WEIGHT GAIN, THERMOREGULATORY RESPONSE AND BLOOD INDICES OF MALE RABBITS FED DIETS WITH *Vernonia amygdalina* LEAF MEAL

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ABSTRACT

Plants with bio-nutrients that can support performance and normal health can be adopted as a feed resource for animals. This study assessed the effect of Vernonia amygdalina Leaf Meal (VLM) on weight gain, thermoregulatory response and blood indices of male rabbits. Dried Vernonia amygdalina leaves was used as a feed resource at 0, 5, 10 and 15 % levels with other feed ingredients to formulate four experimental diets and designated as VLM0, VLM5, VLM10 and VLM15 respectively. Forty heterogeneous male rabbits with an average weight of 1.8kg were allotted to four groups (10 bucks per group) and fed the diets for 84 days. Weight gain, morphometric traits, heart rate, rectal temperature, serum cholesterol, glucose, haematology and organ indices of rabbit bucks were evaluated using standard procedures. All data recorded were analyzed using a one-way analysis of variance. The statistical significance was established at P<0.05. Average daily weight gain and feed intake increased (p<0.05) in rabbit bucks fed the VLM10 diet. Weight gain in bucks fed VLM 15 was significantly (p>0.05) lower than that of bucks fed VLM0 and VLM10. All the rabbits had similar heart rates and rectal temperatures among the groups. Among the morphometric traits assessed body length decreased significantly as VLM increased in the diets. Cholesterol, glucose and platelet count were higher (P<0.05) in rabbits fed the diet without VLM (VLM0). Alanine Aminotransferase and Aspartate Aminotransferase levels increased significantly (P<0.05) by 41% and 51% in rabbits fed the VLM15 diet compared to the VLM0 group. Organ weights were similar among the rabbits, this showed a relatively non-toxic effect of VLM on organ size in the bucks. Vernonia amygdalina leaf meal as a feed ingredient of up to 10% can be adopted as a component of male rabbits' diet without deleterious effects on their overall performance.

Keywords: ***Bitter leaf, Body length, Haematology, Rectal temperature, Weight gain***

INTRODUCTION

Adequate weight, good health and efficient reproduction are essential for profitable livestock production. Commercial production of rabbits is hinged on efficient management practices which include supply of feeds with adequate nutrients. Leaf meals have been identified as suitable replacements for some conventional feedstuffs (Oloruntola *et al.*, 2016). It may help to relatively reduce feed costs, improve the performance of the rabbits and in some cases to prevent or treat some diseases. Most of the plants used as leaf meals are rich sources of bioactive phytochemicals or bio-nutrients (Saxena *et al.*, 2013). *Vernonia amygdalina*, popularly known as bitter leaf is an essential source of leaf meal with both nutritional and therapeutic properties (Arhoghro *et al.*, 2009). It is widely grown in the tropics. Phytochemical analysis of *Vernonia amygdalina* revealed that it contains bioactive compounds which include; oxalate, phylate, tannins, saponins, flavonoid, cyanogenic glycosides, alkaloids, anthraquinone, steroids and phenol (Udochukwu *et al.*, 2015). *Vernonia amygdalina* leaf contains 22.81% crude

protein, 18.17% crude fibre, 16.65% ash, 4.34% crude fat and 38.03% carbohydrate (Usunobun & Okolie, 2016).

Plants with medicinal properties can be a source of exposure to toxic substances depending on their origin and nature (Yuan *et al.,* 2011) and toxicity can have a negative effect on the performance of livestock. The presence of toxins in the body can alert heart rate and rectal temperature which are indicators of the physiological state of an animal. Changes in blood constituents can be used in evaluating the response of farm animals to various physiological conditions (Etim *et al.,* 2014). Liver enzymes (such as Alanine aminotransferase) present in blood serum are physiological markers for a normal or deceased liver (Lala *et al*., 2022).

Bioactive compounds in *Vernonia amygdalina* leaf can have direct or indirect effects on physiological indicators in farm animals. Research shows that extract of *Vernonia amygdalina* reversed induced liver damage in rats (Arhoghro *et al.,* 2009). Dried *Vernonia amygdalina* leaf above 15% significantly increased the mortality rate in West African Dwarf goats (Olosunde & Odeyinka, 2017). Levels of *Vernonia amygdalina* had no detrimental effect on the pancreas, kidneys and heart in broiler chickens; birds fed 75g/kg level had small liver size which could be an indication of liver purification (Ndelekwute *et al.*, 2017). *Vernonia amygdalina* leaf meal at 2% had an optimum impact on growth and physiological responses in broiler chickens (Tokofai *et al*., 2020). Also, *Vernonia amygdalina* leaf meal of up to 10% had no deleterious effect on the reproductive indices of male rabbits (Adeyemi *et al*., 2022).

Unconventional feed ingredients such as *Vernonia amygdalina* leaf meal can serve as a relatively cheap feedstuff for rabbits and it has bioactive components that can have beneficial or adverse effects on farm animals. Despite the fact that up to 10% dietary level of *Vernonia amygdalina* leaf meal can be tolerated by male rabbit breeding stock (Adeyemi *et al.*, 2022), there is no information on the effect of bitter leaf meal on their body and thermoregulatory indices. This study, therefore, assessed the effect of *Vernonia amygdalina* leaf meal on growth indices, morphometric traits, thermoregulatory indicators and blood profile of heterogeneous stock of rabbit bucks.

MATERIALS AND METHODS

Experimental diet, site and animal management

Fresh *Vernonia amygdalina* leaves were harvested from established plots within Osun State, Nigeria. Leaf samples were authenticated and identified at the Botany Department, Obafemi Awolowo University, Ile-Ife with voucher number - Ife18001. The leaves were air dried until brittle and milled to obtain *Vernonia amygdalina* leaf meal (VLM) which was incorporated into the experimental diets to replace wheat bran and pelletized. The experimental diets were designated as VLM0 - diet without VLM (control diet), VLM5 - diet with 5% VLM, VLM5 - diet with 10% VLM and VLM15 - diet with 15% VLM (Table 1). The study was conducted at the Rabbitry unit of the Teaching and Research Farm, Obafemi Awolowo University, Ile-Ife, Nigeria during the rainy season (August – October with an average temperature of 25.1°C). All protocols for animal care and handling in this study were carefully carried out as approved by the Animal Research Ethics Committee of the Department of Animal Sciences, Obafemi Awolowo University, Ile-Ife. Forty rabbit bucks with an average weight of 1818.3±26g were used for this experiment. The rabbits were randomly allocated into four groups with 10 replicates per group in a Completely

Randomised Design and housed individually in cages. The experimental diets were offered to the rabbits at 100g per buck daily. The experiment lasted for eighty-four (84) days.

Table 1: Composition of Experimental diets

INGREDIENTS	VLM0	VLM5	VLM10	VLM15
Maize	9.00	9.00	9.00	9.00
Corn bran	22.5	22.5	22.5	22.5
Rice bran	20.8	20.8	20.8	20.8
Soybean meal	9.90	9.90	9.90	9.90
Palm kernel cake	5.25	5.25	5.25	5.25
Wheat bran	30.8	25.8	20.8	15.8
Vernonia amygdalina Leaf Meal	0.00	5.00	10.0	15.0
Others	1.75	1.75	1.75	1.75
TOTAL	100	100	100	100
Calculated analysis:				
Crude protein (%)	16.1	16.4	16.6	16.9
Crude fibre (%)	9.04	9.19	9.35	9.30
Digestible energy (kcal/kg)	2468	2500	2532	2564

VLM- *Vernonia amygdalina* leaf meal; VLM_0- diet without VLM (control diet), VLM_5- diet with 5% VLM, VLM_{10}- diet with 10% VLM and VLM_{15}- diet with 15% VLM Others: Bone meal =1.10, Salt = 0.32 and Premix = 0.33
Adeyemi *et al*., 2022

Evaluation of weight gain and thermoregulatory response

The bucks were weighed weekly using a digital weighing scale and the body weight gain was estimated from values obtained. Feed intake was estimated by subtracting the leftover feed from the amount of feed offered to the animal daily and the values were recorded in grams. Morphometric traits (abdominal circumference, body length, ear length, head length, and tail length) of the animals were taken in the morning before feeding the rabbits once every two weeks using a tape rule as described by Ogbuewu *et al., (*2011). The heart rate of the rabbits was monitored by placing a hand on the lower left side of the chest to feel the heartbeat. The number of counts for 15 seconds was recorded using a stopwatch; values obtained were multiplied by four to obtain the number of heartbeats per minute. The rectal temperature was measured by gently inserting a clean digital thermometer in the rectum of each rabbit; values were recorded in degree Celsius (°C)

Blood indices

At the end of the experiment, 5 ml of blood was withdrawn from each animal using sterile needles and syringes. A portion was dispensed into labelled EDTA bottles for haematological assessment. The remaining was dispensed into sterile sample bottles without anticoagulant and centrifuged at 3000rpm for 10mins to obtain serum for Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) assessment. Serum levels of cholesterol, glucose, ALT and AST were measured. Randox commercial test kits specified for each parameter was used following manufacturers' instruction and the absorbance was measured using a spectrophotometer. Haematological indices were assessed using Lamb's (1981) procedures.

Organ Evaluation

At the end of the experiment, twenty rabbits randomly selected (5 from each group) were weighed, stunned and sacrificed by jugular severing. The carcasses were eviscerated;

some internal organs (heart, liver, kidney, lungs, spleen and gall bladder) were carefully removed and weighed using a sensitive digital weighing scale. The values were recorded in grams as absolute weight. Paired organs were weighed individually and recorded; both were added together to obtain the paired weight of the organs. Percentage relative weights of the organs were calculated using the formula below:

Relative organ weight= {(Absolute organ weight)/ (Live weight of the rabbit)}x100

Statistical analysis

All data obtained from the study were analyzed using one-way ANOVA. The general linear model (GLM) procedures of SAS (2009) software were used in this statistical analysis. The linear model employed was:

$$Yij = \mu + Si + Eij$$

where,

Yij = observation of the dependent variables *ij* (growth indices, morphometric traits, thermoregulatory response and blood parameters),

μ = fixed effect of the population mean for the variable,

Si = effect of diet (*j* = 4; VLM0, VLM5, VLM10 and VLM15), and

Eij = random error associated with observation *ij* assumed to be normally and independently distributed. Means were separated using Duncan Multiple Range Test (at P<0.05).

RESULTS

The result on body weight gain and some morphometric traits assessed in male rabbits fed dietary levels of *Vernonia amygdalina* Leaf Meal is presented in Table 2. Levels of *Vernonia amygdalina* leaf meal had a significant (P<0.05) impact on weight gain and feed intake of the bucks. The highest average daily weight gain (6.29g) was recorded in bucks fed the 10% VLM with the least (3.21g) in bucks fed 15% VLM. Daily feed intake increased in bucks fed VLM 5-15%; this ranged from 101.01 to 108.50 g compared to that of bucks fed diets without VLM (97g). Among the morphometric traits assessed, only body length of the rabbits reduced (P<0.05) with VLM levels. The highest body length (39.88cm) was recorded in bucks fed 0%VLM diet. Heart rates (Figure 1) and rectal temperatures (Figure 2) were not adversely affected by levels of VLM fed to the rabbits. Heart rate ranged from 124.80 to 126.68 beats per minute while rectal temperature ranged from 38.41 to 38.60 °C; VLM had no significant effect on the thermoregulatory parameters.

Table 3 showed the serum cholesterol, glucose and haematological indices in rabbit bucks fed dietary level of *Vernonia amygdalina* leaf meal. Male rabbits fed VLM10 had lower (P<0.05) cholesterol (76.36mg/dL) compared to those fed other diets. A significantly (P<0.05) higher serum glucose (121.36mg/dL) was recorded in rabbit bucks fed VLM0; which was 27.8, 39.7 and 19.1 % higher than that of bucks feed VLM5, 10 and 15 respectively. Haematological parameters assessed did not increase or reduce significantly (P>0.05) with dietary levels of VLM; however, PCV, haemoglobin and RBC values were lower in bucks fed with diets with 5 and 10% VLM compared with the control group. Platelet count was significantly (P=0.01) lower in bucks fed 10 and 15% VLM diets (13.87 and 16.03 $x10^4$µl respectively) compared with that of bucks fed diet with no VLM ($28.53x10^4$ µl). The values obtained for Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) are presented in Figure 3. Rabbits fed diets with *Vernonia amygdalina* leaf meal had lower ALT and AST levels. Male rabbits fed diets with 15% VLM had the highest ALT level (31.60IU/L) and AST (31.20IU/L) which

were significantly (P<0.05) higher than values recorded in those fed VLM0. The relative weights of some organs in male rabbits fed levels of *Vernonia amygdalina* leaf meal (VLM) as presented in Table 4 showed no significant (P>0.05) effect on the weights of the organs assessed.

Table 2: Weight-gain and Morphometric Traits of Rabbit Bucks Fed *Vernonia amygdalina* leaf meal

Parameters	Experimental Diets				SEM	P value
	VLM0	VLM5	VLM10	VLM15		
Weight Indices:						
Initial weight (g)	1801	1828	1823	1821	52.65	0.9856
Final weight (g)	2291	2155	2352	2091	73.18	0.0619
Total weight gain (g)	490.3^{ab}	327.5^{bc}	528.5^{a}	269.5^{c}	62.8	0.0158
Daily weight gain (g)	5.84^{ab}	3.89^{bc}	6.29^{a}	3.21^{c}	0.75	0.0158
Daily feed intake (g)	97.0^{ab}	101^{ab}	108.5^{a}	106.4^{ab}	2.98	0.0156
Morphometric Traits:						
Abdominal Circumference (cm)	28.8	28.8	29.0	27.9	0.54	0.451
Body Length (cm)	39.9^{a}	37.1^{b}	38.2^{b}	37.8^{b}	0.46	0.011
Ear Length (cm)	11.2	11.7	11.6	11.1	0.12	0.056
Head Length (cm)	11.9	11.4	11.4	11.5	0.13	0.226
Tail Length (cm)	9.85	8.49	9.72	9.97	0.18	0.106

VLM- *Vernonia amygdalina* leaf meal; VLM0- diet without VLM (control diet), VLM5- diet with 5% VLM, VLM10- diet with 10% VLM and VLM15- diet with 15% VLM; a, b, c -Means in the same row with different superscript are significantly (P< 0.05) different SEM – standard error of means

Table 3: Cholesterol, Glucose and Haematological indices in rabbit bucks fed *Vernonia amygdalina* leaf meal

Parameters	Experimental Diets					P value
	VLM0	VLM5	VLM10	VLM15	SEM	
Cholesterol (mg/dL)	120.5^{a}	116.4^{a}	76.4 ^{b}	87.1^{ab}	9.09	0.023
Glucose (mg/dL)	121.4^{a}	87.7^{b}	73.2 ^{b}	98.1^{ab}	5.51	0.017
Packed cell volume (%)	39.0	32.4	31.3	41.0	2.42	0.441
Hemoglobin (g/dl)	13.1	10.8	10.4	13.4	0.80	0.466
Red Blood cell (x10^{6}µL)	6.42	5.25	5.18	6.80	0.42	0.471
White Blood cell (x10^{6}µL)	9.22	7.70	9.62	7.09	0.45	0.167
Platelet (x10^{4}µL)	28.5^{a}	21.0^{ab}	13.9^{b}	16.0^{b}	2.15	0.013
Lymphocyte (%)	66.7	58.0	57.0	66.3	2.85	0.542
Neutrophil (%)	28.3	37.6	39.3	30.3	2.84	0.522
Monocyte (%)	2.67	1.60	1.67	2.25	2.25	0.191
Eosinophils (%)	2.33	2.80	2.00	1.25	0.31	0.289

VVLM- *Vernonia amygdalina* leaf meal; VLM0- diet without VLM (control diet), VLM5- diet with 5% VLM, VLM10- diet with 10% VLM and VLM15- diet with 15% VLM; a, b, c -Means in the same row with different superscript are significantly (P< 0.05) different

TABLE 4: Relative organ weights (%) of rabbit bucks fed *Vernonia amygdalina* leaf meal

Parameters	Experimental Diets				SEM	p value
	VLM0	VLM5	VLM10	VLM15		
Heart	0.20	0.22	0.21	0.20	0.01	0.630
Liver	2.72	2.61	2.49	2.34	0.14	0.238
Kidney	0.43	0.48	0.49	0.50	0.03	0.389
Spleen	0.03	0.03	0.04	0.04	0.01	0.725
Lungs	0.37	0.38	0.40	0.35	0.05	0.967
Gall bladder	0.03	0.02	0.03	0.05	0.01	0.161

VLM- *Vernonia amygdalina* leaf meal; VLM0- diet without VLM (control diet), VLM5- diet with 5% VLM, VLM10- diet with 10% VLM and VLM15- diet with 15% VLM; a, b, c -Means in the same row with different superscript are significantly (P< 0.05) different

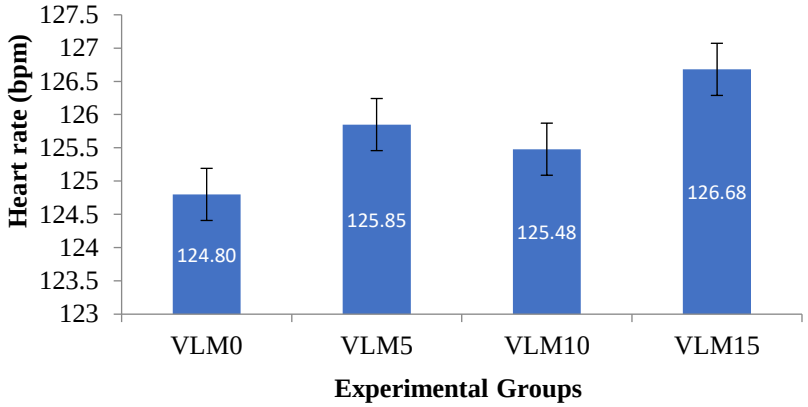

Figure 1: Respiratory rate of male rabbits fed diets with *Vernonia amygdalina* leaf meal

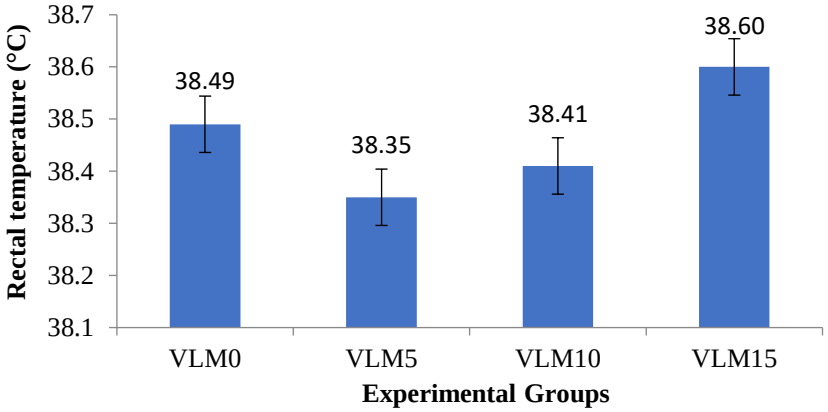

Figure 2: Rectal temperature of male rabbits fed diets with *Vernonia amygdalina* leaf meal

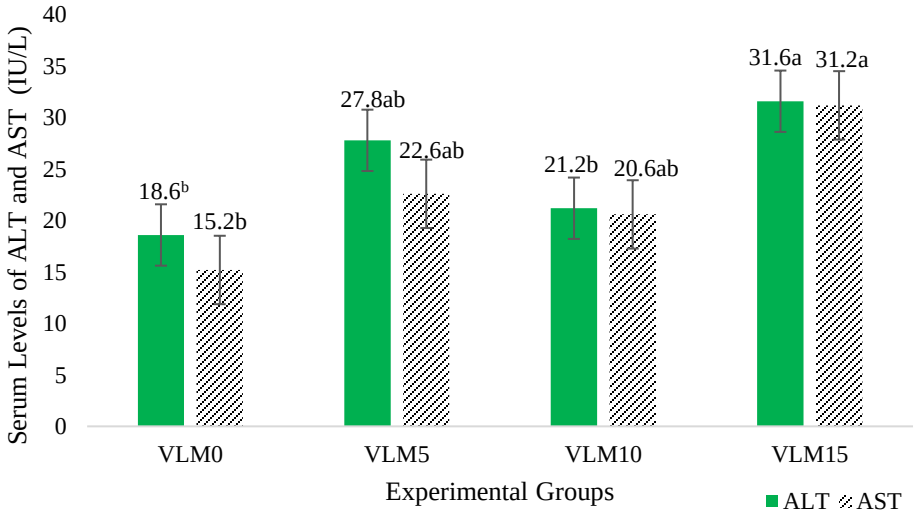

Figure 3: Serum levels of ALT and AST in male rabbits fed diets with *Vernonia amygdalina* leaf meal

DISCUSSION

The weight gained by an animal within a specific period is an important indicator of its growth. Some bioactive compounds can modulate lipid metabolism thereby impacting adipose tissue formation in a positive manner to influence weight gain (Sandner *et al.*, 2020). VLM at 10% may be adopted in feeding adult male rabbits. The increase in daily feed intake of rabbit bucks fed the VLM diet showed that the diet was acceptable and palatable to the animals. It may also imply that the animals consumed more to meet their daily maintenance need. The significantly lower body length recorded in the bucks fed VLM diets (37.1 -38.2 cm) is at variance with the findings of Ogbuewu *et al*, (2011) who reported no significant effect of neem leaf meal on the body length of rabbit bucks. Physiological parameters such as heart rate, pulse rate, rectal temperature and respiratory rates are indicators of an animal's well-being. Heart rates of bucks in this study ranged from 124.80 to 126.68 beats/minute, and rectal temperatures of 38.41 to 38.60°C; this is an indication that VLM had no adverse effect on thermoregulation in the rabbits. The heart rates in this study are lower than 143.56 - 184.95 hbpm but the rectal temperature is similar (37.29 to 38.08 °C) to the values recorded in exotic breeds of rabbit managed in Ibadan, Nigeria (Jimoh and Ewuola, 2018).

Saponin inhibits cholesterol absorption in the intestine causing a hypocholesterolemic effect (Amany-Ali *et al.*, 2019). The decrease in cholesterol levels observed in the VLM groups may be a result of the effect of saponin content in the leaf meal. This corroborates the findings of Oloruntola *et al.* (2016) who reported reduced cholesterol levels in rabbits fed *Alchornea cordifolia* leaf meal-based diets. A decrease in the cholesterol level of broiler chicks administered *Vernonia amygdalina* extract at 50g of dried leaf /L of water has been earlier reported (Oleforuh-Okoleh *et al.*, 2015). Reduction in the serum glucose of male rabbits fed VLM is an indication of the hypoglycemic property of *Vernonia amygdalina* induced by substances such as glycosides that act as alpha-glucosidase inhibitors present in the plant (Yeap *et al.*, 2010). A similar study also reported that dietary levels of water spinach leaf meal reduced serum glucose levels in rabbits (Ahemen *et al.*, 2013). VLM had a non-significant effect on the haematological parameters assessed. A decrease in blood platelet count in bucks fed VLM diets (13.87

- 21.02 x10^4 ul) may be an indication of thrombocytopenia as normal platelet in adult male rabbits range from 30.4 to 65.6 x10^4 ul (Moore et al, 2015). Phytochemical analysis of the *Vernonia amygdalina* leaves yielded two sesquiterpene lactones known as vernolide and vernodalol (Erasto *et al*., 2006). Vernolepin isolated from *Vernonia amygdalina* plant induces anti-platelet activity (Yeap *et al*., 2010). Sesquiterpene lactones in *Capparis decidua* (Forsk.) have been reported to have potent anti-platelet activity (Mohammed *et al*., 2014). The low platelet count recorded in bucks fed with VLM5-15 may be due to the presence of bioactive secondary metabolites and sesquiterpene lactone compounds in VLM. A depressed or elevated level of serum enzymes indicated the alteration of specific organs (Lala *et al*., 2022). Serum levels of Alanine Aminotransferase and Aspartate Aminotransferase increased by 41% and 51% respectively in rabbits fed VLM15. However, the values recorded (ALT -18.6 -31.6; AST -15.2-31.2 IU/L) were lower than the range ALT (49-79 IU/L) and AST (42-98 IU/L) reported by Mitruka and Rawnsley (1981) but higher than the values (ALT -5.00-8.00; AST -7.00-19.00 IU/L) recorded by Ozkan *et al*., (2012) for New Zealand rabbits. The increase in serum ALT and AST concentration in rabbits fed VLM implies that bioactivity substances in the leaf meal can alter the integrity of the liver and kidney or other organs when consumed in higher quantities. Since no shrinkage or enlargement of organs was recorded in this study, histological evaluation of the organs may give a clearer impact on the elevated serum levels of ALT and AST. This is in agreement with the findings of Owen *et al.* (2011) who reported no adverse effects of VLM on the organs of broiler finisher chicken. Decreased organ weight was reported in broiler chicken with an increase in the dietary levels of *Vernonia amygdalina* (Ndelekwute *et al.*, 2017).

CONCLUSION

The study revealed that *Vernonia amygdalina* leaf meal (VLM) can be fed to male rabbits up to 10% in a maintenance diet with no detrimental effects on the morphometric traits. Also, the health status of the rabbits was not compromised as rectal temperature, heart rate, haematological indices and organ weights were similar for the groups. An increase in the level of serum enzymes did not result in shrinkage or enlargement of the organs. The cholesterol and glucose-lowering ability of VLM was elucidated in this study. This suggests that VLM could be included in the diet of mature male rabbits up to 10% without depressing the overall health and performance of the animals.

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