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Serum biochemistry, meat quality and oxidative stability in broiler chicken supplemented with a novel phytogenic feed additive formulated from *P. betle*, *P. nigrum*, *A. lanata* and *C. dactylon*

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ABSTRACT

The present study investigated the effect of a phytogenic feed additive (PFA) formulated with *Aerva lanata*, *Piper betle*, *Cynodon dactylon* and *Piper nigrum* on the haematological and serum biochemical profiles in broiler chicken, and its efficacy to improve quality and oxidative stability of chicken meat. In a six-week experiment, a total of 192 day-old broiler chicks were subjected to four dietary treatments that included, basal diet+ chlortetracycline; only basal diet without chlortetracycline and PFA; and basal diet + 1 or 2% PFA. Each treatment group included six replicates of eight birds per replicate. The results showed improved albumin and cholesterol in serum of birds fed 1% PFA. The meat of birds supplemented 1% PFA showed a significant reduction in drip loss after 1 and 4 days of storage. The lipid peroxidation of breast meat measured as malondialydehyde concentration was not altered by dietary treatments at different periods of storage. Feeding 1% PFA did not induce any toxic effects on liver, kidney and spleen histology. It was concluded that the PFA when used as a feed additive improved serum biochemistry and meat quality in broiler chickens.

Keywords: Broiler chicken, Drip loss, Meat oxidative stability, Phytogenic feed additive, Serum biochemistry

Poultry meat is a major source of animal protein worldwide. Driven by increasing population, changing food habits and the improved economic status, the demand for poultry meat and meat products is projected to increase in the coming years (Flees *et al*. 2021). Chicken meat is highly susceptible to oxidative deterioration that adversely affects the nutritional and economic value of meat (Lanni *et al*. 2019). Further, the use of antibiotic growth promoters in animal production has detrimental effects on meat quality (Valenzuela-Grijalva *et al*. 2017).

Phytogenics are being explored in poultry to improve the overall health and meat quality (Valenzuela-Grijalva *et al*. 2017). Dietary supplementation of phytogenic preparations rich in antioxidant polyphenols and flavonoids improved oxidative stability of chicken patties (Ali *et al*. 2022) and broiler chicken meat quality (Xiu-dong *et al*. 2018).

The leaves of *Piper betle* are rich sources of phytochemicals with antioxidant and hepatoprotective (Autade *et al*. 2023) and growth promoting effects in broilers (Basit *et al*. 2020). The dried fruit of *Piper nigrum* (common name:black pepper) plant contains the phytochemical 'piperine' that possess antioxidant, digestion stimulatory and health promoting properties in broiler chicken (Abou-Elkhair *et al*. 2014). Piperine also improves meat quality in pigs and is the major phytochemical responsible for the beneficial effects of pepper (Sampath *et al*. 2020).

The medicinal plant *Aerva lanata* (L.) (family: Amaranthaceae) has been shown to exhibit antioxidant, hepatoprotective and immunomodulatory properties *in vivo* (Singh *et al*. 2020). *Cynodon dactylon* (family: Poaceae; common name: bermuda grass) is a perennial grass distributed all over the world. The leaves are reported to have various biological activities including reduction of lipid peroxidation, immunomodulation, antioxidant and hypolipidemic functions (Das *et al*. 2021).

The amalgamation of various phytogenic herbs and spices has received attention as a novel approach to promote the synergistic and additive effects of several bioactive compounds in order to achieve maximum desired benefits (Bassole *et al*. 2012). So, for this experiment, a phytogenic feed additive (PFA) was prepared from *P. betle*, *P. nigrum*, *A. lanata* and *C. dactylon* and was hypothesized that the PFA loaded with phytochemicals could act synergistically and improve meat quality and oxidative stability in broilers. The present study, was thus intended to decipher the effect of PFA on serum chemistry in broiler

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chicken and its efficacy to improve quality and oxidative stability of chicken meat. The biochemical, haematological and histopathological studies were carried out to confirm the non-toxic nature of the PFA.

MATERIALS AND METHODS

Chemicals: The phytochemical standards and 1, 1, 3, 3- tetraethoxypropane standard used in the present study were procured from Sigma Aldrich, India. The total protein, albumin, uric acid, cholesterol, glucose, creatinine, aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma-glutamyl-transferase (GGT) kits were procured from Trivitron Healthcare Private Limited, India. All the other chemicals used in the study were of analytical grade.

Formulation of the phytogenic feed additive (PFA): The PFA used for the present study was formulated using *P. betle, A. lanata, C. dactylon* and *P. nigrum*. To prepare the PFA, commercially available black pepper seeds were purchased, dried at 45°C and finely powdered before use. Powders of *A. lanata* and *C. dactylon* (aerial parts) were procured from Jeyam Herbals Ltd, Tamil Nadu, India. Fresh betel leaves were sourced from a farm in Karnataka and chopped into fine pieces after removing the stalks and petioles. For preparing the PFA, equal quantities of all the four constituents were weighed separately. The weighed betel leaves were mixed with distilled water, ground to a uniform paste and homogenized. The homogenized mixture was allowed to settle overnight and filtered through a muslin cloth. The extract thus obtained was mixed thoroughly with *A. lanata*, *C. dactylon* and *P. nigrum*, dried at 35°C and stored at 4°C until used. The thymol, piperine, phytol, eugenol, iso eugenol, methyl eugenol, phenolic acid and flavonoid contents in PFA were determined by LC-MS/MS (Oso *et al*. 2019) and the concentrations were: phytol: 114.01 µg/g, thymol: 61.5 µg/g; piperine: 26.01 µg/g; eugenol: 10.72 µg/g; methyl eugenol: 4.3 µg/g; isoeugenol: 3.6 µg/g; flavonoids (epigallocatechin, quercetin, hesperidin, kaempferol, rutin, catechin, myricetin, epigenin): 0.053 mg/g and phenolic acids (salicylic acid, chlorogenic acid, ferulic acid, protocatechuic acid, gallic acid, 2,4-dihydroxy benzoic acid, gentisic acid): 10.18 mg/g.

Ethical considerations: All animal care and use protocol used in the present study were reviewed and approved by the Animal Ethics Committee of ICAR-National Institute of Animal Nutriton and Physiology, Bengaluru, India.

Location, experimental design and management: The experiment was carried out at the Poultry Unit of ICAR-NIANP Experimental Livestock Unit, Bengaluru, India. A total of 192 (Cobb) day-old broiler chicks were purchased from a commercial hatchery, weighed and assigned to four dietary regimens. A completely randomized design was adapted with six replicates of eight chicks assigned to each of four dietary treatments.

The birds were fed only basal diet or basal diet supplemented with chlortetracycline as antibiotic growth promoter or the PFA as detailed below:

Treatment 1: Basal diet + chlortetracycline, feed grade @ 355 g/metric ton (positive control)

Treatment 2: Basal diet (no antibiotic/PFA, negative control)

Treatment 3: Basal diet + 1% PFA (*P. betle* + *A. lanata* + *C. dactylon* + *P. nigrum)*

Treatment 4: Basal diet + 2% PFA (*P. betle* + *A. lanata* + *C. dactylon* + *P. nigrum)*

The basal diet consisted of a three-phase feeding program: starter feed from d 1–14, grower feed from d 15-21, and finisher feed from d 22–42. The ingredients and nutrient composition of the diets are shown in Table 1. The basal diet was a corn-soybean meal diet formulated to meet the nutritional requirement of growing chicks as recommended by National Research Council (1994). The birds were maintained on a 24-h light schedule and allowed *ad lib.* access to feed and water following standard management conditions. The experiment was conducted for 42 days.

Sample collection: At the end of the study (42 days), from each dietary treatment, 2 birds per replicate were selected and blood samples were collected from the

Table 1. Ingredient and chemical composition of basal diet of experimental birds (Starter:1- 14 d; grower:15-21 d; finisher : 22- 42 d)

Item	Starter	Grower	Finisher
Ingredients (g/kg)			
Maize	562	584.5	625
Soybean meal	380	361	317
Soy oil	16	18	22
Dicalcium Phosphate	17.5	17.5	16
Limestone	10	10	11
Salt (NaCl)	3.5	3.5	3.5
Lysine	4.0	1.0	1.3
Methionine	2.5	2.0	1.5
Threonine	2.0		
Vitamin-minerals premix*	2.5	2.5	2.7
Nutrient and energy compositions			
ME (Kcal/kg)	2996	2992	3048
Crude protein (CP) (%)	22.51	21.90	20.07
Lys (%)	1.5	1.26	1.2
Met (%)	0.55	0.51	0.44
Thr (%)	0.98	0.80	0.72
Tryp (%)	0.23	0.23	0.21
Val (%)	0.90	0.90	0.80
Arg (%)	1.37	1.35	1.21
Ca (%)	1.00	1.01	1.00
P, avail. (%)	0.45	0.45	0.41

*Supplied per kilogram of diet: Vitamin E, 20 IU; cholecalciferol, 1,800 IU; vitamin A, 8,050 IU; vitamin K3, 5.1 mg; niacin, 32 mg; pantothenic acid, 15.3 mg; riboflavin, 8.2 mg; pyridoxine, 3.1 mg; thiamin, 2.4 mg; cobalamin, 0.02 mg; biotin, 0.20 mg; choline chloride, 1,000 mg; Fe, 85 mg; Mn, 68 mg; Zn, 58 mg; Cu, 8.6 mg; folic acid, 1.2 mg; I, 0.27 mg; and Se, 0.20 mg.

wing vein into heparinized vacutainer tubes (Becton Dickinson India Pvt. Ltd., New Delhi, India) and used to study hematological parameters. For serum studies, blood samples were collected in separate vacutainer tubes (without anticoagulant), centrifuged at 3000 rpm for 15 min and the serum separated was stored at -20°C for further use. For meat quality and storage studies, the birds were sacrificed immediately after blood collection and breast muscle (Pectoralis major) tissues were sampled. To determine the chemical composition, a portion of breast meat samples were stored at -20°C until analysis. Subsequently, liver, kidney and spleen tissues were collected for histopathology studies.

Haematological indices: In whole blood samples, the total haemoglobin concentrations, packed cell volume (PCV), total red blood cell (RBC) count and white blood cell count (WBC) were determined. Hemoglobin was estimated by cyanmethemoglobin method (Rodkey *et al*. 1979) and PCV was determined as per the method of (Schalm *et al*. 1975). RBC and WBC counts were determined using hemocytometer.

Serum parameters: The serum total protein, albumin, glucose, cholesterol, uric acid and creatinine concentrations and GGT, AST and ALP activities were measured using standard protocols of commercial laboratory kits.

*Meat colour analysi*s: For meat colour measurements, portion of breast muscles (*Pectoralis major*), each measuring 2.5 × 2.5 × 1.6 cm were cut using an ultra-thin cutter immediately after slaughter and scanned at 300 dpi resolution using a flatbed scanner (Model HP Scanjet 3970, Hewlett-Packard India, Mumbai, India) connected to a computer. The images were then imported into Adobe Photoshop 7.0 software and the mean of the colour parameters such as lightness (L*), redness (a*) and yellowness (b*) values were obtained from the histogram.

Meat pH, drip loss and chemical composition: The *p*H values of breast muscles were measured immediately after slaughter using a *p*H meter by penetration in breast muscle (Glamoclija *et al*. 2015). The drip loss of meat was measured according to the method described by Honikel *et al*. (1998). The dry matter, ether extract, crude protein and ash contents of the meat samples were measured as per the AOAC (2000).

Oxidative stability of meat: The extent of lipid oxidation in meat was determined by measuring the TBA-reacting substances at 1 d ($TBARS_{24h}$), 4 d ($TBARS_{96h}$), and 6 d ($TBARS_{144h}$) of storage at 4°C as per modified method described by Buege and Aust (1978). The concentration of TBARS was expressed as mg malondialdehyde (MDA) per kg meat using a standard curve prepared from 1, 1, 3, 3- tetraethoxypropane.

Histopathology: The liver, kidney and spleen tissues collected after sacrifice were washed with 0.1 M phosphate buffered saline (*p*H 7.4) and fixed in 10% neutral buffered formalin. Sections from each sample were processed to obtain 4 µm thick paraffin sections and were stained with hematoxylin and eosin (HE). The histological sections were examined under an Upright microscope coupled with camera and NIS-D Ver.4.0 software (Model: Eclipse Ci-S, Nikon, Japan).

Statistical analysis: Statistical analysis was first performed using the GLM procedures of SAS statistical software (2000) to examine the treatment effect. A completely randomized design was considered for ANOVA, and the cages were used as experimental units for all measurements. All treatment means were compared using the Tukey's test, and the differences were separated at the statistical level of $P<0.05$. Effect of PFA supplementation levels (0, 1 and 2%) was determined by the "contrast" option of the GLM procedure. Orthogonal polynomial contrasts were also applied to determine the Linear (L) and Quadratic (Q) responses to different levels of PFA supplemented for the phytogenic treatment.

RESULTS AND DISCUSSION

The present study deciphered the effect of the dietary supplementation of a phytogenic feed additive (PFA) prepared from *A. lanata, P. betle, C. dactylon* and *P. nigrum* on hematology, serum biochemistry, meat quality and oxidative stability in broilers. In a previous report, dietary inclusion of the phytogenic blend prepared from *A. lanata, P. betle, C. dactylon* and *P. nigrum* was observed to improve growth, nutrient digestibility and intestinal morphology in broilers. The study recorded the highest body weight gain in birds supplemented with the phytogenic supplement at 1% concentration. Further, a lower feed conversion ratio (FCR) has been reported in birds fed 1% phytogenic as compared to birds fed chlortetracycline as AGP (Oso *et al.* 2019).

The effect of dietary supplementation of PFA on hematological indices of broilers is presented in Table 2. The Hb levels, RBC, WBC and PCV were not affected by dietary treatments.

Hematological indices function as indicators of physiological and health status (Albokhadaim *et al*. 2012). The MCV, MCH and MCHC are corpuscular indices that

Table 2. Effect of dietary supplementation with PFA on haematological indices of broiler chicken

Item	Control (Antibiotic)	Dosage of PFA (%)			SEM	Antibiotic vs PFA	PFA levels	
		0	1	2			Linear	Quadratic
Red blood cell (RBC) ($\times 10^6$ cells/µL)	2.35	2.8	2.67	2.55	0.67	0.55	0.295	0.965
Haemoglobin (Hb) (g/dL)	10.83	12.1	10.83	11.12	0.28	0.46	0.311	0.817
Packed cell volume (PCV) (%)	32.45	36.03	33.65	32.55	0.89	0.49	0.266	0.807
White blood cell (WBC)	235575	247950	239150	239025	2004	0.15	0.102	0.333

Table 3. Effect of dietary PFA on serum biochemistry of experimental broilers

Item	Control (Antibiotic)	Dosage of PFA			SEM	Antibiotic vs PFA	PFA levels	
		0	1	2			Linear	Quadratic
ALP (U/L)	232	126.9	320.1	244.2	42.9	0.5025	0.407	0.279
AST (U/L)	118.8[a]	85.86[b]	23.06[d]	41.67[c]	12.1	<0.0001	0.02	0.95
GGT (U/L)	16.75	17.25	13.38	14.88	0.63	0.0998	0.137	0.062
Total protein (g/dL)	3.23	3.87	4.5	4.64	0.19	0.3496	0.856	0.069
Albumin (g/dL)	1.11[b]	1.14[b]	1.82[a]	1.13[b]	0.02	0.0112	0.105	0.521
Globulin (g/dL)	2.12	2.73	3.39	3.5	0.19	0.9204	0.938	0.534
Albmin/globulin ratio	0.53[a]	0.47[a]	0.32[b]	0.32[b]	0.03	0.0109	0.105	0.466
Creatinine (mg/dL)	0.187	0.163	0.1	0.084	0.02	0.5467	0.392	0.764
Cholesterol (mg/dL)	149.27[a]	116.61[ab]	97.80[b]	108.00[ab]	6.81	0.0229	0.034	0.055
Glucose (mg/dL)	198.9	204.53	203.98	198.61	1.75	0.5178	0.326	0.637
Uric acid (mg/dL)	4.62	5.41	6.12	5.88	0.44	0.6859	0.707	0.667

[a–d]Means in a row bearing different superscripts are significantly different ($P< 0.05$).

exhibit a significant role in revealing anemias and thus could be used in diagnosis and therapy (Buttarello *et al*. 2016). The PCV values help to determine the effect of stressors on health and the oxygen carrying capacity of blood (Ochayi *et al*. 2021). In earlier studies, *P. betle* (Alam *et al*. 2013), *C. dactylon* (Aruldoss *et al*. 2014) and *P. nigrum* (Zodape *et al*. 2020) were reported as hematopoietic in mice, fish and rats respectively. The absence of changes observed in the present study in contrast to previous reports suggests the possible differences in species and the physiological status of the animals. However, the absence of adverse effects on hemtological parameters in the experimental birds indicate the non-toxic nature of the PFA.

The effect of dietary supplementation of PFA on serum biochemical parameters of broiler chickens is shown in Table 3. Dietary treatment significantly affected the serum albumin, albumin/globulin ratio, serum cholesterol and AST concentrations. Broilers fed diet supplemented with 1% PFA recorded the highest ($P<0.05$) serum albumin ($P=0.0112$) and lowest ($P<0.0001$) AST concentrations. Broilers from antibiotic group recorded the highest (*P<0.0001*) AST concentrations. Broilers from antibiotic and control group had significantly higher albumin/globulin ratio than birds fed diet supplemented with 1 or 2% PFA. Dietary supplementation with 1% PFA resulted in reduced serum cholesterol concentration (*P=0.0229*) when compared with antibiotic group. Orthogonal effect of PFA supplemental levels showed a linear reduction in serum cholesterol and AST concentration as the dosage of PFA increased to 1%, but further increased with 2% PFA supplementation.

Liver marker enzymes are studied to assess the liver damage in poultry. Serum concentrations of the liver marker enzymes have been reported to increase under stress and hepatotoxic situations (Tang *et al*. 2022). The lowest serum AST concentrations recorded in broilers fed diet supplemented with 1% PFA in the current study suggests normal liver function. Serum albumin, synthesized exclusively by liver is the single most abundant plasma protein that executes multiple biological functions. In a previous study, Gozlan *et al*. (2017) have reported increased serum albumin in birds fed rosemary leaves rich in antioxidant phenolic acids and flavonoids. In the current study, the concentration of serum albumin was significantly high in birds fed 1% PFA as compared to other treatments. In the general circulation, polyphenol (phenolic acids, flavonoids, etc.) compounds are primarily bound to serum albumin and transported in the blood. The binding of polyphenol compounds to albumin is suggested to determine the delivery of the compounds to cells and tissues as well as their rate of removal from circulation. It is also possible that the unbound metabolites, instead of the bound forms, may enter the cells and execute the functions (Pandey *et al*. 2009). Yet, further experiments need to be conducted in broilers to investigate whether the free polyphenol compounds or the albumin bound forms exhibit the biological activity.

Many phytogenics are known for their hypocholesterolemic properties *in vivo*. In the present study, a significant reduction in serum cholesterol was observed in birds supplemented with 1% PFA as compared to birds fed only basal diet or basal diet with AGP or 2% PFA. The hypocholesterolemic effect of the PFA observed is in agreement with earlier reports that indicate similar results during *in vivo* supplementation of *A. lanata* (Vidya and Udayakumar 2016), *C. dactylon* (Pashaie *et al*. 2017) and *P. nigrum* (Wang *et al*. 2021). In the study by Venkadeswaran *et al*. (2014), eugenol, the active constituent present in *P. betle* was found to prevent Triton WR-1339 induced hypercholesterolemia and its associated toxic effects. Some phenol compounds stimulate PPAR-α and its downstream target enzymes, decrease the available free fatty acids for triacylglycerol synthesis and thereafter the cholesterol biosynthesis (Laka *et al*. 2022). Further thymol inhibits the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, a key regulatory enzyme of cholesterol biosynthesis pathway resulting in reduced cholesterol synthesis (Case *et al*. 1995). The hypocholesterolemic effect observed in the present study in birds fed 1% PFA indicates the possible synergistic

Table 4. Effect of dietary supplementation with PFA on meat colour, meat composition and pH

Item	Control (Antibiotic)	Dosage of PFA (%)			SEM	Antibiotic vs PFA	PFA levels	
		0	1	2			Linear	Quadratic
Meat colour								
L*	62.162[b]	70.89[a]	74.62[a]	77.31[a]	1.426	<0.0001	0.015	0.802
a*	26.02[a]	19.29[ab]	15.18[b]	14.69[b]	1.319	0.002	0.075	0.399
b*	22.74	19.396	21.52	18.71	0.704	0.1496	0.75	0.199
Meat composition (%)								
Moisture	65.09[a]	62.72[b]	61.48[b]	62.06[b]	7.33	0.0221	0.32	0.19
Lipid	0.59	0.55	0.52	0.62	0.1	0.75	0.33	0.11
Protein	56.38	54.4	61.37	54.07	2.22	0.6626	0.965	0.27
Ash	6.67	7.43	8.05	7.93	1.81	0.3725	0.937	0.071
Meat pH	6.17[ab]	6.12[b]	6.20[a]	6.17[ab]	0.011	0.0528	0.031	0.01

[a–d]Means in a row bearing different superscripts are significantly different ($P< 0.05$).

effects of the constituent compounds in the PFA to reduce cholesterol biosynthesis.

The effect of dietary supplementation of PFA on meat colour, proximate composition and pH are presented in Table 4. The breast meat from birds supplemented CTC had the least score (*P<0.0001*) for lightness (*L**). Orthogonal effect of PFA levels revealed a linear increase (*P=0.0150*) in the degree of lightness (*L**) of breast meat with increasing inclusion levels of PFA from 0 to 2%. Broilers fed diet supplemented with PFA had lower (*P=0.002*) redness (*a**) score when compared with antibiotic group which recorded high *a** score. The degree of yellowness of breast meat (b*) was not influenced by dietary treatments. Breast meat from antibiotic group had the highest (*P<0.05*) moisture when compared with other treatments. However, dietary treatment had no effect on the pH, ash, protein, and lipid content of meat.

The PFA used in the present study appeared to have a measurable impact on the degree of lightness (*L**) and redness (a*) of breast meat yield of broilers. Muscle tissues from PFA-supplemented groups showed lower score for redness (*a**) when compared with antibiotic group. The increasing *L** values of breast muscle tissues obtained in the present study with increasing inclusion levels of PFA agreed with previous reports that meat with lower redness (a*) score are normally higher in the degree of lightness (Fletcher *et al*. 1999). High L* and, low a* of meat following PFA supplementation could be attributed to the phytochemical constituents in the PFA. Lighter (higher *L** values) breast fillets of male broiler chickens were also reported by Jiang *et al*. (2007) following dietary supplementation of 40 mg/kg isoflavone. According to Petracci *et al*. (2015), poultry meat has been categorized into lighter (pale, $L^* > 56$), normal ($50 \leq L^* \leq 56$) and darker (dark, $L^* < 50$) meats. Based on these categories, lighter meats were produced from all treatments imposed in the current study.

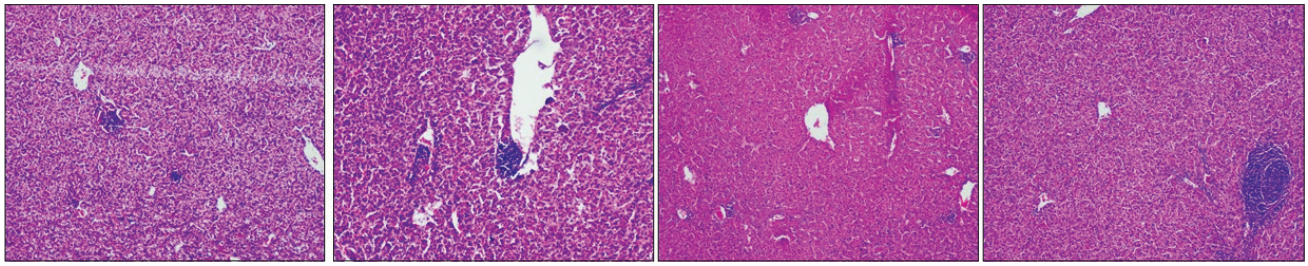
The drip loss of meat and concentration of thiobarbituric acid reactive substances measured as markers of lipid oxidation in meat after 1, 4, and 6 days (24, 96 and 144 hours respectively) of storage are given in Table 5. At day 1 and 4 post slaughter, breast meat from broilers fed diet supplemented with 1% PFA recorded the least (*P<0.01*) drip loss percentage. Orthogonal effect of PFA supplemental levels revealed that in all the postmortem period investigated, drip loss reduced quadratically as the supplemental levels of PFA increased from 0 to 1%, but increased with the 2% PFA. The concentration of lipid peroxidation products in all the groups were comparable. Dietary PFA supplementation did not show any effect on concentration of TBARS in meat samples studied after different days of post-mortem storage.

Drip loss is considered to be one of the most vital functional properties of raw meat and is an important index

Table 5. Effect of dietary supplementation with PFA on drip loss (%) and lipid oxidation status of breast meat

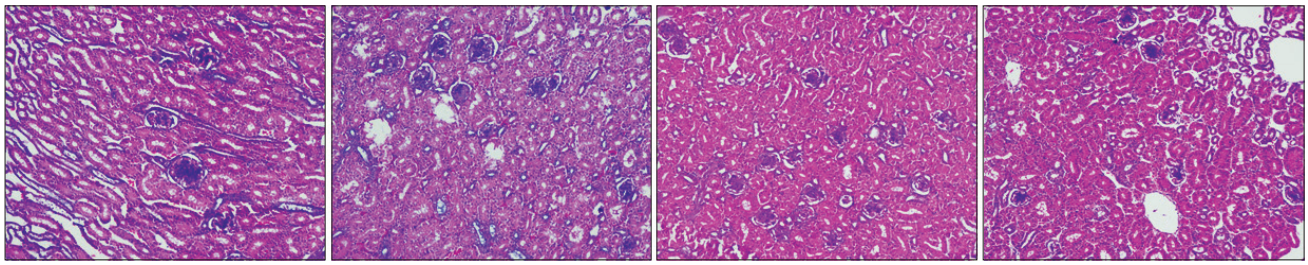
Item	Control (Antibiotic)	Dosage of PFA (%)			SEM	Antibiotic Vs PFA	PFA levels	
		0	1	2			Linear	Quadratic
Drip loss(%)								
24 h post slaughter	2.67[a]	2.44[a]	1.17[b]	2.32[a]	0.17	0.002	0.77	0.003
96 h post slaughter	5.24[a]	5.43[a]	2.95[b]	5.17[a]	0.32	0.007	0.75	0.005
144 h post slaughter	6.53[ab]	6.55[ab]	4.49[b]	7.83[a]	0.43	0.039	0.3	0.02
TBARS (MDA/kg)								
24 h post slaughter	0.38	0.54	0.23	0.11	0.09	0.33	0.06	0.61
96 h post slaughter	0.56	0.33	0.37	0.35	0.07	0.73	0.89	0.88
144 h post slaughter	0.43	0.64	0.32	0.36	0.09	0.63	0.37	0.48

[a–b]Means in a row bearing different superscripts are significantly different ($P<0.05$).



A: Antibiotic B: Negative control C: 1% PFA C: 2% PFA

Fig. 1. Histological study of liver tissues of broilers. Antibitoic (A), Negative control (B): Moderate periacinar lymphocyte infiltration or aggregation; 1% PFA (C): Normal histology; 2% PFA (D): Highest vacuolar degeneration and periacinar lymphocyte infiltration, Hematoxylin and eosin (× 10).



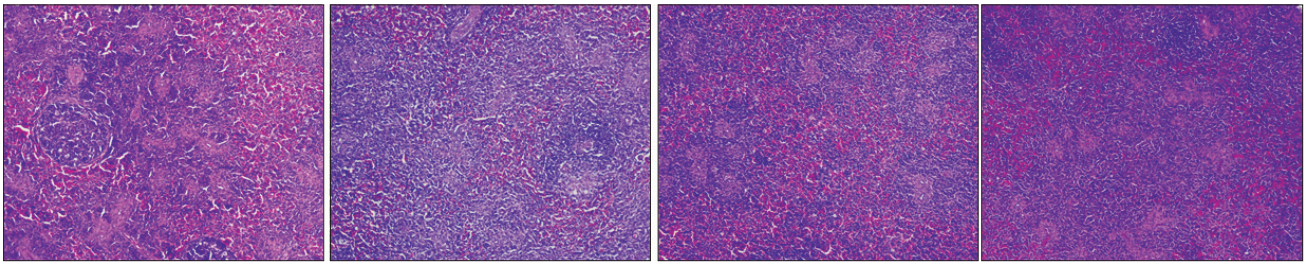
A: Antibiotic B: Negative control C: 1% PFA C: 2% PFA

Fig. 2. Histology of kidney tissues of broilers. Antibiotic (A); 1% PFA (C); 2% PFA (D): Normal histology of kidney; Negative control group (B): Mild atrophic changes with a greater number of nephrons per field (×10).

to evaluate meat quality as well. However, it has been a problem for many years in the chicken meat industry (Otto *et al*. 2006). The process of fluid loss from fresh meat through passive exudation is referred to as muscle drip loss. In meat, the ability of proteins (mainly actin and myosin) to form hydrogen, electrostatic and capillary bonds with water molecules is impaired due to the oxidation of proteins (mainly actin and myosin), which leads to drip loss (Xiong 2022). Loss of moisture leads to loss of physical form and meat quality (Xiu-Dong *et al*. 2018). Dietary supplementation of phytogneics such as chlorogenic acid-enriched extract from *Eucommia ulmoides* leaf (Zhao *et al*. 2019) and flavonoids from *Scutellaria baicalensis Georgi* (Xiu-Dong *et al*. 2018) were reported to reduce drip loss in broilers. In the current investigation, amongst all treatments, the birds fed diets supplemented with 1% PFA exhibited the lowest drip loss after 1 and 4 days of storage period. This result indicate the addition of PFA in broiler diets is able to reduce drip loss upto 4 days of storage after sacrifice. The positive impact on drip loss could be attributed to the interactions of PFA constituents, mainly the phenolic acids and flavonoids, with cellular proteins (Murakami *et al*. 2018), making them less susceptible to oxidation. Further, the reduction in drip loss up to 4 days storage period indicates the benefit to the consumers and industry in terms of higher meat weight and quality.

Lipid oxidation is a spontaneous and unavoidable process that occurs during post-mortem ageing of meat. In spite of various advantages in chicken meat over red meat, a major challenge faced in chicken meat is its high susceptibility to lipid oxidation due to its high concentration of polyunsaturated fatty acids (PUFA) (Mech *et al*. 2019). Though peroxidation is a normal process, it affects nutritional quality of meat and generates compounds that are toxic to humans. Currently, thiobarbituric acid reactive substance (TBARS) is the most commonly used biomarker for quantifying lipid oxidation in meat and meat products. Dietary supplementation of antioxidants is being widely explored as a possible strategy to prevent lipid peroxidation in meat (Nongkhlaw *et al*. 2019, Suganthi *et al*. 2019). However, the results of previous studies were inconclusive. For instance, in a previous report, supplementation of oregano (*Origanum vulgare*) essential oil containing thymol was reported to improve the oxidative stability of meat in pigs (Forte *et al*. 2017). Conversely, the supplementation of dietary marigold xanthophylls, thyme, or synthetic antioxidants showed no positive effect on the TBARS levels in stored broiler meat (Koreleski *et al*. 2007). In our study, there were no significant difference in TBARS concentration amongst different treatments. However, a reducing trend in TBARS value in meat sampled from birds fed 1% PFA was observed after 24 h of storage and this trend could be due to the antioxidant action of phenolic acids, flavonoids, thymol and eugenol.

The histopathological examination of the liver samples of broilers fed different dietary treatemtns are shown in Fig. 1. The liver samples of broilers fed diet containing antibiotic revealed the highest vacuolar degeneration and pericinar lymphocyte infiltration followed by broilers fed only basal diet and 2% PFA. On the other hand, the least liver degeneration and periacinar lymphocyte infiltration was recorded in the liver samples of broilers fed diet supplemented with 1% PFA. The histopathological examination of kidney samples from broilers on negative control revealed mild atrophic changes with a greater number of nephrons per field with shirked glomerulus



A: Antibiotic B: Negative control C: 1% PFA C: 2% PFA

Fig. 3. Histology of spleen tissues of broilers. Antibiotic (A): lymphoid depletion; Negative control (B); 1% PFA (C); 2% PFA (D): Normal histology, (× 10).

while minimal lesions with no significant changes were seen in other treatments (Fig. 2). The histology of spleen tissues of different treatments are shown in Fig. 3. The results revealed lymphoid depletion in spleen samples of broilers fed diet containing antibiotic, while no visible pathological changes were seen in spleen of other treatments. The histopathology studies help to identify the pathological changes induced by the dietary supplements, if any. In the current investigation, liver samples of broilers fed diet supplemented with 1% PFA exhibited the least periacinar lymphocyte infiltration and this indicates normal functioning of hepatocytes and absence of hepatic damage (Saravanan *et al*. 2002). On the other hand, the highest vacuolar degeneration observed in liver of birds fed AGP and only basal diet indicates hepatocellular damage (Ali *et al*. 2021). Further, the noticeable lymphoid depletion in the spleen of broilers fed with chlortetracycline also corroborated with the hepatocellular damage observed in the liver of broilers fed AGP. The pathological changes observed in kidney of birds fed negative control diet implies mild kidney damage that were apparently absent in other treatments. The histopathology results of the present study clearly indicated the absence of toxic effects of PFA on liver, kidney and spleen at 1% inclusion level.

From this study, it is concluded that the supplementation of a phytogenic feed additive prepared from *P. betle*, *P. nigrum*, *C. dactylon* and *A. lanata* to the broiler diet @ of 1% improved serum protein, reduced serum cholesterol and drip loss of meat at 1 and 4 days of storage without inducing adverse effects on hematology and histology of major organs. The above findings of the study indicated the synergistic action of phytochemicals present in the phytogenic feed additive in imparting an advantageous effect on drip loss. Supplementation of the phytogenic feed additive @ 2% did not exhibit additional improvement in any of the parameters studied compared to 1% PFA. However, the phytogenic feed additive, both at 1 and 2% concentrations exhibited limited capacity to improve oxidative stability of meat. Further studies to elucidate the mechanism of action of the phytogenic feed additive as a feed additive and its effects on physiological and nutritional response of chickens under different climatic conditions need to be conducted.

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