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Influence of Bamboo Leaf Extract on Welfare, Immune Response and Antioxidant Status of Bill-trimmed Mule Ducks

Eman Ahmed Negm[1*], Madeha Darwish[2], Ahmed Mohammed[2]

[1]Department of Physiology, Faculty of Veterinary Medicine, Assiut University, Assiut 71526, Egypt.

[2]Department of Animal and poultry behavior and management, Faculty of Veterinary Medicine, Assiut University, Assiut 71526, Egypt.

***Correspondence**
Corresponding author: Eman Ahmed Negm
E-mail address: Emannegm@vet.aun.edu.eg

Abstract

It has been indicated that bamboo leaf extract (BLE) displays physiological activities in mammals. The goal of this research was to evaluate the effects of different doses of dietary supplementation of BLE on behavior, inflammation and antioxidant activity of bill trimmed (BT) mule ducks. One hundred- and twenty-one-day-old mule ducklings were randomly divided on 24 floor pens (5 birds per pen), each pen was assigned at random to one of four dietary treatments that each contained the following: a BLE at 0 (Control), 0 (BT-control), 1.0 (BT-BLE1), and 2.0 (BT-BLE2). At 21 d, all birds were exposed to bill trimming except control group. There was a significant rise in serum TNF-α, INF-γ, IL-6, MDA and homocysteine levels while there was a significant drop in the level of TAC in BT- control group as opposed to the control. Both doses of bamboo leaf extracts significantly decreased TNF-α, INF-γ, IL-6, MDA and homocysteine while significantly enhanced TAC activities in comparison with BT- control group, moreover, BT-BLE2 was more efficient in repairing all blood parameters measured than that of BT-BLE1 group. However, there were no significant differences in the gait score, tonic immobility, and stride length tests between all groups. These results suggest that the BLE could inhibit the negative effects of bill trimming on mule ducks health through improvement of immunity and antioxidant status, regulation of inflammatory reactions with a reduction in homocysteine level.

KEYWORDS
Bill trimming, Mule ducks, Bamboo leaf extract, Homocysteine, Cytokines, behavior, Antioxidant status.

INTRODUCTION

Feather pecking and cannibalism are major problems in the poultry business, leading to stress and financial losses, so, beak trimming is practiced globally to lessen feather damage, skin pecking injuries and death in poultry. Beak trimming is the cutting off roughly one-quarter to one-third of both upper and lower beak or of the upper beak only. There are numerous techniques for beak trimming either by using hot blade, mechanically, infra-red or electrically. Even though beak trimming is done as part of a larger plan, it causes some welfare issues like pain and sensory loss (Riber and Hinrichsen, 2017). Moreover, Beak cutting slows growth and causes significant behavioral changes in birds, such as decreased meal intake, activity, pecking frequency, and power (Janczak and Riber, 2015). The practice of beak trimming caused tension and discomfort, particularly when done aggressively, and this influences feed consumption in days following the beak trimming, may be due to heightened sensitivity resulting from the wound (Gentle, 2011).

Without having an impact on welfare or body weight, bill cutting with scissors effectively decreased aggressive pecking behaviour (Elshafaei *et al*., 2007). Meanwhile, trimming with hot blade considerably decreased aggressive pecking without affecting welfare but effectively declined body weight and activities such as feeding and drinking (Marchant-Forde *et al*., 2008; Lagana *et al*., 2011; Na-Lampang, 2012). In contrast, Guesdon *et al*. (2006) stated that bill trimming by hot blade enhanced body weight gain in laying hens. On the other hand, Sengul *et al*. (2015) reported that neither live weights nor carcass weights of large white turkeys were affected by beak trimming.

Exposure of broilers to artificial or natural stresses, stimulates reactive oxygen species (ROS) formation and inflammatory process generation after beak trimming leading to morphological and/or physiological malfunctions (Voslarova *et al*., 2013). Small protiens called pro-inflammatory cytokines such as interleukin IL-6, tumor necrosis factor (TNF)-α, IL-8, IL-1β and interferon (IFN)-γ are generated from several tissues in response to pathological or physiological stress (Ramadori and Armbrust, 2001). Also, severe release of malondialdehyde (MDA) and other free radicals under stress causing that radicals overcome the antioxidant defense system leading to oxidative stress that could be measured by the total antioxidant capacity (TAC) (Khajehnasiri *et al*., 2013).

One of the most popular and practical strategies to reduce the negative effects of stress is through dietary changes. Antibiotics have been used since its discovery near 1950 as a growth enhancers to make broiler chickens perform better but its repeated usage in poultry ration led to many defects like buildup of antibiotics residue in environment and animal products, resistance of pathogen to antibiotics and imbalance of beneficial microflora in intestine (Nair *et al*., 2018; Ben *et al*., 2019). So, many nations have imposed outright bans or reduction on antibiotics usage in the poultry and livestock industries. Since 2006, antibiotics have

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been banned as growth stimulants in animal diet in the European Union, while the usage of alternative additives (non-antibiotics) have expanded (Alagawany *et al*., 2018).

Plant extracts were consistently used to aid in bird growth and help in disease resistance as it has growth-promoting, antibacterial effects (Kumari *et al*., 2007), immunomodulatory (Fakeye, 2008) and anti-inflammatory properties (Castaldo and Capasso, 2002).

Bamboo plant is widely distributed around the world, since long ago, bamboos and bamboo extract have been utilized in Korea to treat paralysis, sweating, and hypertension. Bamboo extract has been shown to have anti-inflammatory and antioxidant effects (Hu *et al*., 2000 and Jung *et al*., 2005). Also, bamboo leaf extracts (BLE) have been used in China for human medicinal and culinary purposes having a variety of biological functions, such as neutralizing oxygen radicals and boosting immunity, antibacterial, antiviral, and anticancer effects. In addition, they are frequently utilized in medication, cosmetics, anti-aging goods and in human nutrition (Rajendran *et al*., 2004 and Kim *et al*., 2016), However, its applications and functions in raising birds and animals are restricted.

Moreover, Singhal *et al*. (2011) revealed that Bamboo leaf has a high nutritional value and contains essential basic components including ash, crude fibre, crude protein with other minerals as copper, calcium, manganese, magnesium,zinc, potassium, sodium and phosphorous. Interestingly, it was reported that active substances are present in BLE such as flavones, amino acids, glycosides, and phenolic acids, which together provide skin-soothing and astringent properties (Shen *et al*., 2019b).

The production efficiency and meat quality are the primary foci of the existing study on BLE in broilers under normal conditions (Zhang *et al*., 2014); limited research has been conducted on behavior, bone health and biochemical changes in bill trimmed mule ducks. Therefore, the aim of this experiment was to investigate the influence of two concentrations of bamboo leaf extract in ration on welfare and some physiological parameters related to immune response and antioxidant status of bill trimmed mule ducks. We hypothesized that the dietary supplementation of bamboo leaf extract will promote welfare, immune response, anti-inflammatory effect and antioxidant status of bill trimmed mule ducks.

MATERIALS AND METHODS

All processes in the current research were carried out in animal and poultry behavior and management research unit in the Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt. All precautions for using and/or dealing with laboratory animals were taken into consideration and the Ethics Committee of Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt, according to The OIE standards (experiment No. 06/2023/0067).

Bamboo leaf extract

It was brought from Zhejiang Xin Huang biotechnology Ltd, Zhejiang, China and the main contents were polyphenols, silica, crude protein, flavonoids and polysaccharides.

Birds and housing

At one day old, 120 mule ducklings were obtained from a commercial hatchery (El-Salam Company, Cairo, Egypt). The mule ducklings were weighed and separated with nearly same weight into groups of 5 birds each and allocated in to 1 of 24 floor pens (100×100 cm). The lighting program was set to be 30 lx for 8h dark: 16h light during the period of experiment (60 day) (Abdel-Hamid and Abdel-fattah, 2020). The environmental temperature was measured using a digital thermometer that placed at the bird's back. It was fixed at around 32-34°C during the first week before dropping by 3-5°C each week until the fully feathered of ducklings were done at 4 weeks, when it reached 19-20°C (Sari *et al*., 2013). A thermos hygrometer mounted on the wall was used to measure the relative humidity inside. There was a 60 to 70% relative humidity range on average (Coates *et al*., 2000).

Dietary treatments

Four dietary treatments and a thoroughly randomized design were used in the trial. There were six replicate pens (5 birds/pen) for each treatment. The experimental groups were designed as follows, (control) treatment 1: birds were fed basal diet only without bill trimming. Birds of treatment 2 (BT- control): birds were bill-trimmed and fed with the basal diet. Treatment 3 and 4 (BT-BLE1, BT-BLE2): bill-trimmed birds were fed with basal diet added with 1 and 2 gkg^{-1} BLE from day 1 of age till the end of the research, respectively.Small batches of the relevant amounts of basal diet and BLE were mixed before being combined with bigger batches of the appropriate amounts of basal diet until the entire amounts of the various diets were well blended. They were fed with duck mash 17% from day 1 to 8 weeks of age. It was produced by El-Salam Company, Assiut, Egypt (Table 1).

Table 1. Ration formulation of base diet.

Chemical analysis	Finisher diet
Raw protein %	17
Raw fat %	4.15
Crude fiber	3.8
Energy kcal	3000
Component	
Yellow corn %	---
Soya bean meal %	47%
Soya bean oil %	7.5
Mono calcium phosphate %	---
Limestone %	1.3
Food salt %	0.4
A mixture of vitamins and minerals salts %	0.27
Choline %	0.3
DL-methionine %	0.27
L Lysine hydrochloride %	---
Gluten	---

The ration formulation was produced by EL-salam Company Feed Mill. (Assiut, Egypt), and the treatments were the regular diets supplemented with 0 (control), 0 (BT- control), 1 (BT-BLE1), and 2 (BT-BLE2) g kg^{-1}probiotic, respectively. BT- control, BT-BLE1, BT-BLE2 were exposed to bill trimming.

Bill trimming

At 21 days, all birds were subjected to catching, handling and by using LYON beak trimmer (LYON Technologies Inc, CA, USA) about 0.51 cm of their maxilla were trimmed by using hot searing method, except control birds, they were sham trimmed. Ducks that were sham trimmed were similarly caught and held, and then moved near the bill trimmer but we did not cut the bill to control effect of handling (Gustafson *et al*., 2007).

Physiological parameters

At 60 day of age, a 5 mL blood was taken (1 bird per replicate 6 birds per treatment) euthanized by jugular vein cutting and allowed blood to flow nearly for 2 min. Blood sample was collected into a serum separator tube free from anticoagulant and keep for 2 – 3 h to clot at room temperature, centrifuged at 3000 r.p.m for 10 minute and then serum separated to another Eppendorf tube by micropipette and stored at -80°C until the analyses (Parga *et al*., 2001).

ELISA was used (Dynatech Microplate Reader Model MR 5000, 478 Bay Street, Suite A213 Midland, ON, Canada) to detect serum inflammatory marker tumor necrosis factor-alpha (TNF-α), interferon-gamma (INF-γ), interleukin-6 (IL-6) and homocysteine concentrations by ELISA kits from SinoGeneClon Biotech Co., Ltd, No.9 BoYuan Road,YuHang District 311112, Hang Zhou, China.

Serum lipid peroxide Malondialdehyde (MDA) and total antioxidant capacity (TAC) levels were measured using Reagent kits bought from Biodiagnostic, Giza, Egypt.

Behavioral tests

Gait Score

At the end of the experiment, two ducklings per cage (12 ducklings per treatment) were taken and 3-point gait score system was utilized to examine the walking ability (Zero = Ordinary gait, one = Apparent sickness in the gait, and two = Serious sickness in the gait) as illustrated previously (Mohammed *et al*., 2021).

Tonic immobility test (TI)

TI was done in a different room having the same situations as the bird room. The bird was resting in a U-shaped cradle on its back. Then, the bird was held with one hand on its sternum for five seconds while the other held the head and neck. If the chick continued to move after the hand pressure was progressively withdrawn, another induction period was started until the movement. Stopwatch was begun after removal of the hands pressure. After that, the experimenter left, moving away from the bird. Retraining was repeated if the bird righted itself in less than 10 seconds. TI length was deemed to be 0s if TI was not caused after three trials, while the birds were taken from the cradle after 600s if no attempt was made to right themselves (Mohammed *et al*., 2021).

Stride length

A special wooden pole was prepared, and it was used as a run (6×3 m). Then we filled the run with fine sand, and water was sprinkled on it. Each duckling was allowed to move along the run, and we measured the distance between the heels (distal end of the claw) of the left and right footprint (Cooper, 2007).

Statistical analysis

Pens (n = 6/treatment) were used as the experimental units in a randomized block design. The data were reported as mean ± standard error (SEM). The data were analyzed using Prism software (version 8.0.1; GraphPad Software, Inc San Diego, CA, USA), one-way analysis of variance (ANOVA) subjected to the Bonferroni's Multiple Comparison Test. Statistics showed that differences with $p < 0.05$ were significant.

RESULTS

The effect of dietary supplementation of bamboo leaf extract (BLE) on serum TNF-α, INF-γ, IL-6, MDA, TAC and homocysteine activities of bill trimmed mule duck

There was a significant increase in the levels of TNF-α, INF-γ, IL-6, MDA and homocysteine ($P < 0.001$) while there was a significant decrease in the level of TAC ($P < 0.001$) in BT-control group compared to control one (Table 2).

As shown in Table 2, BT-BLE2 significantly lowered TNF-α, INF-γ, IL-6, MDA, and homocysteine ($P < 0.001$) levels in comparison the BT- control birds. While BT-BLE1 significantly lowered IL-6, and homocysteine ($P < 0.001$), TNF-α, MDA ($P < 0.01$) and INF-γ ($P < 0.05$) levels in comparison the BT- control birds. At the same time, there levels (TNF-α, INF-γ, IL-6, MDA, and homocysteine) were decreased in BT-BLE2 than in BT-BLE1 birds ($P < 0.05$).

Table 2. Effect of dietary supplementation of bamboo leaf extract (BLE) on serum TNF-α, INF-γ, IL-6, MDA, TAC and homocysteine activity of bill trimmed mule duck.

Treatment	Control	BT-Control	BT-BLE1	BT-BLE2	P value
TNF-α (ng/L)	486.3 ± 18.85[a]	705.8 ± 5.78 [b]	619.0 ± 1.53 [c]	563.8 ± 9.58 [d]	0.00
INF-γ (pg/ml)	154.9 ± 8.21[a]	361.2 ±18.11 [b]	291.9 ± 4.49 [c]	226.8 ± 12.54 [d]	0.00
IL-6 (ng/L)	96.7 ± 1.5 [a]	136.5 ± 0.50 [b]	114.0 ± 1.89 [c]	105.3 ± 1.42 [d]	0.00
MDA (nmol/ml)	8.4 ± 0.09 [a]	10.3 ± 0.12 [b]	9.6 ± 0.12 [c]	9.0 ± 0.09 [d]	0.00
TAC (mM/L)	1.3 ± 0.07 [a]	0.7 ± 0.03 [b]	0.9 ± 0.02 [c]	1.1 ± 0.09 [d]	0.00
Homocysteine (mM/L)	8.63 ± 0.41 [a]	16.00 ± 0.29 [b]	11.67 ± 0.09 [c]	10.23 ± 0.15 [d]	0.00

TNF-alpha: Tumor necrosis factor-alpha; INF-gamma: Interferon-gamma; IL-6: Interleukin-6; MDA: Malonaldehyde; TAC: Total antioxidant capacity; BT: bill trimmed; BLE: bamboo leaf extract.

Data are presented as Mean ± SE. Values in the same row followed by different superscript ([a, b, c, d]) are significant ($P < 0.05$).

Table 3. The dietary supplementation of bamboo leaf extract effects on gait score.

Treatment	Control	BT-Control	BT-BLE1	BT-BLE2	P value
Gait Score					
0	60.66±10.26	44.16±14.04	60.83±15.91	83.16±11.46	0.25
1	33.00±8.52	49.66±14.24	38.66±15.87	16.5±11.27	0.35
2	5.50±5.50	5.50±5.50	-	-	0.58

Data are presented as Mean ± SE. Values in the same row are not significantly different ($P > 0.05$). BT: bill trimmed; BLE: bamboo leaf extract.

TAC level was higher in BT-BLE1 and BT-BLE2 in comparison the BT- control birds (P < 0.05 and P < 0.001, respectively); at the same time, TAC level increased in BT-BLE2 than in BT-BLE1 birds (P < 0.05).

Leg health parameters

Gait score

The dietary supplementation of bamboo leaf extract effects on gait score is presented in Table 3. The gait score was not influenced by the dietary supplementation of bamboo leaf extract regardless of the dose (P > 0.05).

Tonic immobility

The dietary supplementation of bamboo leaf extract effects on tonic immobility is shown in Figure 1. The tonic immobility test was not affected by the dietary supplementation of bamboo leaf extract regardless of the dose (P > 0.05).

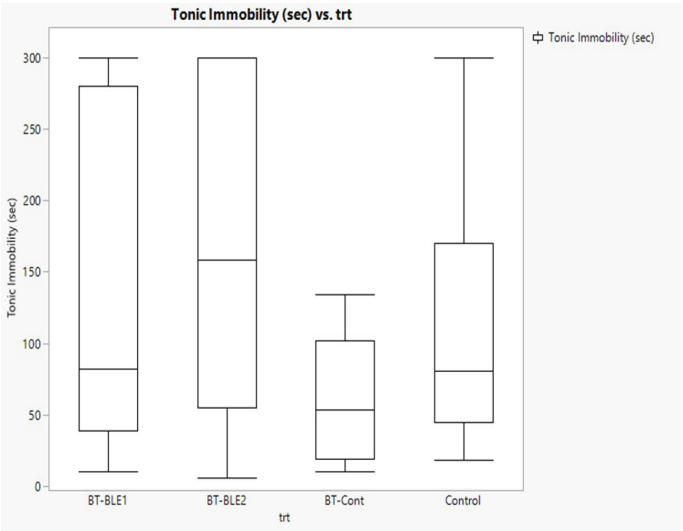

Fig. 1. The dietary supplementation of bamboo leaf extract effects on tonic immobility.
Data are presented as Mean ± SE. Values are not significantly different (P > 0.05). BT: bill trimmed; BLE: bamboo leaf extract.

Stride length

The dietary supplementation of bamboo leaf extract effects on stride length is presented in Figure 2. The stride length was not affected by the dietary supplementation of bamboo leaf extract regardless of the dose (P > 0.05).

DISCUSSION

Beak trimming is a common practice in chicken business to reduce cannibalism, aggression, and feather pecking. The beak is a sophisticated functioning organ with an many sensory receptors and numerous nerves supply. Beak trimming lead to anatomical, physiological, biochemical changes and pain (chronic, acute, or both) due to nerve injury and tissue damage depending on genetic-, lesion-, and age factors (Cheng, 2006).

Our results showed that BT significant increase in serum TNF-α, INF-γ, IL-6, MDA and homocysteine concentrations while significantly decrease the level of TAC in comparison with control group. That is consistent with the findings of Ding *et al.* (2023) who said that BT cause physiological changes due to induction of inflammatory process and stress, demonstrated that the levels of the inflammation-related indices IL-1β, TNF-α, IL-6 and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) were enhanced significantly in the serum of BT chickens referred that to the up-regulated expression levels of spleen IL-1β, MyD88 and NF-κB which stimulated the generation of inflammatory factors.

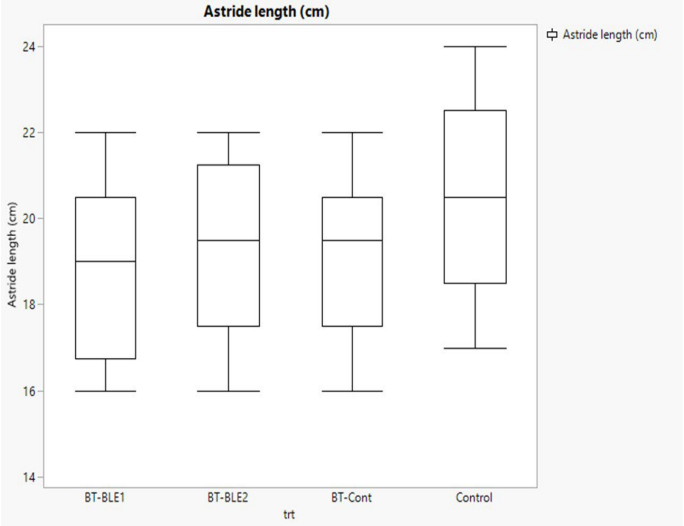

Fig. 2. The dietary supplementation of bamboo leaf extract effects on stride length.
Data are presented as Mean ± SE. Values are not significantly different (P > 0.05). BT: bill trimmed; BLE: bamboo leaf extract.

Moreover, Cheng (2006) explained that following beak trimming, inflamed tissues may produce adenosine triphosphate (ATP), bradykinin and prostaglandins, cytokines as interleukin IL-6, IL-1, histamine, tumor necrosis factor-alpha (TNF-α), and neurotransmitters such as calcitonin-gene related peptide and substance P from injured nerve endings causing behavioral and physiological changes that may stay for many weeks or months.

Also, stress stimulates the excessive production of ROS (reactive oxygen species), MDA with decreasing in TAC levels by inhibiting the electron transport apparatus in the mitochondrial membrane leading to oxidative stress status (Mujahid *et al.*, 2005; Pan *et al.*, 2018).

Nowadays, bamboo leaf extract is excessively utilized in human cosmetics, medicine and food. Also, bamboo shoots provide a multitude of health benefits, including healing cardiovascular diseases, boosting appetite and digestion, anti-inflammatory and antioxidant activities (Tundis *et al.*, 2023). Otherwise, it has not been used in the poultry production. The findings of using the bamboo leaf extract in human medicine have brought great interest for using it as dietary supplement to enhance bone integrity and consequently welfare in bile trimmed mule ducks.

In this research BLE on its two concentration doses (BT-BLE1 and BT-BLE2) succeeded significantly to lower TNF-α, INF-γ, IL-6, MDA, and homocysteine levels while significantly increase TAC in comparison with the BT- control birds. At the same time, the repairing effects in BT-BLE2 birds were better than that in BT-BLE1 birds at all parameters.

These results agree with Shen *et al.*, (2019a) and (2019b) who recorded that BLE supplementation in broilers linearly upgraded the total antioxidant capacity by enhancing glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), catalase (CAT) mRNA and glutathione S-transferase gene expression in breast meat, liver and serum while reduced MDA levels compared to control birds. Explaining that to its active substances containing polysaccharides, flavonoids and polyphenols which have immune regulation, antioxidant, anti-inflammatory and lipid-lowering effects. Besides its ability to stimulate the pathway of nuclear factor erythroid 2-related factor 2 (Nrf2) gene expression to reduce lipid oxidation and boost breast meat's antioxidant capacity since Nrf2 plays a crucial role in cells' reactions toward oxidative stress. Additionally, flavonoids improve the microbiota populations in

the gut, which have been shown to significantly improve well-being and production in birds by regulating their immune systems, preventing bacterial colonization, and fostering digesting and detoxification (Wei *et al*., 2013; Dragana *et al*., 2014).

Moreover, Higa and Panee (2011) discovered that bamboo extract prevents lipotoxicity-induced IL-6 overproduction in adipose cell lines and muscle by blocking of both AP-1 and NF-κB pathways, the two main transcriptional regulatory mechanisms for IL-6. Also, Choi *et al*. (2013) said that treatment with BLE caused inhibition of 63% monocyte adhesion in TNF-α-activated human umbilical vein endothelial cells (HUVECs) and about inhibition of 50% interleukin-6 released from lipopolysaccharide-stimulated monocyte. In addition, Zhao *et al*. (2013) approved that bamboo salt declined the serum proinflammatory cytokines levels as interferon (IFN)-γ, interleukin (IL)-6 and tumor necrosis factor (TNF)-α preventing hepatic damage in Sprague-Dawley rats that done by carbon tetrachloride. Revealed that the unique combination of high minerals content (potassium, calcium, manganese, and magnesium) of bamboo salt and its antioxidant properties showing a highly anti-inflammatory action. implying a possible use for this natural substance as a cheap anti-inflammatory nutraceutical.

So, BLE decreased homocysteine concentration, may be due to that bamboo plant is rich in minerals (Ca, Ph, Mn, mg, Na, K and Fe) and vitamins (thiamine B1, niacin B3, vitamin A, B6, and E) (Nongdam and Tikendra, 2014) as high homocysteine levels mean that there is a vitamin deficiency. Homocysteine is an amino acid containing sulfur group and formed from the metabolism of methionine, an essential amino acid. Vitamins B12, B9 (folate) and B6 break homocysteine down to form other chemicals your body needs (Stehouwer and Guldener, 2005). Hyperhomocysteinemia is an abnormally status of a high serum homocysteine level that has been suggested to be a significant risk factor for a variety of disorders, such as fractures or thrombosis (Van Meurs *et al*., 2004). Many of the major diseases accompanied by high homocysteine level are linked to oxidative stress as it enhances free-radical and oxidative damage to protein (Sibrian-Vazquez *et al*., 2010).

The fast-growing meat birds, involving ducks, demands skeletal strength; but rapid growth may not be conducive to sufficient bone integrity (Robison *et al*., 2015). Bone health problems, include lameness, harm ducks welfare and health growth, as well as production performance (Reiter and Bessei, 2009). Our results indicate that the dietary BLE supplementation had no effect on leg health indicators including gait score, tonic immobility, and stride length tests. Inability to find any influences of the dietary supplementation of bamboo leaf extract could be linked with various factors, like ducklings' age, time of bill trimming, the bamboo leaf extract concentration, and the dietary supplementation period when the previously mentioned parameters were performed.

CONCLUSION

The current results indicated that dietary supplementation of the BLE significantly improves the antioxidant status and the stress response. The current findings could suggest that the use of the BLE, especially at 2.0 g/kg level, could be a useful management strategy for improving welfare and health of bill trimmed mule ducks. Further research is needed to estimate the accurate dose of BLE in the diet.

CONFLICT OF INTEREST

The authors affirm that they do not have any competing interests.

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