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Efficacy of dried *Phyllanthus amarus* leaf meal as an herbal feed additive on the growth performance, hematology and serum biochemistry of growing rabbits

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Abstract
This experiment was carried out to evaluate the effects of dried *Phyllanthus amarus* leaf meal (PLM) as an herbal feed additive on the growth performance, haematology and serum biochemistry of growing rabbits. Fifty (50) 8 weeks bucks cross breed rabbits (Chinchilla × New Zealand White) with an average weight of 505.5g and 508.7g were used. The five dietary treatments comprises of: treatment 1 containing basal diet as control, treatment 2 contained basal diet + 0.25g/kg, treatment 3, 4 and 5 were supplemented with PLM at 4%, 6% and 8% respectively. Clean feed and water were supplied ad libitum during the experiment which lasted for 90 days. The performance criteria covered final live weight, feed intake, feed conversion ratio, water intake and mortality. The final live weight, feed intake, feed conversion ratio and daily water intake were not ($p>0.05$) significantly different among the treatments. Results on haematology showed that PCV and Hb were the only parameters affected ($p<0.05$) by the dietary supplementation of OXY and PAM. Other serum biochemical parameters (Albumin, globulin, glucose, ALP, AST and ALT) were not significantly different ($p>0.05$). Mortality was recorded for rabbits in treatment 1 and 2 with three (3) rabbits each, none was recorded for animals in treatment 3, 4 and 5 respectively. The result of this study showed that PAM are rich in bioactive chemicals that have no negative effect on the general performance and health of rabbits and can be included up to 8% in the animals diet.

Keywords: *Phyllanthus amarus* meal, growing rabbits, performance, haematology, serum biochemistry, mortality

Introduction
The use of herbal plants in Livestock production is becoming an object of increasing interest as the use of antibiotics is becoming ever restricted because of its adverse effect on animals, their residues in animal products and the development of antibiotic resistance in bacteria (Lee *et al*., (2004) [56] and Arun Panda (2009). Recently, studies on herbal/medicinal plants are now becoming more popular (food safety) because drugs of synthetic origin constitute a negative impact on animal health and the environment (Magi and Sahk, 2003) [18].

Herbal plants are rich in bioactive chemicals such as steroids, alkaloids, flavonoids, saponins, phenols and so on. Quantity of these secondary metabolites varies from one species of plants to another. It plays an important role in increasing feed intake and palatability, performance, strengthening the immune system, stimulation of digestive enzymes and stabilizes the eubiosis of intestinal microbiota (Straub *et al*., 2005; Chami *et al*., 2005 and Kroismayr *et al*., 2006). It has a promising future because there are about half million plants around the world, and most of their medical activities have not yet been investigated (Bassam Abdul Rasool Hassan, 2012) [35]. Around 21,000 plant species have the potential for been used as medicinal plants most of which are considered to be very safe for human being and animals. Herbal plants such as ginger, garlic, aloe, turmeric, moringa, neem, Polyalthia longifolia etc have proven to be of high therapeutic value and increase an animal's general performance (Sridhar *et al*., 2014; Subapriya and Nagini, 2005; Umashanker and Shruti, 2011; Zhang *et al*., 2012; Zhai *et al*., 2011; Wallace *et al*., 2010 and Alagbe J.O, 2017) [44, 43, 78]. Among the potential herbal plants is *Phyllanthus amarus* leaf which is found to be loaded with several bioactive chemicals of high pharmacological activities.

Phyllanthus amarus belongs to the family of Euphobiaceae, its leaves are tiny, small with yellow flowers. The genus amarus includes about 6000 species occurring mainly in West Africa, South America and South – Eastern Asia, South America and Philippines (Ekate *et al*., 2013 and Burkill, 1994). The therapeutic efficacy of *A. amarus* has been extensively used in the treatment of several forms of disease conditions in many countries for instance, liver and kidney diseases, fever, Jaundice, prostrate problems and so on (Batra, 2013; Khartoon *et al*., 2004 [57] and Nguyen *et al*., 2012) [42]. Reports on the plant have shown that its various parts possess different biological activities. Pharmacologic studies on the bark and leaves of this plant show effective antimicrobial activity (Chandan *et al*., 2012; Adeolu *et al*., 2013 and Ushie *et al*., 2013) [18, 1, 61], anti-inflammatory function (Oluwafemi and Debiri., 2008; Dada *et al*., 2014) [60, 23], anti-diabetic effects (Evi and Degbeku, 2011) [26], antioxidant (Calixto *et al*., 1998; Lim and Murtijaya, 2007) [16, 58], anticonvulsant activity (Manikkoth *et al.*, 2011) [59], anti-carcinogenic and antitumor (Rajeshkumar *et al*., 2002) [34] and antiviral (Prasad *et al*., 2013 and Salazar *et al*., 2011) [32, 36]. There are several experiment that have been conducted to evaluate the effect of different medicinal

plants in animals, for instance turmeric (Hanan E. Al-Mashhadani, 2015 and Nderi *et al.*, 2014) [52], Olive leaf extract (Tarek *et al.*, 2013) [76], Rosemary leaves and garlic (Amera *et al.*, 2013) [4], Allium cepa (Mahima *et al.*, 2012), Thymus vulgaris (Ashour *et al.*, 2014; Chrenkova *et al.*, 2012), Aloe vera (Elbanna *et al.*, 2012), Neem (Ansari *et al.*, 2013; Hady and Zaki, 2012), Ocimum sanctum (2010) and so on, but there is little information on the effect of supplementing different levels of *Phyllanthus amarus* on the general performance of rabbits.

Therefore this experiment was designed to evaluate the effects of dried *Phyllanthus amarus* leaf meal as an herbal feed additive on the growth performance, hematology and serum biochemistry of growing rabbits.

Materials and Methods

Experimental site

The experiment was carried out at Sumitra Research Farm, Gujarat, India.

Collection of plant materials and preparation

Fresh healthy and mature *Phyllanthus amarus* was obtained within the farm premises; it was authenticated and assigned a voucher PAM 18101. The leaves were thoroughly washed under running tap water and air dried for 14 days to obtain a constant weight and then grind into coarse powder using high capacity grinding machine separately to obtain *Phyllanthus amarus* leaf meal (PAM). It was finally stored in airtight containers at 4^{o}C for further analysis.

Analysis of PAM covered:

- Phytochemical screening were determined according to Harborne (1973) and Trease and Evans (1983).
- The mineral analyses were carried out using Atomic Absorption Spectrophotometer (AAS).
- Proximate analysis of PAM and experimental feed were determined using the methods described by AOAC (1990).

Pre-experimental operations

The experimental pens were thoroughly cleaned and disinfected with Morigad, drinking and feeding troughs were properly washed before the arrival of the animals. Grasses were also cleared and all pen doors and windows were properly fixed to prevent theft and ensure proper ventilation. Separate isolation cage was also provided in the pen to accommodate any isolated animal after arrival.

Animal management

A total of fifty (50), 8 weeks bucks cross breed rabbits (Chinchilla × New Zealand White) with an average weight of 505.5g and 508.7g were used for this experiment. They were individually housed in an all wire cages measuring 45cm×30cm×35cm (width×length×height) and given prophylactic treatment with Ivermectin at 0.5 ml/kg body weight administered intramuscularly (I.M). The rabbits were also allowed two week adjustment period during which they were fed with control diet (morning and evening) before they were placed on the experimental diets. The animals are feed twice daily between 7:30 am and 3:30pm, clean feed and water were provided ad libitum throughout the experimental period which lasted for 90 days.

Experimental design and diet formulation

Rabbits were randomly assigned to five treatments of twelve (10) animals per group, each treatment was replicated ten times with each replicate having a rabbit in a completely randomized design (CRD).

Treatment 1 (control): Basal diet (0% OXY and PAM)
Treatment 2: Basal diet + 0.25g/kg OXY
Treatment 3: Basal diet + 4% PAM
Treatment 4: Basal diet + 6% PAM
Treatment 5: Basal diet + 8% PAM

The basal diet was formulated to meet the nutrients requirements of growing rabbits according to the (NRC, 1977).

Data collection

Daily feed intake (g) was calculated by difference between feed offered and the left over, feed conversion ratio was determined as feed intake divided by body weight gain, water consumption and mortality were recorded daily.

Blood analysis

At the end of the experiment (90 days), three animals were selected from each treatment for haematological and serum biochemical analysis. The animals were bled via the marginal veins and 10 ml of blood was collected. Blood (5ml) meant for haematology were collected into bottles containing Ethylene diamine tetra acetate (EDTA) as anticoagulant while those for serum biochemical indices (5ml) were collected into bottles free from anticoagulant. Pack cell volume (PCV), red blood cell (RBC), haemoglobin (Hb), white blood cell (WBC) and absolute counts of neutrophils, lymphocytes, monocytes and eosinophils which were all computed according to standard techniques as reported by Bush (1991), mean corpuscular volume (MCV), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration were computed according to Jain (1986) [28].

The serum total protein, Albumin and Globulin were computed according to (Doumas and Briggs, 1972) [24]. Alkaline phosphatase, Alanine aminotransferase and alkaline phosphatase were determined according to Reitman and Frankel (1957) [33], glucose were computed according to the procedure of Toro and Ackermann (1975) [47].

Statistical analysis

Data collected were subjected to analysis of variance (ANOVA) as outlined by Snedecor and Cochran (1978) [37]. Where significant differences were observed, treatment means were compared using Duncan's Multiple Range Test as outlined by Obi (1990) [31].

Table 1: Chemical composition of *Phyllanthus amarus* leaf meal (PAM)

Nutrients	% Dry matter
Dry matter	91.12
Crude fibre	15.11
Crude protein	10.22
Ether extract	5.88
Ash	9.11
Nitrogen free extract	50.80

Table 2: Percentage composition (%) of experimental diets.

Ingredients	Treatments				
	1	2	3	4	5
Maize	41.25	41.25	41.25	41.25	41.25
Wheat offal	20.0	19.97	16.0	14.0	10.0
Soya meal	12.0	12.0	12.0	12.0	12.0
Groundnut cake	5.00	5.00	5.00	5.00	5.00
Palm kernel meal	17.0	17.0	17.0	17.0	17.0
Bone meal	3.00	3.00	3.00	3.00	3.00
Limestone	1.00	1.00	1.00	1.00	1.00
Lysine	0.10	0.10	0.10	0.10	0.10
Methionine	0.10	0.10	0.10	0.10	0.10
[1]Premix	0.25	0.25	0.25	0.25	0.25
Salt	0.30	0.30	0.30	0.30	0.30
OXY	0.00	2.50	-	-	-
PAM	0	0.00	4.00	6.00	8.00
100	100	100	100	100	
Calculated analysis					
Crude protein (%)	18.11	16.23	16.19	16.12	16.04
Energy (MEkcal/kg)	2556.5	2559.1	2556.3	2552.6	2552.1

[1]Premix supplied per kg diet :- Vit A, 8,500 I.U; Vit E, 5mg; Vit D3, 3000I.U, Vit K, 3mg; Vit B2, 5.5mg; Niacin, 25mg ; Vit B12, 16mg ; Choline chloride, 120mg ; Mn, 5.2mg ; Zn, 25mg ; Cu, 2.6g ; Folic acid, 2mg ; Fe, 5g ; Pantothenic acid, 10mg ; Biotin, 30.5g ; Antioxidant, 56mg

Table 3: Chemical composition of Experimental diets

Nutrients (%)	Treatments					
	1	2	3	4	5	SEM
Dry matter	89.76	88.67	88.51	88.41	88.31	2.13
Crude protein	18.45	17.72	17.51	17.30	17.22	1.01
Crude fibre	9.91	10.25	10.39	10.44	10.51	0.99
Ether extract	2.31	2.28	2.26	2.25	2.23	0.24
Ash	6.11	5.71	5.62	5.32	5.38	2.07
Nitrogen free extract	63.22	64.04	64.22	64.69	64.66	4.56

Table 4: Phytochemical composition of PAM

Constituents	(%) Composition
Flavonoids	9.11
Phenols	7.21
Alkaloids	1.05
Tannins	2.20
Saponins	1.88
Terpenoids	0.77
Glycosides	1.33

Table 5: Mineral composition of PAM

Minerals	% (mg/100g)
Copper	1.44
Iron	1.01
Zinc	9.02
Calcium	5.04
Magnesium	3.07
Potassium	1.02
Sodium	1.31
Phosphorus	0.56

Table 6: Growth Performance of Rabbits fed varying levels of PAM

	Treatments					
Parameters	**1**	**2**	**3**	**4**	**5**	**SEM**
Number of animals	10	10	10	10	10	-
Initial body weight (g)	508.7	505.8	505.5	507.2	506.5	12.1
Final body weight (g)	1205.1	1203.4	1201.1	1200.9	1200.1	93.41
Final weight gain (g)	696.4	697.6	695.6	693.7	693.6	13.14
T.F.I (g)	5100.7	5100.5	5100.4	5100.2	5100.0	100.1
FCR	4.24	4.24	4.25	4.25	4.25	0.49
DWI (ml/day)	807.4	806.8	806.4	806.3	807.0	8.91
Mortality	2/10	2/10	0/10	0/10	0/10	1.12

[abc] means different superscript along rows differs significantly at $p<0.05$

T.F.I – Total feed intake FCR- Feed conversion ratio DWI – Daily water intake

Table 7: Haematological parameters of growing rabbits fed different levels of PAM and OXY

Parameters			Treatments			
	1	2	3	4	5	SEM
PCV (%)	39.12c	46.44b	47.00b	47.11b	47.71a	2.01
Hb (g/dl)	9.21b	14.70a	14.09c	14.33b	14.51a	1.31
RBC ×109/L)	6.77	6.91	6.29	6.41	6.71	4.11
MCV (fl)	65.02	65.70	68.21	68.40	68.63	7.31
MCH (pg)	21.41	21.01	21.11	21.33	21.61	17.40
MCHC (%)	34.32	34.05	34.88	34.91	35.00	20.11
WBC ×109/L	11.31	11.40	11.70	11.08	11.03	2.11
LYM ×109/L	4.71	4.51	4.70	4.58	4.41	3.57
MON ×109/L	3.13	3.01	3.08	3.12	3.20	2.09

[abc] means different superscript along rows differs significantly at p<0.05

PCV: Pack cell volume; Hb: Haemoglobin; RBC: Red blood cell; MCV: Mean corpuscular haemoglobin; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular haemoglobin concentration; WBC: White blood cell; LYM: Lymphocytes; MON: Monocytes

Table 8: Serum biochemical parameters of growing rabbits fed different levels of PAM and OXY

Parameters			Treatment			
	1	**2**	**3**	**4**	**5**	**SEM**
Albumin (g/dL)	1.91	1.99	2.00	2.01	2.06	0.21
Globulin (g/dL)	2.44	2.31	2.58	2.52	2.50	0.25
Total protein (g/dL)	4.35	4.20	4.58	4.53	4.56	0.50
Glucose (mmol/L)	4.04	4.21	4.02	4.06	4.11	0.41
ALT (U/L)	7.12	7.08	6.91	6.33	6.21	7.11
AST (U/L)	6.08	6.34	6.22	6.03	6.00	6.03
ALP (U/L)	13.13	13.04	11.23	11.15	10.33	6.61

[abc] means different superscript along rows differs significantly at p<0.05

Results and Discussion

Table 1 revealed the chemical composition of *Phyllanthus amarus* leaf meal (PAM).PAM contained 91.12 % dry matter, 15.11% crude fibre, 10.22 % crude protein, 5.88 ether extract, 9.11% ash and 50.80% nitrogen free extract. The current study is in line with the findings of Ekaete *et al* (2013); Taylor L (2003) and Khartoon *et al* (2004) [57] who reported similar results on chemical evaluation of PAM.

The proximate result obtained for the experimental diets is presented in Table 3. The dry matter, crude protein, crude fibre, ash, ether extract, nitrogen free extract and energy in the diet ranges from 88.31% - 89.76%, 17.22 % -18.45 %, 9.91% – 10.54%, 2.31% – 2.25%, 5.71%-6.11%, 63.22% - 64.69% and 2552.1 – 2559.1 (MEkcal/kg) respectively. In the current study crude fibre shows an increasing trend as the inclusion level of PAM increases. This result is in agreement with the findings of Saulawa *et al* (2015) [38] and Salisu Bakura Abdu (2012) [35] when carrot meal was added to the diets of growing rabbits. However, proximate composition in the experimental diets was within the range recommended for growing rabbits NRC (1977); Adaku and Olukosi (1990); Anugwa *et al* (1998) and Ibrahim *et al* (2018) [76].

Table 4 shows the phytochemical analysis of PAM, the bioactive components present are flavonoids (9.11%), phenols (7.12%), alkaloids (1.05%), tannins (2.20%), saponins (1.88%), terpenoids (0.77%) and glycosides (1.33%). This result is in accordance with the reports of Zubair *et al* (2017); Sen and Batra (2013) [42]; Ushie *et al* (2013) [61]; Manikkoth *et al* (2011) [59]; Oluwafemi *et al* (2008) [60]; Lim and Murtijaya (2007) [58]; Leite *et al* (2007) and Khartoon *et al* (2004) [57] on the comparative pharmacognostic studies of Phyllantus species. According to Adesuyi *et al* (2011) and Middleton *et al* (2000) flavonoids are found in some plants and have been reported to exhibit antioxidant, antiviral and anti-inflammatory properties. Saponins are also used as adjuvants in vaccine production (Asl and Hosseinzadeh, 2008) [2]. Adisa *et al* (2010) [3] reported that tannins possess antibacterial and anti-viral activity while saponin plays a significant role in maintaining blood cholesterol levels (Cheeke, 2000) [20]. Phenols play a key role in red blood cell modifier (Adesanya and Sofowora, (1983) [13].

Mineral analysis of PAM reveals that it contained copper, iron, zinc, calcium, magnesium, potassium, sodium and phosphorus at 1.44, 1.01, 9.02, 5.04, 3.07, 1.02, 1.31 and 0.56 (mg/100g) respectively as expressed in Table 5. PAM had a higher level of zinc followed by calcium, magnesium, copper, sodium, potassium, iron and phosphorus respectively. The values obtained are very low compared with the reports of Asaolu *et al* (2009) [5] and Ojewuyi *et al* (2014) [49] on the nutritional composition of Cymbopogon citratus and Polyalthia longifolia leaves respectively. This could be attributed to differences in species of plants, soil types and environmental factors.

Onwuka (2005) [50] and Adeyeye (2000) [12] reported that Minerals is always required for efficient metabolic processes. Calcium and phosphorus are major components of the skeletal system, magnesium is a component of the bone, a cofactor of several enzyme activity and is involved in the transmission of nerve impulses, copper is significant in iron and energy metabolism while sodium and potassium play key roles in the acid-base regulation of the blood and other body fluids Amy E. Halls (2014) [11].

Table 6 shows the performance parameters of growing rabbits fed diets containing OXY and PAM. The final body weight were 1205.1, 1203.4, 1201.1, 1200.9 and 1200.1g respectively for treatment 1, 2, 3, 4 and 5.There were no significant differences (p>0.05) among the treatments in the final body weight gain. Treatment 2 recorded the highest final body weight gain (697.6 g) while treatment 5 recorded the lowest body weight gain (693.6 g).The total feed intake of the rabbits were 5100.7, 5100.5, 5100.4, 5100.2 and 5100.0g for treatment 1, 2, 3, 4 and 5 respectively. The total

feed intake and the feed conversion ratio (FCR) were not ($p>0.05$) significantly different among the treatments. The feed intake in this experiment tends to be higher in the rabbits fed the basal diet (treatment 1) compared with the other groups, but the differences were not statistically significant. This result is in agreement with the reports of Haruna and Muhammad (2018) and Ojabo *et al* (2012) when sweet orange peel meal was supplemented in the diets of growing rabbits but contrary to the reports of Salisu Bakura Abdu (2012) [35]; Banjo *et al* (2012) [64] and Igwebuike *et al* (2016) when feed graded levels of cowpea testa meal. According to Igwebuike *et al* (2016) [53], rabbits fed 25% cowpea testa meal recorded the highest daily body weight and feed intake.

Similarly, Unigwe *et al*. (2016) [62] reported that dietary supplementation of Neem leaf meal at 10%/100kg diet significantly enhanced the final weight gain, feed conversion ratio and the average total feed intake. Gbore *et al* (2010) [51] also disclosed that inclusion of fumonisin /kg at 5.0 and 10.0 mg, significantly ($P<0.05$) reduced the daily dry matter intake (DMI) and final live weight of rabbits. The water intake values obtained are practically the same.

There were a significant ($p<0.05$) differences among the groups in the mortality rate. However, the result showed that the highest mortality was recorded in treatment 1 (10%) and 2 (10%) respectively. None was recorded in treatment 3, 4 and 5 which could be as a result bioactive chemicals produced by the plants (phytochemicals) which have been reported to perform several activities such as antimicrobial (Edeoga, 2005; Subhashini *et al.*, 2010 and Shahid-Ud-Duaula *et al*., 2009) [25, 40, 41], antifungal (Adegoke *et al*., 2010) [8], anti-inflammatory (Khandelwal, 2004).

Results on the haematological parameters of growing rabbit are expressed in Table 7. The values for PCV obtained is between 39.12% - 47.71%, haemoglobin values of 9.21 – 14.51(g/dl) while RBC values are 6.29 – 6.91 ($(\times 10^9/L)$. The PCV and Hb showed a significant ($p<0.05$) differences among the treatment. The values increased from treatment 1 to 5, this is an indication that there is adequate circulation of oxygen (Isaac *et al*., 2013; Soetan *et al*., 2013 and Ugwuene, 2011) [70, 74, 77]. However, all values were within the normal range reported for rabbits by Özkan *et al* (2012) [65]; Ahemen *et al* (2013) [6]; Chineke *et al* (2006) [21]; Hewitt *et al* (1989) [69]; Mitruka and Rawnsley (1977) [67] and Kronfield and Mediway (1975) [66].

The value obtained for MCV is between 65.02-68.63 (fl), MCH values is 21.01-21.61 (%); RBC values 6.29- 6.91 ($\times 10^9/L$) and MCHC are 34.05-35.00 (%). According to Togun *et al* (2007) [75] and Adamu *et al* (2006) [9] a decrease in RBC can be closely related to the nutritional status, physiological and disease condition of an animal. MCV, MCH and MCHC parameters can also be used in disease diagnosis Njidda *et al* (2006) [72] and Addass *et al* (2012) [7]. All the values reported in this experiment were within the normal ranges of rabbits reported by Darina Chodová *et al* (2017) [22], there was statistically no significant difference ($p>0.05$) in all the parameters measured.

WBC values obtained is between 11.03-11.70 ($\times 10^9/L$), lymphocytes are 4.51-4.71 ($\times 10^9/L$) and monocytes 3.01-3.13 ($\times 10^9/L$). The WBC values and its differentials did not differ significantly ($p>0.05$) among the treatment groups. The values of WBC obtained in this study were found to be within the normal physiological range for rabbits as reported by Research Animal Resources (2009).

The serum biochemistry of rabbits fed diets supplemented with OXY and PAM is expressed in Table 8. The total protein values obtained is 4.45 – 4.87 (g/dl) which fall within the normal ranges of 5.40-7.50 (g/dl) previously reported by Medi rabbit, 2011; Çetin *et al.*, 2009 [17] and Archetti, 2008 this shows that the protein reserves across the diet is enough to assist in tissue and cell rebuild after stress but contrary to the findings of Ladipo *et al* (2015) [79] who reported a higher value of between (6.71-6.91g/dl). According to Rajman (2006) [81] plasma protein are used to ascertain an animal's state of hydration and also contributes in nutrition by functioning as a pool of amino acid and other tissue protein. Glucose values obtained is between 4.04-4.21 mmol/L, total protein and serum glucose concentration was similar in all the treatments, this is a signal of good nutritional state in all five groups. Jenkins (2008) [71] reported that an increase in serum glucose level can be attributed to severe stress condition.

Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) values reduced slightly from treatment 1 to 5, however all were within the range of reference values reported for rabbits in previous studies (Tavares *et al*., 2004; Yamada *et al*., 2004 and Jurcik *et al*., 2007) [45, 82, 54] this is a clear indication that the integrity of the liver is being maintained. The AST, ALT and ALP were not significantly different ($p>0.05$) among the treatment. According to Keer *et al* (1982) [56] AST is an enzyme used in amino acid transamination and its high level can be triggered under serious disease and morbid conditions. Similarly, ALT is responsible for the synthesis of non-essential amino acid found in the plasma and various bodily tissues (Carola *et al*., 1990) [19].

Conclusion

It can be concluded from this experiment that PAM can be used as a good substitute for antibiotics (Oxytetracycline) due to rising issues on food safety, PAM can be included up to 8% in the diets of growing rabbits as it does not also pose negative or deleterious effect on the general performance and health status of the animal.

References

1. Adeolu AA, Sunday OO. Anti-inflammatory and analgesics activities of soft drink extract of *Phyllanthus amarus* in some laboratory animals. British Biotechnology Journal. 2013; 3:191-204.
2. Asl MN, Hosseinzadeh H. Review on the pharmacological effects of Glycyrrhiza species and its bioactive compounds. Phytother. Res. 2008; 22:709-724.
3. Adisa RA, Choudhary EA, Adenoye GA, Olorunsogo OO. Hypoglycaemic and biochemical properties of Cnestis ferruinea, African Jou. Complementary Alternative Medicine. 2010; 7:185-194.
4. Amera S, Abd El-Latif Nahed S, Saleh Tamer S. Allam, Emad W. Ghazy. The effects of rosemary and garlic on performance, haematological, biochemical and immunological parameters of broiler chickens. British Journal of Poultry Sciences. 2013; 2(2):16-24:2013.
5. Asaolu MF, Oyeyemi OA, Olanlokun JO. Chemical composition, phytochemical constituents and in vitro biological activity of various extracts of Cymbopogon citratus. Pakistan Journal of Nutrition. 2009; 8(12):1920-1922, 2009.

6. Ahemen T, Abu AH, Gbor V. Haematological and serum biochemical parameters of rabbits fed varying dietary levels of water spinach (Ipomoea aquatic) leaf meal. Advances in Applied Science Research. 2013; 4(2):370-373.
7. Addass PA, David D, Edward A, Zira KE, Midak A. Effect of age, sex and management system and some haematological parameters of intensively and semi intensively kept chicken in Mubi, Adamawa State, Nigeria. Iranian Journal of Applied Animal Science. 2012; 2(3):277-282.
8. Adegoke AA, Iberi PA, Akinpelu DA, Aiyegoro OA, Mboto CI. Studies on phytochemical screening and antimicrobial potentials of *Phyllanthus amarus* against multiple antibiotic resistant bacteria. International Journal of Appl. Res. Nat. Prod. 2010; 3(3):6-12.
9. Adamu S, Thomas A, Iseh NM, Fatihumi MY, Esieno AN. Normal values of haematology values of Nigerian adopted albino rats in Zaria. Proceedings of Nigerian Society of Animal Production, 2006.
10. Archetti I, Titterelli C, Cerioli M, Brivio R, Grilli G, Lavazza A. Serum chemistry and haematology values in commercial rabbits: Preliminary data from industrial farms in northern Italy. Proceedings of 9th World Rabbit Congress, Verona, Italy, 2008, 1147-1152.
11. Amy Halls E. Nutrient requirements for rabbits. Shur-Gain, Nutreco Canada Inc, 2014.
12. Adeyeye EI. Bio-concentrations of macro and trace minerals in four prawns living in Lagos lagoon. Pak. J. Scient. Ind. Res. 2000; 43:367-373.
13. Adesanya, Sofowora. Biological standardization of Zanthoxylum roots for antisickling activity. Planta Medica. 1983; 48:27-33.
14. AOAC. Association of Official Analytical Chemists. Official Methods of Analysis 19th Edition Washington, D.C, 1990, Pp. 69-77.
15. Bush BM. Interpretation of laboratory results for small animals clinicians. Blackwell Scientific Publication London, 1991.
16. Calixto JB, Santos ARS, Filho VC. A review of the plants of the genus Phyllantus their chemistry, pharmacology and therapeutic potential. Medical Research Reviews. 1998; 18(4):225-358.
17. Çetin N, Bekyürek T, Çetin T. Effects of sex, pregnancy and season on some haematological and biochemical blood values in Angora rabbits. Scand J Lab Anim Sci. 2009; 36:155-162.
18. Chandan S, Umesha S, Balamurugan V. Anti-leptospiral antioxidant and DNA damaging properties of *Eclipta alba* and *Phyllanthus amarus*. Open Access Scientific Reports. 2012; 1(4):1-8.
19. Carola R, Harley JP, Noback CR. Human anatomy and physiology, McGraw Hill Incorporation USA, 1990, Pp. 925.
20. Cheeke ON, Nobert HO. Serum biochemical parameters in clinically healthy dogs in Ibadan. Tropical Vert. 2000; 16:3-4.
21. Chineke CA, Ologun AG, Ikeobi CON. Haematological parameters in rabbit's breeds and crosses in humid crosses in humid tropics. Pakistan Journal of Biological Sciences. 2006; 9(11):2102-2106, 2006.
22. Darina Chodová, Eva Tůmová, Helena Härtlová, Alena Fučíková, Zdeněk Volek, Jana Vlčková. Changes of haematological and biochemical indices with age in rabbits with ad libitum and limited feed intake. ACTA VET. BRNO. 2017; 86:29-35. https://doi.org/10.2754/avb201786010029.
23. Dada EO, Ekundayo FO, Makanjuola OO. Antibacteria activities of Jatropha curcas Linn on coliforms isolated from surface waters in Akure, Nigeria. International Journal of Biomedical Science. 2014; 10(1):25-30.
24. Doumas BT, Briggs HG. Serum Albumen Bromocresol Green Binding Standard Methods. Clinical Chemistry. 1972; (7):175-179.
25. Edeoga HO, Okwa DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. Afr. Jour. Biotech, 4(7):685-688. Int'l Jou of Med. Sci., 2005, 142-152.
26. Evi PL, Degbeku K. Antidiabetic activity of *Phyllanthus amarus* schum and thonn and alloxan induced diabetes in male Wistar rats. Journal of Applied Sciences. 2011; 11(6):2968-2973.
27. El E, Gomma DG, Esmaiel NM, Salem MZM, Gomaa SE. *In vitro* screening for antimicrobial activity of some medicinal plants seed extracts, 2016.
28. Jain NC. Schalms veterinary hematology 4th edition Lea and Febiger, Philadelphia, 1986.
29. Harbone IB. A guide to modern techniques to plant analysis. Chapman and hall, New York, USA 2nd Edition, 1973.
30. National Research Council. Nutrients requirements for rabbits, second edition. National Academy of Science, Washington D.C pages, 1977, 10-15.
31. Obi IU. Statistical method of detecting diff between treatments means 2nd Snaap press Enugu, Nigeria, 1990.
32. Prasad PD, Kavimani S, Suba V, Nudu T, Sanatorium T. Antimicrobial activity of the root extracts of *Phyllanthus amarus*. Applied Journal of Hygiene. 2013; 4(1):1039-1043.
33. Reitman S, Frankel S. A calorimetric method for the determination of serum glutamic oxaloacetate and serum glutamic pyruvic transaminase. Animal Journal of Clinical Pathology, 1957, 28-56.
34. Rajeshkumar NV, Joy NV, Kuttan G, Nair MJ, Kuttan R. Antitumor and anticarcinogenic activity of *Phyllanthus amarus* extract. Jour. Ethnopharmacology. 2002; 81(1):17-22.
35. Salisu Bakura Abdu, Grace Esrom Jokthan., Mohammed Rabiu Hassan., Hanwa Yusuf Adamu., Suleiman Makama Yashim and Emmanuel Ikani. Effects of inclusion of carrot leaf meal on performance of growing rabbits. World Journal of Life Sciences and Medical Research. 2012; 2(2):65.
36. Salazar JR, Martinez-Vazquez M, Cespedes CL, Ramirez-Apan T, Nieto-Camacho A, *et al.*, Flores Murrieta, F. Anti-inflammatory and cytotoxic activities of Chichipegnin, peniocerol and macdougallin isolated from Myrtillocactus geometrizans Con. Z Naturforsch C. 2011; 66(1-2):24-30.
37. Snedecor GW, Cochran WG. Statistical method 6th edition Lowa State University press, Amen Lowa, 1978.
38. Saulawa LA, Sabo MN, Garba MG. Performance of weaner rabbits fed diets supplemented with Pawpaw leaf meal. Scientific Journal of Animal Science. 2015; 4(12):187-191.
39. Salanraj P, Sivasakthivelan P. Screening of antibacterial

activity of the medicinal plants *Phyllanthus amarus* against urinary tract infection causing bacteria pathogens. Applied Journal of Hygiene. 2012; 1(3):19-24.
40. Subhashini R, Mahadeva-Rao US, Sumathi P, Gunalan GA. A comparative phytochemical analysis of cocoa and green tea, Indian Journal of Sci. Tech. 2010; 3(2):188-192.
41. Shahid-Ud-Duaula AFM Anwarul, Basher M. Phytochemical screening, plant growth inhibition and antimicrobial activity studies of Xylocarpus granatum. Malaysian Journal of Pharmaceutical Sciences. 2009; 7(1):9-21.
42. Sen A, Batra A. The study of in vitro and invivo antioxidant activity and total phenolic content of *Phyllanthus amarus*: A medicinally important plant. International Journal of Pharmacy and Pharmceutical Sciences. 2013; 5:947.
43. Subapriya R, Nagini S. Medicinal properties of neem leaves. A review Curr. Med. Chem. 2005; 5:149-156.
44. Sridhar M, Suganthi RU, Thammiaha V. Effect of dietary resveratrol in ameliorating aflatoxin B1 – induced changes in broiler birds. Jour. Anim. Physiol. Anim. Nutr, 2014, 10.111/jpn.12260.
45. Tavares FL, Sousa-e-Silva MCC, Santoro ML, Barbaro KC, Rebecchi IMM, Sano-Martins IS. Changes in hematological, hemostatic and biochemical parameters induced experimentally in rabbits by Loxosceles gaucho spider venom. Hum. Exp. Toxicol. 2004; 23:477-486. doi: 10.1191/0960327104ht475oa
46. Tasheen, M, Mishra G. Ethnobotany and diuretic activity of some selected indian medicinal plants. The pharma. Innovation. 2013; 2:112.
47. Toro G, Ackermann P. Practical clinical chemistry 1st edition. Little Brown and Company, Boston, USA, 1975.
48. Tollba AAH, Hassan MSH. Using some natural additives improve physiological and productive performance of broiler chicks under high temperature condition. Poultry Science. 2013; 23:327-340.
49. Ojewuyi OB, Ajiboye TO, Adebanjo Balogun A, Mohammed AO. Proximate compostion and mineral compostion of Polyalthia longifolia leaves. Fountain Journal of Natural and Applied Sciences. 2014; 3(1):10-19.
50. Onwuka GI. Feed analysis and instrumentation: theory and practice Naphthalic prints Surulere Lagos, 2005, 219-230.
51. Gbore FA, Akele O, Bore FA, Akele O. Growth performance, haematology and serum Growth performance, haematology and serum biochemistry of female rabbits (biochemistry of female rabbits (Oryctolagus cuniculus ryctolagus cuniculus) fed dietary fumonisin. Vet. fed dietary fumonisin. Vet. arhiv 80, 431-443, 2010. Rhiv. 2010; 80:431-443.
52. Hanan E, Al-Mashhadani. Effects of different levels of turmeric supplementation on broiler performance, carcass characteristics and bacterial count of broiler chickens. Egyptian Poultry Science Journal. 2015; 35(1): 25-39.
53. Igwebuike JU, Mohammed G, Kwar ID, Abiola OL, Kolo UM. Effect of feeding graded levels of Cowpea testa meal on the growth and economic performance of growing rabbits. Trakia Journal of Sciences. 2016; 2:148-152.
54. Jurcik R, Suvegova K, Hanusova E, Massanyi P, Ryban L, Chrenek P. Evaluation of haematological, biochemical and histopathological parameters of transgenic rabbits. J. Vet. Med. A. 2007; 54:527-531. doi:10.1111/j.1439-0442.2007.00976.x
55. Khanadelwal KR. Practical pharmacognosy. 12th edition, Nirali Prakashan, 2004.
56. Keer GR, Lee ES, Lam EK, Lorimor RJ, Forthofer R, Davis MA. Relationship between dietary and biochemical measure of nutritional status. American Journal of Clinical Nutrition. 1982; 35:294-308.
57. Khartoon S, Rai V, Rawat A. Comparative pharmacognostic studies of three Phyllantus species. Journal of Ethanopharmacology. 2004; 104:79-86.
58. Lim Y, Murtijaya J. Antioxidant properties of *Phyllanthus amarus* extract as affected by different drying methods. Food Science and Technology. 2007; 40(9):1664-1669.
59. Manikkoth S, Dcepa B, Joy AE, Rao S. Anti-convulsant activity of *Phyllanthus amarus* in experimental animal models. 2011; 4:144-149.
60. Oluwafemi F, Debiri F. Antimicrobial effect of *Phyllanthus amarus* and *Parquetina nigrescens* on Salmonella. Afr. Jour. Biomed. Res. 2008; 11:215-219.
61. Ushie O, Neji P, Etim E. Phytochemical screening and antimicrobial activities of *Phyllanthus amarus* stem back extracts. Int. Jour. Modern Biology and Medicines. 2013; 3:101-112.
62. Unigwe CR, Balogun FA, Okorafor UP, Odah IS, Abonyi FO, Olona JF. Effect of neem leaf meal on the growth performance and hematology of rabbits. World Scientific News. WSN. 2016; 55(2016):51-62.
63. Unander DW, Webster GL, Blumberg BS. Usage and bioassays in Phyllantus (Euphorbiaceae) – IV. Clustering of antiviral uses and other effects. Journal of Ethnopharmacology. 1995; 45(1):1-18.
64. Banjo OS, Mako AA, Ettu RO. The replacement of maize with graded level of brewer's dry grain in the diet of grass cutters. Journal of Natural Sciences Research. 2012; 2:8.
65. Özkan C, Kaya A, Akgül Y. Normal values of haematological and some biochemical parameters in serum and urine of New Zealand White rabbits.World Rabbit Sci. 2012; 20:253-259. doi:10.4995/wrs.2012.1229.
66. Kronfield OW, Mediway NC. Blood Chemistry In: Textbook of Veterinary Clinical Pathology. Publ. Williams and Williams Co., Baltimore, 1975, pp. 81-96.
67. Mitruka BM, Rawnsley HM. Clinical Biochemical and Haematological reference values in normal experimental animal. Masson Publ. Co.New York, 1977, pp. 102-117.
68. Medirabbit. Complete blood count and biochemical reference values in rabbits Retrieved from www.medirabbit.com, 22nd July, 2011.
69. Hewitt CD, Innes DJ, Savory J, Wills MR. Normal Biochemical and Haematological Values in New Zealand White Rabbits. CLIN. CHEM. 1989; 35/8:177-179.
70. Isaac LJ, Abah G, Akpan B, Ekaette IU. Haematological parameters of different breeds and sexes of rabbits (Pages 24-27). Proceedings of the 18th

Annual Conference of Animal Science Association of Nigeria, 2013.
71. Jenkins JR. Rabbit diagnostic testing. J. Exot. Pet Med. 2008; 17:4-15. doi:10.1053/j.jepm.2007.12.003.
72. Njidda AA, Igwebuike JU, Isidahomen CE. Haematological parameters and carcass characteristics of weaning rabbits fed graded levels of molasses. Global Journal of Agricultural Science. 2006; 5(7):167-172.
73. Research Animal Resource. Reference values for Laboratory animals: Normal haematological values. RAR websites, University of Minnesota, 2009. Retrieved from http://www.ahc.umn.edu/rar/refvalues.html
74. Soetan KO, Akinrinde AS, Ajibade TO. Preliminary studies on the haematological parameters of cockerels fed raw and processed guinea corn. Proceedings of 38th Annual Conference of Nigerian Society of Animal Production, 2013.
75. Togun VA, Oseni BSA, Ogundipe JA, Arewa TA, Mustapha F. Effect of chronic lead administration on the haematological parameters of rabbits- a preliminary study. Proceedings of the 41st Conference of the Agricultural Society of Nigeria, 2008.
76. Tarek M Shafey, Ibrahim M Al-Ruqaei, Saud I Almufarij. Effect of feeding Olive leaves on the performance, nutrient utilization and carcass characteristics of broiler chicken. Journal of Animal and Veterinary Advances. 2013; 12(6):740-746.
77. Ugwuene MC. Effect of dietary palm kernel meal for maize on the haematological and serum biochemistry of broiler turkey. Nigerian Journal of Animal Science. 2011; 47(5):175-182.
78. Umashanker M, Shruti S. Traditional Indian herbal medicine used as antipyretic, antiulcer, anti-diabetic and anticancer: A review. International Journal. Res. Pharm. Chem. 2011; 1:1152-1159.
79. Ladipo MK, Adu OA, Oyefeso SD, Akinmuyisitan IW. Growth Performance and Blood Profile of Male Rabbits Fed Dietary Cerium Oxide. International Journal of Agriculture, Forestry and Fisheries. 2015; 3(3):87-92.
80. Naderi M, Akbari MR, Asadi-Khoshoei E, Khaksar K, Khajali F. Effect of dietary inclusion of turmeric powders on performance, organ relative weight and some immune parameters in broiler chickens. Poultry Science Journal, 2014. ISSN 2345-6604 (print).
81. Rajman M, Juráni M, Lamošová D, Máčajová M, Sedlačková M, Košťál Ľ, *et al.* The effects of feed restriction on plasma biochemistry in growing meat type chickens (Gallus gallus). Comp Biochem Physiol A. 2006; 145:363-371.
82. Yamada S, Ito T, Tamura T, Shiomi M. Age-related changes in serum/plasma biochemical parameters of WHHLMI rabbits. Exp. Anim. Tokyo. 2004; 53:159-163. doi:10.1538/expanim.53.159