



## Effect of *Eugenia jambolana* and *Psidium guajava* Leaf Meal Mixture Supplementation on Performance, Biochemical Profile and Histopathological Changes of Broiler Chicks

M.A. Zargar, A.K. Pathak\*, S. Rahman, R.K. Sharma and M.I. Daing

Division of Animal Nutrition, Faculty of Veterinary Sciences & AH, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, R. S. Pura, (J & K), INDIA

\*Corresponding author: AK Pathak; E-mail: dranand\_pathak@yahoo.com

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### ABSTRACT

Present study was undertaken in 120 day-old broiler chicks, randomly distributed into 4 dietary treatments (T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> supplemented with leaf meal mixture (LMM) of *Eugenia jambolana* and *Psidium guajava* @ 0, 2.5, 5.0 and 7.5 % of diet, respectively) each having 3 replicates (10 chicks/ replicate) in a complete randomized block design (CRD). Experimental chicks were fed basal diets (starter and finisher) supplemented with graded levels of LMM. Feed intake, body weight gain and feed conversion ratio (FCR) were recorded at weekly intervals. Two birds from each replicate were slaughtered at the end of feeding trial (42 days). Blood samples were collected and serum separated for biochemical profiles. For histopathological examination, representative tissue samples were collected in 10% neutral buffer formalin and then processed for paraffin embedding employing alcohol as dehydrating agent and xylene as clearing agent. Sections were cut at 4-5µm thickness and stained by routine haematoxylin and eosin method. On histopathological examination of liver, kidney, heart and intestine of T<sub>1</sub> and T<sub>2</sub> groups showed normal integrity, mild to moderate histopathological changes in T<sub>3</sub> group, while, T<sub>4</sub> showed drastic histopathological changes. It was concluded that *E. jambolana* and *P. guajava* LMM supplementation (2.5% or even 5%) maintained birds performance, minor histopathological changes and producing healthy low cholesterol broiler meat. The LMM incorporation (2.5%) in the diet of broiler chicks may be recommended as socioeconomic, alternative functional feed resource.

**Keywords:** Biochemical profile; Broilers; Histopathology; Leaf meal mixture

Poultry provides an immense supply of animal protein for the world's human population. It is one of the most profitable businesses in agriculture as it provides nutritious meat and eggs within a short period of time (19<sup>th</sup> Livestock Census, 2012). Poultry play a significant role in conversion of plant materials into high quality protein rich food. Broiler industry has become rapidly developing enterprise among other sectors of poultry production. The rapidly changing patterns of demand for broiler meat and meat products, point to broiler production being an increasing component of the agricultural economies of Asia. The extent to which the rural poor will benefit from these changes depends on how poultry farming can be integrated into developing markets and whether cheaper meat and meat products

benefit the rural poor as consumers as well as producers (Jung *et al.*, 2011).

Tree leaves, leaf meal (LM) and/or leaf meal mixture (LMM) produce wide ranges of active compounds (like Condensed tannins; CT) that provide many beneficial effects (Pathak, 2013; 2017) like natural feed additives, immune stimulator (Pathak *et al.*, 2016; Singh *et al.*, 2015), growth promoter, alternative feed supplements (Pathak *et al.*, 2013a) and various pharmacological effects viz. antioxidants (Daing *et al.*, 2017a,b; Pathak *et al.*,

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2017; Zargar *et al.*, 2016, 2017) anthelmintic (Pathak *et al.*, 2013a,b,c; Singh *et al.*, 2015), antibacterial (Daing *et al.*, 2017a,b) and anticoccidial (Zargar *et al.*, 2016, 2017). Generally, the active compounds do not play an important role in the metabolism of trees, so it is often referred to as plant secondary metabolites (PSM). The PSM have long been known as a source of effective medical therapies and works as important natural feed additives in poultry production.

Moreover, LMM is one of the possible sources of cheap feedstuff that have been incorporated in the diets of poultry as a means of reducing the high cost of conventional feed sources. It was reported that LM or LMM not only serve as protein source but also provide some necessary vitamins, minerals and active compounds like growth promoters and produce low cholesterol healthy meat (Kakengi *et al.*, 2007). Therefore, this raises the possibility that the incorporation of potential source and suitable dose level of CT containing LMM may be an alternative sustainable approach to reduce the cost of production and improve the performance of broiler chicken. Keeping this in view, it is proposed to explore the effect of *E. jambolana* and *P. guajava* LMM on performance, biochemical profiles and organ histopathology of broiler chickens.

## MATERIALS AND METHODS

### Experimental Site

The experimental study was undertaken in the Division of Animal Nutrition, Faculty of Veterinary Sciences & AH, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, R. S. Pura-181102. Fresh leaves of *E. jambolana* and *P. guajava* were harvested from Faculty premises. Both tree leaves were air-dried in the shed for 12-15 days. The dried leaves were micronized with a hammer mill into fine powder, weighed and kept in sterile containers for use later. The fine powder of both tree leaves were thoroughly mixed in proper ratio (50:50) on the cemented floor and suitable LMM was prepared for *in-vivo* trial.

### Experimental Feeds and Nutritional analysis

The basal (starter and finisher) diets were formulated according to BIS (1992) using locally available feed

ingredients to fulfil the nutrient requirements of broiler chicks. The LMM was incorporated into the diets at the rate of 0.0% (T<sub>1</sub>), 2.5 % (T<sub>2</sub>), 5.0 % (T<sub>3</sub>) and 7.5 % (T<sub>4</sub>). The T<sub>1</sub> is control while others (T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub>) are LMM supplemented groups. The ingredient composition of experimental broiler diets is shown in the table 1. The LMM and experimental diets were subjected chemical analysis in triplicates according to the method of AOAC (1995). Nitrogen free extract (NFE) was determined by difference between 100 and the sum of crude protein (CP), ether extract (EE), crude fibre (CF) and total ash (TA) values on dry matter (DM) basis. Calcium (Ca) was determined as per Talpatra *et al.* (1940) and phosphorus (P) content by spectrophotometric method (AOAC, 2000). Condensed tannins (CT) content of LMM was determined by butanol-Hcl method (Makkar, 2000). All chicks were provided fresh clean drinking water and feed *ad-libitum* throughout the experimental period in the morning and evening.

### Experimental birds, design, feeding and management

A total of 120 unsexed day old healthy commercial broiler chicks (Cobb-K strains) were procured from Jammu. Chicks were reared in electric brooder until 7 days of age. Chicks had free access to feed and water throughout and were maintained on a constant 24 hours light schedule. On eighth day, chicks were individually weighed, distributed into 4 treatment groups of 3 replicates with 10 chicks in deep litter pen in a completely randomized block design (CRD). Chicks in control group were fed basal diets without LMM (T<sub>1</sub>), while other 3 treatment groups were fed the basal diet supplemented with *E. jambolana* and *P. guajava* LMM at the rate of 2.5, 5.0 and 7.0 percent in T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> groups, respectively.

Experimental house and brooding pens were thoroughly cleaned, disinfected, fumigated and remain closed for a period of 15 days before the arrival of chicks. All the feeders, drinkers and other utensils were thoroughly washed and disinfected. The brooding and deep litter materials were properly dried. Rice husks used as litter materials were spread on the floor of the pen and a warm temperature of 33-35 °C was maintained in the brooder with electric bulbs before the arrival of chicks and thereafter 7 days of age. On arrival, the chicks were carefully unboxed, weighed and brooded for a period of

7 days before they were randomly allocated to 4 dietary treatments. The temperature of poultry house gradually declined to 27°C by 21 days of age, after which, chicks were maintained at room temperature (25–27 °C). All the chicks were provided same care and good management practices.

### Sample collection and biochemical constituents

Data on the feed intake, body weight gains and FCR were determined on weekly basis. The FCR was computed as feed intake divided by body weight gain. Blood samples were collected on 42 days at the time of slaughter. Two birds were randomly selected from each replicate for bleeding and slaughter. About 4 ml blood from each bird was collected in sterile centrifuge tube for serum collection and it was stored in sample vials at -20°C for estimation of biochemical constituents i.e. glucose (Teitz, 1976), total serum protein (Tietz, 1986), albumin (Doumas *et al.*, 1972), creatinine (Newman and Price, 1999), cholesterol (Roeschlau *et al.*, 1974), triglyceride (Cole *et al.*, 1997) and serum enzymes viz. serum glutamic oxaloacetic transaminase (SGOT; Bergmeyer *et al.*, 1986a), serum glutamic pyruvic transaminase (SGPT; Bergmeyer *et al.*, 1986b), alkaline phosphatase (ALP; Bessey *et al.*, 1946) and lactate dehydrogenase (LDH; Henry *et al.*, 1960). The biochemical profiles were determined by using readymade diagnostic (Erba Diagnostics Mannheim GmbH) kits.

### Histopathological studies

For histopathological examination, representative tissue samples of various visceral and immune organs were collected in 10% neutral buffer formalin and then processed for paraffin embedding employing alcohol as dehydrating agent and xylene as clearing agent. The sections were cut at 4-5µm thickness and stained by routine Harris haematoxylin and eosin method (Suvarna *et al.*, 2012).

### Statistical analysis

Results obtained were subjected to analysis of variance and treatment means were ranked using Duncan's multiple range test. Periodic alterations in feed intake, body weight gain and FCR were analyzed using General linear model multivariate procedure of SPSS (16.0)

software considering replicates as experimental units and the values were expressed as means ± standard error. Degree of freedom of the treatments was portioned into orthogonal polynomial, depicting linear and quadratic trends associated with increasing levels of CT containing LMM supplementation. Significance was declared at P<0.05 unless otherwise stated. Statistical procedures were done as per Snedecor and Cochran (2004).

## RESULTS AND DISCUSSION

### Nutrient and chemical composition of experimental diets

The ingredients composition of experimental diets is presented in the table 1. Various feed ingredients viz. maize, rice polish, soybean meal, meat cum bone meal (MBM), soybean oil, di-calcium phosphate, lime stone powder, salt, mineral mixture and vitamin premix, L-lysine and DL-methionine were procured from local market for formulation of broiler starter and finisher diets as per BIS (1992). In the basal (starter and finisher) diets LMM of *E. jambolana* and *P. guajava* (50:50 ratio) was incorporated @ 0, 2.5, 5.0 and 7.5 % in T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> diets, respectively by replacing maize so as to maintain iso-nitrogenous diets throughout the experimental period. The proximate composition of broiler starter and finisher diets of all experimental chicks were within the range as suggested by BIS (1992).

The OM, CP, EE, CF, NFE, TA, AIA, Ca and P content on (% DM) bases of broiler starter diets were 92.55, 22.93, 4.45, 4.42, 60.75, 7.45, 2.43, 1.67 & 0.76; 92.43, 22.83, 4.40, 4.53, 60.67, 7.57, 2.61, 1.64 & 0.77; 92.11, 22.97, 4.23, 4.69, 60.22, 7.89, 2.78, 1.77 & 0.73 and 91.99, 23.07, 4.64, 4.82, 59.46, 8.01, 2.92, 1.83 & 0.70 percent, whereas, the OM, CP, EE, CF, NFE, total ash, AIA, Ca and P content on (% DM) bases of broiler finisher diets were 92.14, 20.01, 5.04, 4.63, 62.46, 7.86, 2.50, 1.73 & 0.69; 92.08, 20.13, 5.12, 4.38, 62.45, 7.92, 2.43, 1.56 & 0.67; 91.97, 19.81, 5.22, 4.72, 62.20, 8.03, 2.76, 1.83 & 0.63, and 91.88, 19.80, 5.07, 4.83, 62.18, 8.12, 2.91, 1.77 & 0.61 percent, respectively in T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> groups. The ME (Kcal/kg diet) contents were 2818, 2790, 2762, 2734 and 2925, 2897, 2869, 2841, for broiler starter and finisher diets, respectively in T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> groups. The incorporation of LMM of *E. jambolana* and *P. guajava*

**Table 1:** Ingredients composition of experimental broiler diets

Ingredients	Experimental diets							
	Broiler starter				Broiler finisher			
	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>
Crushed maize	51.00	48.50	46.00	43.50	57.00	54.50	52.00	49.50
Rice polish	3.75	3.75	3.75	3.75	6.50	6.50	6.50	6.50
Soybean meal	35.00	35.00	35.00	35.00	27.50	27.50	27.50	27.50
Meat cum bone meal	5.00	5.00	5.00	5.00	4.00	4.00	4.00	4.00
Soybean oil	1.75	1.75	1.75	1.75	2.00	2.00	2.00	2.00
Di-calcium phosphate	1.16	1.16	1.16	1.16	1.00	1.00	1.00	1.00
Lime stone powder	1.00	1.00	1.00	1.00	0.70	0.70	0.70	0.70
Iodised salt	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Premix (Min. and Vit.)	0.47	0.47	0.47	0.47	0.45	0.45	0.45	0.45
Lysine-Hcl	0.31	0.31	0.31	0.31	0.30	0.30	0.30	0.30
DL-Methionine	0.16	0.16	0.16	0.16	0.15	0.15	0.15	0.15
Leaf meal mixture	0.00	2.50	5.00	7.50	0.00	2.50	5.00	7.50

@ 0, 2.5, 5.0 and 7.5% by replacing maize slightly altered the nutrient composition of experimental diets but its incorporation not caused so much difference in the nutritive values of experimental (T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub>) diets.

### Performance parameters

The data of weekly feed intake, body weight gain and feed conversion ratio of all experimental chicks are presented in the table 2. The average feed intake (g) decreased significantly (P<0.05) as the level of LMM increased. Significantly (P<0.05) higher feed intake was recorded in T<sub>1</sub> compared to T<sub>3</sub> and T<sub>4</sub>, whereas, T<sub>2</sub> had an intermediate value between T<sub>1</sub> and T<sub>3</sub>.

The lower feed intake in T<sub>3</sub> and T<sub>4</sub> of LMM supplemented groups might be due to high CT content because CT having astringent property and at higher level it bind with glycol-proteins with some of the salivary protein and other nutrients, such complex causes a sensation in the oral cavity, which greatly reduced palatability and hence tends to depress the feed intake in broiler birds. Similar results have been reported elsewhere (Dube, 1993). Present results are contradictory to the findings of Rahman *et al.* (2013). They had observed that *P. guajava* leaf meal up to 4.5% dietary level had no detrimental effect on feed consumption.

Significantly (P<0.05) higher mean body weight gain (g) were observed in T<sub>1</sub> as compared to T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> groups, while body weight gains between T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> groups

were showed non-significant difference. As the time period of experimental study increased, mean body weight gain of broiler chicks increased significantly (P<0.05). The maximum mean body weight gain of broiler birds was recorded at 5<sup>th</sup> week followed by 4<sup>th</sup>, 6<sup>th</sup>, 3<sup>rd</sup>, 2<sup>nd</sup> and least body weight gain was obtained at 1<sup>st</sup> week. The mean FCR among all four groups ranged from 2.08 to 2.28.

Significantly (P<0.05) better FCR was obtained in un-supplemented (T<sub>1</sub>) group than that of LMM supplemented (T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub>) groups. The FCR when compared among weekly intervals significantly (P<0.05) better FCR was obtained in 2<sup>nd</sup> week (1.40) followed by 1<sup>st</sup> week (1.91), 3<sup>rd</sup> week (2.06), 4<sup>th</sup> week (2.50) and the worst FCR in 6<sup>th</sup> week (2.85). Similarly, El-Deek *et al.* (2009) study found that feeding of guava by-products, raw or treated with the higher levels (6-8 %) showed slightly reduction of broiler body weight gain. Present findings are confirmatory with the results of Shafey *et al.* (2013) who reported that the replacement of higher levels of Olive leaves reduced body weight gain and feed conversion efficiency in broiler chicks. But contradictory with the results of Simol *et al.* (2012), whose study showed that mulberry leaf powder can substitute up to 30% of commercial feed without any adverse effect on the feed intake, growth performance and FCR of broiler chicken.

### Biochemical profile

The biochemical profile of broiler birds supplemented graded level of LMM is presented in table 3. Blood

**Table 2:** Effect of *E. jambolana* and *P. guajava* LMM supplementation on feed intake, body weight gain and FCR of broiler chicks

Periods (in Weeks)	Groups				Mean± SE
	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	
<b>Feed intake (g)</b>					
1st	148.33	141.67	140.00	150.00	145.00 <sup>a</sup> ± 0.39
2 <sup>nd</sup>	285.00	278.33	283.33	281.67	282.08 <sup>b</sup> ± 0.23
3 <sup>rd</sup>	652.00	632.00	611.33	565.00	615.08 <sup>c</sup> ± 2.96
4 <sup>th</sup>	935.00	910.00	903.67	835.33	896.00 <sup>d</sup> ± 3.39
5 <sup>th</sup>	1004.67	980.67	965.00	935.33	971.42 <sup>e</sup> ± 2.31
6 <sup>th</sup>	995.67	975.00	962.67	913.67	961.75 <sup>e</sup> ± 2.76
<b>Mean ± SE</b>	670.11 <sup>c</sup> ± 25.69	652.95 <sup>bc</sup> ± 25.15	644.33 <sup>b</sup> ± 24.74	613.50 <sup>a</sup> ± 23.04	
<b>Body weight gain (g)</b>					
1st	87.33	77.57	82.00	77.39	81.07 <sup>a</sup> ± 1.60
2 <sup>nd</sup>	213.28	201.50	210.92	309.31	233.75 <sup>b</sup> ± 22.79
3 <sup>rd</sup>	326.33	314.33	299.00	202.00	285.42 <sup>c</sup> ± 24.40
4 <sup>th</sup>	438.27	368.83	353.83	370.67	382.90 <sup>e</sup> ± 8.24
5 <sup>th</sup>	459.37	402.60	377.73	404.97	411.17 <sup>f</sup> ± 10.90
6 <sup>th</sup>	349.23	360.20	343.40	329.90	345.68 <sup>d</sup> ± 4.99
<b>Mean ± SE</b>	312.30 <sup>b</sup> ± 10.43	287.51 <sup>a</sup> ± 9.67	277.81 <sup>a</sup> ± 8.71	282.37 <sup>a</sup> ± 24.08	
<b>Feed conversion ratio (FCR)</b>					
1st	1.73	1.91	1.81	2.17	1.91 <sup>b</sup> ± 0.06
2 <sup>nd</sup>	1.37	1.47	1.42	1.33	1.40 <sup>a</sup> ± 0.03
3 <sup>rd</sup>	2.07	2.08	2.07	2.02	2.06 <sup>c</sup> ± 0.05
4 <sup>th</sup>	2.18	2.55	2.60	2.68	2.50 <sup>d</sup> ± 0.09
5 <sup>th</sup>	2.25	2.55	2.69	2.59	2.52 <sup>de</sup> ± 0.05
6 <sup>th</sup>	2.88	2.74	2.90	2.88	2.85 <sup>e</sup> ± 0.04
<b>Mean ± SE</b>	2.08 <sup>a</sup> ± 0.04	2.22 <sup>b</sup> ± 0.04	2.25 <sup>b</sup> ± 0.05	2.28 <sup>b</sup> ± 0.09	

<sup>abcdef</sup>Means with different superscript within a row and column differ significantly (P<0.05).

**Table 3:** Effect on biochemical profile of broiler finisher chicks fed *E. jambolana* and *P. guajava* LMM supplemented diets

Parameters	Group				SEM	P Value
	T1	T2	T3	T4		
Glucose (mg/dl)	190.65	207.38	192.03	204.20	3.06	0.119
Total Protein (g/dl)	5.06 <sup>b</sup>	5.09 <sup>b</sup>	4.40 <sup>a</sup>	4.14 <sup>a</sup>	0.11	0.000
Albumin (g/dl)	3.10 <sup>b</sup>	2.13 <sup>a</sup>	2.12 <sup>a</sup>	2.05 <sup>a</sup>	0.10	0.000
Globulin (g/dl)	1.96 <sup>a</sup>	2.96 <sup>b</sup>	2.28 <sup>a</sup>	2.10 <sup>a</sup>	0.10	0.000
A:G Ratio	1.60 <sup>b</sup>	0.73 <sup>a</sup>	0.96 <sup>a</sup>	0.98 <sup>a</sup>	0.08	0.000
Cholesterol (mg/dl)	147.68 <sup>c</sup>	132.47 <sup>b</sup>	116.45 <sup>a</sup>	111.38 <sup>a</sup>	3.22	0.000
Triglyceride (mg/dl)	53.81 <sup>c</sup>	46.04 <sup>b</sup>	42.78 <sup>b</sup>	31.24 <sup>a</sup>	1.87	0.000
Creatinine (mg/dl)	1.30 <sup>a</sup>	1.42 <sup>a</sup>	1.46 <sup>a</sup>	1.82 <sup>b</sup>	0.06	0.005
SGPT (IU/L)	12.38 <sup>a</sup>	11.94 <sup>a</sup>	13.26 <sup>a</sup>	17.98 <sup>b</sup>	0.65	0.000
SGOT (IU/L)	109.91 <sup>a</sup>	108.44 <sup>a</sup>	108.73 <sup>a</sup>	119.34 <sup>b</sup>	1.76	0.076
ALP (IU/L)	301.60 <sup>b</sup>	273.34 <sup>ab</sup>	287.97 <sup>ab</sup>	258.39 <sup>a</sup>	6.36	0.081
LDH (IU/L)	62.06 <sup>a</sup>	63.41 <sup>a</sup>	64.76 <sup>a</sup>	78.25 <sup>b</sup>	2.41	0.051

<sup>abc</sup>Means with different superscript within a row differ significantly (P<0.05).

glucose level is an indicator of the normal physiology and well being of poultry. An increased or decreased level of blood glucose level is an indicator of stress to the birds. There were no significant differences ( $P>0.05$ ) in blood glucose level among all groups with and without LMM supplementation and the glucose values were within the normal range (Kenako, 1997). However, in present study, analogous glucose level indicates normal physiological condition of all experimental birds. Total protein value was found to be lowest ( $P<0.000$ ) in  $T_3$  &  $T_4$  than that of  $T_1$  &  $T_2$  but the values were statistically similar between  $T_1$  &  $T_2$  and  $T_3$  &  $T_4$ . It clearly indicated that dietary incorporation LMM 2.5 % onward in broiler chicks had adversely affected the serum protein levels. However, total serum proteins were within the normal physiological range established for broiler chicks (Kenako, 1997).

Significantly ( $P<0.000$ ) higher albumin level was recorded in  $T_1$  as compared to  $T_2$ ,  $T_3$  and  $T_4$ . Significantly higher serum globulin level was obtained in LMM supplemented  $T_2$  groups compared to rest of the supplemented and un-supplemented groups. The globulin levels numerically increased in  $T_3$  and  $T_4$  groups than that of  $T_1$  group. Thus the elevated level of serum globulin in LMM supplemented groups clearly indicated that LMM supplementation was having immune stimulating property. The broiler chicks of  $T_1$  group showed significantly ( $P<0.000$ ) higher A:G ratio compared to rest of the supplemented groups but it was statistically similar among supplemented groups ( $T_2$ ,  $T_3$  and  $T_4$ ).

Serum cholesterol and triglyceride values were significantly lower in LMM supplemented groups than that of un-supplemented group. The low cholesterol and triglyceride content observed in the birds fed with CT containing LMM would have been as a result of the hypo-cholesterolemic and hypo-lipidemic properties of CT. Upadhyay (1990) also reported a decline in blood cholesterol levels of broilers and rats fed Neem leaf meal. Present results are in accordance with previous findings (Mahmoud *et al.*, 2013) which affirmed that dietary supplementation of dried guava leaves reduced blood cholesterol and triglyceride levels in broilers.

Creatinine content is an indicator of kidney health status. Serum creatinine levels were significantly ( $P<0.005$ ) higher in  $T_4$  as compared to  $T_1$ ,  $T_2$  and  $T_3$  groups while statistically similar in  $T_1$ ,  $T_2$  and  $T_3$ . The higher creatinine level in  $T_4$  might be due to higher CT level (7.5 %) which

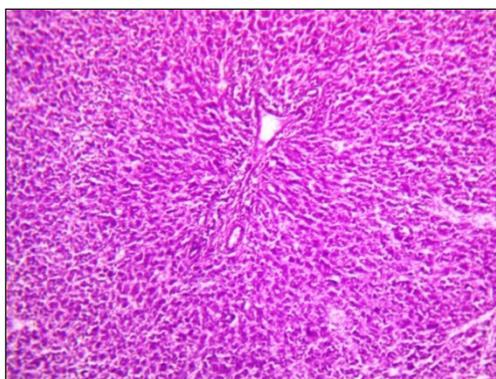
may cause kidney damage.

There was significant difference ( $P<0.001$ ) between the SGPT (IU/L) levels for  $T_4$  and those of  $T_1$ ,  $T_2$  and  $T_3$ . There were no significant differences ( $P>0.05$ ) in SGPT levels among the  $T_1$ ,  $T_2$  and  $T_3$ . Comparatively higher ( $P<0.076$ ,  $P<0.051$ ) SGOT and LDH activities were obtained in  $T_4$  than that of  $T_1$ ,  $T_2$  and  $T_3$  but there were no significant difference among SGOT and LDH levels for  $T_1$ ,  $T_2$  and  $T_3$ . The ALP (IU/L) activity in  $T_1$  group was comparatively higher than that of in  $T_4$  group, whereas,  $T_2$  and  $T_3$  had an intermediate position between  $T_1$  and  $T_4$  which clearly indicated the higher weight gain in  $T_1$  group. Low cholesterol in broilers was also reported by Obikaonu *et al.* (2012). They studied the effects of dietary inclusion of neem leaf meal at 0, 2.5, 5.0, 7.5 and 10% levels on serum biochemical indices of broiler starter and found that blood sugar was raised by the leaf meal but cholesterol, ALP, ALT and AST levels decreased with increase in leaf meal.

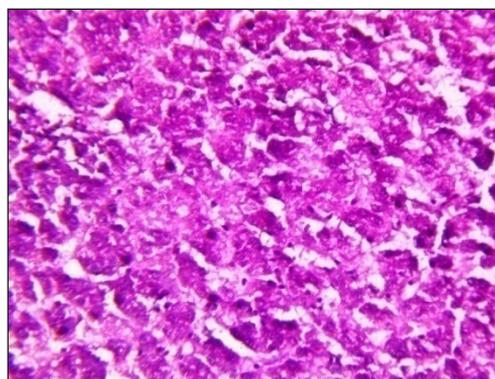
### Histopathological examination

On histopathological examination, liver of  $T_1$  and  $T_2$  groups showed normal hepatocytes arranged in cord pattern with central vein and portal triad (Fig. 1). Liver of  $T_3$  group showed mild to moderate hepatocytic degeneration (Fig. 2), however,  $T_4$  showed mono nuclear cells (MNC's) infiltration, fibrosis and peripheral hepatocytic degeneration (Fig. 3). Kidney of  $T_1$  and  $T_2$  did not show any significant microscopic changes. Nuclei of lining epithelial cells were vesicular and cytoplasm was homogenous, whereas, moderate swelling and degeneration of epithelial cells of tubules occluding the lumen was observed in  $T_3$  group (Fig. 4).

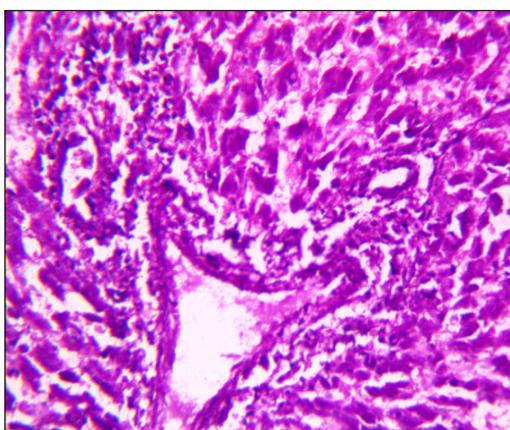
Moreover, kidney of  $T_4$  group showed interstitial haemorrhage, degeneration with vacuolar cytoplasm and necrosis of epithelial cells of tubules (Fig. 5). Heart of  $T_1$  and  $T_2$  groups showed muscle fibres of uniform thickness and minimum of intermyofibril spaces. However, in  $T_3$  group observed thinning of myocardial fibres (Fig. 6), while, moderate necrosis of myocardial fibre was recorded in  $T_4$  group (Fig. 7). Intestine of  $T_1$  and  $T_2$  groups showed normal intestinal villi, although, in  $T_3$  group observed mild degeneration of epithelial cells of intestinal villi (Fig. 8). However, fusion, blunting, desquamation of enterocytes and atrophy of villi with mild necrosis of enterocytes was observed in  $T_4$  group (Fig. 9).



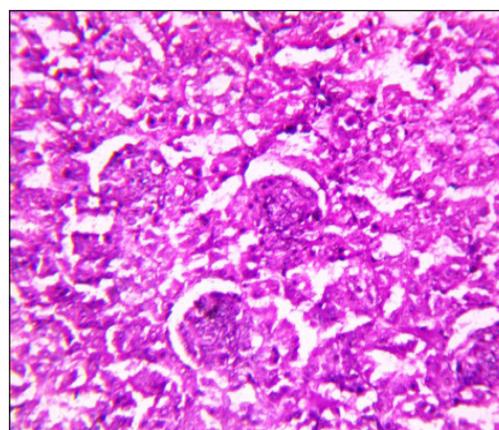
**Fig. 1:** Photomicrograph of liver depicting normal hepatocytes arranged in cord pattern. H&E X 100



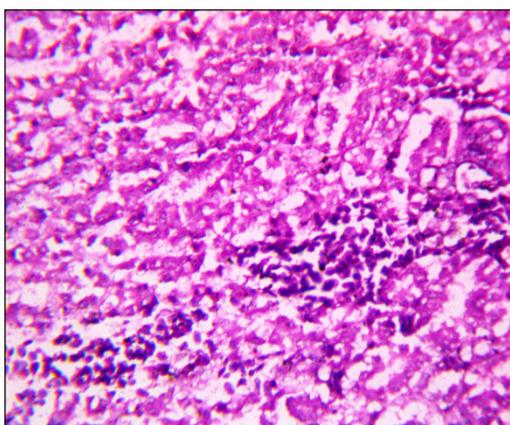
**Fig. 2:** Photomicrograph of liver depicting mild hepatocytic degeneration. H&E X 400



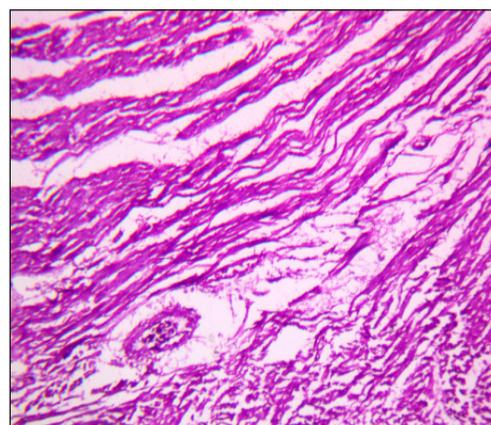
**Fig. 3:** Photomicrograph of liver depicting MNC's infiltration, fibrosis and periportal hepatocytic degeneration H&E X 400



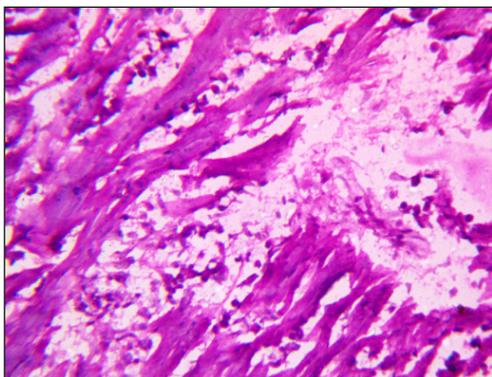
**Fig. 4:** Photomicrograph of kidney depicting swelling and degeneration of epithelial cells of tubules occluding the lumen. H&E X 400



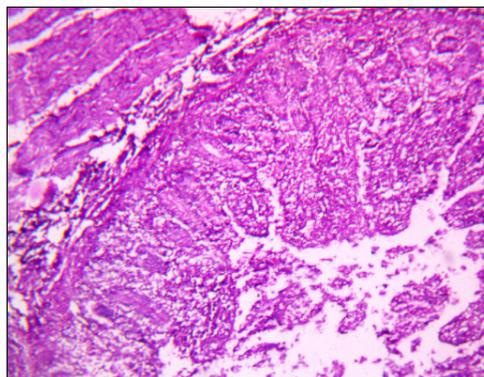
**Fig. 5:** Photomicrograph of kidney depicting interstitial haemorrhages, degeneration and necrosis of epithelial cells of tubules with vacuolar cytoplasm H&E X 400



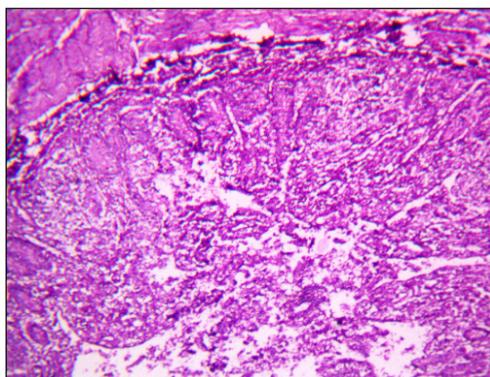
**Fig. 6:** Photomicrograph of heart depicting thinning and necrosis of myocardial fibres H&E X 100



**Fig. 7:** Photomicrograph of heart depicting severe necrosis of myocardial fibres H&E X 400



**Fig. 8:** Photomicrograph of intestine depicting fusion, blunting and desquamation of enterocytes of villi H&E X 100



**Fig. 9:** Photomicrograph of intestine depicting fusion, blunting and atrophy of villi. Note the severe desquamation and necrosis of enterocytes of intestinal villi H&E X 100

The observed changes and lesions in the photomicrographs of the examined vital organs (like liver, kidney heart and intestine) of the experimental chicks of higher level of LMM supplementation might be due to the higher CT level which act as anti-nutritional factors in LMM supplemented diets (5-7.5%). The higher CT level in the diet of chicks cause toxicity and could affect architectural histology of liver, kidney, heart and intestine of experimental chicks. Present findings are confirmatory with the results of previous workers (Adejumo and Ologhobo, 2015; Ewuola, 2009 and Isaac *et al.*, 2017) who also reported lesions on the visceral organs fed toxic substance in the diet of laying hens, rabbits and albino rats, respectively.

## CONCLUSION

It can be concluded that condensed tannins containing leaf meal mixture of *Eugenia jambolana* and *Psidium guajava* (2.5 %) safely recommended as cost effective alternative

feed resource perhaps it can be incorporated up to 5% level in broiler diets without any drastic changes.

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