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Effect of supplementing different levels of peppermint powder on serum biochemical, immune response and meat quality of broilers

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Abstract
A experiment was conducted to evaluate the effect of supplementing different levels of Peppermint powder on biochemical parameters, immunology and meat quality. Hundred sixty (160) day-old broiler chickens (*Gallus gallus domesticus*) of vencobb strain were taken and divided into 4 treatment groups *viz*. T1 (control): Basal diet, T2: T1+0.1% peppermint powder, T3: T1+0.3% peppermint powder and T4: T1+ 0.5% peppermint powder with two replicate in each treatment group. The supplemented birds had significantly lower level of serum cholesterol owing to the antilipidogenic property of peppermint powder. The birds under T4 groups had significantly higher & SRBC response as compared rest three groups. Carcass characteristics for all the treatment groups were non significant.

Keywords: peppermint powder, biochemical, immunology, broilers

Introduction
The Indian Poultry Industry has undergone a paradigm shift in structure and operation. A very significant feature of India's poultry industry is its transformation from a mere backyard activity into a major commercial activity in just about four decades which seems to be really fast. Plants have been a potential source of medicine; though in a crude form, have been used from time immemorial to heal various ailments. The traditional herbal medical system has been practiced globally from ancient times.

The herbal supplements secondary compounds, such as essential oils (EOs), saponins, and tannins, have become a primary source of feed additives and antioxidants to enhance general health conditions in humans and animals. Therefore, the efforts of many researchers directed to evaluate the use of herbal and plant secondary compounds as feed additives for rabbits and poultry, which represent good, fast and cheap sources of white meat. The use of phytogenics as feed additives is gaining importance due to their antimicrobial and stimulatory effects on digestive system (Jamroz *et al.*, 2003; Jang *et al.*, 2004) [10, 11]. Compared with synthetic antibiotics or inorganic chemicals, these plants and their derived products have reported to be less toxic, residue free and thus considered as ideal feed additives in animal production (Hashemi and Davoodi, 2010) [9].

Peppermint leaves contain about 0.5 to 4% essential oils that are composed of 25 to 78% menthol, 14 to 36% menthone, 1.5 to 10% isomenthone, 2.8 to 10% menthyl acetate, and 3.5 to14% cineol (Bupesh *et al.*, 2007; Aziz *et al.*, 2011; Beigi *et al*., 2018) [8, 5, 7]. Khempaka *et al*. (2013) reported that peppermint leaves have beneficial effects on antioxidant activity, abdominal fat deposition, and ammonia production in broilers. Yang *et al*. (2010) [10] reported the effects of using 2% of herbal plants on the immune system in broilers. They concluded that the herb significantly increased antibody titers than the control group, resulting in increased activity of the immune system. Ameri *et.al*. (2015) [4] reported that peppermint can improve immune system in broilers under heat stress. Khurseed *et.al* (2017) [14] reported that supplementation of either raw or enzyme treated mint leaves supplemented diet in the broiler chicken did not reveal any significant difference (p<0.05) in the carcass characteristics such as dressing percentage, yield characteristics of Giblet *viz*. gizzard weight, heart weight and liver weight among different treatment groups.

Materials and Methods

The experimental work was conducted at Instructional Livestock Farm Complex and Department of Livestock Production and Management, College of Veterinary Science and Animal Husbandry, OUAT, Bhubaneswar, Odisha. The experiment was laid out in randomized complete block design (RBD) with 160 day-old broiler chickens (*Gallus gallus domesticus*) of vencobb strain divided into 4 treatment groups with two replicate in each treatment group, having 20 broiler chickens in every replicate. Birds of all treatment groups were housed in deep litter system and managemental conditions were also similar for different treatment groups in the study.

The dietary treatment groups were divided as T1 (Control): Basal diet, T2: Basal diet+0.1% peppermint powder, T3: Basal Diet+0.3% peppermint powder, T4: Basal Diet+0.5% peppermint powder.

Biochemical parameters study

The biochemical analysis was carried out at the end of the experiment. Blood was collected from 3 birds per replicate after slaughtering. Serum biochemical indices like Glucose, Cholesterol, Total Protein, Albumin, Globulin and A:G ratio were determined by using I-chem (Chennai, India) Kit. Serum glucose was estimated by the glucose oxidase (GOD) and peroxidase method (POD). Serum total protein (TP) and albumin were estimated by Biuret and BCG dye binding method. Serum globulin was calculated by subtracting serum albumin from TP and expressed as g/dl blood Serum. The serum albumin value and corresponding globulin values of individuals in each group were utilized to get the A: G ratio. Cholesterol in blood serum was determined by the method of Wybenga *et al.,* (1970) [17]. Serum alkaline phosphatase (ALP) activity was estimated by the method of Kind and King (1954) [15] using diagnostic kit manufactured by Span Diagnostic Limited, Surat, India.

Measure of cellular immunity

At the end of the 6th week of age, three birds from each dietary treatment were injected intra-dermally in the foot pad with 100 micro gram of Phyto-haemaglutinin-P (PHAP) in 0.1 ml of normal saline to measure the cellular immune response by Cutaneous Basophillc Hyper Sensitivity (CBH) test (Edelman *et al.,* 1986). The thickness of foot pad was measured using digital calliper before inoculation and 24 h post inoculation and CBH response was calculated using the formula:

$$\text{CBH response} = \frac{\text{Post injection skin thickness}}{\text{Pre-injection thickness}} \text{ X } 100$$

Measure of humoral immunity

The measure of humoral immunity was carried out as per the method described by Abdallah *et al.* (2009) [1]. Sheep red blood cells (SRBC) were used as test antigens to quantitatively analyse specific antibody response as measure of humoral immunity. At 6th weeks of age, two birds from each replicate in each dietary treatment were immunized intravenously via a wing vein with 0.07 ml packed RBC mixed with 0.93 ml physiological saline (0.9% NaCl) for measurement of primary response. The SRBC were obtained in heparin solution from local sheep (reared at Instructional Livestock Farm, Bhubaneswar, Odisha) and washed three times in physiological saline. Seven days following the antigen challenge, blood samples were collected and serum samples were used to measure humoral immunity. Antibody production to SRBC was measured using microtitration haemagglutination technique with microtiter plate U shape of 96 wells (8 rows X 12 column) according to Bachman and Mashaly (1986) [6] and Kai *et al*. (1988) [12]. All SRBC antibody titres were expressed as log2 of the reciprocal of the highest serum dilution causing agglutination of SRBC.

Carcass Characteristics

The oil gland from vent region, the head from occipital joint and feet from hock joint were severed and removed. Then the weight of the carcass was taken which was recorded as dressed weight with viscera. By a slit opening from the tip of breast bone, abdominal cavity was opened by means of a transverse cut. Then the entire viscera were pulled out. The inedible parts like trachea, esophagus both upper and lower segments, crop, small and large intestine with other portion of intestinal tract, spleen, lungs, ovaries or testis, gall bladder and vent were removed. Serous lining of gizzard, membranes around heart (pericardium) and arteries attached to it were also removed. Then the weight of the carcass was recorded as eviscerated weight. Weight of the carcass along with the edible viscera like liver, heart, gizzard and abdominal fat was recorded as dressed weight. Dressing percentage (dressed weight/live weight × 100) was calculated. The liver, heart, empty gizzard, and different cut-up parts *viz*: Bony cuts, meaty cuts, wings, neck, breast, back, drumstick, thigh were weighed and calculated as a percentage of live body weight.

Statistical analysis

The statistical analysis was performed by using IBM SPSS 22.0computer package., Generalized Linear Model, ANOVA procedure and Duncan's multiple range tests were used (Steel and Torrie, 1980) [16] for comparing between the groups.

Result and Discussion

Serum biochemical parameters

The serum biochemical parameters of the experimental birds under different treatment groups are presented in Table 1. Across all the treatment groups, serum glucose, albumin, globulin, SGPT and SGOT did not vary significantly. The serum glucose level ranged from 195.93 ± 2.88 in T3 group to 203.51 ± 3.65 in T1 birds. Birds from T4 group had similar total protein level as compared to T2 birds (3.24 ± 0.07 vs. 3.23a ± 0.09 g/dl), where was serum total protein level of T1 (3.5 ± 0.06 g/dl) and T3 groups were comparable (3.41 ± 0.11 g/dl). The serum albumin level, which did not vary significantly, ranged between 1.68 ± 0.03 g/dl (T2) to 2.13 ± 0.02 g/dl (T1). There was no significant difference between the level of globulin among all the treated groups. Serum Cholesterol level was significantly higher in T1 birds (142.30 ± 3.04 g/dl) as compared to T4 (105.81 ± 4.26 g/dl) and T2 birds (131.93 ± 4.21 g/dl) and T3 birds (123.28 ± 2.34 g/dl) were comparable. Similarly, comparable results were also observed for SGPT and SGOT levels. The findings of present investigation corroborate with the previous study conducted by Abdel and Lohakare (2014) [2] in which serum biochemical analyses in laying hens fed with various levels of peppermint leaves revealed that serum cholesterol linearly decreased with increasing experimental diet. Al-Fartosi and Al- Rekabi (2014) demonstrated that the phenolic compounds of leaves extract from *Mentha longifolia* and *Mentha spicata* in induced diabetic rats significantly reduced serum cholesterol level

compared to untreated groups. In contrast, the results of the experiment conducted by Akbari and Torki (2014) [3] failed to show the hypocholesterolaemic effects of dietary supplementation of peppermint together with chromium picolinate in broiler chicks.

Table 1: Serum biochemical profile of the experimental birds under different dietary treatments

Parameters	T1	T2	T3	T4
Glucose (mg/dl)	203.51 ± 3.65	197.43±2.51	195.93±2.88	196.36±3.10
Total Protein (g/dl)	3.50 ±0.06	3.23±0.09	3.41±0.11	3.24±0.07
Albumin (g/dl)	2.13±0.02	1.68±0.03	1.94±0.06	1.85±0.4
Globulin (g/dl)	1.37±0.06	1.55±0.05	1.47±0.08	1.39±0.04
cholesterol (mg/dl)	142.30^{c}±3.04	131.93^{b}±4.21	123.28^{b}±2.34	105.81^{a}±4.26
SGPT (U/L)	12.59±0.62	12.63±0.81	12.76±0.72	12.84±0.69
SGOT (U/L)	108.72±6.12	111.28±4.22	109.76±4.20	112.38±6.73

Means bearing different superscripts differ significantly along the rows.

Effect on the immunity status in the experimental birds

Antibody titer against SRBC and CBH response against PHA-P were used as measures to study the immunity status of the layer birds under different dietary treatments. The performance of the experimental birds with respect to the immunity status i.e. antibody titer againt sheep RBC and CBH response is illustrated in Table 2. The birds under T4 groups had significantly higher CBH response (176.80 ± 7.31) as compared rest three groups. Similar trend was also evident in SRBC response, where the T4 birds showed significantly higher titre. The birds under T4 groups had significantly higher CBH response (176.80 ± 7.31) as compared rest three groups. Similar trend was also evident in SRBC response, where the T4 birds showed significantly higher titre.

Table 2: SRBC and CBH response of broiler birds of the experimental broiler birds under different dietary treatments

Parameters	T1	T2	T3	T4
CBH Response	144.87^{a}±8.59	148.43^{a}±10.19	156.20^{a} ±9.77	176.80^{b}±7.31
SRBC Response	5.57^{a} ±0.21	5.89^{a} ± 0.51	6.22^{ab} ± 0.46	6.73^{b} ± 0.42

Carcass characteristics of the experimental birds

The average values of different carcass characteristic *viz*. Dressing yield, eviscerated yield, giblet yield, neck yield, wings yield, breast yield, back yield, thigh yield and drumstick yield are expressed in Table 3. It was observed that the dietary treatment for a period of 5 weeks would not significantly influence all these traits. However as far as dressing yield is concerned T4 birds had slightly higher values among all the groups. In drumstick yield values, performance of T4 birds was numerically better. Giblet yield of T4 birds was slightly better than rest of the groups.

Table 3: Carcass characteristics (percentage body weight) of the experimental birds under different dietary treatments

Parameters	T1	T2	T3	T4
Dressing yield (%)	70.69±6.32	71.44±4.52	71.86±3.31	72.43±3.26
Eviscerated weight (%)	63.56±3.22	63.78±3.19	63.87±4.19	64.23±5.15
Giblet yield (%)	4.42±0.12	4.72±0.21	4.88±0.14	5.03±0.14
Neck yield (%)	7.18±0.27	7.33±0.26	7.67±0.23	8.01±0.18
Wings yield (%)	12.19±0.31	12.32±0.29	12.33±0.37	12.59±0.18
Back yield (%)	18.36±0.50	18.35±0.51	18.61±0.33	18.87±0.13
Breast yield (%)	21.48±0.34	22.68±0.62	23.77±0.36	24.22±0.46
Thighs yield (%)	14.59±0.06	14.68±0.24	14.82±0.06	15.06±0.09
Drumsticks yield (%)	14.68±0.24	14.73±0.23	14.79±0.28	15.18±0.33

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