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Evaluation of Immunomodulatory Activities of Clove Powder in Broiler

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ABSTRACT

The study was planned to evaluate the immunomodulatory effects of clove bud powder in broiler. Total of 60 chicks were divided randomly to 5 groups. Group I served as control and given only basal diet. Group II served as standard control and given basal diet with vitamin E and selenium containing proprietary product in water. Groups III, IV and V were given clove powder at the dose rate of 2.5, 5 and 10 g/kg feed for 35 days, respectively. Cutaneous basophil hypersensitivity (CBH) response was carried out to assess the cell mediated immunity on 14th day of age. Blood was collected on 7th, 21st and 35th day of age and serum was separated to estimate antibody titre against Newcastle Disease Virus vaccine by haemagglutination inhibition (HI) test and biochemical parameters like serum total protein, serum albumin, serum globulin and albumin to globulin ratio (A/G). On 35th day, thin blood smears were prepared to determine differential leucocyte counts microscopically. At the end of the experiment, birds were sacrificed for histopathological examinations. Chicks supplemented with clove powder at 2.5, 5 and 10 g/kg feed and vitamin E & selenium showed significantly higher CBH response. Birds supplemented with clove powder at 5 and 10 g/kg feed and vitamin E & selenium showed significantly increased antibody titre as compared to control birds. The result indicates clove powder has beneficial effect in terms of augmenting the cell mediated as well as humoral immune response in broiler. Clove powder supplementation significantly increased the serum total protein, serum globulin and significantly decreased albumin to globulin ratio. Birds supplemented with clove powder (2.5, 5 and 10 g/kg) in feed showed significantly decreased heterophil to lymphocyte ratio suggesting its beneficial effect on cell mediated immune response. Histopathological evaluation of bursa of Fabricius, spleen and thymus showed no histopathological alteration in birds supplemented with clove powder. Result of the present study revealed that supplementation of clove powder at given doses in feed was possesses immunostimulant effects in broiler.

Keywords: Clove powder, Immunomodulatory, Cell mediated immune response, Humoral immune response, Broiler.

INTRODUCTION

The immune system plays a vital role in the defense against various infectious diseases. The factors which trigger immunity a body's natural defense system include previous infection, various external stimuli and immunization. Once a foreign body enters into body, the collective and coordinated response of specific cells and mediators to the foreign body forms an immune response[1]. The immune system maintains homeostasis in the healthy organisms. The function and efficiency of the immune system is influenced by a variety of extrinsic and intrinsic factors, leading to either immunosuppression or immune stimulation. Biomolecules of synthetic or biological origin that can regulate, suppress, and stimulate any component of adaptive or innate immunity are known as immunomodulators which categorized into three categories: immunoadjuvants, immunostimulants, and immunosuppressants in clinical practices[2]. Clinically used most immunostimulants and immunosuppressants are cytotoxic drugs with serious side effects. There is growing interest in using herbal medicines as multi-component agents to regulate the complex immune system in the prevention of infectious diseases than the treatment of immune-related diseases. It has been reported that phytochemicals such as flavonoids, polysaccharides, lactones, alkaloids, diterpenoids and glycosides present in some plants are involved in the immunomodulatory properties of plants. Therefore, the search for plant-derived natural products as a new guide for the development of effective and safe immunosuppressive and immunostimulatory agents has received enormous research interest.

Clove is an ancient spice used as flavoring agent which belongs to family Myrtaceae. The scientific name of the clove is *Syzygium aromaticum* (*Eugenia caryophyllata*). It is also known as "laving" in Gujarati and "laung" in Hindi. Clove is aromatic, dry, fully grown but unopened flower bud. Trees of clove grow on islands of Indonesia, Tanzania, Sri Lanka, Madagascar, India and Malaysia [3]. In India, it mostly grows in the hilly tracts of Tamil Nadu, Karnataka and Kerala. Clove is amongst the most

essential sources of phenolic compounds, including eugenol (80-90%), eugenyl acetate (15-17%), and beta-caryophyllene (5-12%), alpha-humulene (0.55%), alpha-terpenyl acetate (0.1%), and methyl eugenol (0.2%)[4]. Apart from its use as spice, it is shown to possess a wide range of pharmacological effects such as antimicrobial, antioxidant, anti-inflammatory, analgesic, anticancer and anesthetic effects. Moreover, clove showed insecticidal, mosquito repellant, aphrodisiac and antipyretic activities[5]. It also relieves stomach pain, nausea and vomiting[6]. Safety of clove oil studied in rats & found safe on oral administration[7]. Many scientific reports on combined dietary supplementation of vitamin E and selenium shows improved immune response and performance in broilers[8]. therefore, in present study we used vitamin E and selenium supplementation in positive control birds. There are limited scientific reports on the immunomodulatory effects of clove powder especially in broiler chicken till date. Hence, the present study was undertaken to evaluate the immunomodulatory effect of the clove bud powder at the dose rate of 2.5, 5 and 10 g/kg feed for 35 days in broiler.

MATERIALS AND METHODS

Experimental Birds

The study was conducted on day old Vencobb broiler chicks. Broiler chicks were procured from Venky's India Pvt. Ltd., Anand, Gujarat and were maintained at brooder house of the Poultry Research Station (PRS), Anand under standard managemental conditions. On the day of arrival, chicks were wing banded and weighed individually. After weighing, the chicks were distributed randomly to five treatment groups. Brooding and rearing of chicks were performed in deep litter system at well ventilated brooder house using standard management and health care practices. The experiment was approved by Institutional Animal Ethical Committee (No. 353/VPT/2021) of Veterinary College, Anand. The birds were protected against various diseases like Ranikhet disease and Infectious Bursal Disease by vaccination as followed by PRS, Anand.

Management of Feeding and Watering

Broiler pre-starter, starter and finisher feed were prepared at the feed manufacturing unit of PRS, Anand. All the birds received broiler feed according to the age of birds i.e., 1-7 days (pre-starter), 8-21 days (starter) and 22-42 (finisher) during the entire experimental period. The cinnamon powder at the different doses was mixed with basal feed to make different treatment feeds. Different treatment feeds were offered treatment-wise twice a day during the whole experimental period. Mixing and stirring of feed in the feeder was carried out two times a day and all the precautionary measures were also taken to minimize the wastage of feed. Clean, fresh and cool drinking water was given to all experimental birds ad libitum throughout the experimental period.

Experimental Design

Total of 60 chicks were divided randomly to 5 groups each of 12 chicks. Group I served as control and given basal diet without clove powder and vitamin E and selenium. Group II served as standard control and given vitamin E and selenium containing proprietary product in water at the dose rate of 1.5 grams per 100 birds for first two weeks and 5 grams per 100 birds for next 3 weeks. The remaining groups III, IV and V were given clove powder at the dose rate of 2.5, 5 and 10 g/kg of feed, respectively. Based on the dose rates given for treatment groups, clove powder was mixed in basal diet with the help of mixer during the preparation of broiler diets. This study was conducted for 35 days. Various parameters were studied to evaluate immunomodulatory activity of clove powder in broilers.

Cutaneous basophil hypersensitivity (CBH) response

Cell mediated immunity was assessed by conducting cutaneous basophil hypersensitivity response on 14th day by using classical toe web assay method [9].

Determination of antibody titer against ND virus

Blood sample (1-2 ml) was collected using 2 ml syringe equipped with 26 G needle in plain vials from wing veins of birds from each group on 7th, 21st and 35th day. Samples were centrifuged at 3000 rpm for 5-6 minutes. Serum was separated from blood, transferred to 2 ml centrifuge tubes and stored at -55 ºC until analysis. Antibody titers against ND virus were determine by haemagglutination inhibition (HI) test as described by Buxton and Fraser (1977) with slight modifications [10]. The HI titre was expressed as the reciprocal of the highest dilution of serum, inhibiting agglutination of the RBC. The data of antibody titre was converted into $\log_2$ value and these converted values were subjected to statistical analysis.

Biochemical investigations

Serum was separated from blood samples collected in plain vials on 7th, 21st and 35th day and stored at -55ºC till further biochemical estimations. Total protein (g/dl) and albumin (g/dl) were analyzed using total protein kit **(**Biuret method) and albumin kit (BCG method), respectively. Globulin (g/dl) and Albumin to globulin ratio were calculated.

Hematological investigation

On 35th day, following blood collection immediately thin blood smears were prepared on grease free clean slides for the assessment of differential leucocyte counts (DLC). Thin blood smears dried at room temperature and fixed in alcohol immediately. Later on, blood smears were then stained in field's stain, allowed to dry and examined microscopically for differential leucocyte counts under oil immersion objective (100X). After this heterophil to lymphocyte ratio was calculated.

Histopathological examination

At the end of the experimental period, birds of all groups were slaughtered at chicken shop by butcher under supervision. The birds were offered *ad-libitum* water for 24 hours and kept off feed for 6 hours, prior to slaughter. Tissues like thymus, spleen and bursa of Fabricius were collected in tissue collection bottles containing 10% formalin solution and processed by standard histopathological technique. Sections of tissues were cut and stained with haematoxylin and eosin (H & E) staining method to observe any microscopic alterations.

Statistical analysis

Data were evaluated by one-way-analysis of variance (ANOVA) for a complete randomized design to compare the means of various parameters of immunomodulatory by using SPSS statistics software (version 26.0). Significant differences ($p<0.05$) between different experimental groups were analyzed by Duncan's multiple range test. All values expressed as mean ± S. E.

RESULTS

All birds were observed daily throughout the period of study. All the birds were found active during experimental period and did not reveal any abnormal symptoms attributable to oral administration of clove powder at the dose rate of 2.5, 5 and 10 g/kg feed for 35 days in broiler. There was no mortality during study period.

In present study, the cell mediated immune response was assessed by conducting cutaneous basophil hypersensitivity response (delayed type hypersensitivity) test using phytohemagglutinin-P (PHA-P). The CBH response was assessed with two different doses of

phytohemagglutinin-P (100 µg and 200 µg) where six birds treated with 100 µg and other with 200 µg dose of PHA-P powder of same group. The CBH response in mm (Mean) of different experimental groups have been presented in Table 1.

The results of the present study revealed that the cutaneous basophil hypersensitivity (CBH) response in form of toe web skin thickness was significantly improved in chicks supplemented with clove powder (2.5, 5.0 and 10 g/kg feed) at both the doses (100 and 200 µg) of PHA-P at 12 and 24 hours after injection as compared to chicks of the control group. After 12 and 24 hours of PHA-P injection (100 µg and 200 µg), toe web skin thickness of chicks supplemented with clove powder (2.5, 5.0 and 10 g/kg feed) was similar to the toe web skin thickness of vitamin E & selenium supplemented chicks. It indicates that dietary supplementation of clove powder at different doses stimulates cell mediated immune response in broiler.

Antibodies are produced from the plasma cells and B lymphocytes which plays main role in humoral immune response, where major immunoglobulins are IgG and IgM. They are involved in the opsonization, complement activation and toxin neutralization[16]. In this study, Haemagglutination inhibition (HI) test was carried out to assess humoral immune response against NDV vaccine and HI antibody titer was expressed as $\log_2$ values. The mean $\log_2$ values of HI antibody titer against NDV vaccinated broiler birds in different experimental groups on 7th, 21st and 35th day of experiment have been presented in Table 2.

At the first week of age, non-significant changes in antibody titer were found among the various groups except a group of chicks supplemented with clove powder (10 g/kg feed) showed a significant increase in antibody titre as compared to chicks of control group. However, at third week of age, the antibody titre was significantly increased in birds supplemented with clove powder (5.0 and 10 g/kg feed) in comparison to birds of control group. At fifth week of age, the antibody titre was significantly increased in birds supplemented with clove powder (2.5, 5.0 and 10 g/kg feed) in comparison to birds of control group. Furthermore, at third and fifth week of age, the log2 values of HI antibody titer in birds supplemented with clove powder (5.0 and 10 g/kg feed) were similar to the log2 values of HI antibody titer of vitamin E & selenium supplemented birds suggesting that clove powder had a similar effect as of standard supplement. It indicates that dietary supplementation of clove powder stimulates humoral immune response in broilers.

The result of biochemical estimations as mean values of total protein (g/dl), albumin (g/dl), globulin (g/dl) and albumin to globulin ratio in different experimental groups on 1st, 3rd and 5th week of experiment have been presented in Table 3 & 4. At first week of age, total protein and serum globulin were significantly increased and A/G ratio was significantly decreased in birds supplemented with clove powder at 5 and 10 g/kg feed as compared to birds of control group. At third week of age total protein and serum globulin level were significantly increased and A/G ratio was significantly decreased in birds supplemented with clove powder at 2.5, 5 and 10 g/kg feed and vitamin E & selenium as compared to birds of control group. However, at fifth week of age serum globulin level was significantly increased while A/G ratio significant decreased in clove powder at 5 and 10 g/kg feed and vitamin E & selenium supplemented birds as compared to birds of control group. Also, significant increase in total protein level in birds supplemented with clove powder at 10 g/kg feed and vitamin E & selenium as compared to birds of control group while nonsignificant serum albumin level among groups. Moreover, total protein, serum globulin and A/G ratio in birds supplemented with clove powder at 5 and 10 g/kg feed were similar to the vitamin E & selenium supplemented birds suggesting that clove powder has similar effect as of standard supplement.

Differential Leucocyte Counts was estimated on 5th week of experiment. The results of mean ± S.E. values of differential leucocyte counts in different groups have been presented in Table 5. Birds supplemented with clove powder at 2.5, 5 and 10 g/kg feed and vitamin E & selenium showed significantly increased lymphocyte counts and significantly decreased heterophils and heterophil to lymphocyte ratio (H/L) as compared to birds of control group while monocyte and eosinophil counts did not differ significantly among the different experimental groups. Moreover, lymphocytes, heterophils and heterophil to lymphocyte ratio (H/L) in birds supplemented with cinnamon powder at 2.5, 5 and 10 g/kg feed were similar to the birds supplemented with vitamin E & selenium suggesting that cinnamon powder has similar beneficial effects as of standard supplement.

Histopathological examination of bursa of Fabricius, thymus and spleen from birds of control group and birds supplemented with clove powder at 10 g/kg feed were depicted in figure 1 to 6 respectively. During necropsy there was no appreciable gross changes were observed in bursa of Fabricius, thymus and spleen of any experimental broiler birds. On histopathological examination, normal architecture was observed in the sections of bursa of Fabricius, thymus and spleen from birds of control group, vitamin E and selenium as well as clove powder supplemented birds.

DISCUSSION

The present findings were in accordance with results reported by Tariq *et al*. (2014) who observed significantly improved cutaneous basophilic hypersensitivity response in birds supplemented with 0.5% clove as compared to birds of control group[11]. Similarly, Sethy *et al*. (2017) who observed significantly higher cutaneous basophilic hypersensitivity response in (0.5% and 1.0%) turmeric supplemented chicks[12]. Singh and Doley (2012) reported significantly increased in inter digital fold thickness after and before PHA-P injection in 1% tulsi leaf powder supplemented chicks[13]. Marimuthu *et al*. (2020) also reported that cutaneous basophils hypersensitivity response was significantly ($p<0.05$) increased at 24 h after PHA-P challenge in phytogenic feed supplemented group as compared to both heat stress control and normal control groups in broiler chickens[14]. Parmar *et al.* (2021) observed significantly increased CBH response in birds treated with clove oil at 200, 400 and 800 mg/kg feed compared to birds of control group[15].

CBH is a localize *in vivo* inflammatory response to phytohaemagglutinin (PHA) and has been used to measure cell-mediated immunity. It is primarily a thymus-dependent reaction mediated by T lymphocytes and their products like lymphokines. Cell mediated immune responses are very important to defense against any infectious organisms, foreign antigens and delayed type hypersensitivity reactions[16]. Phytohaemagglutinin P is an excellent T cell mitogen that specifically stimulates T lymphocytes and induces proliferation at the injection site[17]. Also, T cells are involved in the CBH swelling response and secrete cytokines during swelling that recruits more effector cells. However, other white blood cells also affect tissue swelling and secrete additional cytokines that further promote the infiltration or proliferation of more white blood cells and this indicates that the PHA-P swelling response is a cell-mediated immune response [18].

The results with respect to antibody titre against NDV vaccine were in agreement with Similarly, Gandomani *et al.* (2014) also reported significantly ($p<0.05$) high antibody titre against NDV vaccine in laying hens supplemented with 0.2 and 0.4% clove bud powder in diet as compared to control group[19]. Tariq *et al*. (2014) observed significantly improved immunoglobulin levels in birds supplemented with 0.5% clove as compared to birds of control group[11]. Mahrous *et al*. (2017) also reported significantly increased immunoglobulin IgA, IgG and IgM levels in birds supplemented with clove (1.5 g/kg) in feed as compared to birds of control group at the 3rd and 5th weeks of age[20]. Chowdhury *et al.* (2018) also reported that antibody titre against NDV vaccine was greater in clove bud oil (@ 600 mg/kg feed) supplemented group as compared to control group in broiler chickens[21]. Likewise, Al-AL-Mufarrej *et al*. (2019) reported significantly increased HI antibody titre against Newcastle disease and

IBD viruses in 1%, 2% and 4% clove powder supplemented group of birds as compared to birds of control group at 28th day[22]. Parmar *et al*. (2021) reported significantly increased HI antibody titre against Newcastle disease in group of birds supplemented with clove oil (200, 400 and 800 mg/kg feed) as compared to birds of control group at 3rd and 5th week[15].

The result with respect to serum biochemical investigations were in accordance with the findings of Mehr *et al*. (2014) also observed that serum albumin level did not differ significantly in clove bud oil (@ 150, 300 and 450 ppm) supplemented groups as compared to control group in broiler chickens[23]. Similarly, Tariq *et al*. (2014) also observed that non-significant difference in serum albumin level in clove powder supplemented groups as compared to control group[11]. Mahrous *et al*. (2017) reported significantly increased serum total protein and globulin levels in birds supplemented with clove (1.5 g/kg) in feed as compared to birds of control group at the 3rd and 5th weeks of age[20]. Al-Al-Mufarrej *et al.* (2019) also observed that non-significant difference in serum albumin level in clove powder supplemented groups as compared to control group[22]. Hussein *et al.* (2019) also mentioned that serum total protein and globulin levels were significantly ($p<0.05$) increased in clove oil (@ 0.75 and 1.5 mL /kg feed) supplemented groups as compared to control group, while non-significant difference was found in the level of albumin among different groups in quail[24]. Garba *et al*. (2021) who observed significantly increased serum total protein and globulin levels in birds treated with clove (2 g/kg) in feed compared to birds of control group[25]. El-Kholy *et al*. (2021) observed significantly increased serum total protein and globulin levels and significantly decreased A/G ratio in post hatch birds treated with (in-ovo injection of clove extract 0.1 ml/egg) as compared to birds of control group[26]. Parmar *et al*. (2021) observed significantly increased serum total protein, globulin levels and significantly decreased A/G ratio in birds treated with clove oil at 200, 400 and 800 mg/kg feed compared to birds of control group[15]. The present findings were not in accordance with Tariq *et al*. (2017) reported non-significant difference in total protein, albumin, globulin and A/G ratio levels in clove (0.5%) supplemented birds compared to birds of control[11]. Al-Al-Mufarrej *et al*. (2019) also reported non significant difference in serum total protein and globulin levels in 1% and 2% clove powder supplemented group of birds as compared to birds of control group[22]. Arif *et al*. (2022) observed that no significant difference in serum total protein in birds treated with clove powder (0.5%) in feed as compared to birds of control group[27].

The result with respect to hematological investigation were in accordance with the Najafi and Torki (2010) who reported that heterophil to lymphocyte ratio was significantly ($p<0.05$) improved from dietary treatments of clove oil in broiler chicks[28]. Gandomani *et al.* (2014) also reported that heterophil to lymphocyte ratio was significantly ($p<0.05$) decreased in clove bud powder treated groups as compared to control group in fowls[19]. Mustafa and Wasman (2020) who reported significantly decreased H/L ratio supplemented with clove (2 g/kg) in feed as compared to birds of control group. Parmar *et al.* (2021) reported significantly decreased H/L ratio in birds supplemented with clove oil (400 and 800 mg/kg feed) significantly reduced as compared to birds of control group[29]. The present findings were not similar with results reported by Bello *et al.* (2016) reported non-significant difference in H/L ratio in clove extracts (@ 200, 400, and 600 mg/kg in diet) supplemented groups as compared with the control group in fowls[30]. Arif *et al.* (2022) also observed that no significant difference in heterophils, lymphocyte, monocyte, eosinophil and H/L ratio in birds treated with clove powder (0.5%) in feed as compared to birds of control group[27].

The result with respect to the histopathological examination were in agreement with Gupta and Charan, (2007) reported no significant histopathological changes in thymus, spleen and bursa of Fabricius of the broilers supplemented with 600mg *Ocimum sanctum* dried leaves powder per chicken daily for 15 days[31]. Also, Humbal *et al*. (2019) reported no significant histopathological (kidney, liver, spleen and heart) changes in male and female rat administrated of clove oil at dose of 50, 100 and 200 mg/kg body weight, orally[7]. On contrary to results of present study, Parmar *et al.* (2021) found lymphoid cell proliferation in bursa of Fabricius and thymus of birds supplemented with clove oil (400 and 800 mg/kg feed) [15].

Table 1: Effect of dietary supplementation of clove powder for 35 days on CBH response (mm) against phytohemagglutinin-P in broiler birds (Mean ± SE, n=6)

Group	Mean toe web skin thickness (mm)					
	Pre- injection (PHA-P @100 µg)	Post injection (PHA-P @100 µg)		Pre- injection (PHA-P @ 200 µg)	Post injection (PHA-P @ 200 µg)	
		12 h	24 h		12 h	24 h
I	0.68±0.04	1.25±0.09^{a}	1.09±0.04^{a}	0.68±0.06	1.28±0.07^{a}	1.10±0.03^{a}
II	0.69±0.02	1.65±0.11^{b}	1.27±0.06^{b}	0.70±0.04	1.66±0.11^{b}	1.33±0.07^{b}
III	0.72±0.02	1.59±0.16^{b}	1.22±0.05^{b}	0.75±0.03	1.60±0.14^{b}	1.30±0.04^{b}
IV	0.66±0.02	1.66±0.10^{b}	1.24±0.03^{b}	0.72±0.03	1.65±0.07^{b}	1.34±0.10^{b}
V	0.71±0.02	1.69±0.05^{b}	1.29±0.03^{b}	0.71±0.04	1.68±0.06^{b}	1.36±0.04^{b}

Values bearing different superscripts (a, b) within a same column differ significantly from each other ($P<0.05$).

Table 2: Effect of dietary supplementation of clove powder for 35 days on HI antibody titer (log_2 value) against NDV vaccine in broiler birds (Mean ± SE, n=6)

Groups	Antibody titer (log_2 value, Mean ± S.E.) against NDV vaccine		
	1st week (Day 7)	3rd week (Day 21)	5th week (Day 35)
I	3.17±0.31^{a}	4.67±0.21^{a}	4.17±0.31^{a}
II	3.83±0.31^{ab}	5.83±0.31^{c}	5.83±0.31^{c}
III	3.33±0.21^{ab}	4.83±0.40^{ab}	5.00±0.26^{b}
IV	4.00±0.26^{ab}	5.67±0.33^{bc}	5.67±0.21^{bc}
V	4.17±0.31^{b}	6.17±0.31^{c}	6.33±0.21^{c}

Values bearing different superscripts (a, b, c) within a same column differ significantly from each other ($P<0.05$).

Table 3: Effect of dietary supplementation of clove powder for 35 days on serum total protein (g/dl) and albumin (g/dl) in broiler birds (Mean ± SE, n=6)

Groups	Serum total protein (g/dl)			Serum Albumin (g/dl)		
	1st week	3rd week	5th week	1st week	3rd week	5th week
I	2.42±0.16^{a}	2.54±0.08^{a}	3.01±0.12^{a}	1.22±0.05^{c}	1.24±0.05^{b}	1.49±0.10^{ab}
II	2.56±0.06^{ab}	3.13±0.05^{c}	3.92±0.43^{bc}	0.99±0.03^{a}	1.17±0.05^{b}	1.30±0.03^{ab}
III	2.43±0.03^{a}	2.73±0.04^{b}	3.38±0.12^{ab}	1.12±0.01^{b}	1.20±0.04^{b}	1.62±0.18^{b}
IV	2.77±0.05^{bc}	2.86±0.05^{b}	3.54±0.10^{abc}	1.08±0.02^{b}	1.19±0.05^{b}	1.29±0.04^{ab}
V	2.85±0.01^{c}	3.21±0.06^{c}	4.12±0.15^{c}	1.05±0.02^{ab}	1.02±0.03^{a}	1.18±0.12^{a}

Values bearing different superscripts (a, b, c) within a same column differ significantly from each other (P<0.05).

Table 4: Effect of dietary supplementation of clove powder for 35 days on serum globulin (g/dl) and albumin to globulin ratio in broiler birds (Mean ± SE, n=6)

Groups	Serum Globulin (g/dl)			Albumin to globulin ratio (A/G ratio)		
	1st week	3rd week	5th week	1st week	3rd week	5th week
I	1.21±0.14^{a}	1.31±0.07^{a}	1.53±0.10^{a}	1.13±0.22^{b}	0.97±0.08^{d}	1.00±0.10^{b}
II	1.57±0.07^{b}	1.95±0.05^{c}	2.62±0.44^{cd}	0.64±0.04^{a}	0.60±0.03^{ab}	0.55±0.07^{a}
III	1.31±0.02^{a}	1.53±0.05^{b}	1.76±0.07^{ab}	0.86±0.01^{ab}	0.79±0.05^{c}	0.94±0.15^{b}
IV	1.69±0.03^{bc}	1.67±0.07^{b}	2.26±0.11^{bc}	0.64±0.01^{a}	0.72±0.05^{bc}	0.58±0.04^{a}
V	1.80±0.00^{c}	2.18±0.07^{d}	2.93±0.12^{d}	0.58±0.01^{a}	0.47±0.03^{a}	0.41±0.05^{a}

Values bearing different superscripts (a, b, c, d) within a same column differ significantly from each other (P<0.05).

Table 5: Effect of dietary supplementation of clove powder for 35 days on differential leucocyte counts (%) and H/L ratio in broiler birds (Mean ± SE, n=6)

Groups	Heterophiles	Lymphocytes	Monocytes	Eosinophils	H/L Ratio
I	38.33±0.84^{c}	53.83±0.70^{a}	4.50±0.34	3.33±0.33	0.71±0.02^{c}
II	27.33±0.71^{a}	65.50±1.18^{c}	4.17±0.54	3.00±0.52	0.42±0.02^{a}
III	32.17±1.40^{b}	60.00±2.05^{b}	4.50±0.43	3.33±0.42	0.54±0.04^{b}
IV	25.83±0.75^{a}	66.67±0.67^{c}	4.33±0.33	3.17±0.31	0.39±0.01^{a}
V	25.17±0.87^{a}	67.67±1.15^{c}	4.33±0.49	2.83±0.31	0.37±0.02^{a}

Values bearing different superscripts (a, b, c) within a same column differ significantly from each other (P<0.05).

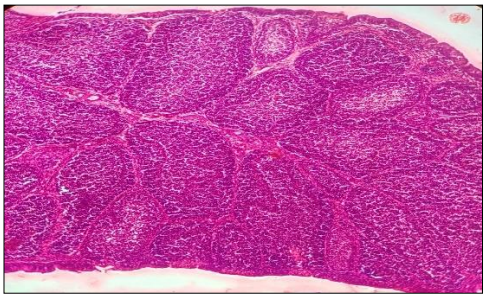

Figure 1: Section of bursa of Fabricius from broiler bird of control group showing normal architecture without any histopathological changes

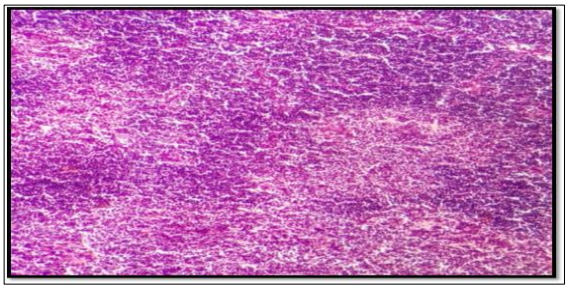

Figure 3: Section of thymus from broiler bird of control group showing normal architecture without any histopathological changes

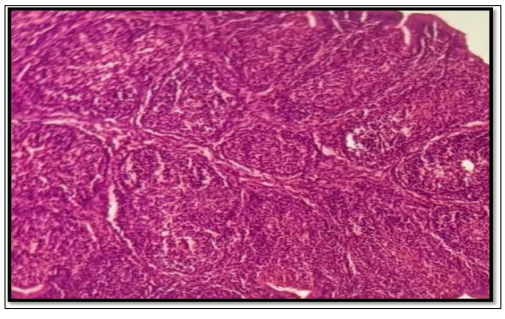

Figure 2: Section of bursa of Fabricius from clove powder @ 10 g/kg feed supplemented group showing normal architecture without any histopathological changes in broiler

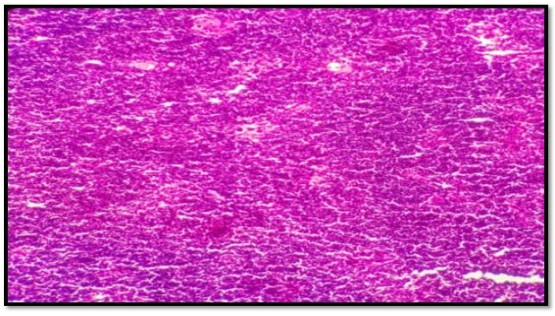

Figure 4: Section of thymus from clove powder @ 10 g/kg feed supplemented group showing normal architecture without any histopathological changes in broiler

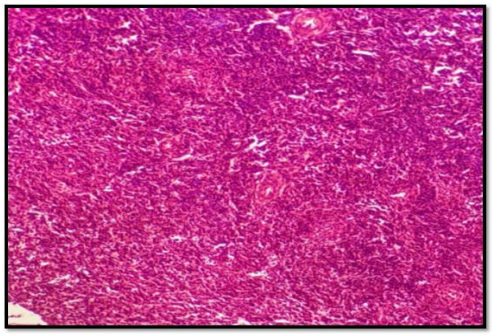

Figure 5: Section of spleen from broiler bird of control group showing normal architecture without any histopathological changes

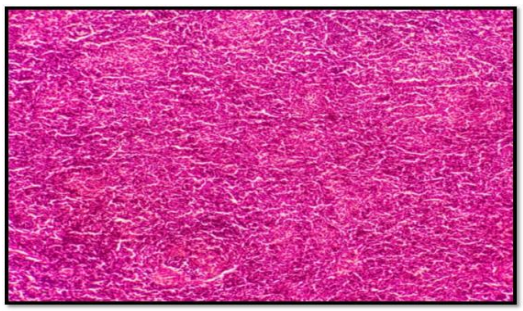

Figure 6: Section of spleen from clove powder @ 10 g/kg feed supplemented group showing normal architecture without any histopathological changes in broiler

CONCLUSION

Based on the finding of present study, it suggests that dietary supplementation of clove powder at 5 and 10 g/kg feed has potential to enhance the cell mediated as well as humoral immune response in broiler. It can be stated that clove bud powder can be used as a natural and safe alternate dietary substance at given dosage in broiler diet without any ill effects on the health of broiler. Clove powder has potential substance as it shows immunostimulant effects.

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Conflict of interest

The authors declared no conflict of interest.

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