Nigerian J. Anim, Sci. 2025, 27 (1): 60 - 72

Haematological and Serum Biochemical Indices of Rabbits Fed Diets Supplemented with Mashed Lime Fruit Juice as an Alternative to Antibiotics

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Targeted Audience: Rabbit Farmers, Animal Nutritionists, Extensionists and Students

Abstract

The study evaluated the haematological and serum biochemical indices of rabbits fed diets supplemented with mashed lime fruit juice (MLFJ) as an alternative to antibiotics. The experiment was conducted with 30 rabbits with an average age of 11 weeks old and were assigned to 6 dietary treatments in a completely randomised design with five replicates each. A basal diet was formulated containing 0, 500, 1000, 1500 and 2000 ml of MLFJ per 100kg representing treatments T1–T5 respectively while T6 contain 55 g/100kg Oxytetracycline. The treatments T1 and T6 served as controls. All data generated were subjected to analysis of variance using General Linear Model procedure of the Statistical Analysis System (SAS) software. Significant differences between treatment means were separated using Duncan Multiple Range Test of the same software. Result showed significant differences (P<0.05) in all blood parameters considered except alkaline phosphatase. Pack cell volume was lowest with rabbits fed 500 ml MLFJ while other levels were higher and similar. Total protein was significantly (P<0.05) higher at 2000 ml but was similar to 0 ml and Oxyt. It was concluded that growing rabbits could tolerate dietary MLFJ up to 2000 ml/100kg without negative effect on haematological and serum biochemical indices.

Keywords: organic acid, lime, antibiotics, haematology, rabbits

Description of Problem

High level of production, least cost feed and efficient feed conversion are the needs of the modern rabbit production and this to a certain extent could be achieved by the use of specific feed additives (1). Antibiotic growth promoters (AGPs) such as Zinc bacitracin among others is among the most used AGP in rabbit feed (2). They stabilised the intestinal microflora and prevented some specific

intestinal pathogens leading to better gain and feed conversion (3). However, despite these good qualities, it has been observed that antibiotics have their negative impact because of their residual effect in both animals and humans leading to public health concern (4, 5). This had necessitated the search for alternatives to AGPs. As alternative to AGPs, organic acids among others have been reported to be efficacious by modifying intestinal pH, improving digestion and absorption of nutrients (6). However, organic acids currently in use are synthetic types which are very expensive. Additionally, farmers may not have access to them, hence the need to explore and exploit natural sources which can compare favourably with AGPs (7).

Fruits are major sources of organic acids especially *Citrus spp* (lime, lemon and grape) which contain citric and ascorbic acids and could serve these purposes. It is reported by (1) that lime fruit juice contains 0.6% ascorbic and 8.02% citric acids. Haematological parameters yield information about the red blood cell population and leucocytes response to stress and pathogens (8). The blood in an animal serves as a transport medium. It transports food materials such as glucose, fatty acids, vitamins and electrolytes from the gastrointestinal tract to body tissues where they are utilised for body building and energy. Increase or decrease in body weight from the previous weight for a specific period is the principal measure of productivity in meat animal and this depends on the quality and to a lesser extent the quantity of feed given (9). This means that the weight, feed and blood are related (10). It has been established that feed components affect blood constituents (11). Haematological parameters can thus be used to assess the effects of the test ingredient. There is dearth of information on haematological and serum biochemical parameters of rabbits fed diets supplemented with mashed lime fruit juice. Therefore, the study assessed the impact of mashed lime fruit juice on haematological and serum biochemical parameters of growing rabbits to ascertain its safety as an alternative to antibiotics.

Materials and Methods

Study location

The study was conducted at the Rabbit Research Unit of Swine and Rabbit Research Programme of the National Animal Production Research Institute, Shika-Zaria, Kaduna state, Nigeria. Zaria is located in the Northern Guinea Savannah ecological zone. The area lies between latitude 10°11' N and longitude 7°8' E, and 650 m above sea level (12). The dry season begins in the middle of October, with dry cold weather that ends in February. This is followed by relatively hot dry weather from March to May, when the rain begins. The mean minimum and maximum daily temperatures are from 14 to 24°C during the cool season and from about 19 to 38.3°C during the hot season. The relative humidity varies between 19 and 35% in the dry season and between 63 and 85% in the wet season. The wet periods are between May and October characterised by annual rainfall ranging from 700 to 1400 mm, (13).

Processing of lime juice

Green lime fruits were harvested from a farm in Gboko Local Government Area of Benue State and some were purchased from Samaru market, Sabon Gari Local Government Area, Kaduna State, Nigeria. The lime fruits were mixed, washed and cut into pieces with a

sharp knife and mashed (ground) using a grinding machine. Cheesecloth was used to extract the juice. The juice was stored in a refrigerator at a temperature of 4 degree Celsius to reduce oxidation of bioactive compounds in the juice.

Experimental design and management of growing rabbits

The experiment was conducted with 30 apparently healthy mixed breed growing male rabbits with an average age of 11 weeks old. The rabbits were assigned to six dietary treatments (T1, T2, T3, T4, T5 and T6) in completely randomized design and T1 and T6 served as negative and positive controls, respectively. Five growing rabbits were assigned to each dietary treatment and each rabbit served as a replicate. The rabbits were treated for internal and external parasites by injecting *Ivermectin* Super® at 0.2 ml/kg live weight and oral administration of coccidiostat (Amprolium® at 1 g to 1 L of drinking water) before the commencement of the trial. They were housed individually in galvanised wire cages of 60 x 60 x 40 cm in a well-ventilated building. The rabbits were fed weighed quantities of the dietary treatments daily in flat bottom earthen feeders at 09:00 hours. Feed leftover and/or wastage was collected, the fur removed, weighed and recorded daily before feeding. Routine management practices were carried out daily. The trial lasted for 56 days.

Experimental diets

A basal diet formulated as shown in Table 1 to which were added 0, 500, 1000, 1500 and 2000 ml of MLFJ per 100 kg feed was added to the basal diet to form treatments T1 – T5, respectively while T6 was formed by the addition of 55 g/100 kg Oxytetracycline to the basal diet as recommended by the manufacturer. Ten kilogrammes of the feeds were compounded for each treatment every 10 days.

Blood sample collection and haematological study

On the last day of the feeding trial, three rabbits per treatment were selected for the evaluation of haematological indices. The blood characteristics were used for evaluation of the health status of animals and the level of their metabolism. Blood samples were collected at 08:00 hours from the external ear veins of each rabbit by vein puncture using 2 ml disposable syringes and 21 G needles. The areas punctured were sterilised with methylated spirit swab and 2 ml of blood samples were collected into labelled universal sterile bottles containing Ethylene Di-amine Tetra Acetic Acid (EDTA) as anti-coagulant for the determination of haematological parameters as described by (14). Parameters that were determined include packed cell volume (PCV), red blood cell (RBC), haemoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cells count, neutrophils, lymphocytes, monocytes, eosinophils and basophils.

Blood sample collection and serum biochemical study

Another 3 ml of blood samples was collected from the same rabbits into EDTA free bottles for serum biochemical analysis as described by (14). The blood was allowed to clot by allowing it to stand for 2 hours at room temperature and then centrifuge using Wintrobes Microhaematocrit for 10 minutes

Table1: Ingredients Composition and Calculated Nutrients Analysis of the Experimental Diet Fed to Growing Rabbits

Ingredients	Quantity (%)
Maize	34.85
Groundnut cake	20.00
Maize offal	22.00
Rice offal	20.00
Bone ash meal	2.00
Common salt	0.30
*Vitamin/mineral premix	0.25
Lysine	0.30
Methionine	0.30
Total	**100.00**
Calculated Nutrient Analysis	
Metabolisable energy, (kcal/kg)	2515.93
Crude protein, %	16.00
Crude fibre, %	12.74
Ether extract, %	4.43
Calcium, %	0.83
Available phosphorus, %	0.41
Lysine, %	0.71
Methionine + cystine, %	0.53

*Biomix broiler starter premix supplies the fol lowing per kg diet: vit. A1000 IU; Vit. D3, 2000 IU ; Vit. E, 5.0mg; Vit. K, 2mg; Vit. B1, 1.8mg; Vit B2, 5.5mg; Niacin, 27.5mg; Pantothenic acid, 0.5mg; Vit. B6, 0.30mg; Vit. B12, 0.015mg; Folic acid, 7.5mg; Biotin, 0.06mg; Choline Chloride, 300mg; Cobalt, 0.2mg; Copper, 3mg; Iodine, 1mg; Iron, 20mg; Manganese, 40mg; Selenium, 0.2mg; Zinc, 30mg; Antioxidant, 1.25mg.

at 2000 rpm to separate blood cells from serum. Samples were preserved at 4°C until analysed at Histology Preparation Laboratory, Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria. Parameters that were determined include; total serum protein, albumin and globulin levels, glucose and cholesterol levels, total bilirubin, conjugated bilirubin, creatine, uric acid, aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase.

Statistical Analysis and Model

Data generated in the study were subjected to Analysis of Variance as described by (15) using General Linear Model procedure of the Statistical Analysis System (16) software package. Significant differences between treatment means were separated using Duncan Multiple Range Test (17) of the same software. Below is the model for the analysis:

$Y_{ij} = \mu + T_i + e_{ij}$

Where,

Y_{ij} = performance of j^{th} rabbit fed i^{th} diet

μ = the overall mean of observations of interest

T_i = the fixed effect of the i^{th} treatment

e_{ij} = random error

Results

Table 2 shows the haematological parameters of growing rabbits fed diets supplemented with mashed lime fruit juice. There were significant differences (P<0.05) in all parameters considered in this study. Pack cell volume (PCV) was lowest with rabbits fed 500 ml MLFJ while other levels were higher and similar. Red blood cell (RBC) was significantly (P<0.05) higher at 1000 ml and was similar to 2000 ml and Oxyt. Rabbits fed 1500 ml had higher RBC compared to those fed 500 ml but were similar to 0, 2000 ml and Oxyt while 0 and 500 ml were also similar (P>0.05). Haemoglobin (Hb) was significantly (P<0.05) higher at 0 ml compared to 500 ml and was similar to 1500 ml while others were similar. For mean corpuscular volume (MCV), rabbits fed 0, 1000, 1500 and 2000 ml of MLFJ were similar while Oxyt had similar values with 500, 1000, 1500 and 2000 ml. For mean corpuscular haemoglobin (MCH), rabbits fed 0, 1500 and 2000 ml had significantly (P<0.05) higher values, while 2000 ml was similar to 500, 1000 ml and Oxyt. For mean corpuscular haemoglobin concentration (MCHC), rabbits fed 500 ml were significantly (P<0.05) superior to 0, 1000, 2000 ml and Oxyt. On the other hand, rabbits fed 1500 ml showed no significant difference (P>0.05) to all levels of MLFJ. White blood cell (WBC) was significantly (P<0.05) higher at 1000 ml. Neutrophils

Table 2: Haematological Parameters of Growing Rabbits Fed Diets Supplemented with Mashed Lime Fruit Juice

	Treatment Levels of Mashed Lime Fruit Juice (ml)							
Parameters	**T1(0)**	**T2(500)**	**T3(1000)**	**T4(1500)**	**T5(2000)**	**T6 (Oxyt 55g/100kg)**	**SEM**	**Reference values**
PCV (%)	35.60^{e}	29.97^{d}	39.43^{a}	37.10^{bc}	38.43^{ab}	38.80^{ab}	1.13	$30\text{-}50^{M\&R}$
RBC ($x10^{6}$l)	5.18^{bc}	4.76^{c}	5.99^{a}	5.46^{b}	5.77^{ab}	5.90^{ab}	0.22	$3.8\text{-}7.90^{M}$
Hb (g/dl)	11.13^{b}	9.60^{c}	12.27^{a}	11.73^{ab}	12.03^{a}	12.13^{a}	0.37	$9.4\text{-}17.4^{M}$
MCV (fl)	68.67^{a}	63.27^{c}	66.13^{ab}	68.07^{ab}	66.70^{ab}	65.70^{bc}	1.28	$50\text{-}75^{M}$
MCH (pg)	21.50^{a}	20.30^{b}	20.50^{b}	21.50^{a}	20.90^{ab}	20.57^{b}	0.45	$18\text{-}24^{M}$
MCHC (g/dl)	31.30^{b}	32.07^{a}	30.97^{b}	31.60^{ab}	31.30^{b}	31.30^{b}	0.33	$27\text{-}34^{M}$
WBC ($x10^{3}$/l)	5.70^{c}	5.87^{c}	12.17^{a}	8.03^{b}	7.07^{bc}	5.70^{c}	1.01	$5.5\text{-}16.50^{H}$
Neutrophils (%)	28.90^{bc}	30.83^{b}	35.20^{a}	35.67^{a}	26.40^{c}	38.03^{a}	1.73	$18.8\text{-}46.4^{Z}$
Lymphocytes (%)	58.90^{ab}	56.07^{bc}	44.10^{e}	51.17^{cd}	63.30^{a}	46.10^{de}	3.23	$43\text{-}80^{M}$
Monocytes (%)	8.40^{b}	9.37^{b}	14.60^{a}	10.37^{ab}	7.13^{b}	10.23^{b}	2.01	$4\text{-}12^{Z}$
Eosinophils (%)	2.93^{cd}	3.37^{cd}	5.87^{a}	4.13^{bc}	2.67^{d}	5.00^{ab}	0.7	$0.5\text{-}3.50^{Z}$
Basophils (%)	0.87^{a}	0.37^{d}	0.23^{e}	0.57^{bc}	0.50^{c}	0.63^{b}	0.05	$0\text{-}0.84^{M}$

abc...e Means with different superscript along rows are significantly different at (P<0.05); Oxyt = Oxytetracycline; SEM = Standard error of means; PCV = Packed cell volume; RBC = Red blood cell; Hb = Haemoglobin concentration ; MCV = Mean corpuscular volume; MCH = Mean corpuscular haemoglobin; MCHC = Mean corpuscular haemoglobin concentration; WBC = White blood cell; M&M = Mitruka and Rawnsley, 1977; M = Medirabbit, 2011; H = Hewitt et al. 1989; Z = Zimmerman et al. 2010.

values were significantly (P<0.05) higher at the Oxyt group but similar to 1000 and 1500 ml. Neutrophils were significantly (P<0.05) higher at 500 ml compared to 2000 ml but were similar to 0 ml. Lymphocytes values were significantly (P<0.05) highest at 2000 ml but similar to 0 ml which was also similar to 500 ml. The lowest value of lymphocytes was obtained at 1000 ml but was similar to Oxyt which was similar to 1500 ml. Rabbits fed 500 ml and 1500 ml also had similar values of lymphocytes. Monocytes were significantly (P<0.05) higher at 1000 ml but were similar to 1500 ml while all others were similar. Eosinophils values of rabbits fed 1000 ml were significantly (P<0.05) higher but were similar to Oxyt. The lowest value was recorded at 2000 ml which was similar to 0 and 500 ml while 1500 ml was also similar to 0 and 500 ml. Basophils values of rabbits fed 0 ml were significantly (P<0.05) higher while those fed 1000 ml had the lowest value. Rabbits fed 2000 ml had significantly (P<0.05) higher value compared to those fed 500 ml but was lower than those fed Oxyt which was similar to those fed 1500 ml.

Table 3 shows the serum biochemical indices of growing rabbits fed diets supplemented with mashed lime fruit juice. There were significant differences (P<0.05) in all parameters measured except ALP. Total protein (TP) of rabbits fed 2000 ml was significantly (P<0.05) higher but was similar to those fed 0 ml and Oxyt. Rabbits fed 0 and 1000 ml had significantly (P<0.05) higher TP compared to those fed 1500 ml but were lower than those fed 0 ml which was similar to 2000 ml and Oxyt. Albumin was significantly (P<0.05) higher at 0 ml but was similar to 2000 ml while the lowest value was at 1500 ml which was similar to Oxyt. Rabbits fed 1000 ml had similar value with 500 ml and Oxyt while 500 and 2000 ml were also similar. Globulin was significantly (P<0.05) higher at Oxyt while 0 ml had the lowest value which was similar to 500 and 1500 ml while others were similar. Glucose was significantly (P<0.05) higher at 0 ml. This was followed by 500, 2000 ml and Oxyt which were similar. The least values were recorded at 500 ml which was similar to 0 ml, and 1500ml was similar to 0 and 2000 ml. Aspartate amino transferase (AST) was significantly (P<0.05) highest in the blood of rabbits fed 1500 ml while the lowest value was recorded in the blood of rabbit fed 1000 ml. AST was significantly (P<0.05) higher in the blood of rabbits fed 2000 ml compared to those fed 0 ml but was significantly (P<0.05) lower in the blood of those fed 500 ml and at Oxyt. Alanine amino transferase (ALT) was significantly (P<0.05) higher in the blood of rabbits fed 1500 ml diet while the lowest value was obtained in the blood of rabbits fed 0 ml diet. ALT was significantly (P<0.05) higher in the blood of rabbits fed 1000 ml compared to those fed 500 ml and Oxyt but was significantly (P<0.05) lower in the blood of rabbits fed 2000 ml diet.

Discussion

Haematological indices are indices and reflections of the effects of dietary treatments on the animal in terms of the type, quality and amounts of the feed ingested and available for the animal to meet its physiological, biochemical and metabolic necessities (18). In this study it was observed that the MLFJ treated groups had better PCV than the negative control except for the 500 ml group and were comparable to the positive control. Meanwhile, all the values were within the normal range (30-50%) for healthy rabbits (19). PCV is an index of toxicity and

Table 3: Biochemical Indices of Growing Rabbits Fed Diets Containing Graded Levels of Mashed Lime Fruit Juice

Parameters	Treatment Levels of Mashed Lime Fruit Juice (ml)						SEM	Reference values
	T1(0)	T2(500)	T3(1000)	T4(1500)	T5(2000)	T6(Oxyt 55g/100kg)		
TP, g/dl	7.94^{abc}	7.47^{c}	7.58^{bc}	6.73^{d}	8.32^{a}	8.14^{ab}	0.29	$5.0\text{-}8.0^{M\&R}$
ALB, g/dl	5.77^{a}	4.99^{bc}	4.73^{c}	4.22^{d}	5.39^{ab}	4.56^{cd}	0.25	$2.7\text{-}5.0^{M}$
GLB, g/dl	2.17^{c}	2.50^{bc}	2.94^{b}	2.5^{bc}	2.92^{b}	3.58^{a}	0.28	$2.5\text{-}4.50^{B}$
GLC, mg/dl	98.26^{a}	83.72^{b}	67.43^{c}	68.07^{c}	86.44^{b}	83.94^{b}	5.28	$8.10\text{-}183.0^{H}$
CHO, mg/dl	70.08^{cd}	64.59^{d}	83.82^{a}	74.06^{bc}	78.22^{ab}	82.30^{a}	3.14	$10.0\text{-}80^{M}$
AST (u/l)	56.33^{d}	70.67^{b}	48.33^{e}	85.67^{a}	68.33^{c}	71.67^{b}	0.53	$10.0\text{-}98.0^{M}$
ALT (u/l)	137.27^{e}	181.30^{d}	187.30^{c}	216.43^{a}	190.83^{b}	180.40^{d}	0.73	$55.0\text{-}260.0^{M}$
ALP (u/l)	52.00	82.67	65.67	80.00	76.67	95.00	25.35	10.0-96.0M

abc...e Means with different superscript along rows are significantly different at ($P<0.05$); SEM = Standard error of means; TP = Total protein; ALB = Albumin; GLB = Globulin; GLC = Glucose; CHO = Cholesterol; AST = Aspartate aminotransferase; ALT = Alanine aminotransferase; ALP = Alkaline phosphatise; M&M = Mitruka and Rawnsley, 1977; B= Burke, 1994; H = Hewitt et al. 1989; Medirabbit, 2011.

reduction in the blood usually suggest the presence of a toxic factor which has adverse effect on blood formation (20). The high PCV observed in this study indicated that mashed lime fruit juice was suitable for use as a feed additive in rabbit production. The results obtained from this research was not in agreement with the work of (21) and (22) where DLP was supplemented in broiler chicken diets and no significant effects were observed in PCV. RBC differed significantly ($P<0.05$) among treatments and all values were within the normal range (3.8-7.90) for healthy rabbits (23). The RBC counts according to (24) are influenced among other factors by nutrition, physical activities and volume. Its reduction indicates anaemia. In this study, there were no clinical signs of anaemic condition. This result was an indication that MLFJ had no negative effect on the RBC instead the test ingredient showed ability to improve this parameter. Hb values for all the MLFJ treated groups except the 500 ml group were superior to the negative control and similar to the positive control. All fell within the normal range (9.4-17.4 g/dl) for healthy rabbits (23). The lowest values recorded by the 1500 ml group in most of the parameters considered as mentioned earlier could not be attributed to the effect of dietary MLFJ because even the groups that received higher doses of the juice were within the normal range for healthy rabbits. Though the erythrocyte constants (MCV, MCH, and MCHC) were significantly ($P<0.05$) different among treatments, they were within the normal range (50-75 fl; 18.7-24 pg; 27-34 g/dl respectively) for healthy rabbits (23). These results were indications that MLFJ had no negative effect on the blood indices. WBC values obtained were within normal range ($5.5\text{-}16x10^{3}/l$) for healthy rabbits (25). An increase in WBC count above normal as reported by (26) is an indication of the presence of exogenous substances and or foreign bodies in the blood. Since the values were all within the normal range, it indicated that the MLFJ did not negatively affect WBC and was safe to be used as a feed additive in rabbit production. The values of neutrophils obtained in this study were higher than those reported by

(22). They were however within the normal range (18.8–46.4%) for healthy rabbits (Hewitt, *et al.*, 1989). This is an indication of the safety of MLFJ in the diet of rabbits. This result is in consonance with the work of (22) when they orally administered lime fruit juice to broiler chickens. Lymphocytes values were within the normal range (43-80%) for healthy rabbits (23); signifying that MLFJ did not pose a threat to the heath of rabbits. This result agreed with the result of. (27) and (22) who reported significant ($P<0.05$) differences in their studies with lemon juice in broiler chickens. Monocytes were within the normal range (4-12%) for healthy rabbits (28). This result disagreed with (27) who observed no significant differences in their study with lemon juice in broiler chickens' diets. Eosinophils were above the normal range (0.5-3.5%) for rabbits (28) at 1000 and 1500 ml and the positive control. Eosinophilia in rabbits can occur when tissues rich in mast cells, such as the skin, lungs, gastrointestinal tract or uterus, are involved in disease. Eosinophilia can indicate the presence of an abscess and may be found during wound healing. In other species, eosinophilia is linked to parasitic diseases, especially when larvae are moving through the tissues, but this is rare in domestic rabbits (8). However, the significantly higher values recorded at 1000 could not be attributed to the dietary MLFJ because even the 2000 ml that received higher dose and the 500 ml that received lower dose of the juice were within the normal range for healthy rabbits. Basophils differed significantly ($P<0.05$) among treatment means with the MLFJ having lower values which were all within the normal range (0-0.84%) for rabbits (23). The higher value recorded at the negative control was above the normal range, an indication that they might have been fighting against some foreign agents in the diet. On the other hand, the lower values obtained from the mashed lime fruit juice treated groups were indications that there were no foreign agents in the diets. This result was in agreement with the values reported by (27) which was a demonstration of the antibacterial effect of MLFJ.

Serum protein according to (29) is mainly synthesised in the liver and played important functions including but not limited to maintenance of blood volume through the colloidal osmotic effect, buffer blood pH, transport hormones and drugs, participate in cell coagulations and in the body defence against foreign agents, catalyse chemical reactions (enzymes) and regulate the metabolism (hormones). The differences observed in TP, albumin and globulin due to the effect of MLFJ had no particular sequence; however, values were comparable to the controls. TP values were within the normal range (5-8.0 g/dl) reported by (19) for healthy rabbits except 2000 ml and Oxyt that were slightly above the normal range. According to (8) the main cause of hyperproteinemia in rabbits is dehydration. The high TP value might be as a result of dehydration due to cases of coccidiosis and diarrhoea. According to (30), coccidiosis in rabbits has two forms, namely hepatic and intestinal form. The affected animals show symptoms of diarrhoea, reduced appetite, dehydration, and weight loss as well as liver and intestinal lesions. Similarly, albumin values for MLFJ and the positive control were within the normal range (2.7-5.0 g/dl) reported by (19) for healthy rabbits except at 2000 and 0 ml. A high serum albumin concentration according to (31) is not a

failure of any specific disease. Although an increased albumin level in conjunction with a high PCV is indicative of dehydration. Globulin values fell within the range of 1.5 to 3.3 g/dl and 2.5-4.50 g/dl reported by (32) and (33) respectively, for healthy rabbits. (34) reported this to be indications of nutritional adequacy of the dietary proteins since serum protein synthesis is related to the amount of available protein in the diet. The finding of this study corroborated the report of (35) that there was no alteration in the normal systemic protein utilisation as well as the proper functioning of the liver and that the experimental animals had good resistance to disease and corresponding high immunity. Glucose was significantly ($P<0.05$) influence by the levels of MLFJ and values were within the normal range (8.10-183.0 mg/dl) as reported by (25). The slight decreased in serum glucose observed in the MLFJ groups was an indication of normal energy metabolism. Cholesterol was significantly affected by MLFJ. However, the treated groups compared favourably with both controls and values obtained were within the normal range (10-80 mg/dl) for healthy rabbits as reported by (23) except 1000 ml and the positive control. The result of this study is contrary to the findings of (36) who reported significant reduction in cholesterol when they studied the effect of *C. aurantifolia* peel essential oil on serum triglyceride and cholesterol in Wister rats. The significant proliferation in the 1000 ml group could not be attributed to the effect of the MLFJ because even the negative control that received no MLFJ and those that received higher doses were within the normal range reported by (23). However, in this study it was not clear what could have been responsible for the occurrence. It might be as a result of human error since the animals were offered the same basal diet and hepatic enzymes values were within normal range for healthy rabbits. The dietary MLFJ caused significant variation ($P<0.05$) in the levels of two hepatic enzymes: AST and ALT across the treatment groups. Although the differences observed in these enzymes had no particular sequence, the significant variation in the concentration of these enzymes is an indication that the nature of the experimental diets was influence by the MLFJ which affected their synthesis. The AST and ALT values for the MLFJ treated groups were significantly ($P<0.05$) higher than 0 ml and were comparable to Oxyt except 1000 and 2000 ml for AST, but all the values were within the normal range (10-98 u/l and 55.0-260.0 u/l respectively) for healthy rabbits (23). The ALP values were not significantly ($P>0.05$) different across the treatments and were within the normal range (10.0-96.0 u/l) for healthy rabbits (23). Biochemical indices provide useful information about the health of the visceral organs especially the liver and the kidney (37). According (38), increase in serum AST, ALT and ALP were an indication of damage caused to the liver and kidney by toxins, which involved cellular destruction. It is important to note that AST, ALT and ALP levels did not reflect the function of the liver; they are only used to detect inflammation due to injury or damage to the liver from other sources (39). Even in conditions where AST, ALT and ALP were highly elevated, the liver was still functioning properly (39). The findings of this study indicated that MLFJ had no negative effect on the liver and kidney of the rabbits. This study agreed with the report of (40) that dietary lime fruit juice had no detrimental effect on the biochemical

functions of growing rabbits.

Conclusion and Application

It was concluded that dietary MLFJ up to 2000 ml/100 kg did not negatively affect haematological and serum biochemical functions of growing rabbits. Therefore, inclusion level up to 2000 ml/100 kg could be incorporated in the diet of rabbits.

Acknowledgement

I wish to express my gratitude to Prof. A.K. Mohammed, the Executive Director, National Animal Production Research Institute (NAPRI), Shika-Zaria, Kaduna state, Nigeria, for releasing the facilities used for the study. The contributions of the staff of Rabbit Unit and Central Laboratory Unit, NAPRI are well appreciated.

References

1. Tsavkar, D. T., Sekoni, A. A., Afolayan, M. and Iyeghe-Erakpotobor, G. T. (2021). Effect of mashed lime fruit juice as an alternative to antibiotics on growth performance, villi morphometry and caecal microflora of growing rabbits. *Journal of. Animal Production Research*, 33(2):21-32.
2. Falcao-e-Cunha, L., Castro-Solla, L., Maertens, L., Marounek, M., Pinheiro, V., Freire, J., Mour, J.L. (2007). Alternatives to antidote growth promoters in rabbit feeding: A review. *World Rabbit Science*, 15: 127-140.
3. Waldroup, A., Kanlawato, S., and Mauromuos-Takos, A. (2005). Performance characteristics and microbiological aspects of broilers fed diets supplemented with organic acids. *Journal of Food Protection*, 58: 68-72.
4. Dibner, J. (2004). Organic acids: can they replace antibiotic growth promoters. *Feed International*, 25(12): 14-16.
5. Paul, S.K., Samanta, G., Halder, G. and Biswas, P. (2007). Effect of combination organic acid salts as antibiotic replacers on the performance and gut health of broiler chickens. *Livestock Research and Rural Development*, 19: 115-120.
6. Adams, C. (1999). Poultry and dietary acids. *Feed International*, 20: 70-72.
7. Ndelekwute, E.K. and Enyenihi, G.E. (2017). Lime juice as a source of organic acids for growth and apparent nutrient digestibility of broiler chickens. *Journal of Veterinary Medicine and Surgery*, 1(1): 1-5.
8. Melilo, A. (2007). Rabbit clinical pathology. *Journal of Exotic Pet Medicine*, 16(3): 135-145. https://doi.org/10.1053/j.jepm.2007.06.002
9. Blood, D.C. and Studdert, V.P. (1999). Saunders comprehensive veterinary dictionary, 2nd Ed. W.B. Saunder, New York, pp. 88.
10. Ajayi, A.F. and Raji, Y. (2012). Haematological and serum biochemical indices of prepubertal male rabbits fed with graded level of blood wild sunflower forage meal mixture. *African Journal of Biotechnology*, 11(35): 8730-8734.
11. Harper, A.F., Rodwell, V.W. and Mayes, P.A. (1979). Review of Physiological Chemistry. 17th Ed.

Lang. Medical, Los Atlos, California p. 9422.

12. Ovimaps, (2019). Ovilocation map; ovi earth imaginary. Dated March, 2019.
13. IARMS, (2019). Institute for Agricultural Research Meteorological Station, weather report, Ahmadu Bello University, Zaria.
14. Baker, F.J., Silverton, R.E. and Palister, C.J. (1998). Baker and Silverton Introduction to Medical Laboratory Technology. 7th Edition. Hutterworth – Heinemann. Linancre House, Jordan Hill Oxford OX2 8OP.
15. Steel, R.G.D. and Torrie, J.H. (1980). Principles and Procedures of Statistics. Sydney. McGraw Hill Int. Books Co; 1980. Sun X. Broiler performance and intestinal alterations when fed drug free diets. M.Sc. Thesis, Virginia Polytechnic Institute and State University, Virginia, USA.2004; 96p.
16. SAS, (2002). Statistical Analysis System Institute Inc. *Users' Guide Statistic Version* 9. Carry, North Carolina, USA.
17. Duncan, D.B. (1955). Multiple Range and Multiple F. test Biometrics of Bacteriology. 1:1-4 Pp 11-45.
18. Ewuola, E.O., Folayan, O.A., Gbore, F.A., Adebunmi, A.I., Akanji, R.A., Ogunlade, J.T, Adeneye, J.A. (2004). Physiological response of growing West African goats fed groundnut shell-based diets as the concentrate supplements. *BOWEN Journal of Agriculture,* 1:61-69.
19. Mitruka, B.M. and Rawnsley, H.M. (1977). Clinical, Biochemical and Haematological References Values in Normal Experimental Animals, Masson Publishing, USA. Inc. New York, pp. 83-109.
20. Oyawoye, E.O. and Ogunkunle, M.O. (1998). Chemical analysis and biochemical effect of raw jack beans on broiler. *Proceedings of the Silver Anniversary Conference of Nigerian Society for Animal Production (NSAP) and the Inaugural Conference of the West African Society for Animal Production (WASAP),* held at Gateway Hotel, Abeokuta, Nigeria. 21th-26th March 1998. 23: 141-142.
21. Nobakht, A. (2013). Effects of different levels of dried lemon (Citrus aurantifolia) pulp on performance, carcass traits, blood biochemical and immunity parameters of broilers. *Iranian Journal of Applied Animal Science,* 3(1): 145-151.
22. Aikpitanyi, K. U. and Imasuen, J. A. (2019). Growth performance and blood profile of broiler chickens as influenced by oral administration of lime fruit (*Citrus aurantifolia*). *Nigerian Journal of Animal Production,* 46 (4):130-136.
23. Medirabbit, (2011). Complete blood count and biochemical reference in rabbits. retrieved from www.medirabbit.com, 4th September, 2021, 2:20 pm.
24. Swenson, M.J. (1990). Duke's Physiology of domestic animals, 9th ed. Comestock publishing Co. UK., pp. 15-40.

25. Hewitt, C. D., Innes, D. J., Savory, J. and Wills, M. R. (1989). Normal biochemical and haematological values in New Zealand White Rabbits. *Clinical Chemistry,* 35 (8): 1777-1779.

26. Ologhobo, A.D., Tewe, O.O. and Adejumo, D.O. (1986). Haematological and Neutral metabolites in Broilers Fed Soyabean Based Rations. *Proceedings of the 11th Annual Conference of Nigerian Society for Animal Production,* ABU, Zaria, pp. 101-103.

27. Kadam, A. S., Lonkar, V. D., Patodkar, V. R., Kolangath, S. M. and Bhosale, T. A. (2009). Comparative efficacy of supplementation of natural (citrous lemon juice), herbal and synthetic vitamin C on the immune response of broiler chicken during summer stress. *Asian Journal of Poultry Science.* 3(3): 57-62.

28. Zimmerman, K.L., Moore, D.M. and Smith, S.T. (2010). Hematology of laboratory rabbits. In: Weiss, D.J. and Wardrop, K.J. (eds). *Schalm's, Veterinary Hematology.* Philadelphia: Lippincott Williams and Wilkins; Pp. 862.

29. Melillo, A. (2013). Application of serum protein electrophoresis in exotic pet medicine. *Veterinary Clinics of North America Exotic Animal Practice,* 16(1): 211-222.

30. Abd El-Ghany, W.A. (2021). Coccidiosis: A Parasitic Disease of Significant Importance in Rabbits. *World Veterinary Journal,* 10 (4): 499-507. DOIhttps://dx.doi.org/10.54203/scil.2020.wvj60

31. Harcourt-Brown, F.M. and Baker, S.J. (2001). Parathyroid hormone haematological and biochemical parameters in relation to dental disease and husbandry in rabbits. *Journal of Small Animal Practice,* 42: 30-36. [PubMed][Google Scholar]

32. Anon. (2004). Biochemistry reference values: http://www.medirabit.com/EN/hematology/blood-chemistry.htm

33. Burke, J. (1994). Clinical care and medicine of pet rabbits. *Proceedings of the Michigan Veterinary Conference, Michigan Veterinary Medical Association., Michigan State University, College of Veterinary Medicine,* held at Radisson Hotel and the Lansing Center, Lansing Michigan, 27th-30th January 1994. Pp. 49-77.

34. Iyayi, E.A. and Tewe, O.O. (1998). Serum total protein, urea, creatiine levels as indices in cassava diets for pigs. *Tropical Veterinary,* 8: 11-15.

35. Jiwuba, P.P., Ikwunze, K., Dauda, E. and Ugwu, D.O. (2016). Haematological and serum biochemical indices of growing rabbits fed diets containing varying levels of *Moringa oleifera* leaf meal. *British Biotechnology Journal,* 15(2): 1-7.

36. Reshef, N., Hayari, Y., Goren, C., Boaz, M., Madar, Z. and Knobler, H. (2005). Antihypertensive effect of sweetie fruit in patients with stage I hypertension. *American Journal of Hypertension,* 18(10): 1360-1363.

37. Jurcik, R., Suvegova, K., Hanusova,

E., Massanyi, P., Ryban, L. and Chrenek, P. (2007). Evaluation of haematological, biochemical and histopathological parameters of transgenic rabbits. *Journal of Veterinary Medicine Association,* 54: 527-531. Doi: 10.1111/j.1439-0442.2007.00976.x.

38. Voss, K.A., Platner, R.D., Bacon, C.W. and Norred, W.P. (1990). Comparative studies of hepatotoxicity and fumonisin and B2 content of water and chloroform/methanol extracts of Fusarium moniliforme strain MRC 826 culture material. *Mycopathologia*, 112: 81-92.

39. Solomon, I.P., Oyebadejo, S.A., Ebenso, I.E. and Otoho, E.A. (2015b). Biochemical effect of *Citrus lemon* juice on the liver of growing rabbits (*Oryctolagus cuniculus*). *Scholarly Journal of Agricultural Veterinary Science,* 2(5): 356-361.

40. Solomon, I.P., Oyebadejo, S.A., Ukpo, E.M. and Joseph, O.S. (2015a). Changes in serum electrolyte, creatinine and urea of fresh Citrus limon juice administered to growing rabbits (Oryctolagus cuniculus). *International Journal of Agricultural Science Research,* 4(8): 180-183.