% *P. buxifolius* powder to commercial feed significantly ($p<0.05$) lower than fat content of yolk egg with supplemented 2.0 and 0.0% *P. buxifolius* powder to commercial feed and fat content of yolk egg with supplemented 2.0% *P. buxifolius* powder significantly ($p<0.05$) lower than fat content of yolk egg with supplemented 0.0% *P. buxifolius* powder to commercial feed.

At the age of quails 75 days, fat content of yolk egg with supplemented 8.0% *P. buxifolius* powder to commercial feed (1.41±0.03%), significantly ($p<0.05$) lower than fat content of yolk egg with supplemented 6.0% (1.53±0.05%), 4.0% (1.93±0.05%), 2.0% (2.42±0.10%) and 0.0%

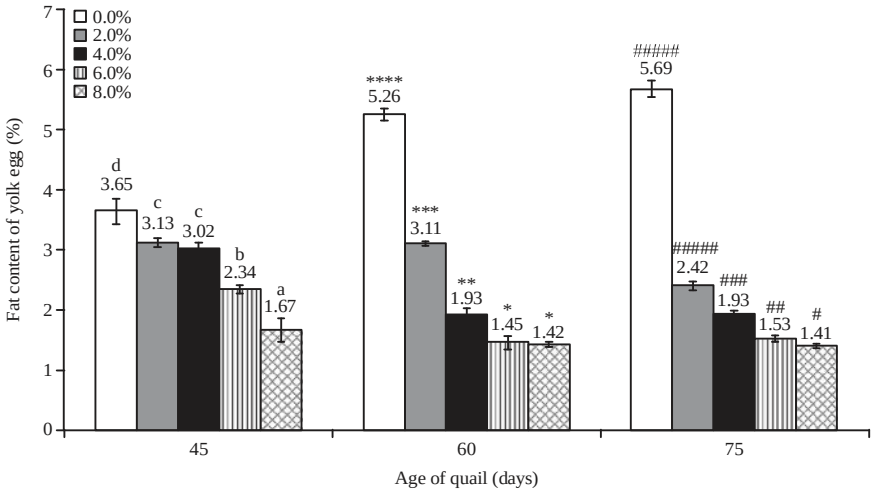

Fig. 1: Effect fed supplementation *P. buxifolius* leaves powder to commercial feed on fat yolk egg quail at the age of 45, 60 and 75 days, values and error bars represent Mean±SD (n = 5), ANOVA was followed by Tukey's test a, b, c and d ($p<0.05$), *,**,***,****($p<0.05$), #,##,###,#####($p<0.05$) within respective groups, 0.0, 2.0, 4.0, 6.0 and 8.0% supplemented *P. buxifolius* powder to commercial feed

(5.69±0.08%) *P. buxifolius* powder to commercial feed. Fat content of yolk egg with supplemented 6.0% *P. buxifolius* powder to commercial feed significantly ($p<0.05$) lower than fat content of yolk egg with supplemented 4.0, 2.0 and 0.0% *P. buxifolius* powder to commercial feed. Fat content of yolk egg with supplemented 4.0% *P. buxifolius* powder to commercial feed significantly ($p<0.05$) lower than fat content of yolk egg with supplemented 2.0 and 0.0% and fat content of yolk egg with supplemented 2.0% *P. buxifolius* powder to commercial feed significantly ($p<0.05$) lower than fat content of yolk egg with supplemented 0.0% *P. buxifolius* powder to commercial feed.

Cholesterol yolk egg: The present study (Fig. 2) showed diet of commercial feed with supplementation *P. buxifolius* powder significantly ($p<0.05$) decreased cholesterol content of yolk egg at the age of quails 45, 60 and 75 days, cholesterol content of yolk egg at the age of quails 45 days which dieted commercial feed with supplementation 8.0% *P. buxifolius* powder (114.42±2.51 mg dL^{-1}) significantly ($p<0.05$) lower than cholesterol in yolk egg quail which dieted commercial feed with supplementation 6.0% (120.50±1.32 mg dL^{-1}), 4.0% (128.28±3.94 mg dL^{-1}), 2.0% (155.00±4.15 mg dL^{-1}) and 0.0% (170.86±6.75 mg dL^{-1}) *P. buxifolius* powder. Cholesterol content in yolk egg quail which diet commercial feed with supplemented 6.0% *P. buxifolius* powder significantly ($p<0.05$) lower than cholesterol content in yolk egg 4.0, 2.0 and 0.0% *P. buxifolius* powder. No significant ($p>0.05$) were observed cholesterol content in yolk egg quail diet commercial feed with supplemented 4.0 and 2.0% *P. buxifolius* powder but the both of supplemented (4.0 and 2.0%) *P. buxifolius* powder significantly ($p<0.05$) lower than cholesterol content in yolk egg quail dieted commercial feed with supplemented 0.0% *P. buxifolius* powder.

Cholesterol content in yolk egg at the age of quails 60 days which dieted commercial feed with supplementation 8.0% *P. buxifolius* powder (155.96±1.25 mg dL^{-1}), significantly ($p<0.05$) lower than cholesterol content in yolk egg quail, which dieted commercial feed with supplemented 6.0% (162.56±1.50 mg dL^{-1}), 4.0% (167.06±0.84 mg dL^{-1}), 2.0% (170.44±0.81 mg dL^{-1}) and 0.0% (182.88±1.40 mg dL^{-1}) *P. buxifolius* powder. Cholesterol content in yolk egg quail which diet commercial feed with supplemented 6.0% *P. buxifolius* powder significantly ($p<0.05$) lower than cholesterol content in yolk egg 4.0, 2.0 and 0.0% *P. buxifolius* powder. cholesterol content were not significantly observed ($p>0.05$) in yolk egg quail dieted commercial feed with supplemented 4.0 and 2.0% *P. buxifolius* powder but cholesterol content both of supplemented (4.0 and 2.0%) *P. buxifolius* powder significantly ($p<0.05$) lower than cholesterol content in yolk egg quail dieted commercial feed with supplemented 0.0% *P. buxifolius* powder.

At the age of quails 75 days, no significant ($p>0.05$) difference was observed between cholesterol content in yolk egg, which dieted commercial feed with supplementation 8.0% *P. buxifolius* powder (180.06±0.81 mg dL^{-1}), 6.0% (182.26±2.32 mg dL^{-1}) and 2.0% (179.88±7.02 mg dL^{-1}) but cholesterol in yolk egg quail these three (8.0, 6.0 and 2.0%) supplemented significantly ($p<0.05$) higher than cholesterol

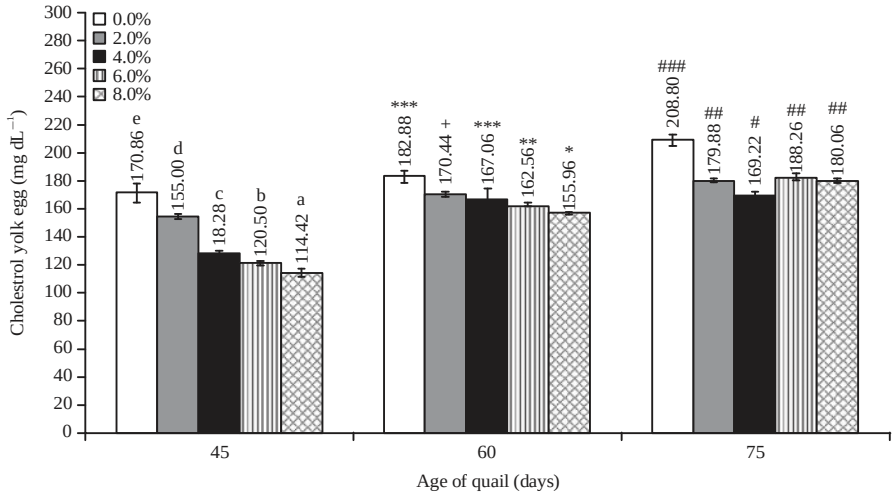

Fig. 2: Effect fed supplementation *P. buxifolius* powder to commercial feed on cholesterol yolk egg quail at the age 45, 60 and 75 days, values and error bars represent Mean±SD (n = 5), ANOVA was followed by Tukey's test a, b, c, d and e $p<0.05$, *,**,***,+ ($p<0.05$), #,##,###($p<0.05$) within respective groups, 0.0, 2.0, 4.0, 6.0 and 8.0% supplemented *P. buxifolius* powder to commercial feed

content in yolk egg quails dieted 4.0% (169.22±2.54 mg dL^{-1}) and significantly ($p<0.05$) lower than cholesterol content in yolk egg quail, which diet commercial feed with supplemented 0.0% *P. buxifolius* powder (208.80±1.41 mg dL^{-1}).

HDL yolk egg: This study (Fig. 3) showed diet of commercial feed with supplementation *P. buxifolius* powder significantly ($p<0.05$) influenced on HDL yolk egg, although no stable data HDL yolk egg obtained between 45, 60 and 75 days of quail. The HDL yolk egg at the age of quails 45 days, which dieted commercial feed with supplementation 8.0% *P. buxifolius* powder (80.98±0.80 mg dL^{-1}) significantly ($p<0.05$) lower than HDL yolk egg quail, which dieted commercial feed with supplemented 6.0% (90.45±3.15 mg dL^{-1}), 4.0% (99.75±1.75 mg dL^{-1}), 2.0% (94.14±0.19 mg dL^{-1}) and 0.0% (88.86±5.09 mg dL^{-1}) *P. buxifolius* powder. No significant ($p>0.05$) difference was observed HDL yolk egg between dieted supplemented 6.0, 2.0 and 0.0% *P. buxifolius* powder but HDL yolk egg quail these three supplementation significantly ($p<0.05$) lower than HDL yolk egg with supplementation 4.0% *P. buxifolius* powder.

At the age of quail 60 days, HDL yolk egg dieted with supplementation 8.0% (90.65±1.04 mg dL^{-1}) *P. buxifolius* powder significantly ($p<0.05$) lower than HDL yolk egg diet with supplementation 6.0% (95.62±1.83 mg dL^{-1}), 4.0% (102.94±1.31 mg dL^{-1}), 2.0% (97.62±1.26 mg dL^{-1}) and 0.0% (87.40±0.84 mg dL^{-1}) *P. buxifolius* powder. No significant ($p>0.05$) different was observed between HDL yolk egg dieted with supplementation 6.0 and 2.0% *P. buxifolius* powder but both HDL yolk egg significantly ($p<0.05$) higher than HDL yolk egg dieted with supplementation 0.0% and significantly ($p<0.05$) lower than HDL yolk egg dieted with supplementation 4.0% *P. buxifolius* powder.

At the age of quail 75 days, HDL yolk egg dieted with supplementation 8.0% (93.99±0.75 mg dL^{-1}) and 2.0% (98.11±1.36 mg dL^{-1}) *P. buxifolius* powder significantly ($p<0.05$) higher than HDL yolk egg diet with supplementation 0.0% (88.02±0.70 mg dL^{-1}) but lower than 6.0% (102.33±0.80) and 4.0% (108.44±1.46%) *P. buxifolius* powder.

LDL yolk egg: This study (Fig. 4) showed diet of commercial feed with supplementation *P. buxifolius* powder significantly ($p<0.05$) influenced on LDL yolk egg at the age 45, 60 and 75 days of quail. The LDL yolk egg at the age of quails 45 days, which dieted commercial feed with supplementation 4.0% *P. buxifolius* powder (41.05±0.69 mg dL^{-1}) significantly ($p<0.05$) lower than LDL yolk egg quail which dieted commercial feed with supplemented 6.0% (44.50±1.02 mg dL^{-1}) and 0.0% (49.12±1.51 mg dL^{-1}) but no significant ($p>0.05$) difference was observed LDL yolk egg 4.0% supplementation *P. buxifolius* powder and LDL yolk egg dieted supplementation 8.0% (43.11±1.18 mg dL^{-1}) and 2.0% (42.77±0.51 mg dL^{-1}) *P. buxifolius* powder.

The LDL yolk egg at the age of quails 60 days which dieted commercial feed with supplementation

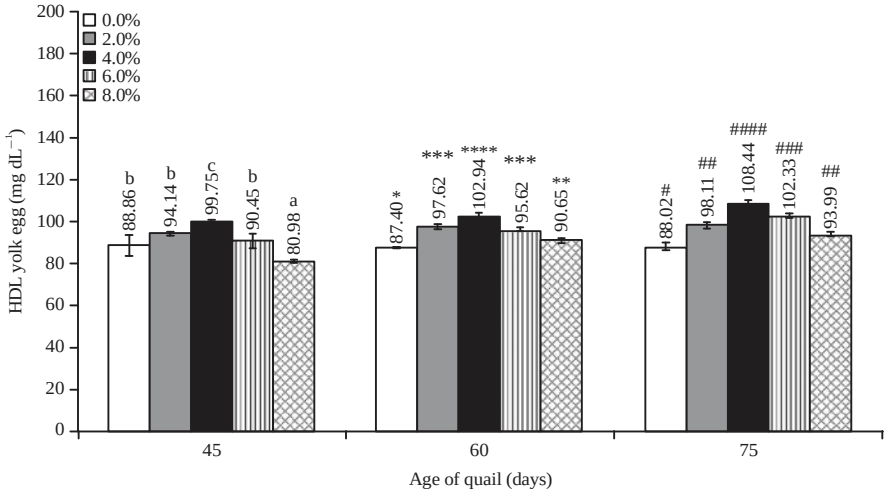

Fig. 3: Effect fed supplementation *P. buxifolius* powder to commercial feed on HDL yolk egg quail at the age 45, 60 and 75 days, values and error bars represent Mean±SD (n = 5), ANOVA was followed by Tukey's test a, b and c ($p<0.05$), *,**,***,**** ($p<0.05$), #,##,###,####($p<0.05$) within respective groups, 0.0, 2.0, 4.0, 6.0 and 8.0% supplemented *P. buxifolius* powder to commercial feed

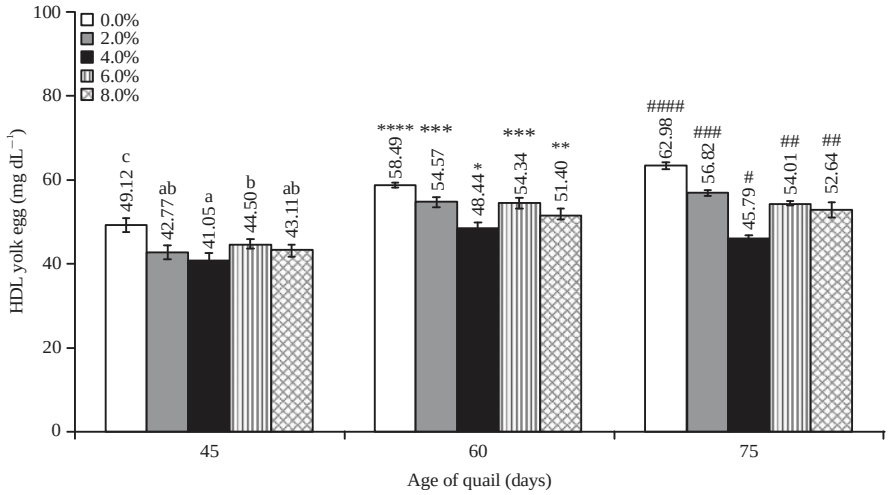

Fig. 4: Effect fed supplementation *P. buxifolius* leaves powder to commercial feed on LDL yolk egg quail at the age 45, 60 and 75 days, values and error bars represent Mean±SD (n = 5), ANOVA was followed by Tukey's test a, b and c ($p<0.05$), *,**,***,**** ($p<0.05$), #,##,###,####($p<0.05$) within respective groups, 0.0, 2.0, 4.0, 6.0 and 8.0% supplemented *P. buxifolius* powder to commercial feed

4.0% *P. buxifolius* powder (48.44±0.63 mg dL^{-1}) significantly ($p<0.05$) lower than LDL yolk egg quail which dieted commercial feed with supplemented 8.0% (51.40±1.39 mg dL^{-1}), 6.0% (54.34±1.23 mg dL^{-1}), 2.0% (54.57±0.89 mg dL^{-1}) and 0.0% (58.49±1.71 mg dL^{-1}) *P. buxifolius* powder. The LDL yolk egg with diet commercial feed with supplementation 8.0% *P. buxifolius* powder significantly ($p<0.05$) lower than LDL yolk egg quail which dieted commercial feed with supplemented 6.0, 2.0 and 0.0% but no significant ($p>0.05$) different was observed LDL yolk egg quail dieted supplementation 6.0, 2.0 and 0.0% *P. buxifolius* powder.

The LDL yolk egg at the age of quails 75 days, which dieted commercial feed with supplementation 4.0% *P. buxifolius* powder (45.79±0.48 mg dL^{-1}) significantly ($p<0.05$) lower than LDL yolk egg quail which dieted commercial feed with supplementation 8.0% (52.64±1.59 mg dL^{-1}), 6.0% (54.01±0.70 mg dL^{-1}), 2.0% (56.82±1.06 mg dL^{-1}) and 0.0% (62.98±1.26 mg dL^{-1}) *P. buxifolius* powder. No significant ($p>0.05$) difference was observed LDL yolk egg with diet commercial feed with supplementation 8.0 and 6.0% *P. buxifolius* powder but both LDL yolk egg supplementation significantly ($p<0.05$) lower than LDL yolk egg quail which dieted commercial feed with supplemented 2.0 and 0.0%.

Interleukin-1β: Supplemented powder leaves of *P. buxifolius* to commercial feed quails significantly ($p<0.05$) influenced on the expression of interleukin-1β (IL-1β) blood quails (Fig. 5) at the age of quails 45 and 75 days. The IL-1β quail at the age 45 days and fed 6.0% powder leaves of *P. buxifolius* (275.4±9.56 pg mL^{-1}) significantly ($p<0.05$) higher than IL-1β 4.0% (204.00±30.29 pg mL^{-1}), 2.0% (179,4±22.70 pg mL^{-1}) and 0.0% (81.40±7.92 pg mL^{-1}) but no significant ($p>0.05$) difference was observed IL-1β at fed 6% powder leaves of *P. buxifolius* and IL-1β at fed 8.0% (240.80±51.95 pg mL^{-1}) powder leaves of *P. buxifolius.* The IL-1β of quails at fed 4.0% powder leaves of *P. buxifolius* significantly ($p<0.05$) higher than IL-1β at fed 2.0 and 0.0%, IL-1β of quails at fed 2.0% powder leaves of *P. buxifolius* significantly ($p<0.05$) higher than IL-1β at fed 0.0% powder leaves of *P. buxifolius*.

The IL-1β quail at the age 75 days and fed 6.0% powder leaves of *P. buxifolius* (391.20±12.52 pg mL^{-1}) significantly ($p<0.05$) higher than IL-1β 8.0% (290.80±19.45 pg mL^{-1}), 4.0% (306,20±18.21 pg mL^{-1}), 2.0% (179.40±22.70 pg mL^{-1}) and 0.0% (82.20±7.40 pg mL^{-1}) powder leaves of *P. buxifolius*. The IL-1β quail at 8.0 and 4.0% powder leaves of *P. buxifolius* significantly ($p<0.05$) higher than IL-1β at 2.0 and 0.0% powder leaves of *P. buxifolius* but no significant ($p>0.05$) difference was observed IL-1β at 8.0 and 4% powder leaves of *P. buxifolius.* The IL-1β quail at 2.0% powder leaves of *P. buxifolius* significantly ($p<0.05$) higher than IL-1β at 0.0% powder leaves of *P. buxifolius*.

Nitric Oxide Synthase (iNOS): Supplemented powder leaves of *P. buxifolius* to commercial feed quails significantly ($p<0.05$) influenced on nitric oxide synthase of blood quails (Fig. 6) at the age of quails 45 and 75 days. The iNOS quail at

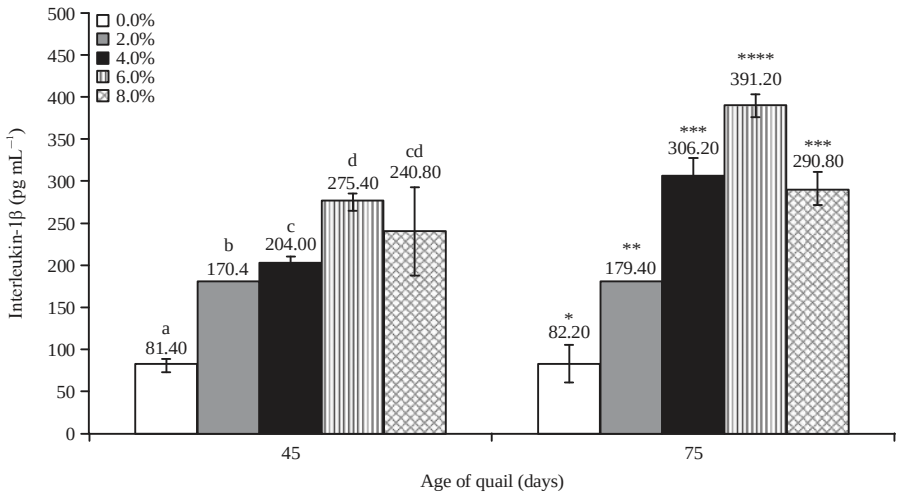

Fig. 5: Effect fed supplementation *P. buxifolius* leaves powder to commercial feed on interleukin-1β blood serum quail at the age 45 and 75 days, values and error bars represent Mean±SD (n = 5), ANOVA was followed by Tukey's test a, b and c ($p<0.05$), *,**,***,**** ($p<0.05$) within respective groups, 0.0, 2.0, 4.0, 6.0 and 8.0% supplemented *P. buxifolius* powder to commercial feed

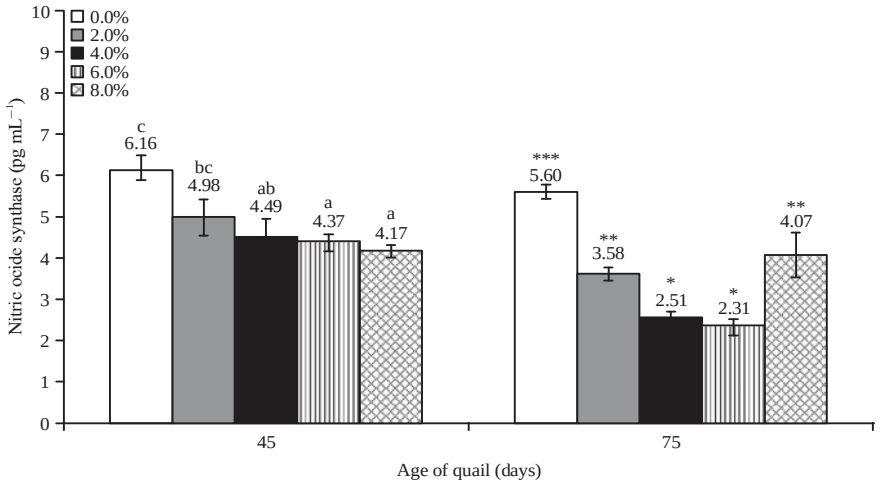

Fig. 6: Effect fed supplementation *P. buxifolius* leaves powder to commercial feed on nitric oxide synthase blood serum quail at the age 45 and 75 days, values and error bars represent Mean±SD (n = 5), ANOVA was followed by Tukey's test a, b and c ($p<0.05$), *,**,***,**** ($p<0.05$) within respective groups, 0.0, 2.0, 4.0, 6.0 and 8.0% supplemented *P. buxifolius* powder to commercial feed

the age 45 days and fed 8.0% powder leaves of *P. buxifolius* (4.17±0.17 pg mL^{-1}) significantly ($p<0.05$) lower than iNOS 2.0% (4.98±0.17 pg mL^{-1}) and 0.0% (6.16±0.28 pg mL^{-1}) but no significant ($p>0.05$) difference was observed iNOS at 6.0% (4.37±0.18 pg mL^{-1}) and iNOS at 4.0% (4.49±0.29 pg mL^{-1}) powder leaves of *P. buxifolius*. No significant ($p>0.05$) difference was observed iNOS at fed 2.0% and iNOS at 0.0% powder leaves of *P. buxifolius*.

The iNOS quail at the age 75 days and fed 8.0% powder leaves of *P. buxifolius* (4.07±0.52 pg mL^{-1}) significantly ($p<0.05$) higher than iNOS at 6.0% (2.31±0.19 pg mL^{-1}) and 4.0% (2.51±0.06 pg mL^{-1}), lower than iNOS at 0.0% (5.60±0.45 pg mL^{-1}) but no significantly ($p>0.05$) difference iNOS was observed at 6.0 and 4.0% powder leaves of *P. buxifolius*.

Total leukocyte: Supplemented powder leaves of *P. buxifolius* to commercial feed quails significantly ($p<0.05$) influenced on total leukocyte of blood quails (Fig. 7) at the age of quails 45 and 75 days. Total Leukocyte Count (TLC) of blood quail

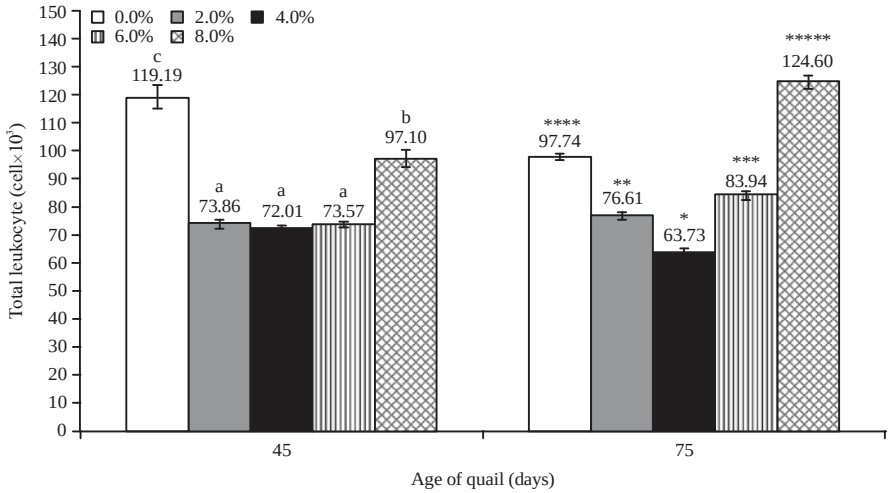

Fig. 7: Effect fed supplementation *P. buxifolius* leaves powder to commercial feed on total leukocyte blood serum quail at the age 45 and 75 days, values and error bars represent Mean±SD (n = 5), ANOVA was followed by Tukey's test a, b and c ($p<0.05$), *,**,***,****,**** ($p<0.05$) within respective groups, 0.0, 2.0, 4.0, 6.0 and 8.0% supplemented *P. buxifolius* powder to commercial feed

at the age 45 days and fed 6.0% (73.57±1.02 cell×10^3), 4.0% (72.01±1.45 cell×10^3) and 2.0% (73.86±1.04 cell×10^3) significantly ($p<0.05$) lower than TLC at 8.0% (97.10±2.77 cell×10^3 and 0.0% (119.19±3.77 cell×10^3) powdered leaves of *P. buxifolius* but no significant ($p>0.05$) difference was observed between TLC 6.0, 4.0 and 2.0% and powdered leaves of *P. buxifolius*.

The TLC of blood quail at the age 75 days and fed 4.0% (63.73±2.03 cell×10^3) significantly ($p<0.05$) lower than TLC at 8.0% (124.60±2.79 cell×10^3), 6.0% (83.94±1.55 cell×10^3), 2.0% (76.61±1.18 cell×10^3 and 0.0% (97.74±1.44 cell×10^3) powdered leaves of *P. buxifolius*. The TLC at 2% powdered leaves of *P. buxifolius* supplementation significantly ($p<0.05$) lower than 8.0, 6.0 and 0.0% powder leaves of *P. buxifolius* supplementation. The TLC at 6.0% powder leaves of *P. buxifolius* supplementation significantly ($p<0.05$) lower than 8.0 and 0.0% and TLC at 8.0% powder leaves of *P. buxifolius* supplementation significantly ($p<0.05$) higher than 0.0% powder leaves of *P. buxifolius* supplementation.

Lymphocyte: Supplemented powder leaves of *P. buxifolius* to commercial feed quails significantly ($p<0.05$) influenced on lymphocyte of blood quails (Fig. 8) at the age 45 and 75 days. Lymphocyte of blood quail at the age 45 days and fed 2.0% (49.60±1.03%) and 4.0% (47.50±0.85%) significantly ($p<0.05$) lower than lymphocyte at 8.0% (57.50±1.53), 6.0% (56.50±1.45%) and 0.0% (59.10±1.07%) in powdered leaves of *P. buxifolius* but no significant ($p>0.05$) difference was observed TLC between 2.0 and 4.0% powder leaves of *P. buxifolius*. No significant ($p>0.05$) difference was observed between TLC 8.0, 6.0 and 0.0% powdered leaves of *P. buxifolius* supplementation.

Lymphocyte of blood quail at the age 75 days and fed 4.0% (68.50±0.78%) significantly ($p<0.05$) lower than lymphocyte at 8.0% (75.30±1.99), 6.0% (74.30±1.45%), 2.0% (78.90±1.89%) and 0.0% (85.80±2.33%) in powdered leaves of *P. buxifolius*. Lymphocyte of blood quail at fed 8.0 and 6.0% significantly ($p<0.05$) lower than lymphocyte at 2.0 and 0.0% (85.80±2.33%) in powdered leaves of *P. buxifolius*. Lymphocyte of blood quail at the fed 2.0% significantly ($p<0.05$) lower than lymphocyte at 0.0% in powdered leaves of *P. buxifolius*.

Monocyte: Supplemented powder leaves of *P. buxifolius* to commercial feed quails significantly ($p<0.05$) influenced on monocyte of blood quails (Fig. 9) at the age 45 and 75 days. No detection monocyte of blood quail at the age 45 days and fed 4.0 and 6.0% powdered leaves of *P. buxifolius* supplementation. Monocyte at fed 8.0% (0.10±0.0002%) significantly ($p<0.05$) lower than monocyte at 2.0 (0.30±0.001%) and 0.0% (0.40±0.001%). Monocyte of blood quail at fed 2.0% significantly ($p<0.05$) lower than monocyte at 0.0% in powdered leaves of *P. buxifolius*. No detection monocyte of blood quail at the age 75 days and fed 4.0 and 6.0% powdered leaves of P. *buxifolius* supplementation. Monocyte at fed 8.0% (0.30±0.002%) and 2.0% (0.30±0.001%) significantly ($p<0.05$) lower than monocyte at 0.0% (0.50±0.002%) powder leaves of *P. buxifolius* but no

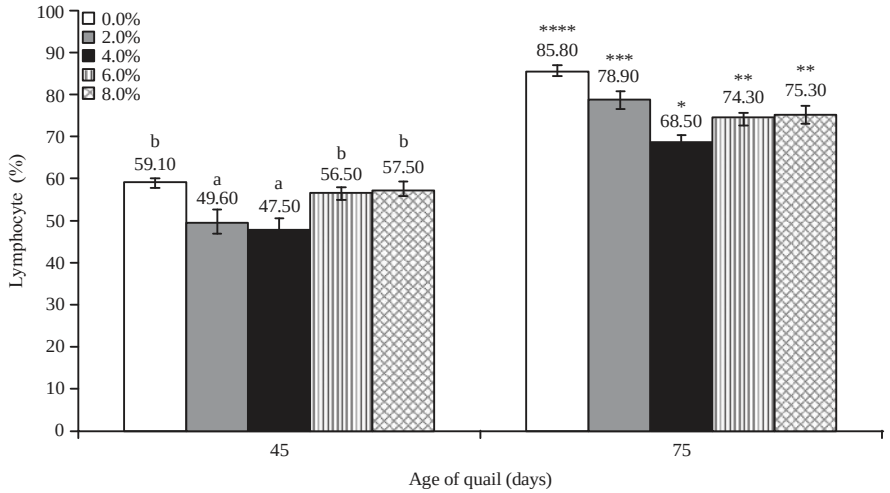

Fig. 8: Effect fed supplementation *P. buxifolius* leaves powder to commercial feed on lymphocyte of blood serum quail at the age 45 and 75 days, values and error bars represent Mean±SD (n = 5), ANOVA was followed by Tukey's test a and b p<0.05, *,**,***,**** (p<0.05) within respective groups, 0.0, 2.0, 4.0, 6.0 and 8.0% supplemented *P. buxifolius* powder to commercial feed

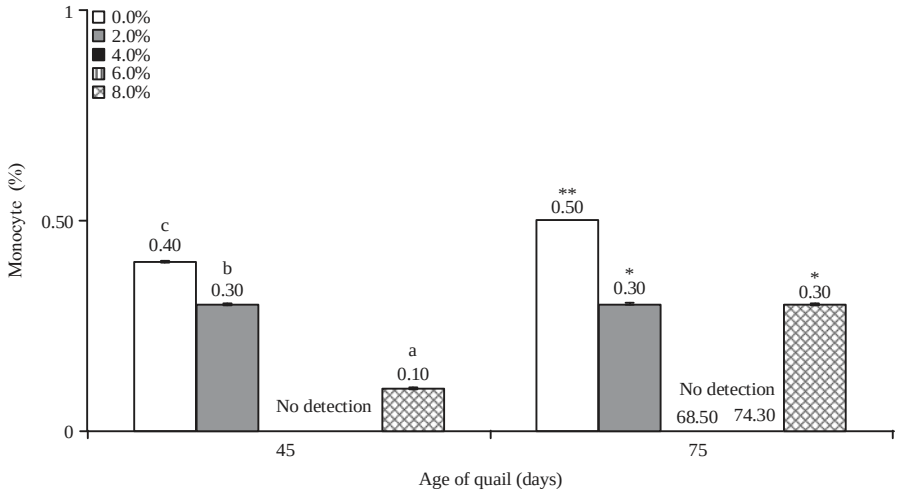

Fig. 9: Effect fed supplementation *P. buxifolius* leaves powder to commercial feed on monocyte of blood serum quail at the age 45 and 75 days, values and error bars represent Mean±SD (n = 5), ANOVA was followed by Tukey's test a and b p<0.05, *,**,***,**** (p<0.05) within respective groups, 0.0, 2.0, 4.0, 6.0 and 8.0% supplemented *P. buxifolius* powder to commercial feed

significant (p>0.05) difference was observed monocyte at 2.0 and 0.0% in powdered leaves of *P. buxifolius* supplementation.

DISCUSSION

This study indicated supplementation of leaf powder of *P. buxifolius* decrease fat and cholesterol content of yolk egg quail. A decrease in egg yolk components were allegedly due to the activity of secondary metabolites such as flavonoids, polyphenols, tannins, saponins, alkaloids, quinones and steroid triterpenoids (Sopandi, 2005; Wardah and Wurlina, 2007) contained in the leaves of *P. buxifolius*. Wardah (2011) reported that *P. buxifolius* leaves also contain soluble fiber such as pectin and high protein. Flavonoids have the capacity as an antioxidant in the body of cattle (Gonzales-Paramez *et al*., 2004) and suppressed the synthesis of fatty acids (Rodrigues *et al*., 2005). Flavonoids and polyphenols also inhibit the enzyme activity of glycerol 3-phosphate dehydrogenase (GPDH) in

adiposit (Hsu and Yen, 2007). The presence of polyphenols and flavonoids in chicken diet significantly reduced hyperlipidemia (Xia *et al*., 2010). Saponins are known to inhibit the absorption of fat by the intestine and excreted through the feces (Dong *et al*., 2007). Tannins in the digestive tract lining the walls of the small intestine so that digestion and absorption of fats do not occur (Matsui *et al*., 2006) significantly reduce hyperlipidemia (Xia *et al*., 2010). *Phyllantus buxifolius* leaf powder is also able to reduce the content of fat and cholesterol in meat broiler, giving 5% leaf powder *P. buxifolius* on broiler for three weeks before harvest significantly reducing intracellular fat, serum leptin and cholesterol levels (Wardah *et al*., 2012). Ethanol extract of powdered leaves *P. buxifolius* of 240 and 320 mg fed daily to chickens was reported to reduce levels of fat and blood cholesterol without causing infection and inflammation (Wardah and Wurlina, 2007). Powder leaves *P. buxifolius* of 5% in broiler feed capable of lowering intracellular accumulation of lipid, serum leptin levels, fat and cholesterol of meat and abdominal fat weight of broiler (Wardah *et al*., 2012).

This study also indicated supplementation leaf powder of *P. buxifolius* to commercial feed can increase the immunity of quail. The high expression of interleukin-1 (IL-1) in the quail given leaf powder of *P. buxifolius* allegedly due to decreased lipid secretion. The fall in lipid secretion allegedly due to the antioxidant ingredients in the form of secondary metabolic components of the flavonoids and tannins. The results of chemical analysis showed that the powder leaves *P. buxifolius* as a feed supplement in this study contain positives of flavonoids and tannins. The reduced secretion of lipids is expected to increase protein synthesis. Increased expression of IL-1 could be expected to lower Low Density Lipoprotein (LDL) and raise High Density Lipoprotein (HDL).

Similarly, the decline in the number of iNOS in quail given *P. buxifolius* leaf powder is also suspected due to the flavonoid the compound in leaf powder *P. buxifolius* to quail consumed as feed supplement. Giving leaf powder up to 8% is not expected to cause animal stress so that cells that express iNOS bit. This is thought to occur because there is inflammation in the body of livestock as a result of the provision of leaf powder *P. buxifolius*. In contrast, cells that stress can cause immune cells much damage, so that the cell iNOS will increase. *Phyllantus buxifolius* leaf powder also has an effect on hematological quail. Hemoglobin is a protein in red blood cells that serve to bind oxygen, if more and more leaf powder supplementation of *P. buxifolius*, then the higher hemoglobin levels, whereas, the administration of 8% leaf powder supplements cause hemoglobin levels fall. Leukocytes indicate the number of white blood cells, increasing white blood cells indicates infection in the body condition of the cattle. Thus, the calculation of platelets, which is the number of blood cells play a role in blood clotting process. The higher the value of platelets in the body of the cattle showed an infection.

Leukocytes is an important component in the immune system. The results showed that the supplementation of *P. buxifolius* leaf powder does not cause an increase in the number of leukocytes in the quail. The number of leukocytes in quail were given 2.0, 4.0 and 6.0% leaf powder of *P. buxifolius* did not differ significantly ($p>0.05$) and lower than the number of leukocytes in quail by 8.0% powder. Provision of 8.0% powder of *P. buxifolius* has increased the number of leukocytes, it is no indication that the cattle were infected, while not increasing the number of leukocytes in quail were given 2.0, 4.0 and 6.0% supplements showed that cattle do not become infected. Conversely, an increase in the number of leukocytes in the organism an indicator of infection in the body organism. The increase in the total number laukosit or white blood cells occurs when an infection.

The number of lymphocytes in the organism related to immune defense mechanisms. An increased number of lymphocytes in the body indicates that the organism occurs antibody excessive defense reaction (Doxey and Nathan, 1989). Administration of 4.0 and 6.0% *P. buxifolius* leaf powder significantly different ($p<0.05$) in increased levels of quail blood lymphocytes compared with other treatments. *Phyllantus buxifolius* leaf powder could be expected to stimulate the bone marrow, lymph and lymph glands to produce lymphocytes, so the number of lymphocytes in quail increased. However, the provision of 8.0% supplements can reduce levels of lymphocytes, indicating that the administration of 8.0% is not effectively influence the increase of antibodies in the animal body.

The results also showed that the percentage of monocytes in the blood quail were given 4.0 and 6.0% leaf powder supplements of *P. buxifolius* no detectable amounts of monocytes, these results differ significantly ($p>0.05$) with other treatments. A decrease in the percentage of monocytes occurs because the immune response involving antibodies and macrophages in the quail as a result of *P. buxifolius* leaf powder supplementation. Increased number of macrophages in the network can cause a reduction in the number of monocytes in the blood circulation (Abbas *et al*., 2011). Acute inflammation caused by infection and tissue damage may

provoke monocytes in the blood circulation moving in large numbers then headed to the damaged tissue. However, these events can also cause monocytes in the blood circulation is reduced (Abbas *et al*., 2011).

CONCLUSION

Leaf powder of *P. buxifolius* potential as feed additive for decreasing fat and cholesterol yolk egg and increasing immunity of quail at 4-6% commercial feed. The use of this medicinal plant as a commercial feed supplementation can reduce quail mortality due to viral infection and may increase the interest of the market for the consumption of qual eggs.

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