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Effect of dietary supplementation of bamboo leaves extract on behaviors, performance and some blood parameters of bill-trimmed mule ducks

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ABSTRACT

Bamboo leaf extract (BLE) has been shown to exhibit physiological actions in mammals. So, the objective of this study was to investigate the effects of dietary supplementation of BLE on performance, behavior, stress indicators, antioxidant activity, and liver and kidney function of bill trimmed mule ducks. One hundred- and twenty- mule ducklings (one day old) were randomly distributed among 24 pens (5 birds / pen), each pen was randomly assigned to one of four dietary groups containing a BLE at 0 (CONT), 0 (BT-CONT), 1.0 (BT-BLE1), and 2.0 (BT-BLE2). Except for the CONT group, all birds were exposed to bill cutting on day 21. Serum corticosterone was analyzed by ELISA kits and other serum physiological parameters were assayed by spectrophotometer. Bamboo leaf extract decreased wall pecking, feather pecking, and aggressive pecking behavior and improved preening ($P < 0.05$) but had no effect on feeding, drinking, walking, standing and resting activities ($P > 0.05$). Serum catalase, superoxide dismutase (SOD), total protein and alkaline phosphatase (ALP) were higher in BLE fed groups and CONT birds than BT-CONT groups ($P < 0.05$). However, there were no significant differences in performance parameters, serum albumin, urea, creatinine, and glucose between all the treatments ($P > 0.05$). Moreover, serum corticosterone was lower in BLE fed groups in comparison with the BT-CONT ($P < 0.05$). These findings imply that the BLE may be able to mitigate the detrimental effects of bill trimming on the health of mule ducks through the regulation of stress reactions, and improvement of antioxidant status.

Introduction

Cannibalism and feather pecking are major behavioral issues that have a negative impact on duck welfare. Moreover, cannibalism outbreaks in duck have been observed early at thirteen days old, and they appear to be linked to the emergence of the first feathers (Knierim *et al*., 2005). Due to the unpleasant and damaging nature of feather pecking and cannibalism, both of which can cause death and economic losses due to the presence of nociceptors in the beak tips. So, duck producers usually trim their birds' beaks and claws within the first three weeks of life (Hester and Shea-Moore, 2003). Beak trimming is a one of the common management technique aimed at lessening the extent of harm caused when bird pecks the skin or the feathers of each other's by removing the sharp points of its lower and upper mandibles (Nicol, 2018). There are several different methods of beak trimming like mechanical, hot blade, electrical and infrared (American veterinary Medical Association, 2010). Additionally, it causes significant behavioral changes such as decreased meal intake, pecking duration, force, and activity, as well as growth rate reductions (Janczak and Riber, 2015). The act of trimming beaks can be uncomfortable and tense, especially if done severely. This can affect the amount of feed consumed in the days following the trimming, especially because of the increased sensitivity of wound (Gentle, 2011). Scissor-trimmed bills effectively decreased aggressive behavior while not affecting welfare or body weight, according to El-shafaei *et al*. (2017). In the meantime, body weight and aggressive pecking was effectively decreased by hot blade cutting without compromising welfare. Beak trimming also decreased the amount of time spent in eating and drinking (Marchant-Forde *et al*., 2008). Moreover, Lagana *et al*. (2011) and Na-Lampang (2012) found that hot blade trimmed bird had a significant decrease in body weight. On the other hand, Guesdon *et al*. (2006) discovered that laying hens' body weight increased when their bills were trimmed with a hot blade. However, Sengul *et al*. (2015) found that beak reduction had no effect on the carcass, live weights, or carcass % of large white turkeys.

Following beak trimming, an inflammatory process occurs that triggers physiological changes, including a rise in corticosterone secretion (Cheng and Muir, 2007; Voslarova *et al*., 2013). Additionally, the production of peptides implicated in eating and food intake are negatively impacted by elevated blood corticosterone levels (Liu *et al*., 2012). It also contributes to behavior changes, in the process of maintaining organic homoeostasis and modifications in the metabolism of lipids, proteins, and carbohydrates (Davis *et al*. 2004). In studies on stress in birds, the heterophil to lymphocyte (H/L) ratio has been shown to be a useful metric (Post *et al*., 2003). Heterophilia induced increases in H/L ratios have been described as a sign of continuous stress (Davis *et al*. 2000). Also, El-Kazaz (2015) discovered that beak-trimmed birds had a H/L ratio that was significantly higher than that of non-trimmed birds.

Bamboo is a plant that is found all over the world. In China, its leaves are utilized in cooking and medicine (Hu *et al*., 2000). Multiple biological actions of bamboo leaf extracts (BLE) have been confirmed, including scavenging oxygen radicals, boosting immunity, and having antiviral, anticancer, and antibacterial properties (Rajendran *et al*., 2004; Kim *et al*., 2016). Although they have been extensively utilized in human feed, anti-aging goods, medications, and cosmetics, their applications in animal production are restricted (Hu *et al*., 2000).

Interestingly, it was reported that BLE contains active ingredients such as flavones, amino acids, glycosides, and phenolic acids, which offer astringent and skin-soothing qualities (Shen *et al*., 2019). A higher silica content promotes the formation of collagen, which gives skin a more radiant and healthy appearance. It also boosts connective tissue, strengthens bones, improves skin suppleness, aids wound healing, also flexibility of joint prevents hair damage, enhances its growth, and relieves eczema. Silica (found in bamboo) serves as glue, sitting inside collagen and giving connective tissues strength, flexibility, and durability (Vasave *et al*., 2019). Moreover, silica creates connections among the protein particles

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that give the skin its inherent capacity to hold onto water, which is critical for cell regeneration and repair. Silicon boosts cell hydroxyproline levels, a crucial amino acid for elastin and collagen formation. Moreover, broiler hens treated with BLE at a dose of 2.0 g/kg showed an increase in the weight gain compared to control ones, increases in BLE dosage resulted with improvements in an average daily weight gain and feed intake, at the same time, feed conversion ratio was significantly decreased (Shen *et al*., 2019).

In addition to the effect on healing of wounds, BLE can increase the antioxidant activity; it significantly increased serum catalase and glutathione peroxidase activity in birds (Shen *et al*., 2019). Chu *et al.* (2013) found that bamboo charcoal increased total protein in fattening pigs. Moreover, Yakubu *et al*. (2009) found that serum ALP of pregnant rabbits fed 250 and 500 mg/kg of aqueous extract of *Bambusa vulgaris* leaves was increased in comparison with control groups. In addition, dietary supplementation of BLE lowered urea nitrogen content in serum of broilers (Shen *et al*., 2020).

BLE research in poultry were restricted on the production performance, immunity and meat quality of broiler chickens under normal conditions (Zhang *et al*., 2014). Research focused on the behavior, anti stress and antioxidant properties of mule ducks is lacking. Although multiple researches reported that BLE is able to affect the metabolism of broiler chickens, the effect of BLE on kidney and liver functions of mule ducks is still not clear. Therefore, This study aimed to determine the effects of two different BLE concentrations on behavior, perfomance, the modification of some stress indicators, antioxident activity and liver and kidney functions of bill trimmed mule ducks.

Materials and methods

All procedures in this study were done in Animal and Poultry Behavior and Management Research Unit at Assiut University, Faculty of Veterinary Medicine, Assiut, Egypt, during the period from March to May (2021). All safety measures for using and/or handling laboratory animals were considered, and the experimental design was approved by the Ethics Committee of Faculty of Medicine, Assiut University (experiment No. 17101934).

Bamboo leaf extract was obtained from China, Zhejiang Xin Huang biotechnology Ltd, Zhejiang, contained flavonoids, polysaccharides and polyphenols.

Housing and birds

One day old mule ducklings (El-Salam Company, Cairo, Egypt) were procured from a commercial hatchery, divided into 1 of 24 floor pens (100×100 cm), each 5 birds with nearly same body weight. The program of lighting was fixed at 30 lx for 16h light: 8h dark for the whole experimental period, which lasted for 60 days (Abdel-Hamid and Abdel-Fattah, 2020). A digital thermometer was used to record the ambient temperature at a bird's back level. The temperature was kept at roughly 32–34°C for the first week, then dropped by 3–5°C each week till reached 19–20°C at the end of the fourth week as the ducklings were fully feathered (Sari *et al*., 2013). A wall-mounted thermo hygrometer was used to determine the humidity level inside the space. The range of the humidity was 60% to 70% (Coates *et al*., 2000).

Dietary treatments

Four treatments, each involved six replicate pens (5 birds/pen). Groups were as follow; birds of treatment 1 (CONT) were fed basal diet only without bill trimming. Birds of treatment 2 (BT-CONT) were fed with the basal diet and subjected to bill-trimming. Birds of treatment 3 and 4 (BT-BLE1, BT-BLE2) were fed on basal diet supplemented (1 and 2 gkg^{-1}), and bill-trimmed from the first day of age until experiment ended.

From the first day of life to eight weeks of age, birds were fed 17% duck mash that was supplied by El-Salam Company, Assiut, Egypt (Table 1).

Table 1. Ration formulation of base diet[1].

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	---

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

Bill trimming

After 21 days, every bird was captured, handled, and had a beak trim except the CONT birds, using a LYON beak trimmer (LYON Technologies Inc., CA, USA) to remove approximately 0.51 cm of the maxilla. Sham trimmed ducks were also captured and brought close to the bill trimmer without cutting the bill (Gustafson *et al*., 2007c).

Behavioral observation

Direct observation of ducklings' behaviour was conducted by scanning technique according to Dawkins *et al.* (2004). Observations were carried out three times per day at 08.00-09.00 am, 12.00-01.00 pm, and 04.00-05.00 pm for three days per week from Monday to Wednesday, through the experiment (from 3 to 8 weeks of age). The behaviors of birds were recorded in accordance with ethogram in Table 2.

Growth performance

Live body weight (LBW): Ducks were weighted per replicate every 2 weeks throughout the experimental period.

Body weight gain (BWG): Every two weeks, the starting live weight was subtracted from the final live weight to determine the mean live body weight gain.

Feed intake (FI): At the conclusion of the two weeks, the residuals were collected, and the quantity of feed taken was computed using the differences.

Feed conversion ratio (FCR): The FCR was computed using the FI and BWG data (FCR=FI/BWG).

Physiological parameters

At 60 days of age, blood sample (5 ml) was collected (1 bird/replicate, 6 birds/treatment) by cutting the jugular vein, which allowed to bleed for approximately 2 min. Blood sample was collected into tube without

anticoagulant to obtain serum and held for 2–3 h at room temperature to clot, then centrifuged for 10 minute at 3000 r.p.m. The separated serum was transferred to Eppendorf tube using micropipette and stored at -80°C until analyses (Parga *et al*., 2001).

Stress indicators

Serum corticosterone level (ng/ml)

Analysis of serum corticosterone concentrations was carried out using the corresponding commercial ELISA kits in accordance with the guidelines provided by the manufacturer (Arbor Assays LLC, Ann Arbor, MI, USA).

Serum glucose (mg/dl)

Serum glucose was measured colorimetrically using a commercial kit (Spectrum glucose diagnostic kits) made by the Egyptian Company for Biotechnology, Cairo, Egypt.

Antioxidant activity

Catalase (U/ml) and superoxide dismutase (SOD) (U/ml) activities were detected by spectrophotometer, colorimetric method by using a commercial kit (Bio diagnostic catalase and SOD capacity kits) obtained from (Egypt, Cairo, Egyptian company for Biotechnology).

Liver function tests

Serum Total protein (g/dl), and albumin (g/dl) levels were detected colorimetrically by using a commercial kit from the Egyptian company for Biotechnology, Cairo, Egypt. Serum Alkaline phosphatase (ALP) activity was recorded by a colorimetric method using a commercial kit (Spinreact, Spain).

Kidney function tests

Serum urea (mg/dl) and creatinine (mg/dl) levels were measured by a colorimetric method using a commercial kit (Diamond diagnostic kits, USA).

Statistical analysis

The nutritional supplement served as the fixed effect in this randomized block design experiment, while the pen (n. = 6/treatment) served as the experimental unit. One-way ANOVA was used to examine the overall effects of the BLE dietary addition on ducks' parameters (SAS Institute Inc., Cary, NC). When a significant difference was found, the means were compared using the Tukey-Kramer test. The data were presented as mean ± SEM, with the statistical significance level was set at $P < 0.05$.

Results

Effect of dietary administration of BLE on behavior of bill trimmed mule ducks

The relationships between the BLE supplementation and behavioral patterns of bill trimmed mule ducks are presented in Table 2. There were insignificant differences in feeding, drinking, resting, standing and walking behaviors between all the treatments ($P > 0.05$). However, compared to control birds, the bamboo leaf extract fed birds had lower wall pecking, feather pecking, aggressive pecking and preening ($P < 0.05$, respectively). But there were no significant differences in wall pecking between the CONT and the BT-CONT birds ($P > 0.05$). There were no statistical differences in these behaviors between BT-BLE1 group and BT-BLE2 group ($P > 0.05$).

Effect of dietary administration of BLE on performance parameters of bill trimmed mule ducks

The effects of BLE on growth performance (BW, BWG, FI and FCR) of bill trimmed mule ducks are presented in Figures 1, 2, 3 and 4. There were no significant differences in BW, BWG, FI and FCR between all treatments ($P > 0.05$).

Table 2. Effect of dietary administration of BLE on behavior of bill trimmed mule ducks.

Treatment	CONT	BT-CONT	BT-BLE1	BT-BLE2	*P*-value
Feeding	16.05±1.57	15.97±1.57	16.67±1.57	16.15±1.57	0.99
Drinking	15.30±1.08	16.28±1.08	17.58±1.08	16.11±1.08	0.53
Resting	35.56±2.66	41.34±2.66	44.39±2.66	42.08±2.66	0.15
Standing	61.09±2.68	54.87±2.68	52.53±2.68	54.52±2.68	0.16
Walking	3.76±0.76	3.44±0.76	3.51±0.76	3.13±0.76	0.95
Wall pecking	6.30±0.60^{a}	4.00±0.60^{a}	1.15±0.60^{b}	1.41±0.60^{b}	0.00
Feather pecking	7.56±0.29^{a}	2.34±0.29^{b}	0.17±0.29^{c}	0.83±0.29^{c}	0.00
Aggressive pecking	9.65±0.52^{a}	2.00±0.52^{b}	0.22±0.52^{b}	0.0±0.52^{b}	0.00
Preening	15.49±1.13^{c}	19.01±1.13^{bc}	25.49±1.13^{a}	23.30±1.13^{ab}	0.00

$^{a, b \text{ and } c}$ Mean ± SE with different superscripts in the same row differ ($P < 0.05$). (n = 6 / treatment; and the data were collected from 30 birds / treatment; 5 birds/pen x 6 pens / treatment).

Table 3. Effect of dietary administration of BLE on stress indicator (Glucose and Corticosterone) and antioxidant activity (Catalase and SOD) of bill trimmed mule ducks.

Treatment	CONT	BT-CONT	BT-BLE1	BT-BLE2	*P*-value
Glucose (mg/dl)	124.67±12.06	145.50±12.06	138.83±12.06	160.67±12.06	0.24
Corticosterone (ng/ml)	0.135±0.021^{c}	1.825±0.021^{a}	0.612±0.021^{b}	0.127±0.021^{c}	0.00
Catalase (U/ml)	61.30±1.99^{a}	25.90±1.99^{c}	28.38±1.99^{bc}	36.63±1.99^{b}	0.00
SOD (U/ml)	27.80±0.825^{a}	16.80±0.825^{c}	23.13±0.825^{b}	27.10±0.825^{a}	0.00

$^{a, b \text{ and } c}$ Mean± SE with different superscripts in the same row differ ($P < 0.05$). (n = 6 / treatment; and the data were collected from 24 birds / treatment;1 birds/pen x 6 pens / treatment).

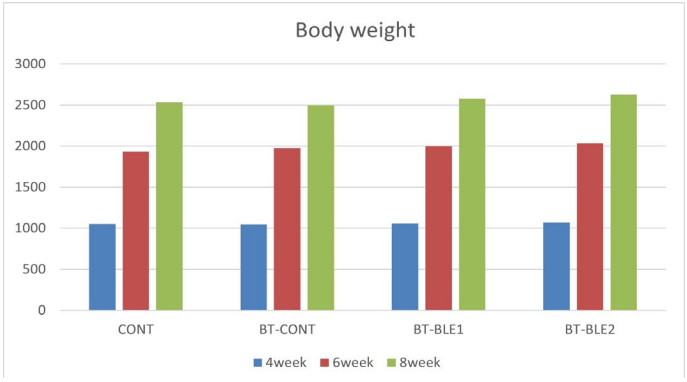

Figure 1. Effect of dietary administration of BLE on body weight of bill trimmed mule ducks. Mean ± SE. (n = 6 / treatment; and the data were collected from 30 birds / treatment; 5 birds / pen x 6 pens / treatment).

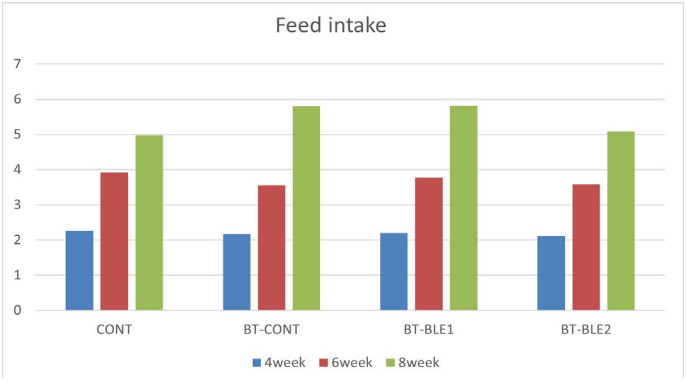

Figure 3. Effect of dietary administration of BLE on feed intake of bill trimmed mule ducks. Mean ± SE. (n = 6 / treatment; and the data were collected from 30 birds / treatment; 5 birds / pen x 6 pens / treatment).

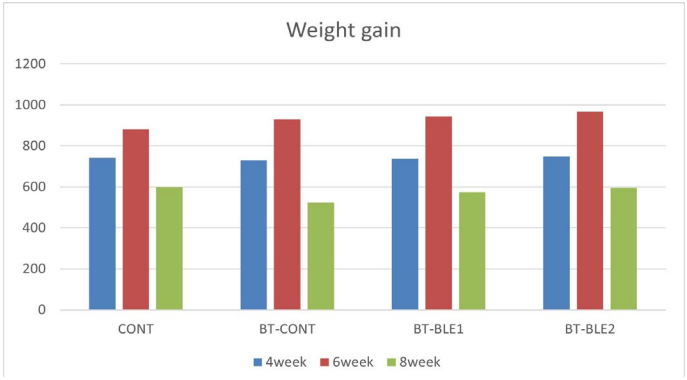

Figure 2. Effect of dietary administration of BLE on body weight gain of bill trimmed mule ducks. Mean ± SE. (n = 6 / treatment; and the data were collected from 30 birds / treatment; 5 birds / pen x 6 pens / treatment).

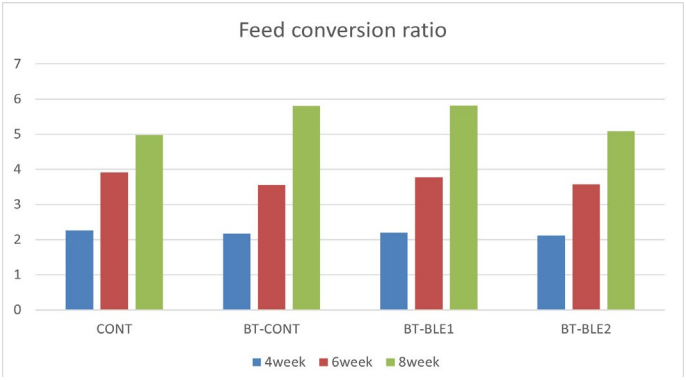

Figure 4. Effect of dietary administration of BLE on feed conversion ratio of bill trimmed mule ducks. Mean ± SE. (n = 6 / treatment; and the data were collected from 30 birds / treatment; 5 birds / pen x 6 pens / treatment).

Effect of dietary administration of BLE on stress indicators of bill trimmed mule ducks

Effects of BLE on stress indicators of bill trimmed mule ducks are presented in Table 3. Serum corticosterone level was lower in BLE fed groups in comparison with the BT-CONT ($P < 0.05$), but there were no significant differences between the BT-BLE2 and the CONT birds ($P > 0.05$). There were no significant differences in serum glucose levels among all treatments ($P > 0.05$).

Effect of dietary administration of BLE on antioxidant activity of bill trimmed mule ducks

Effects of BLE on antioxidant activity of bill trimmed mule ducks are presented in Table 3. There was significant decreases in serum catalase activity in bill-trimmed birds compared with the CONT birds ($P < 0.05$). At the same time, serum catalase activity was significantly higher in the BT-BLE2 in comparison with the BT-CONT birds ($P > 0.05$); however, there was no significant difference between the BT-CONT and the BT-BLE1 groups ($P > 0.05$). Serum SOD activity was significantly higher in BLE fed birds than BT-CONT birds ($P < 0.05$). However, there was no significant difference between the BT-BLE2 and the CONT birds ($P > 0.05$), at the same time, BT-BLE2 had a higher activity of SOD compared with BT-BLE1 ($P < 0.05$).

Effect of dietary administration of BLE on liver function tests of bill trimmed mule ducks

Serum total protein level was higher in bamboo fed group and CONT birds than BT-CONT groups ($P < 0.05$). There was no significant difference in albumin level between all treatments ($P > 0.05$). Serum globulins were higher in BT-BLE1 than BT-CONT groups ($P < 0.05$). There were no significant differences in albumin/globulin ratio between all treatments ($P > 0.05$). Alkaline phosphatase (ALP) activity was higher in BT-BLE1 group than CONT and BT-CONT groups ($P < 0.05$). There were no statistical differences in alkaline phosphatase (ALP) activity between BT-BLE1 group and BT-BLE2 group ($P > 0.05$), as shown in Table 4.

Effect of dietary administration of BLE on kidney function tests of bill trimmed mule ducks

Effects of BLE on kidney function tests of bill trimmed mule ducks are

Table 4. Effect of dietary administration of BLE on liver and kidney function tests of bill trimmed mule ducks.

Treatment	CONT	BT-CONT	BT-BLE1	BT-BLE2	*P*-value
Total protein (g/dl)	5.33±0.126^{b}	4.78±0.126^{c}	5.85±0.126^{a}	5.43±0.126^{ab}	0.00
Albumin (g/dl)	2.86±0.232	3.14±0.232	3.01±0.232	3.38±0.232	0.45
Globulins (g/dl)	2.47±0.225^{ab}	1.64±0.225^{b}	2.84±0.225^{a}	2.03±0.225^{ab}	0.01
Albumin/ Globulin ratio	1.38±0.2664	2.11±0.2664	1.09±0.2664	1.75±0.2664	0.07
Alkaline phosphatase (U/l)	1474.33±73.16^{b}	1133..67±73.16^{c}	1769.67±73.16^{a}	1485±73.16^{ab}	0.00
Urea (mg/dl)	10.67±1.16	12.50±1.16	11.67±1.16	14.67±1.16	0.13
Creatinine (mg/dl)	0.48±0.10	0.46±0.10	0.38±0.10	0.59±0.1	0.69

$^{a, b \text{ and } c}$ Mean± SE with different superscripts in the same row differ ($P < 0.05$). (n = 6 / treatment; and the data were collected from 24 birds / treatment;1 birds/pen x 6 pens / treatment).

presented in Table 4. There were no significant differences in serum urea and creatinine levels between all the treatments (P > 0.05).

Discussion

Despite, beak trimming was used as a main strategy to decrease peck wounds and death in poultry; it caused some welfare issues pain and sensory loss. Moreover, it caused significant behavioral changes in birds, such as decreased feed intake, pecking force, activity, and duration, as well as growth rate reductions. Bamboo leaf extract contains silica which supports collagen production, resulting in healthier skin, increases connective tissue, aids in wound healing. Moreover, it has been confirmed to have many beneficial properties, such as improving immunity, antioxident activity, anticancer, and antibacterial activities (Riber and Hinrichsen, 2017).

In the current study, there were no significant differencee in ingestive behavior (feeding and drinking) and locomotion (standing, resting and walking behaviors) between trimmed and non-trimmed ducks. These results agree with Gentle and Mckeegan (2007) who recorded an insignificant effect of beak trimming on ingestive behavior just after bill trimming and after 6 weeks in layer hens. However, these results disagree with Araújo *et al*. (1997) who found that bill trimming declined drinking frequency, feeding frequency and time when compared to non-trimmed birds.

However, bill trimmed ducks had a lower percentage of wall pecking, feather pecking, and aggressive pecking behaviors compared to non-trimmed ducks, but the differences disappeared between the CONT and BT-CONT groups. Also, BLE fed birds had a lower percentage of wall pecking, feather pecking, and aggressive pecking compared to BT-CONT birds. These results agree with Farm Animal Welfare Council (2007) who reported that beak trimming is used to minimize or prevent interbred aggressiveness, pecking and cannibalism. Also, agree with Gustafson *et al*. (2007a) and Gustafson *et al*. (2007b) who found sign of feather pecking in the trim pens of ducks, but skin damage and feather pecking were more extensive in the non-trim pens of ducks, and trim ducks spent less time engaging exploratory pecking than non-trim ducks. The reason for our results is that bill trimming was a painful procedure for ducklings; it was conceivable that they might have difficulty using their bill until the wound is completely healed. The bird is also handicapped, it must learn to feed itself with a partly missing member (Gentle *et al*., 1982).

Preening has been considered as a comforting behavior in poultry (Hinde, 1970). In current study, compared to BT-CONT birds, the BLE fed birds had higher preening; however, the differences had disappeared between the CONT and the BT-CONT birds. These results agree with Ahmed, (2022) who recorded no significant variations in preening across trimmed and non-trimmed mule duck, and disagree with Sandilands and Savory (2002) who concluded that while sitting, bill-trimmed laying pullets spend more time preening toward the preen gland and at the back. This contrasts with Grigor *et al*. (1995) who revealed that turkeys that had beak trimming showed a significantly reduced preening time along 12-week noticeable period than controls. The reason for this result is that bamboo had a powerful antioxidant effect, and lowered blood stress indicators, so bamboo leaf extract fed ducks made more preening behavior because they feel more comfort.

The current results clarified that compared to non-trimmed ducks; the bill trimmed groups had a non-significant effect on growth performance. Also, compared to BT-CONT groups, BLE showed an insignificant effect on growth performance. These agree with Ruttanavut *et al*. (2009) who reported that bamboo fed Aigamo ducks had a non-significant increase in body weight gain compared to control ducks.

However, these results disagree with Chu *et al*. (2013) who found that bamboo charcoal increased the feed efficiency and growth performance in fattening pigs. The lack of significance in growth performance of ducks fed different levels of BLE compared to CONT and BT-CONT could be partly attributed to the effect of bill trimming on feed intake. These results agree with Davis *et al*. (2004) who found that mild trimming decreased feed intake and growth in Single Comb White Leghorn hens. Disappearance of the difference in the performance parameters may be returned to the dose of the BLE and more studies are needed to determine the proper dose that can improve the growth performance of mule ducks.

Ducks' physiological changes are initiated by the stress and inflammatory processes that follow bill cutting. Active elements in bamboo leaf extract include flavonoid and polyphenols, which have anti-inflammatory, wound healing, and antioxidant benefits. The results of this study proved that BLE may enhance ducks health that exposed to bill trimming stress.

Various research has proved that the hypothalamic pitutary adrenal axis (HPA) is included in stress (Zhang *et al*., 2020). The release of corticosterone is related to pain development in animals in response to various external and internal stimulations (Cheng and Muir, 2007). In our study, corticosterone was lower in BLE fed groups in comparison with the BT-CONT. Nevertheless, the differences disappeared between BT-BLE2 birds and the CONT birds. This result agrees with Voslarova *et al*. (2013) who found that bill-trimmed birds exhibited higher plasma corticosterone concentrations than non-trimmed birds. Moreover, Cheng and Muir (2007) reported that stress and inflammatory processes occur because of bill trimming cause physiological changes, as an increase in corticosterone secretion. Corticosterone was lowered in BLE fed groups because it eliminated the negative effects of bill trimming stress by alleviating inflammatory process associated with bill trimming (Vasave *et al*., 2019).

In our study, there were no significant differences in serum glucose levels between all treatments, these findings agree with Shen *et al*. (2019) who found that there was no significant change in blood glucose levels between BLE fed groups and control groups in broiler chicken. However, Soumya *et al*. (1016) found that the extracted bamboo oil had a hypoglycemic effect and supplementation of 1 ml/kg body weight of bamboo oil showed a severe decline in glucose level from 254.04±2.08 to 92.6±1.63 in rats.

Stress increases the production of reactive oxygen species (ROS) by deactivating the electron transport assemblies of the mitochondrial membrane (Mujahid, *et al*., 2005). In the current study, serum catalase and superoxide dismutase activites were higher in the BLE groups in comparison with the BT-CONT birds. These results agree with Shen *et al*. (2019) who found that BLE supplementation significantly increased serum catalase, glutathione peroxidase and total antioxidants in broiler chickens, which could be attributed to the powerful antioxidant activity of BLE which was reported to improve ROS scavenging, inhibit lipid peroxidation, and improve antioxidant capability by translocating and activating nuclear factors to increase the expression of many enzymes of the antioxidant defense system (Guo *et al*. 2010).

The current results clarified that serum total protein level and ALP activity were higher in BLE fed groups and CONT birds than BT-CONT groups. However, there were no significant differences in albumin level between all treatments. These results agree with Chu *et al*. (2013) who found that bamboo charcoal increased total protein in fattening pigs. Moreover, Yakubu *et al*. (2009) found that serum ALP activity of pregnant rabbits fed 250 and 500 mg/kg of aqueous extract of *Bambusa vulgaris* leaves was increased in comparison with control group. However, these results disagree with Anigboro (2018) who found that there was a significant reduction in serum ALP activity in mice treated with aqueous leaf extract of *Bambusa vulgaris* in comparison to control group. The reason of these results may be attributed to the improvement in normal health and metabolic status of birds following BLE dietary supplementation.

The function and health of the kidneys are confirmed by measuring serum urea, creatinine and uric acid levels, which are used as diagnostic tools to provide indication of renal dysfunction in birds (Lierz, 2003). In the current results, there were no significant differences in urea and creatinine levels between all treatments. These results disagree with An-

igboro (2018) who found that serum urea, creatinine and uric acid levels were significantly reduced in mice treated with aqueous leaf extract of *Bambusa vulgaris* in comparison to control groups. However, Yakubu *et al*. (2009) found that dietary supplementation of 250 and 500 mg/kg of aqueous extract of *Bambusa vulgaris* leaves increased serum levels of creatinine and uric acid in pregnant rabbits in comparison to control group. Absence of the differences in the blood urea and creatinine levels could be attributed to the dose of the BLE which may be not enough to induce a marked effect on blood urea and creatinine levels.

Conclusion

Dietary supplementation of the BLE enhance stress response and the antioxidant status, as proved by raised levels of catalase and SOD with declined corticosterone. Our records suggest that use of the BLE, especially at 2.0 g/kg level, could be a helpful management strategy for enhancing health and welfare of bill trimmed mule ducks. Future studies are needed to detect the accurate dose of BLE in diet.

Conflict of interest

The authors have no conflict of interest to declare.

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