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Original Article

Impact of Phytobiotic Growth Promoters Supplementation on the Morphology and Biometry of the Vital Organs in Broilers

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ABSTRACT

Supplementation of Cv (clove) and Ts (tulsi) augments the growth rate and gut health of broilers. However, studies on their impact on the vital organs and male reproductive system remain scarce. Therefore, the current study aimed to investigate the impact of Cv and Ts supplementation on hematological profiles, as well as the heart, lungs, liver, kidney, and testes of broilers, both morphologically and biometrically. Sixty one-day-old broiler chicks, divided into four homogenous groups (control-T0 and three supplement groups-T1, T2, and T3), were fed 0.5% Cv and 2% Ts (T1), 1% Cv and 3% Ts (T2), and 1.5% Cv and 4% Ts (T3) with drinking water for 21 d. Organ samples were collected on d 14 and d 28 for macroscopic and microscopic (hematoxylin and eosin stain) investigation. Cv and Ts didn't affect the hematological profile, organ weight gain, and their general histoarchitectures. However, the histomorphometric investigation showed an increased (p<0.05) parabronchus surface area of the lungs, size of the renal glomeruli, the diameter of the proximal and distal convoluted tubules of the kidney in the T1 and T2 groups, and size of the seminiferous tubules of the testes in group T1. These findings suggest that unlike antibiotic or steroid growth promoters (GPs), Cv and Ts have beneficial impacts on the vital organs as well as on the male gonad of broilers from the morphologic and biometric perspectives.

INTRODUCTION

Broiler farming is a major sector across the globe, with broiler meat being among the most popularly consumed protein sources. The stakeholders of the poultry industries in Bangladesh are currently moving towards safe organic eggs and meat, as well as better health and living conditions. By 2030, 373 million tons of meat will be produced worldwide, an increase of nearly 44 million tons, with 84% of the growth taking place in emerging countries (OECD/FAO, 2021). This rise has been especially apparent in nations like China, the USA, and Brazil, which are among the biggest broiler producers in the world (OECD/FAO, 2021). Despite its expansion, broiler production has encountered difficulties, including worries about animal welfare, environmental impacts, and food safety. These challenges have prompted the desire to develop alternative, environmentally friendly production techniques, such as the production of organic and free-range broilers.

The use of antibiotic growth promoters (GPs) in poultry has been linked to the development of antibiotic resistance, while steroid GPs cause severe immune suppression, let alone their residual effects on human health (Vidovic & Vidovic, 2020; Islam *et al.*, 2022c, Islam *et al.*, 2023b). To replace synthetic GPs like antibiotics or steroids for the production of organic meat, stakeholders and researchers are working to build a unique, phytobiotic-based feeding approach for broilers

(Islam *et al.*, 2022c; Islam *et al.*, 2023a). Organic GPs are additives to broiler feed that boost growth and increase feed efficiency (Dhama *et al.*, 2014; Islam *et al.*, 2023a). The use of antibiotics to promote growth performance has been a common practice for many years (David *et al.*, 2012). However, there has been an increasing interest in developing alternative solutions due to increasing concerns regarding the development of antibiotic resistance and associated human health risks (Wierup, 2001; Islam *et al.*, 2023a).

Organic growth boosters may be found in the form of yeast derivatives, essential oils, pre- and probiotics, and herbal extracts (Agostini *et al.*, 2012; Laudadio *et al.*, 2012; Dhama *et al.*, 2014; Chowdhury *et al.*, 2018; Thuekeaw *et al.*, 2022). These ingredients are thought to promote gastrointestinal health and functionality, improve nutrient absorption, and strengthen broilers' immune systems, all of which contribute to enhancing growth and feed efficiency (Agbola *et al.*, 2015; Alagawany *et al.*, 2021; Islam *et al.*, 2023a).

Due to the many health advantages of Cv, it has been utilized in traditional medicine for millennia (Batiha *et al.*, 2020; Arif *et al.*, 2022). It has been found to have analgesic, anti-inflammatory, antibacterial, and antioxidant effects (El-Maati *et al.*, 2016; Batiha *et al.*, 2020). Eevuri & Putturu (2013) reported that traditional Indian medicine has been exploited. Ts, also known as holy basil, has been valued as a sacred plant in Hinduism for hundreds of years. Chaudhary *et al.* (2018) explored its therapeutic characteristics and validated its potential health benefits. It has been traditionally used for its medicinal properties in humans, but its potential benefits for livestock, particularly broilers, have also been explored in recent studies (Baliga *et al.*, 2012; Eevuri & Putturu, 2013; Islam *et al.*, 2023a). These researches have shown the benefits of Ts supplementation on broiler development, immunological function, and general health status.

Ts supplementation in broiler diets improves feed efficiency, weight gain, and carcass traits, while also reducing oxidative stress (Agostini *et al.*, 2012; Wati *et al.*, 2015; Chowdhury *et al.*, 2018; Chakma *et al.*, 2020; Islam *et al.*, 2023a). Many scientific researches have shown that eugenol, a major active ingredient of both Cv and Ts, has antibacterial effects against various pathogens, such as Escherichia coli and Staphylococcus aureus (El-Maati*et al.*, 2016; Milind & Deepa, 2011; Islam *et al.*, 2023a). The earlier study findings showed that feeding 2-5% Ts to broilers promotes antioxidant and anti-stress activities, as well as improving broiler growth performance (Bhosale *et al.*, 2015; Hasan *et al.*, 2016; Khalil *et al.*, 2017). In another study, Al-Mufarrej *et al.* (2019) fed 1-6% Cv to broilers and found that feeding clove at a concentration of 2% or more negatively affects broiler growth performance. Islam *et al.* (2023a) used the same combinations of Cv (0.5-1.5%) and Ts (2-4%) in broiler to investigate their effects on growth performance and gut health. Herbal supplements, like other alternative medicinal therapies, should be given prime focus so that farmers can gain maximum production without harmful effects (Saxena, 2008). However, no study has been conducted to elucidate the role of Cv and Ts on the heart, lungs, liver, kidney, and testes of broilers and therefore, it remains ambiguous. Gross morphometric and biometric attributes can be an effective tool to study the impacts of Cv and Ts supplementation on these organs in broilers. In this context, the current study was conducted to investigate how the supplementation of Cv and Ts affects the aforementioned organs, both morphologically and biometrically.

MATERIALS AND METHODS

Statement of the experiment

The study was conducted on sixty (60) healthy male broiler chicks (one-day-old, Cobb-500 strain) to investigate changes in vital organs (heart, lungs, liver, kidney, and testis), both at the macroscopic and microscopic levels, if any. The experimental trial and laboratory works were conducted at the Department of Anatomy and Histology, Bangladesh Agricultural University (BAU), Bangladesh, following the proper guidelines set by the institutional "Animal Welfare and Experimentation Ethics Committee", BAU [AWEEC/BAU/2021(05)].

Experimental shed preparation

The broiler chicks were housed in an acclimatized shed, with the provision of sufficient artificial light and ventilation and standard bio-security measures. The shed was fumigated using 17.5 g of $KMnO_4$ and 35 ml of 37% formaldehyde. The broiler cages were disinfected with a 3% potassium permanganate spray and the feeding trays and drinking pots were cleaned regularly with 1% bleached water.

Preparation of Clove bud powder and Tulsi extract

Cv (*Syzygium aromaticum*) buds were ground into a very fine powder using an electric grinder and stored in an airtight container. An aqueous extract of Ts

(*Ocimum sanctum*) was prepared from fresh leaves by blending them in water. Finally, the required amount of Cv powder and water were added to prepare different combinations, i.e., 0.5% + 2%, 1% + 3%, and 1.5% + 4% of Cv and Ts, respectively.

Broiler management

Standard temperature (brooding temperature: 35°C for the first three days followed by a gradual decrease of 2-3°C daily until reaching 21°C, rearing temperature: 21°C) and relative humidity (50-60%) were maintained throughout the study period. Following the seven days of the acclimatization period, broiler chicks (N=60) were randomly distributed to four experimental groups (15 broiler chicks in each group). Dimension of the pen: 5×4 square meters. Broilers were fed a commercial broiler starter diet (pellet form) up to d 14, and then shifted to a grower diet (feed composition is given in supplementary table 1). 24 hours of light were provided on the first three days, followed by a 30-minutes reduction of the light period each day up to d 28, with the provision of adequate ventilation. The experimental feeding trial was conducted from d 8 to d 28.

The control group (T0) received the commercial diet and normal fresh drinking water without any additional supplementation of Cv or Ts. The treatment groups received 0.5% Cv and 2% Ts (T1), 1% Cv and 3% Ts (T2), and 1.5% Cv and 4% Ts (T3) with drinking water for 21 d (from d 8 to d 28).

Sample collection

Blood samples were collected from the wing vein to analyze the hematological profile of broilers. Heart, lungs, liver, kidney, and testis samples were collected from five broilers (sacrificed by the cervical subluxation method) from each group on d 14 and d 28 of the experiment. As part of the macroscopic study, the weight of each organ was then recorded using a high-precision electric balance (AND FGH Series Digital Balance, Korea). The final live weights of broilers of different experimental groups are given in supplementary table 2. The relative weights (%) of the organs were calculated using the following formula:

$$\text{Relative weight (\%)} = \frac{\text{Organ weight} =}{\text{Body weight} =} \times 100$$

Table 1 – Effects of Clove and Tulsi on the hematological profile of 28-days-old broilers.

Parameters	Treatments[1] T0	T1	T2	T3	SEM	*p*-Value
RBC (million/mm³)	2.74	2.60	2.75	2.69	0.22	0.904
WBC (×10³/µl)	16.77	17.10	16.9	16.67	1.34	0.989
Hb (g/dl)	7.03	6.40	7.33	7.00	0.41	0.224
PCV (%)	25.67	24.33	24.67	23.67	3.42	0.144
ESR (mm in the first hour)	2.33	2.67	2.33	2.00	0.71	0.827
Neutrophil (%)	66.33	66.00	67.60	65.67	1.73	0.685
Eosinophil (%)	2.33	1.60	2.67	2.33	0.85	0.701
Lymphocyte (%)	28.60	29.33	27.67	28.40	1.31	0.567
Monocyte (%)	2.50	2.00	2.33	2.67	0.58	0.627

[1]T0: represents the control group; T1, T2, and T3 groups represent supplementation of 0.5% Clove + 2% Tulsi, 1% Clove + 3% Tulsi, and 1.5% Clove and 4% Tulsi, respectively.

Microscopic investigation

The collected tissue samples were fixed with 10% neutral buffered formalin for 72 hours. The samples were processed for paraffin section preparation and stained with Hematoxylin and Eosin stain, following the standard procedure described by Sultana *et al.*

Table 2 – Effects of Clove and Tulsi on the histomorphometric attributes of the heart (heart wall thickness) and lungs (surface area of lung parabronchus) in broilers.

Attributes	Treatments[1] T0	T1	T2	T3	SEM	*p*-value
Thickness of heart wall						
D 14	1194.01	1218.28	1252.13	1149.90	98.09	0.765
D 28	1432.82	1486.31	1565.16	1345.39	119.02	0.322
Surface area of lung parabronchus						
D 14	98044.80^{b}	102947.05^{ab}	107849.29^{a}	96083.91^{b}	3907.96	0.022
D 28	112751.52^{c}	123536.45^{b}	140204.07^{a}	112418.17^{c}	4733.82	0.001

[a,b,c]Values with different alphabetic superscripts within a row differ significantly ($p<0.05$).

[1]T0: represents the control group; T1, T2, and T3 groups represent supplementation of 0.5% Clove + 2% Tulsi, 1% Clove + 3% Tulsi, and 1.5% Clove and 4% Tulsi, respectively.

(2020). The histological features were examined under a photomicroscope (Model: IS.1153.EPL, Euromex, Netherlands). Morphometric measurements, i.e., heart wall thickness (μm), lung parabronchus surface area (SAP, $μm^2$), glomeruli size and the diameter of the proximal (PCT) and distal convoluted tubules (DCT) of the kidney (μm), and the size of the seminiferous tubules (SST) of the testis (μm) were measured using Image Focus Alpha software. A total of five microscopic fields from each tissue section (five tissue sections for each organ) were evaluated for each organ from each group.

Statistical analysis

All the data sets obtained in the current study were statistically analyzed using the IBM Statistical Package for the Social Sciences-V22, following a completely randomized design. At first, the normality of the data sets was tested by Levene's test. Comparisons between the experimental groups were made using the one-way ANOVA test, followed by post-hoc Duncan's Multiple Range Test (DMRT) and Least Significant Difference (LSD) Test. Differences were considered significant when *p*-value was less than 0.05. On the other hand, *p*-values greater than 0.05 but less than 0.1 ($0.05< p<0.1$) were considered as a tendency to increase or decrease.

RESULTS

Effects of Clove and Tulsi on the hematological profile

The hematological profile of 28-days-old broilers supplemented with Cv and Ts are presented in Table 1. The experimental groups had no significant difference ($p>0.05$) in RBC (mil/mm^3), WBC ($\times 10^3/μl$), Hb (g/dl), PCV (%), ESR (mm in the first hour), neutrophil (%), eosinophil (%), lymphocyte (%), and monocyte (%) levels. The RBC, WBC, Hb, PCV, ESR, neutrophil, eosinophil, lymphocyte, and monocyte levels were found to be between 2.6-2.75 million/mm^3, 16.67-17.10 × $10^3/μl$, 6.40-7.33 g/dl, 24.33-25.67 %, 2-2.67 mm in the first hour, 65.67-67.70 %, 1.60-2.67 %, 27.67-29.33 %, and 2-2.67 %, respectively.

Effects of Clove and Tulsi on the relative weights of different organs

The relative weights of the heart, lungs, liver, kidney, and testes of different experimental groups are shown in Figure 1. No significant difference ($p>0.05$) in heart weight was observed among the experimental groups on d 14 or d 28. However, relative heart weights tended to be higher in the broilers of groups T0 and T3 on d 28.

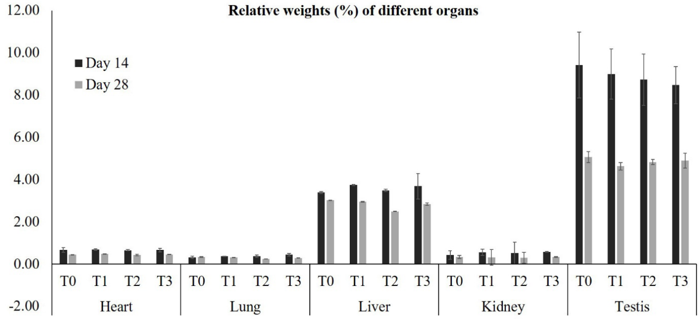

Figure 1 – The effects of clove and tulsi on the relative weights (%) of the vital organs (heart, lungs, liver, kidney, and testis) of broilers. Data are presented as the mean ± SEM.

On d 14, the lungs of the different experimental groups had no substantial difference ($p>0.05$) in their relative weights. However, they differed markedly ($p<0.01$) on d 28, where the broilers of group T0 had the highest relative lung weight and the T2 had the lowest.

The livers, kidneys, and testes of different groups had no significant difference ($p>0.05$) in their relative weights on d 14 or d 28.

Effects of Clove and Tulsi on the heart

Macroscopic attributes

The hearts revealed a normal macroscopic appearance without any visible alteration in both the control and the phytobiotic-supplemented groups. They appeared triangular in shape, bright red in color with a slight greyish tinge, having yellowish-white coronary fat at their base.

Microscopic attributes (heart wall thickness)

The microscopic details of the heart are shown in Figure 2. Microscopically, the hearts were composed of three distinct layers – endocardium, myocardium, and epicardium. No histoarchitectural alteration was observed in any of the experimental groups. Histomorphometric investigation of the heart wall thickness also showed no noticeable variation among the groups on d 14 and d 28 (Table 2).

Effects of Clove and Tulsi on the lungs

Macroscopic attributes

The lungs of the experimental groups appeared wedge-shaped and reddish-pink in color. No visible gross abnormality was observed, either in shape or color.

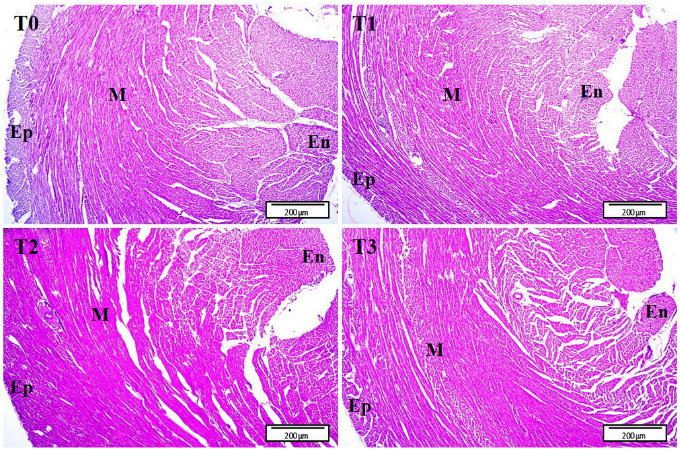

Figure 2 – Histoarchitecture of the heart wall showing the effects of clove and tulsi (hematoxylin-eosin stain). Ep – Epicardium, M – Myocardium, and En – Endocardium. Magnification – 40X, Scale bar – 200 µm.

Microscopic attributes (surface area of lung parabronchus)

The microscopic details of the lungs are shown in Figure 3. Microscopically, the lung parenchyma was composed of tubular parabronchi separated by thin fibrous septa. The parabronchi were somewhat pentagonal in shape. Blood vessels were observed in between the parabronchi. However, no noticeable histoarchitectural alteration was found in the lungs of the experimental groups. The histomorphometric investigation of the SAP showed significant variation ($p<0.05$) among the experimental groups on both d 14 and d 28. The T2 group had the highest SAP, followed by T1, T0, and T3 on d 28 (Table 2).

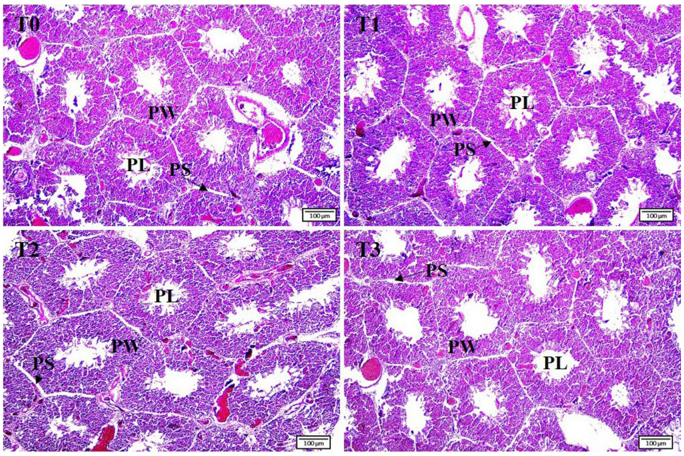

Figure 3 – Histoarchitecture of the lungs showing the effects of clove and tulsi (hematoxylin-eosin stain). PW – Parabronchial wall, PL – Parabronchial lumen, and PS – Parabronchial septum. Magnification – 100X, Scale bar – 100 µm.

Effects of Clove and Tulsi on the liver

Macroscopic attributes

The livers of the experimental groups had normal size and shape, without any noticeable alteration.

Microscopic attributes

The histoarchitecture of the liver is shown in Figure 4. The liver of the experimental groups exhibited normal histological architecture. The hepatic parenchyma was characterized by hexagonal hepatic lobules containing polyhedral hepatocytes arranged in hepatic cords and central veins.

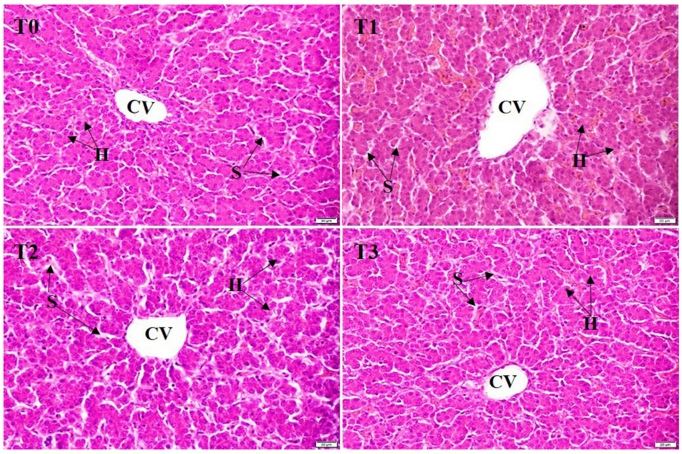

Figure 4 – Histoarchitecture of the liver showing the effects of clove and tulsi (hematoxylin-eosin stain). CV – Central vein, H – Hepatocyte, and S - Sinusoid. Magnification – 400X, Scale bar – 20 µm.

Effects of Clove and Tulsi on the kidney

Macroscopic attributes

The kidneys of the experimental groups appeared quadrangular in shape and dark brown in color. No visible gross abnormality, either in shape or color, was observed.

Microscopic attributes (glomerular size and tubular diameter)

The microscopic details of the kidney are shown in Figure 5. Microscopically, the kidney exhibited normal

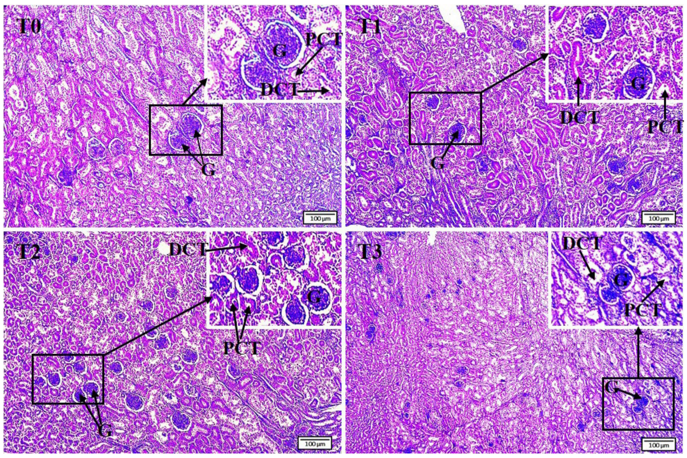

Figure 5 – Histoarchitecture of the kidney showing the effects of clove and tulsi (hematoxylin-eosin stain). G – Glomerulus, PCT – Proximal Convoluted Tubule, and DCT – Distal Convoluted Tubule. Magnification – 100X, Scale bar – 100 µm.

histoarchitectures with regularly distributed glomeruli throughout the cortex. The glomeruli were surrounded by Bowman's capsule, with Bowman's space in between them. The PCTs were lined by simple cuboidal epithelia with apical brush borders. The DCTs showed smooth apical surfaces with comparatively wider lumens. No noticeable histoarchitectural alteration was found in the kidneys of the experimental groups.

The histomorphometric investigation of the size of the glomerulus as well as the diameter of the PCTs and DCTs showed no mentionable variation ($p<0.05$) among the experimental groups on d 14. However, substantial differences ($p<0.05$) in the glomerulus size as well as the diameter of PCTs and DCTs were observed on d 28, with the T2 group having the largest diameter, followed by T1, T3, and T0 (Table 3).

Table 3 – Effects of Clove and Tulsi on the histomorphometric attributes of the kidney (diameter of the proximal convoluted tubules and distal convoluted tubules) in broilers.

Attributes	Treatments[1]				SEM	*p*-value
	T0	T1	T2	T3		
Size of the glomeruli (mm²)						
D 14	0.08	0.09	0.09	0.08	0.007	0.893
D 28	0.09^{b}	0.11^{a}	0.12^{a}	0.10^{ab}	0.008	0.031
Diameter of the proximal convoluted tubules						
D 14	165.25	173.51	170.21	166.90	7.07	0.659
D 28	176.82^{b}	194.33^{a}	199.14^{a}	190.27^{ab}	7.96	0.048
Diameter of the distal convoluted tubules						
D 14	279.84	288.23	305.53	285.44	13.29	0.262
D 28	302.23^{b}	319.94^{b}	354.41^{a}	311.29^{b}	14.79	0.007

[a,b]Values with different alphabetic superscripts within a row differ significantly ($p<0.05$).

[1]T0: represents the control group; T1, T2, and T3 groups represent supplementation of 0.5% Clove + 2% Tulsi, 1% Clove + 3% Tulsi, and 1.5% Clove and 4% Tulsi, respectively.

Effects of Clove and Tulsi on the testis

Macroscopic attributes

No gross visual macroscopic changes were observed in the testes of the experimental groups. Uniform size, shape, and color were found in both the control and treated groups of broilers.

Microscopic attributes (size of the seminiferous tubules)

The microscopic details of the testes are presented in Figure 6. Microscopically, the testes were enclosed with a fibrous capsule (tunica albuginea). Numerous seminiferous tubules containing spermatogenic cells were distributed throughout the testicular parenchyma. Single or cluster Leydig cells were observed in between the seminiferous tubules. The broilers of different experimental groups showed no noticeable histoarchitectural alteration. In the histomorphometric study, no mentionable variation ($p>0.05$) in the SSTs was found among the experimental groups. However, SSTs differed substantially ($p<0.05$) on d 28. The SST size was highest in group T2, followed by T1, T0, and T3 (Table 4).

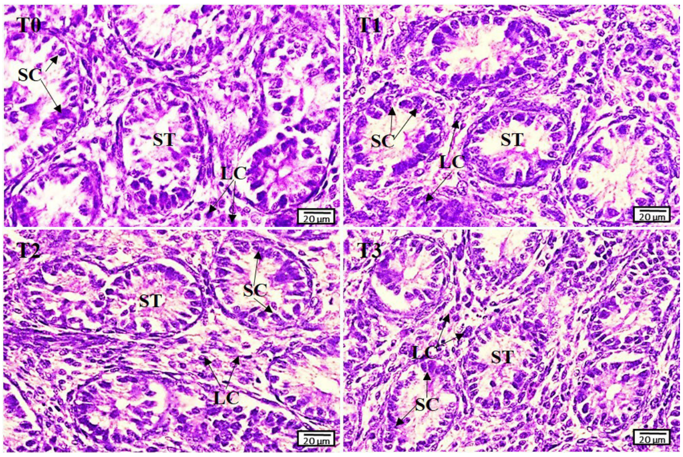

Figure 6 – Histoarchitecture of the testes showing the effects of clove and tulsi (hematoxylin-eosin stain). ST – Seminiferous tubules, SC – Spermatogenic cell, and LC – Leydig cell. Magnification – 400X, Scale bar – 20 µm.

DISCUSSION

Cv and Ts have been proven to be effective phytobiotic feed additives that improve growth performance and gut health in poultry (Agostini *et al.*, 2012; Al-Mufarrej *et al.*, 2019; Islam *et al.*, 2023a). However, their effects on the vital organs of broilers need to be explored to get a clear insight into their efficacy. Morphologic and biometric evaluation of the vital organs can be an important tool in this context (Islam *et al.*,2021; Islam *et al.*, 2022a, b; Sultana *et al.*, 2021; Sultana *et al.*, 2022). Therefore, the current study investigates the impact of Cv and Ts on the morphologic and biometric attributes of the vital organs (heart, lungs, liver, kidney, and testes) of broilers for the first time.

Table 4 – Effects of Clove and Tulsi on the histomorphometric attributes of the testes (size of the seminiferous tubules) in broilers.

Attributes	Treatments[1] T0	T1	T2	T3	SEM	*p*-value
Size of the seminiferous tubules						
D 14	183.83	189.35	196.70	180.16	10.78	0.455
D 28	202.22^{b}	215.86^{ab}	228.17^{a}	196.37^{b}	12.13	0.046

^{a,b} Values with different alphabetic superscripts within a row differ significantly ($p<0.05$).

[1]T0: represents the control group; T1, T2, and T3 groups represent supplementation of 0.5% Clove + 2% Tulsi, 1% Clove + 3% Tulsi, and 1.5% Clove and 4% Tulsi, respectively.

Effects of Clove and Tulsi on the hematological profile

The maintenance of optimal blood cellular profile is crucial to broiler. The hematological profile analysis in broilers fed Cv and Ts showed no mentionable effect on blood cellular constituents. Erythrocyte is one of the major cellular constituents that transport nutrition and oxygen (Hb) around different body parts (Odunitan-Wayas *et al.*, 2018). Nath *et al.* (2012) reported that supplementation of tulsi, clove, and black pepper does not affect broiler hematological indices (TEC, PCV, Hb, and ESR). Hasan *et al.* (2016) reported higher PCV % while ESR % was comparatively lower than the previous report. Neutrophil, eosinophil, lymphocyte, and monocyte counts were also lower than the earlier report by Baudouin *et al.*, 2021. WBCs play a critical role in the body's defense and are indicators of stress response (Glenn & Armstrong, 2019). The decrease in these parameters might be linked to the composition of the supplied feed and environmental factors (Baudouin *et al.*, 2021). However, the current study results strongly suggests that supplementation of Cv and Ts has no harmful or toxic effect on the hematological profile of broilers.

Effects of Clove and Tulsi on the relative weights of different organs

The morphologic and biometric features of the vital organs of broilers have been widely studied due to the potential negative physiological effects associated with genetic selection for growth (Chen *et al.*, 2017; Harash *et al.*, 2019). In avian species, the liver is larger than in mammals compared to body size. The size and color of the liver depend on the broiler's age and body weight (Akers & Denbow, 2013; Denbow, 2015). The morphologic and biometric investigation of the testes was done as it is the primary organ for male reproduction, being responsible for producing sperm and male hormones. Fragaso *et al.* (2013) observed that male fertility is directly linked to testicular weight. The present study findings highlight that Cv and Ts supplementation had no negative impact on broiler heart, lung, liver, kidney, and testis. The relative weights of the heart, right lung, liver, and right kidney found in the current study ranged between 0.45-0.69%, 0.25-0.39%, 2.5-3.02%, and 0.30-0.35%, respectively. The relative weights of the heart, lung, liver, kidney, and testis have been found to be 0.52±0.07% (Gaya *et al.*, 2007), 0.36±0.02% (Azizian & Saki, 2021), 2.6% (Nath *et al.*, 2012), 0.609±0.02% (Xiao *et al.*, 2011), and 0.81±0.14 (Mahmood *et al.*, 2011), respectively, which is in line with the findings of our current study.

Effects of Clove and Tulsi on the macroscopic and microscopic characteristics of the heart

The histoarchitectural integrity of the heart is crucial for maintaining its normal function. The myocardium, which is the muscular layer of the heart, is the most important component of the heart. According to the study reports conducted by Harash *et al.* (2019), broilers have increased myocardial cell size compared to the non-broiler chicken, which is accompanied by increased myofibril size, number, and fibrosis of the heart wall. The implications of these histological changes in broiler hearts are significant. Chen *et al.* (2017) reported that the increase in myocardial cell size and fibrosis can lead to reduced cardiac function and increased susceptibility to heart failure. The use of steroid GPs in broilers causes severe morphologic and biometric alterations like suppression of cardiomyocyte development (Rudolph, 2000; Islam *et al.*, 2022a). In the current study, the hearts of the different experimental groups supplemented with Cv and Ts showed normal histoarchitectures (Eurell & Frappier, 2006, Islam *et al.*, 2022a), without any visible alterations. Therefore, phytobiotic-based (Cv and Ts) dietary inclusion can be a safer option in this context.

Effects of Clove and Tulsi on the macroscopic and microscopic characteristics of the lungs

The histological examination of broiler lungs from both the morphologic and morphometric aspects can provide important information about the presence and distribution of pathological changes, such as inflammation, fibrosis, and cellular proliferation (Islam *et al.*, 2022b). The parabronchi of broiler chickens are composed of air capillaries and blood capillaries. Air capillaries are lined with simple squamous epithelium, while blood capillaries are lined with endothelial cells. Eurell & Frappier (2006) elucidated that the close proximity of the air and blood capillaries facilitates efficient gas exchange. The histological investigation of the lungs showed normal histoarchitectures in the broiler (Eurell & Frappier, 2006). However, the findings of the current study showed that the surface area of the lung parabronchus notably increased in the broilers supplemented with 1% Cv and 3% Ts. This might be beneficial in reducing or eliminating hypoxic stress in broilers through the augmentation of oxygen absorption in the lungs and increased availability of oxygen at the cellular level (Olkowski *et al.*, 2005; Özyiğit *et al.*, 2015).

Effects of Clove and Tulsi on the macroscopic and microscopic characteristics of the liver

Avian liver is histologically different from that of mammals, having less connective tissue and lacking true hepatic lobulation (Eurell & Frappier, 2006; Mushawwir *et al.*, 2021). The liver is a vital organ due to its detoxifying activities within the body, as reported by Zhao *et al.* (2019). Denbow (2015) reported that the synthesis, storage, and distribution of lipids, carbohydrates, and proteins are regulated by the liver, which thus plays critical roles in digestion and metabolism. Hence, a healthy liver is a must for maintaining the health of an organism. Duma *et al.* (2006) noted that drugs are commonly responsible for hepatic damage depending on their dose and duration of administration. Residues of antibiotics and steroids reportedly accumulate in the liver, which is accompanied by severe liver damage, according to earlier study reports (Amine *et al.*, 2020; Sultana *et al.*, 2022; Ulomi *et al.*, 2022). Histological investigation of the liver in different experimental groups also showed no alteration in their histoarchitecture. Therefore, Cv and Ts can be safer alternatives to antibiotic or steroid growth promoters.

Effects of Clove and Tulsi on the macroscopic and microscopic characteristics of the kidney

Histologically, broiler kidneys are similar to those of other avian species, but they have some unique features that reflect their rapid growth and high metabolic rate, as noticed by Nabipour *et al.* (2009). The avian kidney consists of two distinct regions: the cortex and the medulla. The cortex contains glomeruli and proximal and distal convoluted tubules, while the medulla contains the loops of Henle and collecting ducts. The glomeruli are responsible for filtering blood, and the tubules and ducts are responsible for reabsorbing useful substances and excreting waste products (Eurell & Frappier, 2006; Nabipour *et al.*, 2009). Therefore, maintaining structural integrity is indispensable to preserve proper renal functionality. Synthetic growth promoters like antibiotics or steroids reportedly damage broiler kidneys, hence phytobiotic-based feeding practices can be an effective solution to this problem (Amine *et al.*, 2020; Sultana *et al.*, 2021). The present study showed no visible alteration in the histoarchitectures of the kidneys of the experimental groups. However, the size of the glomerulus, and the diameter of both PCT and DCT substantially increased on d 28 in the broilers supplemented with 1% Cv and 3% Ts, indicating a higher capacity of filtration and resorption of water and electrolytes (Nabipour *et al.*, 2009).

Effects of Clove and Tulsi on the macroscopic and microscopic characteristics of the testes

The testes of broiler chickens are composed of seminiferous tubules, which are responsible for the production of sperm, and interstitial tissue, which produces testosterone (Metayer *et al.*, 2019). The seminiferous tubules are lined with germ cells that undergo a complex process of spermatogenesis to produce mature sperm. The interstitial tissue contains Leydig cells, which produce testosterone, a male sex hormone that plays a crucial role in the growth and development of broiler chickens, as reported by Mfoundou *et al.* (2022). Histological investigation of the testes also showed no noticeable change in their histoarchitectures. However, SST substantially increased in broilers fed 1% Cv and 3% Ts. Research has shown that the size of the seminiferous tubules is positively correlated with sperm count and sperm quality, both of which are important factors in male fertility. Larger seminiferous tubules have been associated with higher sperm counts, as well as a greater proportion of morphologically normal and motile sperm (Fragaso *et al.*, 2013; Metayer *et al.*, 2019; Mfoundou *et al.*, 2022).

Research on phytobiotic-based dietary inclusion is getting immense popularity in recent years due to the severe health risks associated with supplementing antibiotic or steroid GPs to broilers. Following these studies, Cv and Ts have been proven to be effective phytobiotic supplements in promoting growth performance, gut health, and meat quality in poultry species. This study reported the impact of Cv and Ts on the vital organs and male gonads of broilers for the very first time. The results suggest that Cv and Ts have no toxic or negative impact, but rather exert some beneficial impacts like increased parabronchial surface area, size of the glomerulus, diameter of the kidney tubules, and size of the seminiferous tubules. However, further study is recommended to investigate the serum biochemical indices linked to these organs, in order to elucidate the overall health and functionality of these organs.

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