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EFFECT OF SPICES AND SWEET VIOLET EXTRACTS TO REPLACE ANTIBIOTICS AND ANTIOXIDANTS IN FEED ON BROILER PERFORMANCE, HEMATOLOGY, LIPID PROFILE AND IMMUNITY

S. Waheed[1], A. Hasnain[1], A. Ahmad[1], O. M. Tarar[2], Z. Yaqeen[2] and T. M. Ali[1]

[1]Department of Food Science and Technology, University of Karachi, Pakistan
[2]Pakistan council of Scientific and Industrial Research (PCSIR), Karachi, Pakistan
Correspondence Author E-mail: drshahidwaheed@yahoo.com

ABSTRACT

A research trial was conducted on 120 broilers, dividing them in eight feeding groups of 3 replicates of 5 birds. Ration F1 was Positive control (PC) diet with antibiotic and synthetic antioxidants. Ration F2 was negative control (NC). Other diets were having extracts as following: F3 was NC+ fenugreek, F4 was NC +black cumin seed, F5 was NC + sweet violet, F6 was NC + ajwain, F7 was PC + ajwain, fenugreek and sweet violet and F8 was NC diet+ ajwain, fenugreek and sweet violet. All the treatments with extracts had significant difference ($p \leq 0.05$) in weight gain and feed conversion ratio (FCR) as compared to NC diet. Dressing percentage and breast meat improved in F6, F7 and F8 treatments. Pancreas, liver and thymus size significantly reduced ($p \leq 0.05$) in diet F7 and F8. Bursa weights were significantly higher in F3, F4 and F6 ($p \leq 0.05$) compared with F2. Hemoglobin, platelets and WBC increased significantly in F3, F4, F5 and F8 group ($p \leq 0.05$), Cholesterol reduced in F5, F6 and F8 and triglyceride in F3, F5, F6, F7 and F8 group as compared to F2. There was non-significant difference ($P > 0.05$) in RBC, lymphocyte, MCH and MCV counts. Treatments with natural extracts significantly ($p \leq 0.05$) increased ND titre.

Key words: broiler, plant extracts, antioxidants, polyphenols, organ size.

INTRODUCTION

Antibiotics effects on cross resistance of microbes, and their tissue residues polluting food (Woodford *et al*., 2000). Botanical sources rich in antioxidant and anti-bacterial potential gain special attention as alternate replacers (El-Deek *et al*., 2012, Sharma *et al.,* 2013). Polyphenols act as antifungal, antibacterial and antiviral components in body (Orhan *et al*., 2010).Plant phenolic extracts role in regulation of gut microflora, reducing pathogens and enhancing intestinal health is well documented (Cardona *et al*., 2013). Aromatic plants like sweet violet (*Violet odorata*) have numerous biological activities derived from polyphenol rich extracts in it (Muhammad and Saeed, 2011). Sweet violet extracts are rich in antioxidants (Ebrahimzadeh *et al*., 2010), antidyslipidemic and help in controlling hypertension by vasodilation (Siddiqi *et al*., 2012). Fenugreek (*Trigonella foenum*) is an important spice and exhibits its antioxidant effects (Bhanger *et al*., 2008) by radical scavenging and antimutagenic activities during in vitro tests (Dash, 2011).Using fenugreek in broiler diets improved antioxidant status of birds (Abbas, 2010). Ajwain (*Trachyspermum ammi*) is a source of therapeutically active polyphenol structures (Ranjan *et al*., 2011). Carvacol and thymol present in it are very effective in controlling *Clostridium perfringens* challenged chickens (Du *et al*., 2015). Black cumin seed (*Nigella sativa*) is rich in therapeutic, pharmacological, nutritional, antimicrobials and antioxidant bioactive polyphenols (Longato *et al*., 2015). Fenugreek powder and garlic powder combination can improve immunity, ND titre, immunoglobulin, white blood cells(WBC), red blood cells(RBC), and haematocrit counts (Motamedi and Taklimi, 2014).

Green tea extracts may modestly lower SUA level and decreases uric acid clearance. Green tea extract also significantly elevated serum antioxidant capacity with a positive dosage effect (Jatuworapruk *et al*., 2014). Plant derived extracts are considered an anti-viral resources, not only fighting viral attacks but also improving immunity (Zarezade *et al.,* 2013). Application of green tea by-products had been very effective in Influenza challenged chicken (Lee *et al*., 2012) and improve humeral immunity against New castle disease (ND). Keeping in view all these potentials of plant extracts and active polyphenolic biomolecules, this research plan was initiated to see the role of these extracts as antibiotic and synthetic antioxidant alternates in broiler feed.

MATERIALS AND METHODS

Extract Preparation from Fenugreek seed, black cumin seed and ajwain: Black cumin seed, ajwain seeds and fenugreek seeds were purchased from local market. 200 grams of each were grinded to pass through 30 mesh sieve. These samples were extracted using method of

Kim and Lee (2002). The seed powders were soaked in flask with 1000 mL of 80% methanol, sonicated for 24 hours, filtered with whatman no.2 filter paper, took the residue on filter and sonicated for more 24 hours and extracted once more (1000 mL each time). The filtrates were transferred to the round bottom flasks and methanol was evaporated in rotary evaporator under vacuum at 40°C until the volume was reduced to 300mL. The volume was then made with 400 mL with water to standardize crude liquid contents.

Extract Preparation from sweet violet dried flowers: Sweet violet dried flowers were purchased from local market. This sample was extracted using method of Kim and Lee (2002). 200 grams of the dried sweet violet flowers were soaked in flask with 1000 mL of 80% methanol, sonicated for 24 hours, filtered with whatman no.2 filter paper, took the residue on filter and sonicated for more 24 hours and extracted once more (1000 mL each time). The filtrates were transferred to the round bottom flasks and methanol was evaporated in rotary evaporator under vacuum at 40°C until the volume was reduced to 300 ml. The volume was then made with 400 ml with water to standardize crude liquid contents.

Feed Formulation and mixing: Broiler starter and grower mash rations were prepared using commercially available ingredients in the market. Formulation was done on computerized software to fulfil all nutrients needs of Hubbard broiler (Table 1). Corn, soybean meal, rice polishing were major ingredients supplemented with amino acids, minerals and vitamin sources to meet ideal requirement of broiler diet. All diets were having same ingredients and nutritional profile, differing only in antioxidants or antibiotics status as follows.

Ration F1: standard or control diet with lincomycin (4.4%) 100 mg/kg, vitamin E 40 mg/kg and synthetic antioxidants, i.e. SELDOX (Ethoxiquine, BHA, BHT and citric acid) 150mg/kg.

Ration F2: negative control (NC), no antioxidant or antibiotics added in feed.

Ration F3: without antibiotics/ antioxidants, supplemented by fenugreek crude extracts 1mL per kg diet.

Ration F4: without antibiotics / antioxidants, supplemented by black cumin seed crude extracts 1 mL per kg diet

Ration F5: without antibiotics or antioxidants, supplemented by sweet violet crude extracts 1 mL per kg diet.

Ration F6: without antibiotics or antioxidants, supplemented by ajwain extracts 1ml/Kg feed

Ration F7: with lincomycin and antioxidants supplemented by ajwain, fenugreek and sweet violet extracts, each 0.5 mL/kg feed.

Ration F8: without antibiotics/antioxidants, supplemented by equal amount (0.5 Ml) of each extract from fenugreek, ajwain and sweet violet.

Birds and management: 120 mixed sexes, Hubbard broiler chicks were purchased from local commercial hatchery. Chicks were kept in an open house on floor and 24 pens having five birds each, were divided by wire mesh. There were eight feeds F1, F2, F3, F4, F5, F6, F7 and F8 and each ration was randomly fed in three different pens. Standard conditions for ventilation, feeding and drinking were adopted during 0-5 weeks. Birds were vaccinated for Newcastle Disease (ND) at 4th and 19th day. Record of feed intake and bird weight was noted after each week. Growth performance of broiler chickens were evaluated in terms of weekly feed intake, body weight and feed conversion Ratio (FCR) as influenced by dietary Green tea extract and its fractions. At the end of trial, birds were slaughtered and carcass percentage was calculated. Similarly, internal organ weights were compared among the feed treatments.

Blood collection: At the end of experiment, one bird from each pen was picked for blood collection. One sample was collected in tube with anticoagulant heparin for CBC test in 3 mL syringe from wing veins (brachial vein). Similarly, another sample from same bird was taken and almost 3 ml blood collected in a test tube without coagulant. This clotted blood sample was further used in lipid profile, uric acid and ND titre estimates. Samples were stored at 4 °C till reached in laboratories for analysis.

Blood testing (Hematological profiles): Blood test for analysis of selected indices like haemoglobin (Hb), hematocrit (HCT), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), red blood cell (RBCs) counts, total white blood cell (WBCs) counts, platelet counts, packed cell volume (PCV), and mean corpuscular volume (MCV) were conducted. These tests were performed using Hematological analyser Sysmex XP-100.

Detection of Newcastle disease virus antibodies (ND Titers): Newcastle Disease (ND) HI titre was determined by the method described in OIE Terrestrial Manual (Health, (2012). A 2-fold serial dilution of serum was made in a 96-well micro litre plate with V-shaped bottom, containing 25 µL of buffer with 7.2-7.4 pH and 25 µL of serum in all wells. 25 µL of ND virus antigen were added to all wells except those in the last row (the controls). Serum dilutions ranged from 1:2 to 1:48. The antigen serum mixture was incubated for 10 min at 37°C. 50 µL of 0.5% erythrocyte suspension were then added to each well and the wells were re-incubated for 30 min. A positive serum, a negative serum, erythrocytes and antigens were also included as controls. The highest

dilution of serum causing complete inhibition of erythrocyte agglutination was considered at the end.

Uric acid testing: Uric acid in blood samples was estimated by direct colorimetric method described by Prencipe (1978), (Trinder's enzymatic assay).

Lipid profile in blood: Total cholesterol was estimated applying method of Zak (1954), triglycerides by use of enzymes as described by Bucolo (1973), and HDL cholesterol by method of Naito (1984).

Data Analysis: Average values on eight treatment diets, fed to three replicates of five birds each, were subjected to One Way Analysis of variance (ANOVA), and significant differences at $p<0.05$ between the mean values were observed using Duncan's multiple range test using SAS version 9.1 (SAS Institute, Inc., 2004, USA). Standard errors values were also calculated and included in data.

RESULTS

Broiler weight, feed consumption and FCR: In the present study, broiler chicken fed different diets had significant difference ($p \leq 0.05$) in weight gain, feed consumption and feed conversion ratio (FCR.). Average Weights of the chicken after 35 days (Table 2) was maximum in ajwain extract fed group (1914) and negative control F2 group had least weight (1502). Feed consumption differed significantly in different treatments and was maximum in F6 group (Table 2). Negative control group had worst FCR (1.79) and all other groups were efficient convertors of feed into meat. Broilers fed ajwain extracts in group F6 had best ($p\leq0.05$) FCR (1.51).

Carcass %age and breast meat yields: Dressing percentage differed significantly among diets and the negative control diet fed group had least (58.8%) carcass weights. Combined botanical extracts in feed F7 had maximum ($p\leq0.05$) carcass (61.4%) gain (Table 2) and breast meat yields (38%). At a glance, Negative control group had worst ($p\leq0.05$) breast meat (30%), which improved by using botanical crude extracts in other feeds.

Broiler organ weights: Pancreas weights of the slaughtered broilers differed among treatments significantly ($p\leq0.05$). Negative control diet group F2 had maximum pancreas weight (0.31%) and mixed extracts fed (Table 3) group F7 had least weight (0.10%). Similar pattern for weights of liver seen in F2 group (3.44%) and reduced to minimum (2.85%) in F7 group. Data revealed significant reduction of liver weights in other groups with extracts ($p\leq0.05$). Spleen weights differed in treatments significantly ($p\leq0.05$) and maximum (0.24%) weight seen in F3 group. Weights of gizzard, heart and thymus were also significantly different ($p\leq0.05$) in different group of birds (Table 3).

Thymus weights reduction in F6 (0.24%) and F8 (0.22%) groups leads towards significant ($p\leq0.05$) effect of botanical extracts. Size of bursa differed significantly ($p\leq0.05$) and was improved maximum (0.35%) in F4 group with black cumin seed extracts.

Haemoglobin: Effect of supplementation spice extracts in feed resulted in increased haemoglobin (table 4) in fenugreek(F3),Black cumin seed (F4), sweet violet (F5) and F8 group ($p\leq0.05$) as compared to negative control group (F2).There was decreased Hb in ajwain groups (F6 and F7) as compared to NC group F2.Hemoglobin levels were increased from 10.2 gm/dL in F2 and attained figures of 11.35, 10.5, 10.45, and 11.15 g/dL respectively in F3, F4, F5, and F8 groups of broilers.

WBC: WBC counts were higher in birds fed fenugreek, black cumin seed or sweet violet extracts as compared to negative control diet without antioxidants (Table 4) F1 group and F8 group birds, also got significantly better WBC counts when compared to F2. This significant difference ($p\leq0.05$) justifies the presence of immunity boosting polyphenols extracts in fenugreek, black cumin seed or sweet violet.

RBC, Lymphocytes, Neutrophils, MCH, MCHC, MCV: There was no significant difference ($P>0.05$) in RBC, lymphocyte, MCH and MCV counts of birds in different groups (Table 4).

Hematocrit, MCHC and neutrophil counts: Hematocrit values were significantly different ($P\leq0.05$) among different diets with spice extracts. Similarly, MCHC and neutrophil values differed among treatments but these values are non-conclusive in favour of any treatment.

Platelets: Astonishing outcome of this trial was scattered trend in platelets counts (Table 4). As a matter of fact, platelets were significantly improved ($p\leq0.05$) by addition of fenugreek, black cumin seed or sweet violet extracts. However, there was decline in platelets in groups F6, F7 and F8, all having ajwain extracts.

Lipid profile and uric acid: Results of blood samples taken for lipid profile are presented in table-5. Cholesterol level in blood of chicken fed negative control feed (group F2) was 112 mg/d L. Group F1 had (110), F3 (115), F4 (123), and was F7 (112) mg/dL cholesterol in blood. It is evident that all the feeding sweet violet, ajwain or combined botanicals extracts in treatments (F5, F6, F8) resulted in significant reduction ($p\leq0.05$) of cholesterol content in blood. Remarkable difference was observed in triglycerides content in blood during application of all spice extracts as in groups, F3, F5, F6, F7 and F8 in feeds of trial chicken. Group, F4, which was

fed diet with black cumin seed extracts, had highest triglycerides content in blood sample (46 mg/d L).

ND titres: Remarkably better titres of ND achieved in blood all the treatments except F8 group. Natural extracts in general had significantly better ($p \leq 0.05$) titers as compared to F2, which was negative control diet fed group lacking antioxidants.

There was no difference in High density lipoproteins (HDL) of broiler blood ($p \leq 0.05$) in all extracts application via feed. A reduction in the levels of LDL was seen in groups of broilers fed sweet violet and ajwain extracts in treatments F5, F6 and F8 as compared to negative control group F2. However, diets with fenugreek, black cuminseed and F7 group birds attained 72, 70 and 70 IU/L, LDL levels in blood. This significant increase ($p \leq 0.05$) in treated broilers with the mentioned extracts differs with negative and positive control groups (F1, F2). Uric acid in broiler blood differed in all diets ($p \leq 0.05$) significantly, supplemented with different extracts.

Table 1. Composition and nutrient profile of broiler starter and broiler grower rations (Hubbard standards).

Ingredient name	Broiler starter Inclusion %	Broiler Finisher Inclusion %	Profile Nutrients	Broiler starter	Broiler finisher
			Metabolizable energy		
Corn (yellow maize) 90 % DM	22.75	30	AME (Kcal/kg)	2950	3100
Rice broken 90 % DM	31.25	31	Crude protein	22.5	20.4
Soybean meal Argentina	30.35	32.6	Calcium	1	0.9
Canolla meal	10	0	Available phosphorus %	0.5	0.4
Limestone/ Marble	1.3	1.4	Digestible lysine %	1.23	1.06
MDCP 21%	0.7	0.9	Digestible Meth+cys %	0.912	0.821
Sodium bicarbonate.	0.15	0.2	Digestible arginine %	1.40	1.3
Salt (NaCl)	0.19	0.2	DigestibleTryptophan %	0.249	0.23
Choline chloride 70%	0.2	0.2	Digestible Threonine %	0.822	0.72
Soybean oil	2.0	2.6	Digestible isoleocine %	0.83	0.781
Lysine sulphate	0.315	0.165	Digestible valine %	0.946	0.878
D. L methionine	0.26	0.25	Linolic acid %	1.69	2.06
L-Threonine	0.125	0.08	Sodium %	0.192	0.186
Phytase (Phyzyme) 10000 TPT	0.010	.005	Chloride %	0.177	0.181
** Vitamin/ mineral premix	0.4	0.4	Pottasium%	0.905	0.85
TOTAL	**100**	**100**			

** Vitamin and mineral premix provided per kilogram of diet: vitamin A, 15.000 IU; cholecalciferol, 3.000 IU; vitamin E*, 50 mg; vitamin K3, 3 mg; vitamin B1, 3 mg; vitamin B2, 8 mg; niacin 60 mg; vitamin B6, 4 mg; vitamin B12, 20 µg; Ca-D- pantothenate, 15 mg; Folic acid, 1.5 mg; biotin, 0.2 mg; Mn, 80 mg; Zn,80 mg; Fe, 60 mg; Cu, 10 mg; Se, 0.2 mg, Iodine 1mg
*(only positive control feed)

Table 2. Performance of broilers fed crude botanical extracts in feed, 0- 35 days.

	Type of feed	Average weight (grams)	Average feed consumed (grams)	Feed conversion ratio (fcr)	Dressing carcass%	BM % of total live weight	BM % of total meat
F1	positive control (PC) antibiotic + antioxidant	1820 ± 2.64 (b)	2889 ± 1.15 (d)	1.58 ± 0 (d)	60.7 ± 0.1 (e)	23 ± 0.57 (c)	31 ± 0 (a)
F2	Negative control, (NC) no antibiotic + no antioxidant	1502 ± 1.15 (a)	2690 ± 2.88 (a)	1.79 ± 0.01 (f)	58.8 ± 0.11 (a)	23 ± 1.15 (c)	30 ± 0.57 (a)
F3	no antibiotic + no antioxidant +Fenugreek extracts 1ML/KG	1865 ± 2.88 (bc)	2858 ± 4.61 (c)	1.53 ± 0.01 (b)	59.6 ± 0.11 (b)	22.6 ± 0.34 (b)	34 ± 1.15 (b)
F4	no antibiotic + no antioxidant +black cumin seed ext. 1ML/KG	1867 ± 4.04 (bc)	2897 ± 1.15 (e)	1.55 ± 0 (c)	59.99 ± 1.88 (c)	22.5 ± 0.28 (b)	31.5 ± 0.28 (ab)
F5	no antibiotic + no antioxidant +Sweet violet extracts 1ML/KG	1878 ± 4.61 (bc)	2905 ± 2.88 (e)	1.65 ± 0.01 (e)	60.76 ± 0.79 (e)	21.88 ± 0.06 (a)	31 ± 1.15 (a)
F6	no antibiotic + no antioxidant +Ajwain extracts1ML/KG	1914 ± 2.3 (c)	2899.33 ± 2.6 (e)	1.51 ± 0 (a)	60.7 ± 0.4 (e)	22.5 ± 1.44 (b)	32.5 ± 0.86 (ab)
F7	Antibiotic + antioxidant+ (ajwain + sweet violet + fenugreek)	1820 ± 2.88 (b)	2869 ± 2.3 (d)	1.53 ± 0.01 (b)	61.4 ± 0.8 (f)	29 ± 1.15 (e)	38 ± 1.15 (d)
F8	No antibiotic + no antioxidant + (ajwain, sweet violet + fenugreek)	1795 ± 2.88 (b)	2757 ± 4.04 (b)	1.53 ± 0 (b)	60.3 ± 0.17 (d)	24 ± 1.73 (d)	34.5 ± 0.28 (bc)

Means ± SEM within a column with different letters differ significantly at ($P \leq 0.05$).

Table 3. Organ size comparison of broilers fed crude botanical extracts in feed, 0- 35 days.

Group	Type of feed	Pancreas % weight of live broiler	Gizzard % weight of live broiler	Bursa % weight of live broiler	Liver % weight of live broiler	Heart % weight of live broiler	Spleen % weight of live broiler	Thymus % weight of live broiler
F1	positive control (PC) antibiotic + antioxidant	0.18 ± 0 (b)	1.8 ± 0.05 (b)	0.29 ± 0 (e)	3 ± 0.05 (b)	0.54 ± 0.02 (c)	0.07 ± 0 (a)	0.32 ± 0.01 (c)
F2	Negative control, (NC) no antibiotic + no antioxidant	0.31 ± 0 (d)	2.05 ± 0.02 (e)	0.18 ± 0.01 (b)	3.44 ± 0.02 (d)	0.51 ± 0 (a)	0.1 ± 0 (b)	0.36 ± 0.01 (e)
F3	no antibiotic + no antioxidant +Fenugreek extracts 1ML/KG	0.25 ± 0 (c)	1.75 ± 0.02 (a)	0.26 ± 0.01 (d)	2.97 ± 0.01 (b)	0.5 ± 0 (a)	0.1 ± 0 (b)	0.39 ± 0 (f)
F4	no antibiotic + no antioxidant +black cumin seed extr. 1ML/KG	0.24 ± 0 (c)	1.96 ± 0.03 (c)	0.35 ± 0 (f)	3.17 ± 0.01 (c)	0.53 ± 0.01 (bc)	0.24 ± 0.02 (e)	0.35 ± 0.01 (d)
F5	no antibiotic + no antioxidant +Sweet violet extracts	0.25 ± 0 (c)	1.99 ± 0.02 (cd)	0.18 ± 0 (ab)	3.2 ± 0 (c)	0.63 ± 0.01 (d)	0.14 ± 0.02 (c)	0.35 ± 0.01 (d)

	1ML/KG							
F6	no antibiotic + no antioxidant +Ajwain extracts1ML/KG	0.24 ± 0.01 (c)	1.81 ± 0 (b)	0.2 ± 0 (c)	2.92 ± 0.01 (b)	0.52 ± 0.01 (a)	0.1 ± 0.01 (b)	0.24 ± 0.01 (b)
F7	antibiotic + antioxidant + (ajwain, sweet violet +fenugreek extracts)	0.1 ± 0.01 (a)	2.1 ± 0.05 (e)	0.17 ± 0.01 (a)	2.85 ± 0.02 (a)	0.62 ± 0.02 (d)	0.13 ± 0.01 (c)	0.32 ± 0.01 (c)
F8	no antibiotic + no antioxidant + (ajwain, sweet violet +fenugreek extracts)	0.18 ± 0 (b)	2 ± 0.05 (de)	0.18 ± 0 (ab)	2.93 ± 0.01 (b)	0.63 ± 0.01 (d)	0.18 ± 0.02 (d)	0.22 ± 0.01 (a)

Means ± SEM within a column with different lower case letters are significantly different at ($P \leq 0.05$).

Table 4. Haematological profile of broilers fed crude botanical extracts in feed, 0- 35 days.

Group	FEED TREATMENT	HGLBN m/dL	HEMTCRT %	RBC 10–12/L	MCV FL	MCH PG	MCHC %	WBC 10–9/L	Lymcytes %	Netrphl %	Platelets 10–9/L
F1	positive control (PC) antibiotic + antioxidant	10.75 ± 0.02 (cde)	29.5±0.28 (b)	2.25±0.14 (a)	129±0.57 (a)	46±0.57 (a)	35 ± 0.57 (a)	255 ± 2.88 (cd)	92 ± 0.57 (c)	6.5 ± 0.05 (a)	16500 ± 57.73 (h)
F2	Negative control, (NC) no antibiotic + no antioxidant	10.2±0.05 (bc)	28.5±0.28 (ab)	2.25±0.14 (a)	127±0.57 (a)	44±0.57 (a)	33 ± 0.57 (a)	248 ± 4.61 (b)	94 ± 1.73 (a)	3 ± 0.05 (a)	11500 ± 57.73 (d)
F3	no antibiotic + no antioxidant + Fenugreek extracts 1ML/KG	11.35±0.02 (e)	32.5±0.28 (ac)	2.6±0.34 (a)	124.16±2.3 (a)	43 ± 0.57 (a)	34.5 ± 0.28 (a)	264 ± 1.15 (e)	94.53 ± 2.54 (b)	3.5 ± 0.05 (a)	14500 ± 57.73 (f)
F4	no antibiotic + no antioxidant +black cumin seed extr. 1ML/KG	10.5±0.28 (bcd)	29.5±0.28 (b)	2.35±0.2 (a)	127±2.88 (a)	44 ± 0.57 (a)	34 ± 0.57 (a)	252 ± 1.15 (bc)	94.46 ± 1.88 (a)	3 ± 0.05 (a)	15000 ± 57.73 (g)
F5	no antibiotic + no antioxidant +Sweet violet extracts 1ML/KG	10.45±0.31 (bcd)	28.5±0.28 (ab)	2.2±0.11 (a)	130±0.57 (a)	46.5 ± 0.86 (a)	35.5 ± 0.28 (a)	250 ± 0.57 (bc)	94 ± 1.89 (b)	3.5 ± 0.05 (a)	12500 ± 57.73 (e)
F6	no antibiotic + no antioxidant +Ajwain extracts1ML/KG	9.3±0.17 (a)	27±0.28 (a)	2±0.02 (a)	130.5±0.28 (a)	44 ± 0.57 (a)	34 ± 0.57 (a)	238 ± 0.57 (a)	95 ± 1.73 (a)	3 ± 0.05 (a)	6500 ± 57.73 (a)
F7	antibiotic + antioxidant + (ajwain, sweet violet + fenugreek extracts)	10±0.28 (b)	30±1.15 (b)	2±0 (a)	130±2.88 (a)	44 ± 0.57 (a)	34 ± 0.57 (a)	240 ± 0.57 (a)	95 ± 1.73 (a)	3 ± 0.05 (a)	10000 ± 115.47 (c)
F8	no antibiotic + no antioxidant + (ajwain, sweet violet + fenugreek extracts)	11.15±0.08 (dc)	32±1.15 (c)	2.6±0.34 (a)	125±2.88 (a)	43 ± 0.57 (a)	34 ± 0.57 (a)	259 ± 0.57 (d)	94.46 ± 2.28 (b)	3.5 ± 0.05 (a)	9000 ± 57.73 (b)

Means ± SEM within a column with different lower case letters are significantly different at ($P \leq 0.05$).

Table 5. Lipid profile, uric acid and ND titre of broilers fed crude botanical extracts in feed, 0- 35 days.

	FEED TYPE / RESULT	CHLSTRL mg/dL	TRIGLCRD mg/dL	HDL IU/L	LDL IU/L	URIC ACD mg/dl	ND TITRE
F1	Positive control (PC) antibiotic + antioxidant	110 ± 5.77 (c)	27.33 ± 2.6 (d)	41 ± 1.73 (a)	33 ± 1.73 (a)	2.5 ± 0.05 (c)	4.5 ± 0.05 (c)
F2	Negative control, (NC) no antibiotic + no antioxidant	112 ± 1.15 (c)	32 ± 1.15 (f)	40 ± 1.15 (a)	65 ± 1.15 (d)	2 ± 0 (a)	4 ± 0.05 (b)
F3	no antibiotic + no antioxidant + Fenugreek extracts 1ML/KG	115 ± 2.88 (c)	18 ± 1.15 (d)	39 ± 1.15 (a)	72 ± 1.15 (e)	2.3 ± 0.05 (b)	6 ± 0.05 (e)
F4	no antibiotic + no antioxidant + black cumin seed extr. 1ML/KG	123 ± 1.73 (d)	46 ± 2.3 (a)	43 ± 1.73 (a)	70 ± 1.15 (e)	4.3 ± 0.05 (e)	6.5 ± 0.05 (f)
F5	no antibiotic + no antioxidant +Sweet violet extracts 1ML/KG	96.5 ± 4.07 (b)	27.66 ± 1.45 (f)	40 ± 1.15 (a)	50.5 ± 0.28 (c)	4.5 ± 0.05 (f)	5 ± 0.05 (d)
F6	no antibiotic + no antioxidant + Ajwain extracts1ML/KG	82.5 ± 1.44 (a)	13 ± 1.73 (b)	41 ± 1.73 (a)	39 ± 1.15 (b)	3.3 ± 0.11 (d)	5 ± 0.05 (d)
F7	antibiotic + antioxidant + (ajwain, sweet violet + fenugreek extracts)	112 ± 1.15 (c)	11 ± 1.15 (ab)	39 ± 1.15 (a)	70 ± 1.15 (e)	2.15 ± 0.03 (ab)	7.5 ± 0.28 (g)
F8	no antibiotic + no antioxidant + (ajwain, sweet violet + fenugreek extracts)	102 ± 1.15 (b)	23 ± 1.73 (e)	42 ± 1.15 (a)	55 ± 2.88 (c)	7.1 ± 0.07 (g)	3 ± 0.05 (a)

Means ± SEM within a column with different lower case letters are significantly different at ($P \leq 0.05$).

DISCUSSION

In current trial, using botanical extracts instead of synthetic antioxidants and antibiotics proved much effective in safeguarding chicken health and growth. The results correspond with Biswas and Wakita (2001), trial on broiler FCR and carcass parameters using Japanese green tea powder. This concept of replacement of botanical extracts with antibiotics was well documented in a research by Al–Attar and Abu-zeid (2013). They revealed the fact that advantage of these extracts over antibiotics is that they do not harm to physiological functions of animal, rather they alleviate the negative effects of drugs. Similarly Dash (2011), reported similar findings that methanolic extracts derived from Fenugreek have excellent antibacterial function. Gunasegaran *et al*. (2011) documented their lab work proving that ajwain is very effective against Salmonella.

Broiler growth and better health in this trial is due to stress fighting capabilities of plant polyphenolic extracts. These facts were demonstrated by Krishnaiah *et al*. (2011) that Plant seed, leaf or flower extracts rich in polyphenols can fight against any oxidative degeneration. This fact is also clear from this research outcome where botanical extracts compensated the absence of most potent commercial antioxidants like BHA, BHT and ethoxyquine. It is in accordance with Sahin *et al.* (2008), work on quails who interpreted the use of Epigallocatechin -3-gallate as a modulator of nuclear transcription in liver tissues of heat stressed quails. Results to improve FCR and carcass yield are not astonishing as flavanoid rich extracts are antifungal, antiviral and antibacterial, in body as Orhan *et al*. (2010) confirmed. Vandeputte *et al*. (2008) showed that fractions like catechins, pyrogallol and flavonoids decrease production of quorum-sensing based virulence factors in bacteria like aeruginosa, Pseudomonas and Vibrio harveyi.

Current trial results show that the plant extracts use in diets of broiler had much better effect to reduce stress of working on organs. This was reflected in reduced weights of liver, pancrease and thymus of the chicken given natural extracts of plants rich in antioxidants (Table 3). Khan *et al*. (2009) demonstrated that in broilers Fenugreek seeds or extracts feeding helps in controlling liver weight. Amamnueysit *et al*. (2010) studied on organ weights, which showed purple corn anthocyanins as heart weight controller in broilers. Umar *et al*. (2012) proved that ajwain antioxidant potential alleviates stress and toxic effects of chemicals like collagen and it is considered anti- inflammatory in rats. Bursa fabricious is the source of beta immunity in broilers and its size was improved during natural extracts application during this trial. Azeem *et al*. (2014) reported similar verdict that black cumin seed is one of the best replacer of antibiotics and works finally as immune booster. In these results of current study, organ sizes (weights) of spleen and gizzard are significantly different among their mean values but are not conclusive in favour of all botanical extracts used in treatments.

Blood physiology is affected by using botanical extracts. Improvement was observed here in this research and Hemoglobin, WBC and platelets were elevated by spice and sweet violet applications (Table 4). Ahmed *et al*. (2015) explained that tea and ginger extracts can increase RBC, WBC, PCV and neutrophil percentage in alloxen induced diabetic rabbits. Such scientific fact is also supported by study on omumseed by Ishtiaq *et al*. (2013), which explains that antioxidant activity at 100-250 microgram/100 micro liter is 74-95% which is comparable to BHA (78-97%). Replacement of BHA with natural antioxidants like omumseed extracts can avoid liver damage risk associated with synthetic chemicals.

Present data was collected from the broilers blood which was raised at 35°C, lacking antibiotics and antioxidants. Broiler hematology changes during extreme temperature and microbial or oxidative stress were reduced and plant phenolics in feed were capable to combat all these challenges. When negative control feeds were supplemented with plant derived extracts fed to broilers, Hemoglobin, Hematocrit, lymphocytes, RBC and total WBC improved (Table 4). Very relevant models of such findings in recent times are frequent. Al-Shavi *et al*. (2014), demonstrated that poison like Methotraxate treated rats' blood testing results show decline in Hb, HCT, MCHC, MCH, RBC and total WBC. Green tea extracts use during this phase of toxic application result in increased Hb, HCT, MCHC, MCH and RBC. During similar work by Elelaimy *et al*. (2012) on rats with stress due to chlorpyrifos poisoning, were fed botanical extracts of eugenol which defended the fatal changes in haemotology by post poisoning rehabilitation of humeral immunity by elevated Immunoglobulin (IgG), lymphocytes viability, neutrophil phagocytic function and WBC increase. Improved status of HDL and reduction in LDL fulfills the required parameter in broiler blood, fed different spice extracts (Table 4). Lipid lowering effect can be best explained by Zang *et al*. (2014) studies where gene expression of fat transportation and metabolic enzymes like carnitine palmitoyltransferase I (CPT-I), acyl CoA oxidase 1 (ACOX1) and peroxisome proliferator-activated receptor-α (PPAR α) in liver adipose and abdominal tissues is much improved by tea polyphenols. Similar lipid catabolic enzymes upregulation in body tissues was observed by Huang *et al* (2013) which results in lowered serum triglycerides, LDL and total cholesterol. Sweet violet and spice extracts reduced cholesterol and triglycerides due to active biomolecules having flavonoids and other polyphenols. This remarkable improvement in reducing cholesterol was observed in

purified supplementations of polyphenols in feed by Kamboh *et al*. (2013). During our current trial, spices and sweet violet extracts resulted in reduced cholesterol and triglycerides.Velićanski *et al*. (2014) demonstrated that bioflavonoids increase the potential of antioxidants in plasma which reflect lipid reduction in serum and breast muscles.

There are some results contrary to most of findings or an exception to majority of scientific fact. Cholesterol and triglycerides were not significantly reduced ($p>0.05$) while supplementing feed of broilers with black cumin seed extracts (Table 5). Similarly, LDL in broiler blood reduced in F5, F6 and F8 treatments as compared to the negative control group (Table 5). Some values like MCV, MCH, RBC, lymphocytes and HDL had non-significant differences among all treatments. Sustaining the optimum haematological contents in absence of antibiotics and antioxidants is itself an achievement. However, these findings are not exceptions, as some groups working on broilers narrated no difference in lipid profile even using polyphenol rich sources. Shirzadegan *et al*. (2014) while conducting a trial on broilers even after two weeks use of green tea powder, PCV, LDL, HDL, total cholesterol and triglyceride contents had non-significant differences. Similar findings of Shomali *et al*. (2012) research outcome disclosed non-significant difference of polyphenols application in blood parameters of broilers.

During our studies using broiler, platelets were improved by spices extracts feeding. Such potential botanical polyphenol rich compounds can improve platelets. Audomkasok *et al*. (2014) used safflower and mulberry extracts during Plasmodium berghei infection in mice and narrated anti-hemolytic effects. Similarly, polyphenols improve immunity and defend the viral attack in influenza challenged chicken. Immunity improvement is clearly visible in our scientific work as WBC, ND titre and bursa size improved using spice extracts. These findings confirmed previous results of Srikhun *et al* (2010)

Conclusions: Plant seed, leaf methanolic supplementation in feed justify their antioxidant and antimicrobial presence and combat the challenges of fast growing broiler needs to safeguard immunity. Antibiotics and antioxidants use is severe concern in feed, food and ultimately human organs. Exploring natural solutions to replace drugs is recommended after this trial and feed will be more secure while using such botanical extracts as alternate.

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