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Research Article
Effect of Noni (*Morinda citrifolia*) Fruit Flour on Antioxidant Status and Hematological Indices of Laying Japanese Quail

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Abstract

Background and Objective: Nutrition has been reported to have a huge role in maintaining the pro-oxidant-antioxidant balance. Therefore, a study was conducted to evaluate the effect of Noni Fruit Flour (NFF) on antioxidant status and hematological indices of layer quail birds. **Materials and Methods:** A total of 400, 4 weeks old quail birds were used in this study in a Complete Randomized Design (CRD). The birds were randomly assigned into four treatment groups of P0, P1, P2 and P3 with 25 birds treatment^{-1} replicated 5 times of 5 birds each. The birds in first group (P0) were given basal feed without noni, while as other groups were supplemented with 0.25% (P1), 0.50% (P2) and 0.75% (P3) NFF. **Results:** There was a non-significant ($p>0.05$) reduction in malondialdehyde (MDA) level of quails due to NFF supplementation. A linear reduction in blood MDA levels was observed with increase in level of NFF, with highest reduction (0.340 ± 0.04 nmol mL^{-1}) in group fed 0.75% NFF compared to control (0.390 ± 0.041 nmol mL^{-1}). A statistically non-significant increase in total number of erythrocytes and hemoglobin was found with increase in NFF level. Highest erythrocyte number ($3.54\pm0.395\times10^{6}$ mm^{-3}) and hemoglobin (11.81 ± 4.03 g dL^{-1}) were found in group fed 0.75% NFF. **Conclusion:** Addition of NFF in diet of quail birds had beneficial effect in reducing the oxidative stress and oxidative damage as reflected by reduced MDA levels and increased erythrocyte number and hemoglobin concentration.

Key words: Erythrocytes, hemoglobin, malondialdehyde, noni fruit, 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

Birds are subjected to various management stresses viz., heat and cold stress, vaccination, etc. This study in oxidative stress leading to an increased formation of substances known as free radicals, which in turn exert undesirable effects on tissues of the body[1]. Malondialdehyde (MDA) is the direct product of lipid peroxidation developed after radical attack and thus is an indicator of the extent of cell damage[2]. The MDA has been reported to cause fragmentation of destruction of cell membrane structure, deoxyribonucleic acid (DNA) and accelerate apoptosis[3].

Nutrition plays a huge role in maintaining the pro-oxidant-antioxidant balance[4]. Noni (*Morinda citrifolia* L.) is a native plant of Indonesia, well known for having good health beneficial effects. Noni fruit (Fig. 1) production in Indonesia continues to increase[5] in the years 2003-2009 by 1.9-16.2 t. Nagalingam *et al.*[6] and Ramesh *et al.*[7] have summarized various active ingredients present in raw and half baked noni fruit respectively as shown in Table 1. Noni is rich in glucoside, acetic acid, benzoic acid, glucose, heptanoic acid, linoleic acid, polysaccharides, potassium, scopoletin, selenium, sodium, seronine, calcium, L-arginine, iron and alanine[8]. Noni also contains vitamin A, C, B and a variety of active components such as terpenoid, damnacanthal, xeronine, proxeronine, anthraquinone, coumarin and scopoletin which are important to improve the cell function of the body[9]. Noni has been considered very important to control many diseases, particularly atherosclerosis and dyslipidemia[10]. Noni is one of the traditional medical plants which contain active antioxidant ingredients like selenium which has an important function to activate glutathione peroxidase and neutralize free radicals which attack fat molecules in the body[11,12]. Noni juice has been reported to inhibit free radicals and having antioxidant activity more powerful than vitamin C, pycnogenol and grape seed[9]. Molecular modifications in various tissues results in an imbalance between protective antioxidants (antioxidant defenses) and increased production of free radicals. This is the initial oxidative damage known as oxidative stress and noni has been reported to reduce this oxidative damage[13].

Table 1: Active components in noni fruit

Component	Noni fruit	Noni fruit (Half-baked)
Flavonoid	Much	Much
Alkaloid	Exist	Much
Terpenoid	Much	-
Saponin	Exist	Exist
Steroid	Much	Much
Tannin	Much	Much

Fig. 1: Noni fruit

Sally[14] has reported that noni has the ability to reduce blood cholesterol, triglyceride and LDL in mice but scanty reports in this regard are available. Noni juice has been reported to inhibit free radicals and having antioxidant activity more powerful than vitamin C, pycnogenol and grape seed[9].

As no literature regarding the effect of noni fruit flour on antioxidant status of quail birds was found, the present study was therefore undertaken. Further, in order to assess the nutritional status and clinical investigation, WHO recommended the evaluation of various blood parameters which provides useful information[15,16], therefore haematological indices were also evaluated.

MATERIALS AND METHODS

Methodology: The Japanese quail birds (*Coturnix coturnix japonica*) were obtained from Quail Breeding Faculty of Animal Husbandry, Universitas Padjadjaran. This study trial was conducted at the Laboratory of Poultry Nutrition, Non Ruminant and Feed Industry, Faculty of Animal Husbandry, Universitas Padjadjaran. The adaptation period of 1 week was given to the birds, following which a trial was conducted that lasted 7 weeks. The birds were offered the maintenance ration throughout the study. A total of 400, 4 weeks old quail birds were used in this study in a Complete Randomized Design (CRD). The birds were randomly assigned into four treatment groups of P0, P1, P2 and P3 with 25 birds treatment^{-1} group replicated 5 times of 5 birds replicate^{-1}. The birds in the first group (P0) were given basal feed without noni, while as other groups were supplemented with 0.25% (P1), 0.50% (P2) and 0.75% (P3) noni flour. The feed and potable water were supplied *ad libitum* throughout the experimental period of 7 weeks, with strict adherence to all the conventional management practices.

Processing of noni fruit: Noni fruit was processed as per traditional method. The unripe noni fruits were collected, washed and cut approximately into 1 cm pieces. They were then dried in hot air oven at 50°C for about 12 h until the moisture content reached below 5%. The material obtained was then ground and flour of noni fruit was obtained and used in the study.

Parameters recorded: Blood samples were randomly collected from 20 quails per replicate at the end of study. The samples were analyzed at the Laboratory of Physiology and Biochemistry, Faculty of Animal Husbandry, Universitas Padjadjaran. The parameters recorded were MDA and haematological indices viz., erythrocytes and hemoglobin levels. The MDA was estimated using a modified test method thiobarbituric acid (TBA) by spectrophotometry as per Zainuri and Wanandi[17]. About 400 mL collected blood sample was treated with 200 mL of 20% trichloroacetic acid (TCA). The vortex was then applied to the resultant mixture and afterwards centrifuged at 5000 rpm for 10 min. The supernatant formed was collected and to it 400 mL of 0.67% TBA was added. The sample was again mixed thoroughly with vortex and incubated in water bath at a temperature of 96°C for 10 min. The mixture was then removed and allowed to cool at room temperature. The absorbance was then read at a wavelength of 530 nm and MDA level was expressed as nmol mL^{-1}. The total erythrocytes and haemoglobin content were estimated using hematology analyzer (KT-6200 VET) and expressed as 10^6 mm^{-3} and g dL^{-1}, respectively.

Statistical analysis: Data collected were subjected to analysis of variance (ANOVA) as per Steel and Torrie[18] and where ever means difference existed, they were compared using Duncan's multiple range test[19] with 5% significant level.

RESULTS AND DISCUSSION

There was a significant ($p<0.05$) effect of dietary supplementation of noni fruit flour on reduction in the MDA level of quail birds (Table 2). A linear reduction in MDA levels of blood of birds was observed with increase in the level of noni fruit flour with highest reduction (0.340 ± 0.04 nmol mL^{-1}) in the group fed 0.75% noni fruit flour in the diet compared to control group (0.390 ± 0.041 nmol mL^{-1}). Following oxidative metabolism, free radicals are formed in the body, which are known as Reactive Oxygen Species (ROS). If the amount of these ROS produced exceeds beyond the antioxidant capacity (enzymatic and non-enzymatic) of the body, oxidative stress occurs. This increases the level of MDA in the blood as a sign of oxidative stress[20].

In the present study, reduction in MDA level in the blood of birds was observed, indicating that there was less oxidative stress induced as a result of dietary supplementation of noni fruit flour. Several other studies on humans have shown antioxidant activities of noni fruit both *in vitro* and *in vivo*[21,22]. Zin *et al.*[23] also reported that noni fruit possesses effective antioxidant activity. Likewise, Anantharaj *et al.*[24] also reported that noni showed antioxidant activity in crossbred calves. Gupta and Singh[25] attributed the antioxidant effect of noni to the presence of various antioxidants in it, mainly the lipid soluble ones, which might exert a profound effect on lipid peroxidation inhibition and generation of free radicals. They further concluded that antioxidant activity of noni fruit juice may be contributed by several compounds of different polarity present in it and part of the antioxidant activity may be due to lipid soluble polyphenols, α-tocopherol, β-carotene and anthraquinones. Dussossoy *et al.*[26] attributed noni's antioxidant activities to phenolic compounds, iridoids and ascorbic acid present in it.

The results of various haematological indices have been shown in Table 2. A statistically non-significant increase in the total number of erythrocytes was found with increase in the dietary concentration of noni fruit flour in the birds. Highest value of total erythrocytes $3.54\pm0.39\times10^6$ mm^{-3} was found in the group fed 0.75% noni fruit flour in the diet. A similar trend was observed in the hemoglobin content. Hazilawati *et al.*[27] reported that dietary supplementation of *M. citrifolia* resulted in normal levels of erythrocytes and haemoglobin and decreased incidence of anaemia in early stages of leukemic rats. Hematological values in birds get influenced by many factors viz., age, gender, hypoxia, environmental conditions and hormones[28]. The erythrocytes are the principal means of delivering oxygen from lungs to

Table 2: Effect on noni fruit flour on MDA level, total erythrocytes and hemoglobin content of quail birds

	Treatments			
Parameters	P0	P1	P2	P3
MDA (nmol mL^{-1})	0.390 ± 0.04	0.381 ± 0.04	0.341 ± 0.04	0.340 ± 0.04
Total erythrocytes (10^6 mm^{-3})	3.320 ± 0.37	3.450 ± 0.37	3.510 ± 0.27	3.540 ± 0.39
Hemoglobin (g dL^{-1})	9.640 ± 1.80	10.300 ± 2.91	10.580 ± 0.80	11.810 ± 4.03

body tissues by means of hemoglobin and have important role in various physiological and metabolic processes[29,30]. Moreover, Cimen[31] reported that erythrocytes are highly susceptible to oxidative damage due to high cellular concentration of oxygen and hemoglobin, a potentially powerful promoter for the oxidative processes. Thus it could be hypothesized that noni fruit flour might have reduced the oxidative stress in quail birds, which in turn decreased the oxidative damage compared to control.

Further, vitamin C has a role in inhibiting the cell death induced by oxidative stress[32] and noni has been reported to be a rich source of vitamin C, containing 32.43 mg ascorbic acid (vitamin C)/100 mL[33]. Moreover, vitamin C plays a role in accelerating the uptake of iron and its transfer via bloodstream to the bone marrow where it is used in the formation of hemoglobin[34]. Mulyawati[35] has reported that consuming iron along with vitamin C increases hemoglobin levels compared to the group fed iron without vitamin C. Thus, the presence of vitamin C in noni fruit could be hypothesized as a possible reason for improved erythrocyte count and hemoglobin content in noni fruit flour supplemented groups.

CONCLUSION

Addition of noni fruit flour in the diet of quail birds had beneficial effect in reducing the oxidative stress and oxidative damage as reflected by the reduction in MDA levels and increase in the number of erythrocytes and concentration of hemoglobin. Thus, dietary inclusion of noni fruit flour is recommended for the welfare of quail birds.

SIGNIFICANCE STATEMENTS

Since poultry birds are subjected to various management stresses which results in oxidative stress leading to undesirable effects on different tissues of body. Nutrition has been associated in having the role in alleviating the oxidative stress. Therefore, nutrition related manipulation was designed for this study. Previously, the effect of noni fruit in quail birds was not known as per the literature available. The results showed that noni fruit flour has a role in alleviating the oxidative stress of birds and it will be an important guide in future with regard to mitigating the harmful effects of oxidative stress by dietary supplementation of noni fruit flour. The future study could be focused on the impact of consumption of noni fruit fed birds on oxidative status in human population.

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