World Rabbit Sci. 2018, 26: 277-285
doi:10.4995/wrs.2018.10182

PERFORMANCE, HAEMATO-BIOCHEMICAL INDICES AND ANTIOXIDANT STATUS OF GROWING RABBITS FED ON DIETS SUPPLEMENTED WITH *MUCUNA PRURIENS* LEAF MEAL

OLORUNTOLA, O.D.*, AYODELE, S.O.†, ADEYEYE, S.A.‡, AGBEDE, J.O.§

*Department of Animal Science. Adekunle Ajasin University. Akungba-Akoko. Nigeria.
†Department of Agricultural Technology. The Federal Polytechnic. Ado Ekiti. Nigeria.
‡Department of Animal Health and Production. The Federal College of Agriculture. Akure. Nigeria.
§Department of Animal Production and Health. The Federal University of Technology, Akure, Nigeria.

Abstract: The effects of dietary *Mucuna pruriens* leaf meal (MLM) supplementation on rabbits' performance, haemato-biochemical indices and antioxidant status outside their thermal neutrality zone (21 to 25°C) were evaluated. One hundred and twenty 35-d old crossbreed (Chinchilla×New Zealand) rabbits weighing 694±5 g were allotted to 4 treatments (30 rabbits/treatment; 3 rabbits/replicate). A basal diet (crude protein: 16.9%, crude fibre: 17.6%, digestible energy: 2671 kcal/kg) was divided into 4 equal portions i.e. diets 1, 2, 3 and 4, supplemented with 0, 4, 8 and 12 g MLM/kg, respectively, and pelleted. The average body weight in rabbits fed on diets 3 and 4 was higher compared to those fed on diet 1 (control) at 91 d of age (+228 and +262 g, respectively; *P*=0.01). Within 35 to 91 d, the average daily weight gain in rabbits fed on diets 3 and 4 was higher compared to those fed on the control diet (+4.1and +4.8 g/d, respectively; *P*=0.01). The dressing-out percentage of rabbits fed on diets 3 and 4 increased (*P*=0.05) compared to those fed the control diet. At 63 d and 91 d of age, the white blood cell level of rabbits fed on diet 4 increased significantly compared to those fed the control diet (+5.05×10^9 and +5.32×10^9/L, respectively). At 63 and 91 d of age, the cholesterol level of rabbits fed on diets 3 (–1.0 and –1.16 mmol/L, respectively) and 4 (–1.10 and –1.21 mmol/L, respectively), were significantly lower compared to those fed on the control diet. The aspartate aminotransferase (AST) concentration in rabbits fed on diet 4 was reduced compared to those on control diet at 63 d of age (–33.68 IU/L; *P*=0.02). At 63 d and 91 d of age, compared to control, the activities of glutathione peroxidase in rabbits fed on diets 3 (+35.77 and +49.09 mg protein, respectively) and 4 (+54.52 and +55.02 mg protein, respectively) increased significantly, while catalase activities in rabbits fed diet 4 (+217.7 and +209.5 mg/g, respectively) also increased significantly. It could be concluded that dietary MLM supplementation enhanced the rabbits' performance, reduced serum AST and cholesterol and improved the antioxidant status.

Key Words: *Mucuna pruriens*, rabbits, performance, antioxidant status, slaughter traits, health status.

INTRODUCTION

Changes in climate and rising ambient temperatures in various African and sub-Saharan regions have been reported as one of the causes of economic losses to livestock farmers (Tawfeek *et al.,* 2014). Ambient temperature outside the thermal neutrality zone (21 to 25°C) for rabbits represents a heat-stress condition and high heat stress could cause a reduction in growth performance, reproductive rates and meat quality (Marai *et al.,* 2002; Attia *et al.,* 2017; El-Desoky *et al.,* 2017 , Marco-Jiménez *et al*., 2017). Heat stress also promotes increased free radical generation, which leads to the formation of reactive oxygen species (ROS) and induced cellular oxidative stress (Tawfeek *et al.,* 2014). Excessive free radical production in the animal's body can produce a negative effect on biological activities (Halliwell and Gutteridge, 1989). Dietary manipulation has been identified as the most affordable method of alleviating the negative effect of high ambient temperature in the tropics, as the high cost of cooling animal pens is unaffordable

Correspondence: O.D. Oloruntola, olugbenga.oloruntola@aaua.edu.ng. Received *May 2018* - Accepted *August 2018.*
https://doi.org/10.4995/wrs.2018.10182

for farmers (Konca *et al.,* 2009). Therefore, the use of diets rich in natural antioxidants as a means of removing excessive free radicals from the animal's body and consequent alleviation of high-temperature negative effects on animal production is becoming popular (Liu *et al.,* 2010; Kone *et al.,* 2016; Zeweil and Elgindy, 2016; Li *et al.,* 2018).

Mucuna pruriens belongs to the family Fabaceae. The plant is among the under-utilised wild legumes and is widespread in the world's tropical and subtropical regions. Mucuna has bioactive compounds which make it useful for activity against bacteria (Kumar *et al.,* 2009). *Mucuna pruriens* has been reported to have antivenom, antidiabetic, antioxidant and neuroprotective properties (Kumar and Muthu, 2010; Suresh *et al.,* 2013; Yadav *et al.,* 2017). However, *Mucuna pruriens* leaf meal (MLM), like most other leaf meals, contains some phytochemicals (Duke, 1995; Yadav *et al.,* 2017) which may alter blood profiles of animals fed diets containing MLM (Ansari *et al.,* 2012, El-Gindy and Zeweil, 2017).

Studies on the effect of dietary *Mucuna pruriens* leaf meal on performance, blood profile and anti-oxidative status of growing rabbits are rare. Therefore, this work investigated the performance, blood indices and antioxidant status of growing rabbits fed on diets supplemented with 0, 4, 8 or 12 g of MLM/kg.

MATERIALS AND METHODS

Experimental site

The experiment was carried out at the Rabbit Unit, Agricultural Technology Department Teaching and Research Farm (ATDT&RF), The Federal Polytechnic, Ado Ekiti, Nigeria from October to December in 2017. The rabbit house had a mean temperature and mean relative humidity of 29.6°C and 77.5%, respectively.

Feeds, chemical analyses, animals and experimental design

Mucuna pruriens leaves harvested fresh within the premises of ATDT&RF, The Federal Polytechnic, Ado Ekiti, Nigeria were chopped, air-dried under the shed for 7 d, milled with a 2 mm hammer mill to MLM and analysed for tannin (Van-Burden and Robinson, 1981), terpenoid (Ferguson, 1956), cardiac glycoside (Ferguson, 1956), saponin (Shad *et al.,* 2013), steroid (Edeoga *et al.,* 2005), alkaloid (Harborne, 1973), flavonoid (Shad *et al.,* 2013), Ferric reducing antioxidant property (FRAP) (Pulido *et al.,* 2002) and 2, 2-diphenyl-1-picrylhydrazyl hydrate (DPPH) (Gyamfi *et al.,* 1999) (Table 1). A basal diet was formulated to support optimal rabbit growth (de Blas and Mateos, 2010) (Table 2). The basal diet was divided into 4 equal portions and designated diets 1, 2, 3 and 4. Diets 1 to 4 contained 0, 4, 8 and 12 g MLM/kg, respectively. The diets were pelleted to 4 mm diameter and 8 mm in length and then analysed for chemical composition. AOAC (1990) procedures were used to determine crude protein (988.05), crude fibre (962.09) and acid detergent fibre (973.18) in diets. Neutral detergent fibre and acid detergent lignin level were determined as described by Mertens (2002) and Robertson and Van Soest (1981), respectively. Gross energy was determined using a combustion calorimeter (Model: e2k combustion calorimeter, www.cal2k.com). Digestible energy was estimated at 0.65 of the gross energy (Xiccato and Trocino, 2010). The recommendations and guidelines for applied nutrition and experiments in rabbits were followed in management of the rabbits (Fernández-Carmona *et al.,* 2005). One hundred and twenty 35-d old crossbreed (Chinchilla×New Zealand) weaner rabbits of equal sexes and weighing 964±5 g were allotted to 4 dietary treatments on (30 rabbits/treatment; 3 rabbits/replicate). The rabbits were housed in wire meshed cages, accommodated in a well-ventilated pen, offered water and experimental diet *ad libitum* and the pen was cleaned and disinfected daily for 56 d of the experiment.

Table 1: Chemical constituents of *Mucuna pruriens* leaf meal.

Parameters (mg/g)	Quantity
Phytochemicals	
Tannin	3.08±0.01
Terpenoid	12.40±0.04
Cardiac glycoside	9.91±0.01
Saponin	30.72±0.52
Steroid	9.51±0.01
Alkaloid	12.12±0.38
Flavonoid	91.15±0.33
Antioxidant parameters	
FRAP	38.35±0.04
DPPH (%)	21.17±0.52

FRAP: Ferric reducing antioxidant property; DPPH: 2, 2-diphenyl-1-picrylhydrazyl hydrate.

Experimental procedure

The rabbits and feed were weighed at 7-d intervals after the beginning of the experiment and the average daily weight gain (ADWG), average daily feed intake (ADFI) and the feed conversion ratio (FCR) were calculated, respectively, for post-weaning period (35-63 d), finishing period (64-91 d) and whole fattening period (35-91 d). Blood samples were collected from 10 rabbits/experimental group at 63 and 91 d of age from overnight fasted selected rabbits, for determination of haematological indices, serum biochemical indices and serum concentration of lipid peroxidase (LPx), superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT). The method described by Burnett *et al.* (2003) was used for the blood collection. The dorsal surface of the pinna was swabbed with cotton wool impregnated into 70% isopropanol and then rubbed with petroleum jelly. A brooding bulb was placed at a height of approximately 45 cm above the ear to supply heat to enhance the ear vein dilation. Thereafter, about 10 mL of blood were collected from the prominent ear vein of the rabbits using a nineteen gauge 3.8 cm needle. The blood samples meant for serum antioxidant enzymes/serum biochemical and haematological indices determination were dispensed into plain (no anticoagulant) and anticoagulant (ethylenediaminetetra-acetic acid) bottles, respectively. At 91 d, after the blood sample collection, one rabbit was selected from each replicate, weighed, tagged, stunned and euthanised as described by Blasco *et al.* (1993). The skin, legs, head, and intestines were removed and the dressing-out percentage (DP) was then calculated. The weights of major internal organs (liver, heart, lung, kidney, and spleen) were determined separately and expressed as the percentage of slaughter weight.

Table 2: Ingredients and chemical composition (%) of the basal diet.

Ingredients	
Maize	8.00
Wheat offal	8.00
Soybean meal	16.1
Maize husk	22.0
Cassava peels	22.0
Brewers dried grain	21.7
Bone meal	1.10
Premix	0.25
Methionine	0.20
Lysine	0.10
Salt	0.25
Vegetable oil	0.30
Chemical composition	
Crude protein	16.88
Crude fibre	17.59
Neutral detergent fibre	39.17
Acid detergent fibre	17.54
Acid detergent lignin	3.68
Gross energy (kcal/kg)	4108
Digestible energy (kcal/kg)	2671

Blood analysis

The haematological indices (WBC, white blood cells; RBC, red blood cells; Hbc, haemoglobin concentration; and PCV, packed cell volume) were determined on the day of collection by Shenzhen Mindray Auto Haematology Analyzer (Model Bc-3200, Shenzhen Mindray Biomedical Electronics Co. Hamburg 20537, Germany). The serum biochemical (TP, total protein; creatinine; cholesterol; AST, aspartate aminotransferase; and bilirubin) were determined with a Reflectron® Plus 8C79 (Roche Diagnostic, GonbH Mannheim, Germany), using Reflectron kits. Concentrations of serum GPx and SOD activity were determined as described by Rotruck *et al.* (1973) and Misra and Fridovich (1972), respectively. The method described by Aebi (1974) was used to determine CAT activity, while lipid peroxidase was determined with the Ohkawa *et al.* (1979) method.

Statistical analysis

A completely randomised design with the following model: $X_{ij=}\mu+\alpha_i+\epsilon_{ij}$ was adopted in this study. Where $X_{ij=}$ any of the response variables; μ=the overall mean; α_i=effect of the i^{th} treatment (i=diets 1, 2, 3 and 4) and ϵ_{ij}=random error due to experimentation. All data collected in this study were subjected to analysis of variance using SPSS statistical software package 2011, version 20. The differences between treatment groups were determined by Duncan's multiple range, while statistical significance was assessed at $P<0.05$.

RESULTS

Growth performance and carcass traits of rabbits

The effects of dietary MLM supplementation on growth performance of growing rabbits are shown in Table 3. At post-weaning period (days 35 to 63), MLM supplementation did not significantly ($P>0.05$) influence the rabbits' average body weight (ABW), ADWG, ADFI, and FCR. At the finishing period (days 64 to 91), only the ABW at 91 d was affected by the MLM supplementation, such that ABW in rabbits fed on diets 3 and 4 was significantly higher (+228 and +262 g, respectively; $P=0.01$) when compared to ABW recorded for rabbits fed on diet 1 (control). Over the entire feeding trial (days 35 to 91), compared to control, the average body weight gain (ABWG) was higher in the rabbits fed diet 3 and diet 4 (+231 and +267 g, respectively; $P<0.01$). Similarly, the ADWG in rabbits fed on diets 3 and diet 4 was significantly higher when compared to those fed on the control diet (+4.1 and +4.8 g/d, respectively; $P=0.01$). The ADFI were not affected by the MLM supplementation, while FCR values tend ($P=0.09$) to be reduced with MLM supplementation.

Table 4 shows the effect of dietary MLM supplementation on carcass and relative internal organs of growing rabbits at day 91 of age. The slaughter weight (SW) of rabbits fed on diets 3 and 4, significantly increased compared to the rabbits fed on the control diet (+228 and +262 g, respectively; $P=0.04$). Similarly, the DP of rabbits fed on diets 3 and 4 was higher compared to the rabbits fed on the control diet (+4.4 and +5.0 percentage points, respectively; $P=0.05$). MLM supplementation did not significantly ($P>0.05$) influence the relative weights of the liver, heart, lung, kidney, and spleen.

Haematological indices

The effect of including MLM in rabbit diets on haematological indices is presented in Table 5. At 63 d of age, the WBC level of rabbits fed on diet 4 was significantly higher compared with the rabbits fed on the control diet ($+5.05\times10^9$/L; $P=0.05$). At 91 d of age, the WBC level of rabbits fed on diet 4 significantly higher when compared to those rabbits fed on the control diet ($+5.32\times10^9$/L; $P=0.05$). However, the RBC, Hbc, and PCV were not ($P>0.05$) influenced by the MLM supplementation at 63 d and 91 d of age.

Table 3: Effects of *Mucuna pruriens* leaf meal (MLM) supplementation on performance of growing rabbits.

	Diet 1 0 g MLM/kg	Diet 2 4 g MLM/kg	Diet 3 8 g MLM/kg	Diet 4 12 g MLM/kg	SEM	*P*-value
No. of rabbits	30	30	30	30		
Post-weaning period (35-63 d)						
Average body weight at 35 d (g)	697	694	694	691	5	0.99
Average body weight at 63 d (g)	1346	1438	1489	1536	29	0.09
Average daily weight gain (g/d)	23.2	26.6	28.4	30.1	1.0	0.07
Average daily feed intake (g/d)	62.2	64.7	63.5	63.6	1.5	0.96
Feed conversion ratio	2.70	2.44	2.24	2.12	0.09	0.11
Finishing period (64-91 d)						
Average body weight at 91 d (g)	2093[a]	2151[a]	2321[b]	2354[b]	37	0.01
Average daily weight gain (g/d)	26.7	25.5	29.7	29.2	1.0	0.45
Average daily feed intake (g/d)	75.1	75.8	76.8	78.0	0.5	0.26
Feed conversion ratio	2.89	2.99	2.58	2.70	0.10	0.52
Whole fattening period (35-91 d)						
Average body weight gain (g)	1396[a]	1458[a]	1627[b]	1663[b]	39	0.01
Average daily weight gain (g/d)	24.9[a]	26.0[a]	29.1[b]	29.7[b]	0.7	0.01
Average daily feed intake (g/d)	68.7	70.3	70.2	70.8	0.8	0.83
Feed conversion ratio	2.76	2.70	2.41	2.38	0.06	0.09

Means within a row with different letters are significantly different ($P<0.05$); SEM: Standard error of the mean.

Table 4: Effects of *Mucuna pruriens* leaf meal (MLM) supplementation on carcass and relative internal organs (% slaughter weight) of growing rabbits at 91 d of age.

	Diet 1 0 g MLM/kg	Diet 2 4 g MLM/kg	Diet 3 8 g MLM/kg	Diet 4 12 g MLM/kg	SEM	*P*-value
No. of rabbits	10	10	10	10		
Slaughter weight (g)	1977[a]	2036[a]	2206[b]	2239[b]	37	0.04
Dressing-out percentage	57.5[a]	58.7[a]	61.9[b]	62.5[b]	0.7	0.05
Liver	2.69	2.69	2.72	2.58	0.14	0.96
Heart	0.25	0.23	0.24	0.24	0.02	0.98
Lung	0.42	0.42	0.42	0.42	0.02	0.97
Kidney	0.45	0.46	0.44	0.44	0.02	0.99
Spleen	0.03	0.03	0.04	0.04	0.01	0.98

Means within a row with different letters are significantly different ($P<0.05$); SEM: Standard error of the mean; Dressing-out percentage=Hot carcass weight/slaughter weight×100.

Serum metabolites

The effect of dietary MLM supplementation on serum metabolites of growing rabbits is shown in Table 6. At 63 d, the cholesterol level of rabbits fed on diets 3 and 4, was lower compared to those rabbits fed on the control diet (–1.0 and –1.1 mmol/L, respectively; $P=0.05$). The AST concentration in rabbits fed on diet 4 was significantly lower compared to those rabbits on the control diet (–33.68 IU/L; $P=0.02$). At 91 d, the serum cholesterol level was lower in rabbits fed on diets 3 and 4 when compared to those rabbits fed the control diet (–1.16 and –1.21 mmol/L, respectively; $P=0.03$). The TP, creatinine, and bilirubin were not significantly ($P>0.05$) affected by MLM supplementation at 63 d and 91 d of age.

Activities of antioxidant enzymes

The effect of including MLM in rabbit diets on activities of antioxidant enzymes are presented in Table 7. At 63 d, compared with the control, the activities of GPx in rabbits fed on diets 3 (+35.77 mg protein) and 4 (+54.52 mg protein) significantly ($P=0.01$) increased, while CAT activities in rabbits fed diet 4 (+217.66 mg/g) increased significantly ($P=0.02$). At 91 d of age, compared with the control, GPx activities in rabbits fed diets 3 (+49.09 mg protein) and 4 (+55.02 mg protein) increased significantly ($P=0.03$). The lipid peroxidase and superoxide dismutase were not ($P>0.05$) influenced by MLM supplementation at 63 d and 91 d of age. The CAT activities in rabbits fed diet 4 (+209.46 mg/g) significantly ($P=0.03$) increased compared with the control.

Table 5: Effects of *Mucuna pruriens* leaf meal (MLM) supplementation on haematological indices of growing rabbits.

	Diet 1 0 g MLM/kg	Diet 2 4 g MLM/kg	Diet 3 8 g MLM/kg	Diet 4 12 g MLM/kg	SEM	*P*-value
No. of rabbits	10	10	10	10		
Observations at 63 d old						
White blood cells ($\times10^9$/L)	7.04[a]	9.71[ab]	9.24[ab]	12.09[b]	0.70	0.05
Red blood cells ($\times10^{12}$/L)	3.88	4.98	5.29	5.83	0.42	0.45
Haemoglobin conc. (g/dL)	14.52	13.98	14.20	13.38	0.60	0.95
Packed cell volume (%)	45.00	44.00	46.33	43.00	1.67	0.93
Observations at 91 d old						
White blood cells ($\times10^9$/L)	7.10[a]	9.98[ab]	9.84[ab]	12.42[b]	0.69	0.02
Red blood cells ($\times10^{12}$/L)	4.11	5.49	5.69	6.28	0.42	0.37
Haemoglobin conc. (g/dL)	14.67	15.00	15.20	15.38	0.48	0.97
Packed cell volume (%)	44.33	46.67	49.33	50.68	1.44	0.46

Means within a row with different letters are significantly different ($P<0.05$); SEM: Standard error of the mean.

Table 6: Effects of *Mucuna pruriens* leaf meal (MLM) supplementation on serum metabolites of growing rabbits.

	Diet 1 0 g MLM/kg	Diet 2 4 g MLM/kg	Diet 3 8 g MLM/kg	Diet 4 12 g MLM/kg	SEM	*P*-value
No. of rabbits	10	10	10	10		
Observations at 63 d old						
Total protein (g/dL)	7.09	7.79	7.85	7.80	0.53	0.96
Creatinine (µmol/L)	100.40	95.85	93.87	99.01	6.14	0.99
Cholesterol (mmol/L)	2.21^{b}	1.51^{ab}	1.19^{a}	1.11^{a}	0.17	0.05
Aspertate aminotransferase (IU/L)	95.27^{c}	84.09^{bc}	67.32^{ab}	61.59^{a}	4.90	0.02
Bilirubin total (µmol/L)	9.20	9.35	9.19	9.06	0.74	0.94
Observations at 91 d old						
Total protein (g/dL)	6.89	7.84	8.08	8.10	0.56	0.89
Creatinine (µmol/L)	98.24	93.17	96.47	100.01	6.88	0.99
Cholesterol (mmol/L)	2.24^{b}	1.47^{ab}	1.08^{a}	1.03^{a}	0.18	0.03
Aspertate aminotransferase (IU/L)	91.34	90.09	80.68	82.21	3.01	0.54
Bilirubin total (µmol/L)	9.69	9.46	8.92	9.01	0.79	0.98

Means within a row with different letters are significantly different ($P<0.05$); SEM: Standard error of the mean.

DISCUSSION

The consequences of exposure of rabbits to ambient temperature outside their thermal neutrality zone (21 to 25°C) are impaired growth, feed intake and utilisation (Brewer and Cruise, 1994; Marai *et al.,* 2002), and heat stress is also one of the factors causing oxidative stress in the tropics (Kumar *et al.,* 2011). Previous studies had identified the intestinal mucosa damage and increased muscle protein hydrolysis being caused by temperature-induced free radicals as the possible cause of thermal stress-induced impaired growth performance (Yuan *et al.,* 2007; Jimoh *et al.,* 2017, Li *et al.,* 2018). In this study, dietary supplementation of rabbit diets with MLM at 8 or 12 g/kg caused increased average body weight gain and average daily weight gain of the rabbits when compared to the control. This suggests that dietary MLM supplementation of diets could serve as a growth performance enhancer in rabbits raised under tropical high ambient temperature. The phenolic compounds present in MLM as detected in this study might have demonstrated high antioxidant and free radical scavenging activities, which help to maintain the intestinal mucosa integrity, thereby promoting growth performance (Yuan *et al.,* 2007). Furthermore, the use of leaf meal as the phytogenic growth promoter in the animal is on the increase (Valenzuela-Grijalva *et al.,* 2017); in particular, the antioxidant and anti-inflammatory activity of some phytochemicals in the leaf meal is of great interest due to their ability to suppress the metabolism of inflammatory prostaglandins. Some of the phytochemicals (e.g. flavonoids and terpenoids) detected in MLM in this study

Table 7: Effects of *Mucuna pruriens* leaf meal (MLM) supplementation on antioxidant enzyme activities of growing rabbits.

	Diet 1 0 g MLM/kg	Diet 2 4 g MLM/kg	Diet 3 8 g MLM/kg	Diet 4 12 g MLM/kg	SEM	*P*-value
No. of rabbits	10	10	10	10		
Observations at 63 d old						
Lipid peroxidase (nmol/mol x10^{6})	15.18	16.93	16.64	16.06	1.08	0.96
Superoxide dismutase (m/mg)	81.33	92.00	91.33	94.66	2.73	0.37
Glutathione peroxidase (mg protein)	84.8^{a}	99.6^{ab}	120.6^{bc}	139.3^{c}	7.2	0.01
Catalase (mg/g)	379.8^{a}	500.3^{ab}	499.7^{ab}	597.5^{b}	27.9	0.02
Observations at 91 d old						
Lipid peroxidase (nmol/mol x10^{6})	14.88	16.36	15.94	15.68	0.90	0.96
Superoxide dismutase (m/mg)	84.33	92.00	92.67	94.33	2.64	0.61
Glutathione peroxidase (mg protein)	90.3^{a}	105.3^{ab}	139.4^{bc}	145.4^{c}	8.5	0.03
Catalase (mg/g)	388.8^{a}	506.0^{ab}	515.1^{ab}	598.2^{b}	27.8	0.03

Means within a row with different letters are significantly different ($P<0.05$); SEM: Standard error of the mean.

possess anti-inflammatory activity (Muanda *et al.,* 2011). These phytochemicals may over-express antioxidant enzyme, thereby down-regulating the inflammatory process. The reactive oxygen radicals produced during the process of food digestion in the digestive tract can attack the intestinal mucosa surface and thereby affect the normal process of nutrient absorption. Therefore, the anti-inflammatory and antioxidant activities in the intestinal mucosa may result in maintenance of healthy gut morphology (Kamel, 2000), improved increased nutrient absorption and enhanced growth (Cardoso *et al.,* 2012) as recorded in this study. Previously, Li *et al.* (2018) reported improved daily weight gain, average feed intake in rabbits' whose diets were supplemented with 1 or 5 g/kg *Eucommia ulmoides* leaves, while Ayodele *et al.* (2016) reported improved feed conversion ratio in rabbits fed on 5 or 10% alchornea leaf meal inclusion diets. However, further studies are required to study the effect of MLM on the morphology of the rabbits' intestinal mucosa.

Phytogenetic additives can produce antioxidant effects, which may result in health conditions for the animals and improved growth of target tissues (Valenzuela-Grijalva *et al.,* 2017). In addition, relative weights of the internal organ of animals may increase abnormally in response to the presence of toxins in their diet (Ayodele *et al.,* 2016). In this study, the increased SW and DP observed in rabbits fed on 8 and 12 g/kg MLM supplemented diets suggests that MLM promoted the growth of edible portion of the rabbits more than offal, and that some of the phytochemicals present in MLM exert direct or indirect effects on animal metabolism, probably by modulating animal metabolism in favour of increasing the development of edible portions of the rabbits (Jiang *et al.,* 2007; Devi *et al.,* 2015; Valenzuela-Grijalva *et al.,* 2017). The stability of the rabbits' relative weight of internal organs (liver, heart, lung, kidney, and spleen) across the varying levels of dietary MLM supplementation as observed in this study also suggests the uncompromised health status of the rabbits.

Oxidative stress caused as a result of high ambient temperature could result in pathological changes such as tissue damage and adverse effects on blood indices (Avellini *et al.,* 1995; Adekonla and Ayo 2009). The stability of blood indices such as RBC, Hbc, and PCV in rabbits across the various dietary treatments in this study is an indication that MLM supplementation supports or does not interfere with normal haemopoiesis processes. However, the observed rise in WBC in rabbits fed MLM supplemented diets, especially 12 g/kg supplemented diet in this study, may be the product of immunostimulatory activities of MLM. The WBC counts possess phagocytic function and biomarkers for immune functions. An immunostimulatory activity in animals is one of the biological activities being associated with phytogenic feed additives (Valenzuela-Grijalva *et al.,* 2017). Similar results were reported in broiler chickens fed 2.5 g/kg *Azadirachta indica* leaf meal (Ansari *et al.,* 2012) and in rabbits fed 1 g/kg alchornea leaf meal (Oloruntola *et al.,* 2016a).

The reduction of serum cholesterol levels in rabbits fed on 8 and 12 g/kg in this study suggests that MLM supplementation interferes with the uptake and catabolism of cholesterol in the rabbits. According to Lording and Friend (1991), decreased uptake of cholesterol or increased loss or cholesterol catabolism is among the causes of hypocholesterolaemia. Phytochemicals in the phytogenic feed additives were reported to exhibit various biological activities that can influence the functions of the intestinal tract (Valenzuela-Grijalva *et al.,* 2017). In particular, saponin, one of the detected phytochemicals in MLM, was linked to the reduction of cholesterol uptake in the gut (Yilkal, 2015). Recently, Oloruntola *et al.* (2016a,b) recorded reduction of serum cholesterol level in rabbits and broiler chickens fed on diets containing 50 or 100 g/kg alchornea leaf meal. The reduction of serum AST in this study suggests that MLM has protective and therapeutic properties, as abnormally rising AST concentration indicates liver and biliary system disease, skeletal muscle disease, myocardial injury/diseases, haemolytic disorder and haemolysis (Lording and Friend, 1991). This is further supported by the stability of the TP, creatinine and bilirubin concentration in the rabbits fed the various experimental diets supplemented with varying levels of MLM. The antioxidant property of MLM and the presence of some phytochemicals at the therapeutic level (Sies, 1997; Yadav *et al.,* 2017) may be responsible for these observations in this study.

High ambient temperature and, consequently, heat stress promote increased free radical generation and lipid oxidation. This condition enhances the generation of free radicals, which if not adequately removed could cause irreversible damage to cells (Tawfeek *et al.,* 2014; Meineri *et al.,* 2017). The importance of antioxidant enzymes, particularly in animals being raised under high ambient temperature, in eliminating the oxygen free radicals being induced by excessive heat has been reported (Masella *et al.,* 2005; Li *et al.,* 2018). Results from this study showed that dietary MLM supplementation could increase the levels of blood GPx and CAT in rabbits, implying that heat stress-induced oxidative destruction could be reduced in rabbits by dietary MLM supplementation. This observation

suggests that dietary MLM is rich in antioxidant and could play a significant role in improving the health of rabbits. The antioxidant potential of this supplement used in this study may be related to the concentration of phenolic substances (tannin, flavonoids), ferric reducing antioxidant property and 2,2-diphenyl-1-1-picrylhydrazyl hydrate in MLM.

CONCLUSION

The dietary MLM supplementation increased the final live weight of rabbits, average daily weight gain and dressing-out percentage, exerted the immunomodulatory effect by increasing white blood cells, reduced the serum aspartate aminotransferase and cholesterol and increased serum glutathione peroxidase and catalase.

REFERENCES

Adekonla A.Y., Ayo J.O. 2009. Effect of road transportation on erythrocyte osmotic fragility of pigs administered ascorbic acid during the harmattan season in Zaria, Nigeria. *J. Cell Anim. Biol., 3: 4-8.*

Aebi H. 1974. Catalase estimation In (ed. HV Bergmeyer). Methods of Enzymatic Analysis. *Verlag Chemic, New York Academic Press, New York, US. https://doi.org/10.1016/B978-0-12-091302-2.50032-3*

Ansari J., Khan S.H., Hag A., Yousaf M. 2012. Effect of the level of *Azadirachta indica* dried leaf meal as phytogenic feed additive on growth performance and haemato-biochemical parameters in broiler chicks. *J. Appl. Anim. Res., 40: 336-345. https://doi.org/10.1080/09712119.2012.692329*

AOAC. 1990. Official methods of analysis of the Association of Official Analytical Chemists, *15th ed. AOAC, Washington, D.C. USA.*

Attia Y., Bakhashwaina A.A., Bertu N.K. 2017. Thyme oil (*Thyme vulgaris* L) as a natural growth promoter for broiler chickens reared under hot climate. *It. J. Anim. Sci., 16: 275-282. https://doi.org/10.1080/1828051X.2016.1245594*

Avellini L., Silverstrelli M., Gaitti A. 1995. Training-induced modifications in some biochemical defenses against free radicals in equine erythrocytes. *Vet. Res. Com., 19: 179-184. https://doi.org/10.1007/BF01839296*

Ayodele S.O., Oloruntola O.D., Agbede J.O. 2016. Effect of *Alchornea cordifolia* leaf meal inclusion and enzyme supplementation on performance and digestibility of rabbits. *World Rabbit Sci., 24: 201-2016. https://doi.org/10.4995/wrs.2016.3933*

Blasco A., Ouhayoun J., Masoero G. 1993. Harmonization of criteria and terminology in rabbit meat research. *World Rabbit Sci., 1: 3-10. https://doi.org/10.4995/wrs.1993.189*

Brewer N.R., Cruise L.J. 1994. The Biology of the Laboratory Rabbit. 2nd Edition. *In: Patrick J. Manning, Daniel H. Ringler, and Christian E. Newcomer (eds.). Elsevier Inc. pp: 483.*

Burnett N., Mathura K., Metivier K.S., Holder R.B., Brown G., Campbell M. 2003. An investigation into haematological and serum chemistry parameters of rabbits in Trinidad. *World Rabbit Sci., 14: 175-187. https://doi.org/10.4995/wrs.2006.556*

Cardoso V.D.S., Lima C.A.R.D., Lima M.E.F.D., Dorneles L.E.G., Danelli M.D.G.M. 2012. Piperin as a phytogenic additive in broiler diets. *Pesq. Agropec. Bras., 47: 489-496. https://doi.org/10.1590/S0100-204X2012000400003*

de Blas C., Mateos G.G. 2010. Feed formulation. *In: Nutrition of the Rabbit. 2nd Edition (eds. C.de Blas and J. Wiseman). CAB International. The United Kingdom. pp. 229. https://doi.org/10.1079/9781845936693.0222*

Devi S.M., Park J.W., Kim I.H. 2015. Effect of plant extract on growth performance and insulin-like growth factor 1 secretion in growing pig. *Rev. Bras. Zootec., 44: 355-360. https://doi.org/10.1590/S1806-92902015001000003*

Duke A.T. 1995. Handbook of Medicinal Herbs. 3rd ed. *CRS Press. London, pp. 220.*

Edeoga G.O., Okwu D.E., Mbaebie B.O. 2005. Phytochemical constituents of some Nigerian medicinal plants. *Afr. J. Biotechnol., 4: 685-688. https://doi.org/10.5897/AJB2005.000-3127*

El-Desoky N.I., Hashem N.M., Elkomy A., Abo-Elezz Z.R. 2017. Physiological response and semen quality of rabbit buck supplemented with Moringa leaves ethanolic extract during summer season. *Animal., 14: 1-9. https://doi.org/10.1017/S1751731117000088.*

El-Gindy Y.M., Zeweil H.S. 2017. Effects of parsley supplementation on the seminal quality, blood lipid profile and oxidant status of young and old male rabbits. *World Rabbit Sci., 25: 215-223. https://doi.org/10.4995/wrs.2017.6532*

Ferguson NM. 1956. A textbook of pharmacology. *McMillan Company. New Delhi. 191.*

Fernández-Carmona J., Blas E., Pascual J.J., Maertens L., Gidenne T., Xiccato G., Garcia J. 2005. Recommendations and guidelines for applied nutrition experiments in rabbits. *World Rabbit Sci., 13: 209-228. https://doi.org/10.4995/wrs.2005.516*

Gyamfi M.A., Yonamine M., Aaniya Y. 1999. Free radical scavenging action of medicinal herbs from Ghana: *Thonningia sanguine* on experimentally induced liver injuries. *General Pharmacol., 32: 661-667. https://doi.org/10.1016/S0306-3623(98)00238-9*

Halliwell B.E., Gutteridge J.M.C. 1989. Lipid Peroxidation: a Radical Chain Reaction, Free Radical in Biology and Medicine, 2nd ed. *Oxford University Press, New York, NY, 188-218.*

Harborne J.B. 1973. *Phytochemical methods*, *Chapman and Hall Ltd, London, 49-188 pp.*

Jiang Z Y., Jiang SQ., Lin YC, Zi PB, Yu DQ, Wu TX. 2007. Effect of soybean isoflavone on growth performance, meat quality and antioxidation in male broiler. *Poultry Sci., 86: 1356-1362. https://doi.org/10.1093/ps/86.7.1356*

Jimoh A.O., Ewuola E.O., Balogun A.S. 2017. Oxidative stress marker in exotic breeds of rabbits during peak of heat stress in Ibadan, Nigeria. *J. Adv. Bio. Biotech., 12: 1-9. https://doi.org/10.9734/JABB/2017/30437*

Kamel C. 2000. Natural plant extracts: Clinical remedies bring modern animal production solutions. *In: 3rd Conference on sow feed manufacturing in the Mediterranean Region. 31-38.*

Konca Y., Kirkpinar F., Mert S., Yurtseven S. 2009. Effect of dietary ascorbic acid supplementation on growth performance, carcass, bone quality and blood parameters in broilers during natural summer temperature. *Asian J. Anim.Vet. Advs., 4: 139-147. https://doi.org/10.3923/ajava.2009.139.147*

Kone A.P., Cinq-Mars D., Desjardins Y., Guay F., Gosselin A., Saucier L. 2016. Effects of plant extracts and essential oils as feed supplements on quality and microbial traits of rabbit meat. *World Rabbit Sci., 24: 107-119. https://doi.org/10.4995/wrs.2016.3665*

Kumar A., Rajput G., Dhatwalia V.K., Srivastav G. 2009. Phytocontent screening of *Mucuna* seeds and exploit in opposition to pathogenic microbes. *J. Biol. Environ. Sci., 3: 71-76.*

Kumar D.S., Muthu A.K. 2010. Free radical scavenging activity of various extracts of whole plant of *Mucuna pruriens* (Linn): An *in-vitro* evaluation. *J. Pharm. Res., 3: 718-721.*

Kumar S., Kumar A.B.V., Meena K. 2011. Review: Effect of heat stress in tropical livestock and different strategies for its amelioration. *J. Stress Physiol. Biochem., 7: 45-54.*

Li S., Zhao M., Jiang T., Lv W., Gao S., Zhou Y., Miao Z. 2018. Growth performance and antioxidant status of growing rabbits fed on diets supplemented with *Eucommia ulmoides* leaves. *World Rabbit Sci., 26: 35-41. https://doi.org/10.4995/wrs.2018.7864*

Liu H.W., Dong X.F., Tong J.M., Qi Z. 2010. Alfalfa polysaccharides improve the growth performance and antioxidant status of heat-stressed rabbits. *Livest. Sci., 131: 88-93. https://doi.org/10.1016/j.livsci.2010.03.004*

Lording P.M., Friend S.C.E. 1991. Interpretation of laboratory results. *Aust. Vet. Pract., 21: 188-192.*

Marai I.F.M., Habeeb A.A.M., Gad A.E. 2002. Rabbit's productive, reproductive and physiological performance traits as affected by heat stress: A review. *Livest. Prod. Sci., 78: 71-90. https://doi.org/10.1016/S0301-6226(02)00091-X*

Marco-Jiménez F., García-Diego F.J., Vicente J.S. 2017. Effect of gestational and lactational exposure to heat stress on performance in rabbits. *World Rabbit Sci., 25: 17-25. https://doi.org/10.4995/wrs.2017.5728*

Masella R., Benedetto R.D., Varì R., Filesi C., Giovannini C. 2005. Novel mechanisms of natural antioxidant compounds in biological systems: involvement of glutathione and glutathione-related enzymes. *J. Nutr. Biochem., 16: 577-586. https://doi.org/10.1016/j.jnutbio.2005.05.013*

Meineri G., Giacobini M., Forneris G. 2017. Evaluation of physiological parameters of the plasma oxidative status in rabbits. *J. Appl. Anim. Res., 45: 315-319. https://doi.org/10.1080/09712119.2016.1190734*

Mertens D.R. 2002. Gravimetric determination of amylase-treated neutral detergent fibre in feeds with refluxing in breakers of crucibles: Collaborative study. *J. AOAC. Int., 85: 12187-1240.*

Misra, H.P., Fridovich, I. 1972. The univalent reduction of oxygen by flavins and quinines. *J. Biol. Chem., 247: 188-192.*

Muanda F., Kone D., Dicko A., Soulimani R., Younos C. 2011. Phytochemical composition and antioxidant capacity of three Malian medicinal plant parts. *J. Evid. Based Complementary Altern. Med., 2011: 21-28. https://doi.org/10.1093/ecam/nep109*

Ohkawa H, Ohishi N, Yagi K. 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem., 95: 351-358. https://doi.org/10.1016/0003-2697(79)90738-3*

Oloruntola O.D., Ayodele S.O., Agbede J.O, Oloruntola D.A, Ogunsipe M.H., Omoniyi I.S. 2016a. Effect of *Alchornea cordifolia* leaf meal and enzyme supplementation on growth, haematological, immunostimulatory and serum biochemical response of rabbits. *Asian J. Biol. Life Sci., 5: 190-195.*

Oloruntola O.D., Ayodele S.O., Agbede J.O., Oloruntola D.A. 2016b. Effect of feeding broiler chicken with diets containing *Alchornea cordifolia* leaf meal and enzyme supplementation. *Arch. Zootec., 65: 489-498.*

Pulido R., Bravo L., Saura-Calixto F. 2002. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. *J. Agric Food Chem., 48: 3396-3402. https://doi.org/10.1021/jf9913458*

Robertson J.B., Van Soest P.J. 1981. The detergent system of analysis. *In: James W.P.T., Theander O. (eds.) The analysis of dietary fibre in food. Marcel Dekker, New York. 123-159.*

Rotruck J.T., Pope A.L., Ganther H.E., Hafeman D.G., Hoekstra W.G. 1973. Selenium: Biochemical role as a component of glutathione peroxidase. *Science, 179: 588-590. https://doi.org/10.1126/science.179.4073.588*

Shad M.A., Nawaz H., Rehma T., Ikram M., 2013. Determination of biochemicals, phytochemicals and antioxidative properties of different part of *Cichorium intybus* L.: A comparative study. *The J. Anim. Plant Sci., 23: 1060-1066.*

Sies H. 1997. Oxidative stress: oxidants and antioxidants, *Exp. Physiol., 82: 291-295. https://doi.org/10.1113/expphysiol.1997.sp004024*

Suresh S., Prithiviraj E., Lakshmi N.V., Ganesh M.K., Ganesh L., Prakash S. 2013. Effect of *Mucuna pruriens* (Linn.) on mitochondrial dysfunction and DNA damage in epididymal sperm of streptozotocin induced diabetic rat. *J. Ethnopharmacol., 145: 32-41. https://doi.org/10.1016/j.jep.2012.10.030*

Tawfeek S.S., Hassanin K.M.A., Youssef I.M.I. 2014. The effect of dietary supplementation of some antioxidants on performance, oxidative stress, and blood parameters in broilers under natural summer conditions. *J. World's Poult. Res., 4: 10-19.*

Valenzuela-Grijalva N.V., Pinelli-Saavedra A., Muhlia-Almazan A., Domínguez-Díaz D., González-Ríos H. 2017. Dietary inclusion effects of phytochemicals as growth promoters in animal production. *J. Anim. Sci. Tech., 58: 8. https://doi.org/10.1186/s40781-017-0133-9*

Van-Burden T.P., Robinson W.C., 1981. Formation of complexes between protein and Tannin acid. *J. Agric. Food Chem. 1: 77.*

Xiccato G., Trocino A. 2010. Energy and protein metabolism and requirements. *In: C. de Blass, J. Wiseman (eds.).Nutrition of the rabbit, 2nd edition. CAB International, United Kingdom. pp. 83. https://doi.org/10.1079/9781845936693.0083*

Yadav M.K., Upadhyay P., Purohit S., Pandey B.L. Shah H. 2017. Phytochemistry and pharmacological activity of *Mucuna pruriens*: A review. *Int. J. Green Pharm., 11: 69-73. https://doi.org/10.22377/ijgp.v11i02.916*

Yilkal T. 2015. Important anti-nutritional substances and inherent toxicants of feeds. *Food Sci. Quality Man., 36: 40-47.*

Yuan S., Chen D., Zhang K., Yu B. 2007. Effect of oxidative stress on growth performance, nutrient digestibilities and activities of antioxidative enzymes of weaning pigs. *Asian-Aust. J. Anim. Sci., 20: 1600-1605.*

Zeweil H.S., Elgindy Y.M. 2016. Pomegranate peel as a natural antioxidant enhanced reproductive performance and milk yield of female rabbits. *World Rabbit Sci., 24: 207-212. https://doi.org/10.4995/wrs.2016.4025*