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A Comparative Effect of Clove Essential Oil with Multi-strain Probiotic and Antibiotic on Blood Biochemical Profile in Broiler

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ABSTRACT

Background: An experiment was conducted to compare the blood biochemical profile of broiler chicken feeding three different types of feed additives.

Methods: 180 day old broilers chicks of Ven Cobb 400 strain were distributed randomly into 4 groups (T_0, T_1, T_2 and T_3) having 45 chicks in each group on the basis of their body weight. Each group divided into 3 replicates of 15 chicks in each. Rations are computed as per BIS (2007) to meet the nutrient requirement and other three groups (T_1, T_2 and T_3) were offered the same standard ration of the control group but supplemented with zinc bacitracin @ 55 mg/kg (T_1), probiotic @ 150 mg/kg (T_2) and clove oil @ 400 ppm (T_3).

Result: The result of the experiment showed significant difference ($p<0.05$) in the level of Glucose in T_3 group whereas, protein, albumin, globulin, A:G ratio, ALT and AST did not differ significantly ($p>0.05$). Probiotic @ 150 mg/kg and clove oil @ 400 ppm supplemented groups showed significant ($p<0.05$) better cholesterol level compared to control and zinc bacitracin @ 55 mg/kg.

Key words: Chicken, Cholesterol, Glucose.

INTRODUCTION

Emergence of antibiotic resistant bacteria has created the necessity of replacement of antibiotic with other products like herbal essential oils in broiler farming. Among the herbal essential oil, clove oil extracted from the clove plant (*Syzygium aromaticum*), can be best suitable option in poultry industry. The major components of clove oil are eugenol (78%), 13% β-caryophyllene (Prashar *et al*., 2006) and lesser amounts of other components such as benzyl alcohol *etc*. Clove oil is known to have antiseptic and digestion stimulant (Kamel, 2001), strong antimicrobial and antifungal (Ehrich *et al*., 1995), analgesic and anti-inflammatory (Feng *et al*., 1987), anesthetic and anticarcinogenic (Prasad *et al*., 2004), antiparasitic and antioxidant (Dragland *et al*., 2003) properties.

Keeping in view to the beneficial effects of clove essential oil, the experiment was designed to study and compare the effects of clove oil with antibiotic and probiotic on blood biochemical profile of broiler chicken.

MATERIALS AND METHODS

The experiment was conducted in the experimental poultry shed, Department of Animal Nutrition, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam during the period of November 2019 to December 2019.

One hundred eighty (180) day old commercial broiler chicks were procured from the local hatchery. Based on similar body weight, chicks were divided randomly into 4 groups with 3 replicates (15 birds per replicate). The chicks were offered *ad libitum* pre-starter, starter and finisher rations to meet the nutrient requirement as per BIS (2007) by using commonly available ingredients. The drinking water was provided *ad libitum* and light was given for 12-14 hrs every night. The experimental diets were designated as diet T_0 (control with no feed additives), T_1 (zinc bacitracin @ 55 mg/kg), T_2 (Multi-strain probiotic @ 150 mg/ Kg which contain 2×10^9 CFU, *Bifidobacterium bifidum, Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus casei, Lactobacillus plantarum, Streptococcus faecium, Streptococcus thermophilus, Aspergillus oryzae, Torulopsis spp*) and

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T_3 (essential oil Clove @ 400 mg/kg). Chicks were reared under intensive deep litter system.

Haematological parameter

For estimation of haematological parameter like haemoglobin (g/dl), WBC (m/mm^3), RBC (m/mm^3), MCV (fl), HCT %, MCH (pg), Lymphocyte (%), Monocyte (%) and Neutrophil (%), about 2 ml of blood was collected aseptically with anticoagulant from 3 birds of each group and the haematological parameter was estimated in "Automatic Haematolyzer".

Blood biochemical profile

A total of 12 birds from each group were selected for collection of blood samples on 21st (starter phase) and 42nd (finisher phase) days of the experiment by puncturing the wing vein with sterile needle and syringe. The blood samples were centrifuged at 1500 rpm for 15 minutes and serum was stored at -20^{o}C for further analysis. The serum samples were analysed for glucose, total protein, albumin, globulin, cholesterol, triglyceride, AST and ALT using commercial kits manufactured by Aspen Laboratories as per standard procedure.

Statistical analysis

The hematological and biochemical parameters of broilers corresponding to clove essential oil, zinc bacitracin antibiotic and multi-strain probiotic supplementation were compared by performing analysis of variance using SAS 9.3. The one and two way analysis of variance (ANOVA) was used to compare the means at 5% and 1% level of significance according to Duncan's multiple range tests (1955).

RESULTS AND DISCUSSION

Blood haematological profile

The haematological parameters comprising of haemoglobin (g/dl), WBC (m/mm^3), RBC (m/mm^3), MCV (fl), HCT%, MCH (pg), MCHC (g/dl), Lymphocyte%, Monocyte% and Neutrophil% were studied on 42nd day of the experimental period and mean (± SE) values have been presented in Table 2.

The mean values of haematological parameters had been barely accelerated in the treatment groups. Still, the values were within the normal ranges. The haematological parameters among the groups did not varied significantly; however, the improvement in haemoglobin and RBC level was noticed in the experimental groups. Tabari *et al*. (2018) also reported significant improvement in Hb, WBC and PCV level in broiler chicken fed with clove aqueous extract. The effect may be due to improvement in feed intake and digestion. The active component of clove oil (ugeonol) is considered as digestion stimulatory factor (Cabuk *et al*., 2003) in addition to their anti-microbial properties, which might have resulted in greater efficiency in feed utilization. Similar findings were observed by Tariq *et al*., (2014) who reported that, haemoglobin concentration, packed cell volume, total erythrocyte count and total leucocyte count were not affected by 0.5% Aloe Vera (*Aloe barbadensis*), 0.5% clove (*Syzigium aromaticum*) and 0.25% Aloe Vera and 0.25% clove combination.

Table 1: Composition of broiler feed.

Nutrients	Pre-starter	Starter	Finisher
Dry matter	91.23	92.03	92.55
Crude protein	22.98	22.06	20. 01
Ether extract	5.7	5.63	5. 16
Crude fiber	4.53	4.78	4.95
Calcium	1.03	0.92	0.89
Phosphorus	0.71	0.72	0.63
ME (Kcal/Kg)	3001.23	3115.65	3183.55
Lysine	1.24	1.29	1.25
Methionine	0.5	0.5	0.5

Blood biochemical profile

Serum blood glucose level (Table 3 and Fig 1) on the 21st day of experiment was found as 135.3±2.86, 165.44±1.61, 165.45±1.97 and 136.94±0.87 mg/dl for T_0, T_1, T_2 and T_3 groups, respectively and the respective values on 42nd day was recorded to be 147.66±2.83, 180.24±3.64, 181.48±3.13 and 145.1±1.2 mg/dl. Results showed that there was no significant ($p>0.05$) difference between T_1 and T_2 and T_0 and T_3. But in clove supplemented groups significantly ($p<0.05$) reduced glucose level was observed as compared to other treatment groups. Similar results were also reported by Tabari *et al*. (2018), who observed significantly ($p<0.05$) lower serum glucose level in broiler chicken treated with clove aqueous extract as compared to control. Sabu and Kuttan (2002) reported that, aqueous extract of clove (*Eugenia caryophyllus*) exhibits an anti hyperglycemic activity in rats without affecting basal plasma glucose concentration. This effect may be due to polyphenol-rich clove extract, which increase glucose consumption by muscle cells. Mohammadi *et al*. (2014) also observed that the dietary supplementation of clove essential oil decreased glucose concentration compared to the control.

Serum total protein, albumin, globulin and albumin-globulin ratio (Table 3) did not show any significant difference between the groups at 21st and 42nd days of experiment, the findings were in accordance with the findings of Tariq *et al*. (2014).

The total serum cholesterol (Table 3 and Fig 1) and triglyceride (Table 3) concentration on 21st days were 129.68±1.21, 130.02±0.72, 120.13±4.41 and 116.58±3.8 and 121.03±2.11, 122.87±2.2, 122.31±1.75 and 121.78±0.64 for T_0, T_1, T_2 and T_3 groups, respectively. On 42nd days serum cholesterol and triglyceride concentration were 155.97±7.29, 154.58±7.17,138.04±2.36 and 133.46±3.35 and 153. 98±9.95, 154.05±10.8, 153.90±10.03 and 153.18±8.53 for T_0, T_1, T_2 and T_3 groups, respectively.

The supplementation of clove essential oil and multi-strain probiotic significantly ($p<0.05$) reduced blood

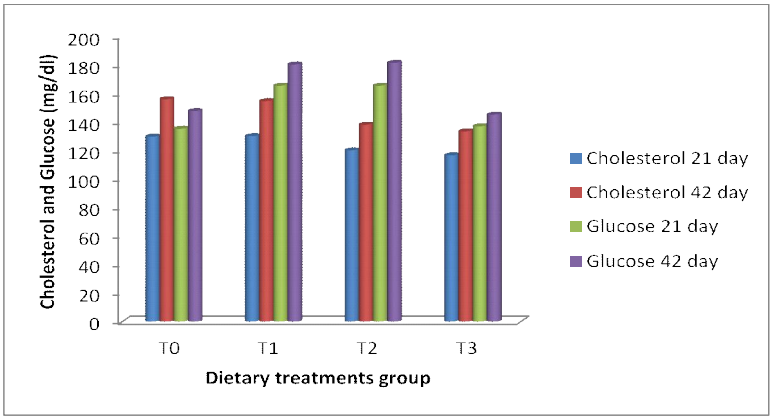

Fig 1: Graphical representation of blood glucose (mg/dl) and serum cholesterol (mg/dl) level.

Table 2: Blood haematological profile of broiler chicken in different experimental groups on 42nd day.

Attributes	Dietary treatment				SEM	P value
	T_0	T_1	T_2	T_3		
Hb (g/dl)	11.57±0.24	12.13±0.19	12.72±0.29	12.41±0.67	0.24	0.27
WBC (m/mm^3)	40.79±1.09	43.5±0.5	45.01±2.57	40.13±1.89	1.14	0.22
RBC (m/mm^3)	2.49±0.05	2.69±0.15	2.7±0.1	2.7±0.22	0.05	0.68
MCV (fl)	129.67±0.49	132.57±1.22	130.5±0.36	129.77±2.14	0.67	0.39
HCT %	31.7±0.87	33.54±0.33	35.87±0.76	33.25±1.85	0.86	0.13
MCH (pg)	44.77±1.09	47.78±0.71	46±0.55	47.13±1.37	0.66	0.22
Lym %	71.8±0.4	68.47±2.43	70.57±4.53	74.57±0.37	1.27	0.45
Mon %	3.73±0.15	3.7±0.12	3.89±0.21	3.53±0.15	0.07	0.48
Neu %	24±0.15	24.93±5.08	22.71±1.63	22.02±0.34	0.65	0.86

Table 3: Serum blood biochemical level of broiler chicken in different experimental groups during the entire experiment.

Attributes	Dietary treatments				SEM	P-value
	T_0	T_1	T_2	T_3		
21st Day						
Glucose (mg/dl)	135.3^a±2.86	165.44^b±1.61	165.45^b±1.97	136.94^a±0.87	8.47	3.44 E-06
Total protein (g/dl)	4.48±0.22	4.58±0.26	4.57±0.33	4.58±0.39	0.02	0.99
Albumin (g/dl)	2.49±0.23	2.58±0.15	2.59±0.22	2.55±0.1	0.02	0.98
Globulin (g/dl)	1.98±0.01	2.00±0.19	1.98±0.53	2.03±0.41	0.01	0.99
A:G	1.26±0.12	1.31±0.14	1.52±0.41	1.37±0.31	0.05	0.91
Cholesterol (mg/dl)	129.68^a±1.21	130.02^a±0.72	120.13^{ab}±4.41	116.58^b±3.8	3.39	0.03
Triglyceride (mg/dl)	121.03±2.11	122.87±2.2	122.31±1.75	121.78±0.64	0.39	0.89
ALT (U/L)	0.04±0.01	0.05±0.01	0.04±0.01	0.05±0.01	0.002	0.65
AST (U/L)	0.16±0.01	0.16±0.01	0.17±0.01	0.17±0.00	0.007	0.10
42nd Day						
Glucose (mg/dl)	147.66^a±2.83	180.24^b±3.64	181.48^b±3.13	145.1^a±1.2	9.97	1.72E-05
Total protein (g/dl)	5.49±0.22	5.52±0.1	5.53±0.08	5.53±0.2	0.01	0.99
Albumin (g/dl)	3.4±0.27	3.46±0.1	3.47±0.06	3.48±0.2	0.02	0.98
Globulin (g/dl)	2.09±0.06	2.06±0.02	2.06±0.01	2.05±0.01	0.01	0.82
A:G	1.63±0.17	1.68±0.06	1.69±0.02	1.7±0.11	0.01	0.97
Cholesterol (mg/dl)	155.97^a±7.29	154.58^a±7.17	138.04^{ab}±2.36	133.46^b±3.35	5.72	0.04
Triglyceride (mg/dl)	153.98±9.95	154.05±10.8	153.90±10.03	153.18±8.58	0.20	0.99
ALT (U/L)	0.03±0.01	0.03±0.01	0.03±0.00	0.03±0.00	0.002	0.90
AST (U/L)	0.23±0.01	0.23±0.01	0.23±0.01	0.23±0.0 2	0.01	0.60

^{abc} Means bearing different superscripts differ significant (P≤0.05).

cholesterol level as compared to control and antibiotic, whereas, serum triglyceride concentration did not show any significant effect among the groups.

Tabari *et al*. (2018) observed significant decrease of cholesterol concentration in broiler chicken fed with clove aqueous extract. The main component of clove (*Eugenia caryophyllus*) essential oils that inhibit hepatic 3-hydroxy -3 methylglutaryl co-enzyme (HMG- COA) reductase activity, which is a key regulatory enzyme in cholesterol synthesis and leads to hypocholestrolemic condition (Shimaa, 2015). Clegg and Mbada (1980) opined that a 5% inhibition of HMG-COA reductase lowers the serum cholesterol level by 2% in poultry. Jin and Cho (2011) also found that cold-pressed clove oil (CCPO) reduced the serum cholesterol and triacylglycerol levels in hyperlipidemic zebra fish model by 68% and 80%, respectively. Khaksar *et al*., (2012) also reported that the supplementation of thyme essential oil decreased the serum total cholesterol, triglyceride and glucose in Japanese quails.

Some researchers also reported that supplementation of probiotic reduced the serum cholesterol (Kalavathy *et al*., 2003) level in broiler chicken. The probiotic preparation containing some of the microorganisms that utilized the cholesterol present in the gastrointestinal tract for their own metabolism and reduced the amount of cholesterol absorption (Mohan *et al*., 1995). Fukashima and Nakano (1995) reported the inhibitory effect of probiotic microorganism on hydroxymethyl glutaryl co-enzyme A (HMG-COA) reductase enzyme involved in the cholesterol synthesis. However, the most important mechanism by which probiotic eliminates cholesterol probably be through reducing lipid absorption in intestine by binding bile acids, which results in increased cholesterol elimination and hepatic synthesis of new bile acid (Zhang *et al*., 2003).

No significant differences were observed in the blood liver enzyme profile (Table 2) between the groups on 21st and 42nd day of experiment. The finding was in accordance with that of Mahrous *et al*., (2017) in broiler chicken fed with different levels of clove buds (0.5 g, 1 g and 1.5 g/kg) at 3rd and 5th weeks of age.

CONCLUSION

The result of the present experiment revealed that there were significantly ($P<0.05$) better response in blood bio-chemical profile on broiler chicken due to supplementation of clove oil and probiotics. Thus clove oil can be incorporated as alternative growth promoter (as essential oil) in broiler chicken ration for better production and optimum health status.

However, further more elaborate studies with large number of birds is appreciated to ascertain the suitable amount of clove oil for dietary supplementation in broiler chicken rations for better response and health.

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