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EFFECT OF DIETARY SUPPLEMENTATION OF *BAMBOO* LEAF EXTRACT (BLE) ON INTESTINAL BACTERIAL PROFILE AND DISEASE RESISTANCE OF BILL TRIMMED MULE DUCK

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ABSTRACT

Bamboo leaf extract plays a significant role in enhancing immunity and promotingdisease resistance of birds and can be used as a feed supplement in avian husbandry. Beak trimming causes inflammation reactions and stress, leads to immune suppression and decreases disease resistance. The objectives of the current study were to investigate the effect of the extract of bamboo leaves on the enhancement of stressed bird resistance. Four groups (6 birds per group) were used; group 1: not bill trimmed and fed basal diet only without extract. Group 2: bill trimmed and had only a basal diet without extract. Groups 3 and 4: bill trimmed and fed basal diet plus bamboo extract 1 and 2 g per Kg respectively), a blood sample was collected on day 60 of the experiment to determine the Heterohil / Lymphocyte ratio as an indicator for bird resistance. The total bacterial count of lactobacilli and bifidobacteria wasdetermined. The pathogenic bacteria were isolated and identified by phenotypic examination and were confirmed by PCR. H/L ratio was lower in the bamboo supplied groups (3 & 4) than in groups 1 & 2. The groups (3 & 4) showed a significant increase in Lactobacillus count than groups 1 and 2. The result of the antibacterial test suggested antibacterial action of the extract against the isolated GramGram-negative bacteria more than GramGram-positive bacteria. The current results imply that the bamboo leaf extract could potentially be used as a substitute for synthetic agents that are used to solve bill trimming stress problems.

Keywords: Bamboo, H/L ratio, bill-trimming, lactobacilli and antibacterial activity

INTRODUCTION

The poultry meat had been preferred by the majority of Egyptian consumers over other animal proteins due to their remarkable growthbenefitsthatsupportconsumers'health, and theirlowprice (Hosny, 2006). In poultry husbandry, feather pecking andcannibalism are frequent problems that cause financial losses. Beak trimming means that nearly 25% to 33% of the upper beak only or with the lower beak of the bird is removed (American Veterinary Medical Association, 2010 and Riber & Hinrichsen, 2017). Stress

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caused by beak trimming has been shown to cause decreased production performance, inflammation, immunological suppression, different infections and mortality, all of which have a negative effect on the production's return (Schwean-Lardner *et al.,* 2016; Ranjan *et al.,* 2019 and Li *et al.,* 2020).

Many synthetic growth promoters, such as antibiotics, have been used in poultry diets to enhance broiler chicken behavior and reduce the prevalence of microbial diseases. However, frequent use of these antibiotics has led to problems like disruption of normal flora, antibiotic resistance, and antibiotic residue in animal products (Nair *et al.,* 2018 and Ben *et al.,* 2019). Consequently, the antimicrobials use in the poultry industryhas been restricted in various countries. As a result, there has been a rise in the seeking ofnatural alternative dietary supplements that improve growth (Alagawany *et al.,* 2018).

Plant extracts have emerged as one of the most promising natural feed additives because of their growth-promoting, anti-inflammatory, antibacterial, and immunomodulatory properties (Kumari *et al.,* 2007). The plant extracts are potent dietary supplements due to many compounds, including flavonoids and bioactive polysaccharides, that have been shown to promote avian growth, immune response and disease resistance through antiviral, antibacterial and antioxidant effects (García-Lafuente *et al.,* 2009;Park and Jhon, 2010 and Alagawany *et al.,* 2018).

Bamboo is one of the most productive and medicinal plants (Kim *et al.,* 2011 and He *et al.,* 2014). Bamboo extract is rich in polysaccharides and flavonoids that have antioxidant capacity and boost animal immunity, extensive antibacterial activity, and anti-cancer effect (Kim *et al.,* 2016 and Ge *et al.,* 2020). The bamboo crude extract showed the most cytotoxicity against the MCF-7 cell line (IC_{50} = 38.8 µg/mL) and inhibited 63.4% of the cell growth at the highest conc. of 100 µg/mL, while it was safe against normal cells (Abdelhameed, R. F. *et al.,* 2020).

Antibacterial action of Bamboo shoots was reported against *Klebsiella pneumoniae, Enterococcus faecalis, Staphylococcus epidermidis, Bacillus subtilis,* and *Enterobacter aerogenes.* (Ambika & Rajagopal. 2017) *Escherichia coli, Bacillus cereus* and *Staphylococcus aureus* (Tanaka *etal.,* 2011 and 2013). Moreover, the fibers of bamboo shoot shell have prebiotic potential that supports the lactic acid bacteria growth (lactobacillus and bifidiobacteria) and increases their capacity to ferment substrates due to its high content of polysaccharides (Azmi *et al.,* 2012; Gang *et al.,* 2020 and Wu *et al.,* 2020). Competition of Bifidiobacteria and lactobacilli with enteric pathogens for nutrients leads to prevention of pathogen invasion by intensive attachment to the mucosa of the intestine, and provokes mucosal defence mechanism (Symonds *et al.,* 2012).

Enhancing the immune system of the host is a potent approach to combat the infections and mortality in birds. The bamboo leaf extract as a dietary supplement could possess immune-provoking functions in birds as it improves the IL-2 and IFN-γ production in blood and their gene expressions in the spleen, which playsa powerful role in the defense mechanism of host by magnified action on natural killer (NK) and T cells (Zhang *et al.,* 2015 and Gang *et al.,* 2020).

The host disease resistance in birds can be dependent on the heterophil to lymphocyte ratio (H/L) (Zhang *et al.,* 2023). So, the preference for immune function should be an important trait for the commercial breeding standards in the poultry industry, but it is difficult due to the absence of accurate indicators that can reflect the immune status of birds (Fulton 2004).

Heterophil to lymphocyte ratio (H/L) has a major heritability level and complicated

mechanism of genes related to immunity, that supportsthe selection for promoting chicken disease resistance based on Heterophil to lymphocyte ratio and its applicationas a marker for the avian immunity status (Campo & Davila 2002 and Zhang *et al.,* 2023).

Consequently, the current research was designed to determine outcomes of the extract of bamboo leaves as a dietary supplement for mule ducks exposed to beak trimming stress to enhance immunity and promote bird resistance against pathogenic bacteria and increase beneficial flora.

MATERIALS AND METHODS

This study was conducted in the Animal and Poultry Behavior and Management Research Unit and Department of Microbiology and Immunology at Assiut University, Faculty of Veterinary Medicine, Assiut, Egypt. All safety measures for handling laboratory animals and the microbiology lab. Biosafety were considered, and the ethical approval (No. 0620250292) of the experiment was designed by the Ethics Committee of the Faculty of Veterinary Medicine, Assiut University.

1-The bamboo extract

The mass spectrometer detector and the gas chromatograph (at the Central Laboratories Network, National Research Centre, Cairo, Egypt) were used to analyze the bamboo extract that was obtained from Pure Original Ingredient, Utah, USA. The main components were flavonoids, polyphenols and polysaccharides. As was supplied in a supplementary Figure1 and supplementary tables (1-3) in a recent study by Fouad*et al.*(2025).

Birds and Dietary Treatments

2-The experiment

The mule ducklings of the experiment were supplied from the company of El-Salam in Cairo, Egypt. A total of 120 birds at the age of one day old from a commercial hatchery, all housing management measures are considered (same body weight, ambient temperature, lighting and humidity) (Coates *et al.,* 2000; Sari *et al.,* 2013 and Abdel-Hamid & Abdel-Fattah, 2020).

Twenty-four birds free from pathogenic bacteria were selected, and four groups (6 birds/group) were used; group 1: not bill trimmed and fed a basal diet only without bamboo extract. Group 2: bill trimmed and fed a basal diet without bamboo extract. Groups 3 and 4: bill trimmed and fed basal diet with bamboo extract (1 and 2 g per kg-1), and bill-trimming was made for birds of groups 2,3& 4 fromthe 1st day of age to the experiment end. Birds were fed 17% duck mash from the 1st day to 8 Ws according to Matoq, S. *et al.* (2024).

3-Beak trimming:

Birds in groups 2, 3 &4 were subjected to beak trimming after 21 days of experiment according to Gustafson *et al.*(2007) to cut nearly 0.51 cm of the maxilla.

4- Detection of Heterohil/ Lymphocyte (H/L) ratio as an index for disease resistance:

-Blood collection:

EDTA test tubes were used to collect blood samples from all birds in all groups to determine the Heterohil / Lymphocyte (H/L) ratio onthe 60day. Blood films were constructed, and Giemsa stain was applied for staining. One hundredWBCs were enumerated, and then we calculated heterophil/lymphocyte ratios according to Parga *et al.*(2001).

The following equation was used for the calculation of H/L ratios:

$$\text{H/L ratio} = \frac{\text{The count of heterophil cells}}{\text{The count of lymphocytes}}$$

5-Microbial assessment:

1-Sample collection:

The caecal contents of ducks were collected aseptically from all birds. The samples were

transferred in an icebox to the microbiology lab and analyzed within a few hours of collection **(Sieuwerts *et al.,* 2008)**.

2-Isolation and Estimation of Total Bacterial Count of Lactobacilli and : (Amaliah *et al.,* 2018).

One Gram of cecum content wascollected and homogenized in 0.9 % sterilized saline (W/V:1/10) under aseptic conditions for microbiota count.

MRS and MRS supplemented with l-cysteine, respectively, were used for isolation and enumeration of lactobacilli and bifidio-bcteria. The plates of Lactobacillus and Bifidiobacterium were incubated anaerobic-ally at 37°C for 48h under anaerobic conditions (Taye, Y. *et al.,* 2021). The isolated bacteria were identified by the morphology of colonies, microscopic examination and biochemical tests. Finally confirmed by PCR using a set of species-specific primers listed in Table (1). Each bacterial count was calculated from plates containing 30 to 300CFU (Levy-Pereira *et al.,* 2018). Then we counted colonies, transformed the colonycount to log and log CFU g^{-1} was the final expression of total bacterial count (Adel *et al.,* 2016).

CFU/g* = (*count of Colonies* × *Factor of Dilution*) / *Volume of Culture plated* (*ml)

3-Isolation and identification of Gram +ve and Gram -ve aerobic bacteria according to Manual of Clinical Microbiology: (Murray, P. R., 2003 and Versalovic, J., 2011)

In 9 ml buffered peptone water 1g of each sample were homogenized (High Media, India), then kept for overnight at 37- 42 °C in incubator and streaked onto a set of agars (Blood Agar, Nutrient agar, Mannitol Salt Agar, Bile azide esculin agar, MacConkey agar, Eosin and methylene blue agar (EMB), aeromonas pseudomonas selective media, Bisthmus sulphite agar and Xylose-Lysine-Deoxycholate (XLD)) to allow the growth of bacteria under aerobic conditions for 24-48 hrs. The media are obtained from (Oxoid, UK) and (High Media, India).The isolated bacteria were identified by morphology of colonies, microscopic examination and biochemical tests (catalase, oxidase, coagulase, citrate, urease, TSI, IMVC and motility test) and confirmed by PCR using the mentioned species-specific primers in Table (1).

3-Molecular confirmation of isolated bacteria by PCR: (Ramadan H. et al 2016)

1-DNA extraction:

One mL of sterile distilled water was used for the suspension of 3 to 5 colonies of each sample. The resuspended bacterial cells were destroyed by heat for 15-20 minAt 100°C, and then were centrifuged at 15,000 rpm for 15 min. DNA obtained from supernatant was preserved at -20°C until used for PCR. The DNA concentration was detected using a nanodrop.

2- Amplification:

The thermal cycler (BioRadT100, USA) was used for PCR. The Amplification was prepared in a total of 20 µl volume.The PCR mixture was composed of: 12.3µl of free nuclease water, 2 µl of 10X Taq PCR Buffer, 1.6 µl of dNTP Mixture, 1 µl of forward Primer, reverse Primer was 1 µl of, 2 µl of DNA and 0.1 µl Taq DNA Polymerase. Different amplification conditions of the PCR reaction are made according to each primer pair.

3-Electrophoresis and imaging of PCR products:

Agarose gel (1.5%) stained with ethidium bromide in 1x TBE buffer was used for the detection of PCR products. The ladder was supplied from Fermentas, Thermo, Germany. The gel was visualized under ultraviolet light, imaged by a gel documentation system and computer software was used to analyze the data.

Table 1: Primers used for PCR in the study.

	Sequence (5′ to 3′)	Band size	Ref.
Lactobacillus spp (16s rRNA)	TGGAAACAGRTGCTAATACCG GTCCATTGTGGAAGATTCCC	232 bp	**(Byun *et al.*, 2004)**
Bifidiobacterium spp (16s rRNA)	GGGTGGTAATGCCGGATG CCACCGTTACACCGGGAA	523 bp	**(Kok *et al.*, 1996)**
Salmonella spp (inv A gene)	GCT GCG CGC GAA CGG CGA AG TCC CGG CAG AGT TCC CATT	389 bp	**(Cocolin *et al.*, 1998 & Ferretti,*et al.*, 2001)**
Pseudomonas spp (OprL gene)	ATG GAA ATG CTG AAA TTC GGC CTT CTT CAG CTC GAC GCG ACG	504 bp	**(Xu *et al.*, 2004)**
Enterococci spp (16s rRNA)	TACTGACAAACCATTCATGATG AACTTCGTCACCAACGCGAAC	112 bp	**(Ke *et al.*, 1999)**
S. aureus (nuc gene)	GCGATTGATGGTGATACGGTT AGCCAAGCCTTGACGAACTAAAGC	270 bp	**Louie *et al*.2002**
E. colispp (phoA gene)	CGATTCTGGAAATGGCAAAAG CGTGATCAGCGGTGACTATGAC	720 bp	**Hu*et al.*, 2011**

Nuc: nuclease, *phoA*: alkaline phosphatase, *OprL*: outer membrane proteins**,** *inv A*: invasion protein, bp:base pair

4-*Invitro* antibacterial effect of bamboo extract by Agar well diffusion assay: (Hossain*et al., 2022*)

The Muller Hinton agar (MHA) plate surface wasinoculated by spreading a volume of the microbial inoculum over the entire agar surface. Then a circular hole (6–8 mm) was made aseptically into the agar using a sterile cork borer, and a suitable volume (20–100 µL) of 1% and 2% conc of the extract solution was applied to the well. The agar plate was then incubated under proper conditions (37 °C for 24h) then the zone of inhibition (ZOI) wasmeasured.

5-Statistical analysis:

One-way ANOVA was used to analyze the collected data and comparingthe means was made by the Tukey-Kramer test.The differenceswere considered statistically significant when$P<0.05$; and the results were reported as mean ± SE.

RESULTS

1-Effect of dietary supplementation of bamboo leaf extract (BLE) Heterophile /Lymphocyte ratio of bill-trimmed mule ducks.

Heterophil-lymphocyte ratio was lower in birds of groups 3 &4 than in groups1 and 2 ($P <0.05$). Atthe same time, the group 1 birds had a lower heterophil lymphocyte ratio than the group2. There were no statistical differences in heterophil lymphocyte ratio between groups 3 &4 ($P > 0.05$) Table (2).

Table 2: Effect of dietary supplementation of bamboo leaf extract (BLE) Heterophil/Lymphocyte ratio of bill trimmed of mule duck.

Treatment	Group 1	Group 2	Group 3	Group 4	*P*-value
H/L ratio	0.303±0.028[b]	0.579±0.028[a]	0.146±0.028[c]	0.072±0.028[c]	0.0001

[a,b,c]Mean± SE with different superscripts in the same row differ (P < 0.05).
Group 1, No bill-trimming and fed only a basal diet;
Group 2, Bill-trimmed supplied only the basal diet;
Group 3: Bill-trimmed and fed the basal diet + 1 gr of bamboo extract.
and Group 4, Bill trimmed and fed the basal diet +2 gr of bamboo extract.
H/L ratio means heterophile lymphocyte ratio.

2-Estimation of total bacterial count of lactobacilli and bifidiobacteria of bill-trimmed mule duck:

Table (3) showed that bamboo shoots fed to groups 3and 4 showed significant increasein Lactobacillus count, more than groups 1 and 2.

The count of lactobacilli in the bamboo-fed groups was 7.01±0.02 and 7.31±0.04 in groups 3and 4, respectively. Whereas the groups 1 and 2 recorded 6.70±0.07 and 6.63±0.06, respectively. However, Bifidiobacteria were not detected in all experiment groups

Table 3: Estimation of total bacterial count of lactobacilli and bifidiobacteria.

Treatment	Group 1	Group 2	Group 3	Group 4
Lactobacillus	6.70±0.07c	6.63±0.06c	7.01±0.02b	7.31±0.04a
Bifidobacteria	ND	ND	ND	ND

Data expressed as (mean ± SD) of 6 replicates. **ND:non-detected
Within the same column, means with different letters are significantly different (p ≤ 0.05).
Group 1, No bill-trimming and fed only a basal diet;
Group 2, Bill-trimmed supplied only the basal diet;
Group 3: Bill-trimmed and fed the basal diet + 1 gr of bamboo extract.
and Group 4, Bill trimmed and fed the basal diet +2 gr of bamboo extract

3-Isolation of both GramGram+ve and Gram -ve aerobic bacteria from bill-trimmed mule duck:

The pathogenic bacteria flourished in group 2, that exposed to bill trimming and fed the basal diet without extract supplementation.This can suggest the stress effect of bill trimming on the immune status of the bird.The bamboo leaf extract effects on isolated bacteria are presented in Tables (5-9). The isolated Gram -ve bacteria were *E.coli, Klebsiella spp, Salmonella spp, Proteus spp* and*Pseudomonas spp* and Gram +ve bacteria were *staphylococci spp and enterococci spp*.

The result of bacterial isolation showed variation between groups (P < 0.05) in Gram negative bacteria. It was noticed that pathogenic Gram-negative bacteria (except *E. coli*) as *Pseudomonas, Proteus* and *Salmonella* were less in groups 3&4 than the groups 1&2,whichsuggests the antibacterial effect of bamboo leaf extract on Gram-ve bacteria, even with the lowest amount in group 3. However, no significant differences (P>0.05) were presented in isolated Gram positive bacteria between all the groups that suggest the absence of antibacterial effect of bamboo leaf extract on Gram +ve bacteria (*S.aureus, S.epidermidis, enterococci*) as isolated from all birds in all groups by nearly percent that may be due to absence of antibacterial activity or the need for raising the dose of bamboo extract.

Table 4: Effect of dietary supplementation of *bamboo* leaf extract (BLE) on the number of isolated Gram +ve and Gram -ve bacteria from bill-trimmed mule duck.

Treatment	Group 1	Group 2	Group 3	Group 4	*P*-value
No. of isolated Gm+ve *spp.*	2±0.23	1.83±0.23	2.17±0.23	2.17±0.23	0.7065
No. of isolated Gm-ve *spp.*	2±0.42^{ab}	2.83±0.42^{a}	2.17±0.42^{ab}	1±0.42^{b}	0.0463

^{a,b,ab}Mean± SE with different superscripts in the same row differ (P < 0.05).
Group 1, No bill-trimming and fed only a basal diet;
Group 2, Bill-trimmed and fed only the basal diet;
Group 3: Bill-trimmed and fed the basal diet + 1 g of bamboo extract.
and Group 4, Bill trimmed and fed the basal diet +2 g of bamboo extract.

Table 5: Isolatedbacteriafromducks in group 1.

Sampleno.	Gm +ve bacteria	Gm -ve bacteria
N1	S. aureus Enterococcus spp.	*E. coli spp.*
N2	*S. aureus* *Enterococcusspp.*	*E.coli spp.* *Klebsiella*
N3	*S. epidermidis* *Enterococcusspp*	*E.coli spp.* *Klebsiella*
N4	*S. aureus* *Enterococcusspp* *S. aureus*	*E.coli spp.* *Klebsiella*
N5	*Enterococcusspp* *S. aureus*	*E.coli spp.* *Pseudomonasspp*
N6	*Enterococcusspp.*	*E.coli spp.*

Group 1, No bill-trimming and fed only a basal diet.

Table 6: Isolated bacteria from ducks in group 2.

Sample no.	Gm+ve bacteria	Gm-ve bacteria
C 1	*S. aureus* *Enterococcus spp.*	*E. coli spp.* *Pseudomonas spp* *Salmonella spp.*
C 2	*S. aureus* *Enterococcus spp.*	*E. coli spp.* *Proteus spp.* *Salmonella spp*
C 3	*S. aureus* *Enterococcus spp.*	*E. coli spp.* *Klebsiella spp.*
C4	*S. aureus* *Enterococcus spp.*	*E. coli spp.*
C 5	*S. aureus* *Enterococcus spp.*	*E. coli spp.* *Pseudomonas spp.* *Proteus spp*
C 6	*S. aureus*	*E. coli spp.* *Klebsiella spp.* *Pseudomonas spp* *Proteus spp.* *Salmonella spp*

Group 2, Bill-trimmed and supplied only the basal diet;

Table 7: Isolated bacteria from ducks in group 3.

Sample no.	Gm+ve bacteria	Gm-ve bacteria
L 1	*S. aureus* *Enterococcus spp.*	*E. coli spp.*
L 2	*S. aureus* *Enterococcus spp.*	*E. coli spp.* *Klebsiella spp* *Proteus spp.* *Salmonella spp*
L 3	*S. aureus* *Enterococcus spp.*	*E. coli spp.* *Proteus spp*
L4	*S. epidermidis* *Enterococcus spp.*	*E. coli spp.*
L 5	*S. aureus* *Enterococcus spp.*	*E. coli spp.* *Klebsiella spp* *Pseudomonas spp.* *Proteus spp*
L6	*S. aureus* *S. epidermidis* *Enterococcus spp*	*Klebsiella spp.*

Group 3: Bill-trimmed and fed the basal diet + 1 g of bamboo extract.

Table 8: Isolated bacteria from ducks in group 4.

Sample no.	Gm+ve bacteria	Gm-ve bacteria
H 1	*S. aureus* *Enterococcus spp.*	*E. coli spp.*
H 2	*S. aureus* *S. epidermidis* *Enterococcus spp.*	*E. coli spp.*
H 3	*S. aureus* *S. epidermidis* *Enterococcus spp.*	*E. coli spp.*
H4	*S. aureus*	*E. coli spp.*
H 5	*S. aureus* *S. epidermidis* *Enterococcus spp.*	*E. coli spp.*
H6	*Enterococcus spp.*	*Klebsiella spp.*

Group4: Bill trimmed and fed the basal diet +2 g of bamboo extract

4-Molecular confirmation of isolated bacteria by PCR:

Using the previously mentioned primers in Table 1 for confirmation of *Lactobacilli, Bifidiobacteria, S.aureus, Salmonella spp, Pseudomonas spp, E.coli and Enterococci,*PCR yielded different products with different size bands specific for each primer that were observed in gel documentation figures (1-6).

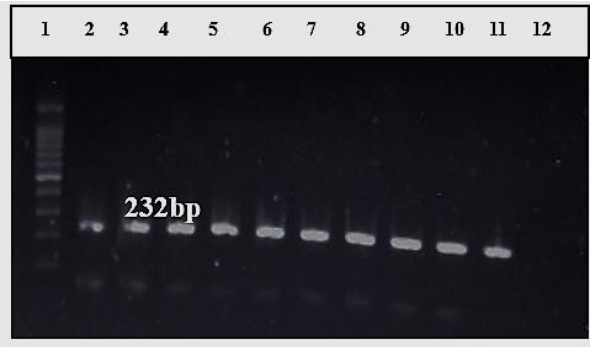

Fig1: Agarose gel electrophoresis of amplified *16srRNA gene of lactobacilli*,: Lanes 1: Marker lane 100-2000 bp DNA ladder; Lanes 1-10: positive amplification of 232 bp of *16srRNA gene*, lane 11: positive control and lane 12: negative control

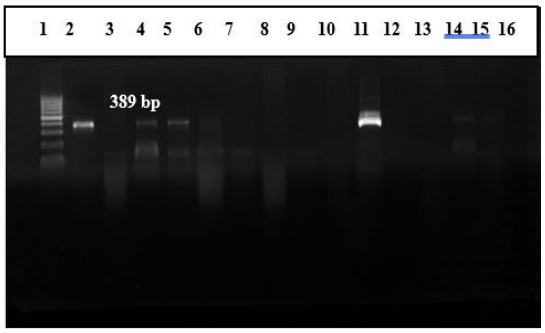

Fig2: Agarose gel electrophoresis of amplified *invA gene of Salmonella spp*: Lanes 1: Marker lane 100-1000 bp DNA ladder; Lane2: positive control and Lane 3: negative control and lanes (4,5,11&14): positive amplification of 389 bp of *invA* gene.

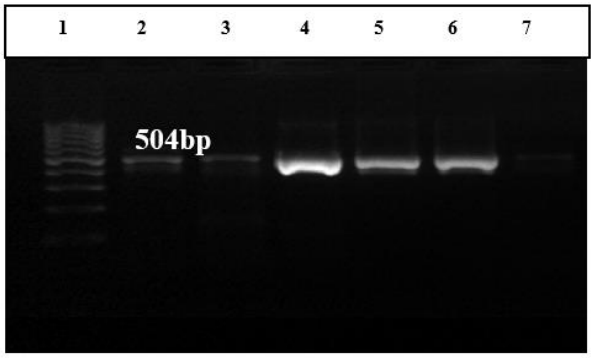

Fig3: Agarose gel electrophoresis of amplified *oprL gene of Pseudomonas*,: Lanes 1: Marker lane 100-1000 bp DNA ladder; Lanes 1-7positive amplification of 504 bp of *OprL* gene.

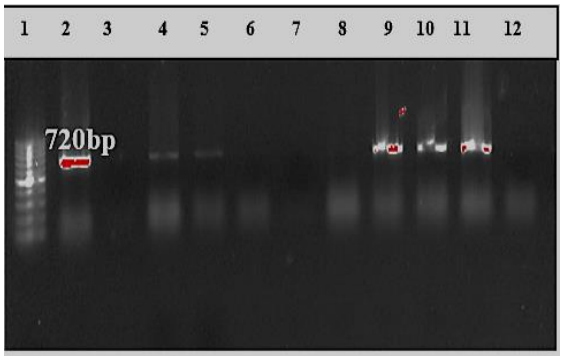

Fig4: Agarose gel electrophoresis of amplified *PhoA gene of E.coli*: Lanes 1: Marker lane 100-1000 bp DNA ladder; Lanes 2: positive control and lane 3: negative control; lanes (4,5,9,10,11) positive amplification of 720 bp of *PhoA* gene.

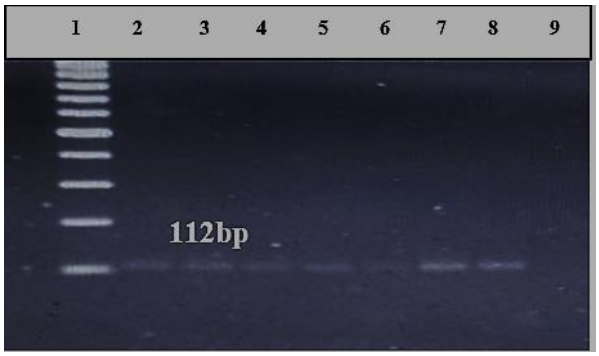

Fig5: Agarose gel electrophoresis of amplified *16srRNA gene of Enterococci*,: Lanes 1: Marker lane 100-1000 bp DNA ladder; Lanes 2-7: positive amplification of 112 bp of *16srRNA of Enterococci* gene; lane 8:positive control and lane 9;negative control.

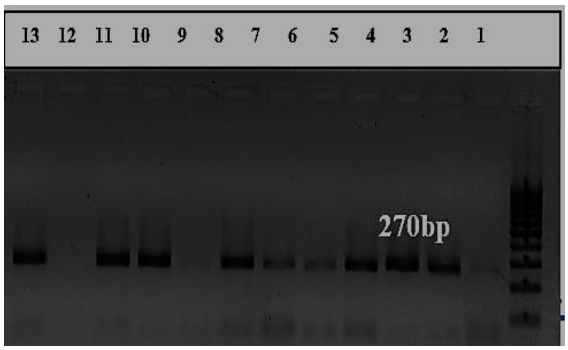

Fig6: Agarose gel electrophoresis of amplified *nuc gene of S.aureus*: Lanes 1: Marker lane 100-1000 bp DNA ladder; Lanes (2-8,10&11): positive amplification of 270 bp of *nuc* gene; lane 12: negative control and lane 13:positive control.

5-*Invitro* antibacterial effect of bamboo extract by Agar well diffusion assay: (Hossain *et al., 2022*)

The antibacterial effect of bamboo extract against *E.coli, Pseudomonas, Salmonella, S.aureus, S.epidermidis* and *enterococci* at conc of 1% and 2% showing an antibacterial effect against*Gram-negative bacteria* (Table 9).

Table 9: The ZOI 1% and 2% concof the extract against both isolated gm-ve and GM+ve bacteria.

ZOI/ conc.	*E. coli*	*Klebsiella*	*Salmonella spp.*	*Pseudomonas spp.*	*S. aureus*	*S. epidermidis*	*Entero-cocci*
1%	22 mm ± 1	31 mm ± 2	31 mm ± 1	30 mm ± 2	7mm ± 0.5	9 mm ± 1	13 mm ± 0.5
2%	27 mm ± 1	33 mm ± 1	35mm ± 1.5	34 mm ± 2.5	11 mm ± 1	14 mm ± 1	18 mm ± 2

All of the results of the effect of dietary supplementation of bamboo leaf extract on H/L ratio, lactic acid count and pathogenic bacteria in bill-trimmed mule duck were summarized in Figure 7.

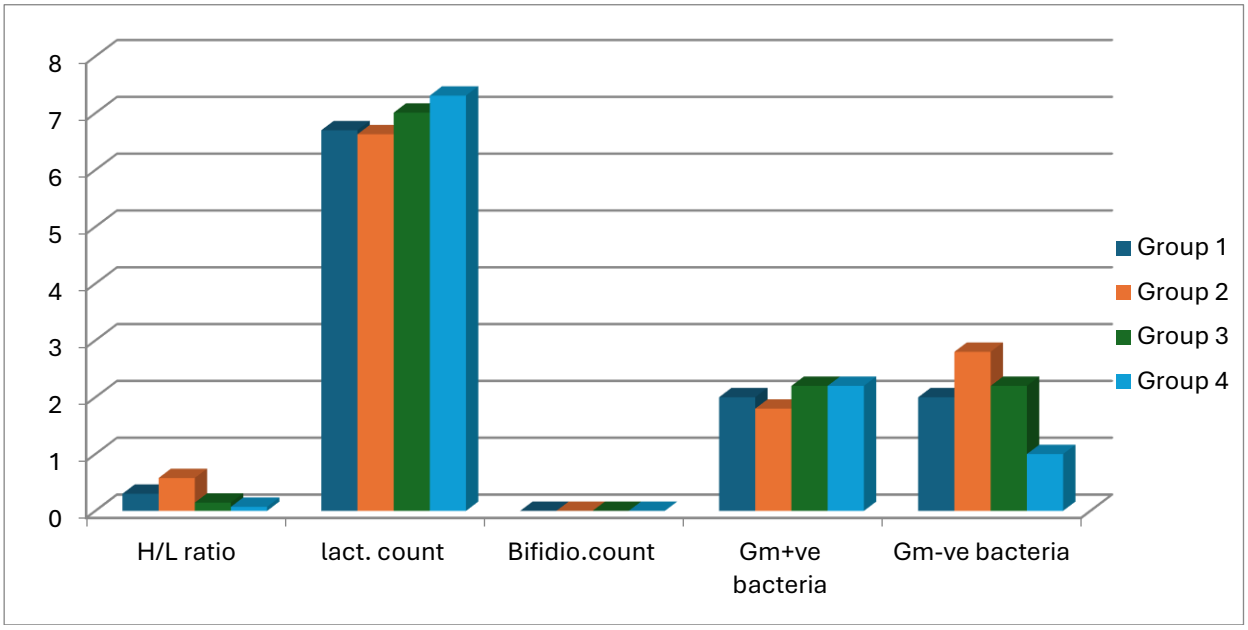

Fig 7: Effect of dietary supplementation of bamboo leaf extract on H/L ratio, lactica acid count (lactobacilli and bifidiobacteria) and pathogenic bacteria (Gram positive and Gram negative) in bill-trimmed mule duck. (Group 1, No bill-trimming and fed only a basal diet; Group 2, Bill-trimmed supplied only with the basal diet; Group 3: Bill-trimmed and fed the basal diet + 1 g of bamboo extract. and Group 4, Bill trimmed and fed the basal diet +2 gr of bamboo extract.)

DISCUSSION

Beak trimming was considered as an important step of an inclusive strategy toreduce aggressive peck cannibalism and death in poultry, However, it causes stress due to pain that has been shown to cause inflammation and change in intestinal bacteria profile that affect birds and lead to immune suppression, influence of diseases and finally death resulted in economic losses (Schwean-Lardner *et al.,* 2016; Riber & Hinrichsen, 2017; Ranjan *et al.,* 2019 and Li *et al.,* 2020).

Recent studies haverecorded that bamboo leaf extract food additiveswerea promising natural feed supplement which enhances immune-response, antioxidant capacity

regulation (Wedler*et al.,* 2014; Lodhi *et al.,* 2016 and Ge *et al.,* 2020). Additionally, bamboo leaf extract has powerful antibacterial action and can be used to treat a variety of bacterial infections. Moreover, it improves the beneficial gut bacteria community by acting as substrates due to the high content of polysaccharides, which promote the fermentation process in the colon by increasing the *Lactobacillus spp* and *Bifidobacterium spp.* number (Tanka *et al.,* 2011 &2013;Durgesh & Wasnik, 2014 and Vasave *et al.,* 2019).

The current study focused on the outcomes of usingthe bamboo leaf extract as an antibacterial and prebiotic feed additive that increases the disease resistance of birds, improves the beneficial bacteria, and supports immune response.

Quantification of leucocytes is the most uncomplicated measure to evaluate the chicken's robustness and immunity status using blood films (Ken and Evans 2000). The most abundant immune cells in avian blood are heterophils and lymphocytes (Palacios *et al.,* 2009 and Lee, 2006;). The heterophils participate mainly in natural and adaptive defence mechanisms to stand against pathogenic microbes, and lymphocytes are responsible for antibodyproduction and cellular immunity (Wigley, 2013).

The heterophil to lymphocyte ratio is related to the potency of innate and adaptive avian immunity (Krams *et al.,* 2012 and MacColl *et al.,* 2017).

Thiam *et al.*(2022a and 2022b) showed that H/L was an indicator to select the resistant birds to *Salmonella typhimurium* and *Salmonella Enteritidis* in chickens. Thiam *et al.*(2021) mentioned that the H/L was correlated with *Salmonella enteritidis* clearance through the intestinal immune response and the fowls with low H/L had improved intestinal immune defense mechanism and observed a great relation of the H/L ratio with intestinal expression of IFN-γ and IL-1β, which allow to predict the intestinal immune status in vivo in fowls and considered the H/L ratio a trait for bird disease resistance. The level of H/L ratio could cause a decline in antibody production and immune suppression through high levels of secreted corticosterone (Rouhalamini & Salarmoini, 2014). In the current study, the Heterophil/lymphocyte ratio was lower in the bamboo leaf extract-fed groups than in groups 1 and 2 ($P<0.05$). This agreed with El-Kazaz (2015), who reported that the H/L ratio in non-bill-trimmed ducks was lower than bill-trimmed ducks. These results can be attributed to the antioxidant and antagonistic effect to inflammation of bamboo leaf extract, which was confirmed by Hu *et al.*(2000), Guo *et al.* (2008) and Ni *et al.*(2013). On the contrary, our result disagreed with Dennis *et al.*(2009) and El Shafaei *et al.*(2017), who revealed that there wasnosignificant difference between the bill-trimmed ducks and non-trimmed birds in heterophil lymphocyte ratio.

Bamboo leaf extract is a potential source of polysaccharides that possess prebiotic properties, whichstimulate the multiplication of *Lactobacillus spp.* and *Bifidobacterium spp.* (Azmi *et al.*, 2012). In the present study, groups 3 & 4 (supplied with bamboo extract) showed significant differences ($P<0.05$) in Lactobacillus count than groups 1 and 2, which recorded a decrease in the total count of Lactobacillus. However, Bifidiobacteria were not detected in all experiment groups. The prebiotic activity of bamboo was recorded in different studies (Azmi *et al.,* 2012; He *et al.,* 2016 and Chen *et al.,* 2018) that agree with our result regarding lactobacilli and disagree with the results of bifidiobcteria.

Pathogenic bacteria are implicated inmajor economic losses in duck breeding. The mortality rates in ducks due to bacterial diseases are higher than viral diseases that have been expanded worldwide (Friend, 1999 and Enany, 2018). Various studies detected the in vitro antibacterial activity of

the bamboo plant to fight Gram+ve and Gram-ve bacteria (Cushnie *et al.,* 2014; Mabhiza *et al.,* 2016 and Ambika & Rajagopal, 2017). Interestingly, in this study, the Gram -ve bacteria (except *E. coli*) were more less in the groups supplemented with bamboo extract. While the Gram +ve bacteria (*S.aureus, S.epidermidis and enterococci*) were isolated from all groups without a significant difference (P>0.05). The present result of isolated bacteria and *invitro* antibacterial test showed activity against *Pseudomonas spp, Klebsiella and Salmonella spp.* (Table 9) could suggest the antibacterial effect against Gram -ve bacteria more than Gram +ve bacteria. Recent studies of the antibacterial effect of bamboo in vitro supported our results as they showed that the bamboo species are more efficacious against Gram-ve bacteria than against Gram+ve bacteria (Gokarneshan &Khan, 2020; Anselmo-Moreira *et al.,* 2021 and Angeline *et al.,* 2021). Anselmo-Moreira *et al.*(2021) indicated that the higher lipophilicity of the studied bamboo is related to the antibacterial constituents and that they are more effective against Gram-negative bacteria. Moreover, high content of flavonoids operates as an antibacterial agent byinhibiting the production of nucleic acid, energy metabolism of bacteria and damaging of the cytoplasmic membrane (Xie *et al.,* 2014). Concerning the isolation of the *E. coli and S. aureus* and *S. epidermis* in the current study, the Bamboo leaf extract supplemented groups did not show an antibacterial action that can kill *E. coli and S. aureus* and *S. epidermis*. These results agreed with some studies that proved that inert bamboo fiber was inactive against *S. aureus and E. coli* (Xi and Qin, 2012; Tanaka *et al.,* 2013 and Gokarneshan &Khan, 2020). Different results of antibacterial activity were confirmed by various studies (Singh *et al.,* 2010; Zhang *et al.,* 2010 and Owolabi & Lajide, 2015), whichcould be due to differences in the method of extraction,type of extract and the hygroscopicity of the extract (Tanaka *et al.,* 2013;Gokarneshan & khan, 2020). It was reported that the extracts of bamboo leaves in ethanol and aqueous phases were not effective against species of Staphylococcus (*S. aureus, S. subfava and S.epidermis*), and Gram-ve bacteria (Parekh and Chanda, 2008). However, Naidu (2012) and Singh *et al.*(2012) observed that the methanol extract of bamboo plant species could be a bactericidal agent for *E. coli, P. aeruginosa, S. aureus,* and *S. epidermis.* Finally, a recent study highlighted the effect of the concentration of the dose on the antibacterial effect as was detected that the methanol extract of bamboo plant species did not have effect at dose of 10 mg/ml against *E. coli* and *S. aureus* and began to give moderate effect from concentration of 20 mg/ml which is directly proportional to the increase in the dose (Angeline *et al.,* 2021).

CONCLUSION

The current results imply that the bamboo leaf extracts could be used as a natural feed additive candidate due to potent antibacterial activity, prebiotic effect and moreover enhancing immunity. Other medicinal applications and future research arerecommended for studying the factors that determine the antibacterial property and the roles of the extract of bamboo leaves on duck health, especially with the increasing demand for organic animal products without antibiotics and synthetic chemicals.

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تأثير المكمل الغذائي لمستخلص اوراق نبات الخيزران علي بكتريا الامعاء ومقاومة الامراض في بط البغال المشذبة المنقار

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يلعب مستخلص أوراق الخيزران دورًا مهمًا في تعزيز مناعة الطيور ومقاومتها للامراض ويمكن استخدامه كمكمل غذائي طبيعي في تربية الطيور. يؤدي تقليم المنقار إلى تفاعلات التهابية وحدوث الإجهاد التي بدورها قد تؤدي إلى كبت المناعة وتقليل مقاومة الطائر للأمراض. كانت أهداف الدراسة الحالية هي معرفة تأثير مستخلص أوراق الخيزران في تعزيز مقاومة الطيور المجهدة. تم استخدام أربع مجموعات (٦ طيور لكل مجموعة)؛ المجموعة ١: لم يتم تقليم المنقار وتغذيته على العليقة الأساسية فقط بدون مستخلص. المجموعة ٢: المنقار قلص واتبع نظامًا غذائيًا أساسيًا فقط بدون مستخلص. المجموعتان ٣ و ٤: تم تقليم المنقار وتغذيته على غذاء أساسي بالإضافة إلى مستخلص الخيزران (1 و ٢ جم لكل كجم، على التوالي)، وتم جمع عينة الدم في اليوم ٦٠ من التجربة لتحديد نسبة خلايا الهيتيروفيل / الخلايا الليمفاوية (H / L) كمؤشر لمقاومة الطيور. تم تحديد العدد البكتيري الكلي للعصيات اللاكتوبسيلس والبيفيديو بكتريا وتم عزل البكتيريا المسببة للأمراض وفحصها عن طريق الفحص المظهري وأخيراً تم التأكد من ذلك بواسطة اختبار تفاعل البلمرة المتسلسل. كانت نسبة H / L أقل في المجموعات التي تم تغذيتها بمستخلص أوراق الخيزران مقارنة بطيور المجموعة ١ والمجموعة ٢. أظهرت المجموعتين ٣ و ٤ نباتات التي تم تغذيتها علي الخيزران على زيادة معنوية في عدد بكتيريا اللاكتوبسيلس مقارنة بالمجموعة ١ والمجموعة ٢. وأظهرت نتيجة اختبار التاثيرالمضاد للبكتريا نشاطا مضاداً للبكتيريا سالبة الجرام المعزولة مقارنة بالبكتريا موجبة الجرام المعزولة. تشير النتائج الحالية إلى أنه من الممكن استخدام مستخلص أوراق نبات الخيزران كبديل للمواد الصناعية التي تستخدم لحل مشاكل إجهاد تقليم المنقار.