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Biomedicine and Nursing

MARSLAND PRESS
Multidisciplinary Academic Journal Publisher

Performance, Haematology And Serum Biochemical Parameters Of Growing Grass Cutters (*Thyronoyms Swinderianus*) Fed Phyllantus Amarus And Pilogstigma Thonngii Leaf Meal Mixture As Partial Replacement For Soya Bean Meal

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Abstract: The objective of the present study was to evaluate the performance, haematology and serum biochemical parameters of growing grasscutters (*Thrynomys swinderianus*) fed *Phyllantus amarus* (PAM) and *Pilogstigma thonngii* leaf meal (PTM) mixture as partial replacement for soya bean meal. A total of 35 weaned grasscutters of mixed sex between 5-6 weeks with an average weight of 436.1 and 437.0 g were divided into five groups of 7 animals each and randomly assigned to 5 experimental diets each animal served as a replicate in a completely randomized design. The dietary treatments include a control diet with no PATML, T2 (10.0% PATML), T3 (20.0 % PATML), T4 (30.0 % PATML) and T5 (40.0 % PATML) respectively. Feed and water were offered *ad libitum* throughout the experiment which lasted for 12 weeks. The data obtained was used to evaluate the growth, haematological parameters (PCV, RBC, Hb, MCV, MCH , MCHC, WBC and its differentials), serum biochemical indices (Albumin, globulin, total protein, creatinine, cholesterol, calcium, phosphorus, ALP, AST and ALT) and fatty acid composition. Average weight gain, average daily feed intake and feed conversion ratio were influenced by the dietary treatments ($P<0.05$). Haematological and serum parameters were significantly ($P<0.05$) different among the treatments and were within the normal physiological range for grasscutters. It could be concluded that replacement of soya meal with 40 % PATML does not have any deleterious effect on the performance and blood profile of the animal.
[Alagbe, J.O., Agubosi, O.C.P and Liu, S.D. **Performance, Haematology And Serum Biochemical Parameters Of Growing Grass Cutters (*Thyronoyms Swinderianus*) Fed Phyllantus Amarus And Pilogstigma Thonngii Leaf Meal Mixture As Partial Replacement For Soya Bean Meal**. *Biomedicine and Nursing* 2021;7(3):21-31]. ISSN 2379-8211 (print); ISSN 2379-8203 (online). http://www.nbmedicine.org 4. doi:10.7537/marsbnj070321.04.

Keywords: Weaner grasscutters; *Phyllantus amarus*; *Pilogstigma thonngii*; haematology

INTRODUCTION

Success in livestock production is impossible without giving enough attention to nutrition and improving wholesome feed in adequate quantity daily. In fact, nutrition is one of the important cardinals of management, but scarcity of feed resources has been a hindrance in the production of livestock products to meet the animal protein requirements of human and other industrial needs (Unigwe *et al*., 2016). Onu *et al.* (2004) reported that protein intake in developing countries is low due to high cost of feeding with the accompanying high cost of production. To achieve breakthrough in production and maximize profit there is a need to employ the use of agricultural by products and plants that are cheaper, safe and available to feed animals (Alagbe, 2016). Some plants have been reported to perform multipurpose roles of supplying proteins, vitamins, minerals and as a phytobiotics (Alagbe and Omokore, 2019). Among the potential underexplored plants are *Phyllantus amarus* and *Piliostigma thonningii*.

Grass cutter (*Thrynomys swinderianus*) is a wild herbivores rodent, which lives mainly on grass and other succulent forages (Wogar and Agwunobi, 2011). It is an explored in most areas of Africa as a source of animal protein and it significantly contributes to both local and foreign earnings of most African countries (Asibey, 1974). Feeds containing 2-20 % crude protein have been reported to suitable for grass cutters (Meduna, 2002); they are prolific and contain low cholesterol in their meat which makes them safe for consumption.

Phyllantus amarus belongs to the family of Euphobiaceae. It is commonly found in forest areas, arid land, savannah areas, leached and exhausted soil in many countries including India, China, Cuba and Philippines among others (Zubair *et al*., 2016; Burkill, 1994). Omokore and Alagbe (2019) reported that

Phyllantus amarus leaves contained dry matter (91.2 %), crude fibre (15.11 %), crude protein (10.22 %), ether extract (5.88 %), ash (9.11 %) and nitrogen free extract (50.80%). Reports on the plant have revealed that the various parts are loaded with bioactive chemicals or secondary metabolites (Zubair *et al*., 2016) which confers it the ability to perform several biological activities such as anti-inflammatory functions (Adeolu and Sunday, 2013), antimicrobial (Evi *et al*., 2011), anti-bacterial (Dada *et al*., 2014), antioxidant (Chandan *et al*., 2012), antidiabetic (Khartoon *et al*., 2004; Oluwafemi and Debiri, 2008), antiviral (Prasad *et al*., 2013), anticonvulsant (Manikkoth *et al*., 2011) and anticancer (Rajashkumar *et al*., 2002).

Piliostigma thonningii belongs to the family Caesalpiniaceae. It has large two-lobed simple leaves and without thorns or spines and perennial in nature (Jimoh and Oladiji, 2005). Phytochemical screening of the leaves revealed the presence of phenol, alkaloids, saponin, flavonoids and steroids as secondary metabolites (Jimoh and Oladiji, 2005). Alagbe *et al.* (2019) reported that the leaves of *Piliostigma thonningii* contain dry matter, ether extract, crude protein, crude fibre and ash at 91.20 %, 0.31%, 11.21 %, 14.22% and 7.22 % respectively. Ighodaro et al. (2012) also evaluated the leaves to have 10.09 % crude protein, 2.81 % ether extract, 6.10 % ash, 5.23 % crude fibre and 72.17 % carbohydrates. The plant parts (seed and leaves) play a vital role as an antimicrobial (Lina, 2017), antiviral (Nakayama *et al*., 1993), antifungal (Oyagade, 1999), antibacterial (Salazar *et al*., 2011), anti-inflammatory (Sofowora, 1993), antihelminthic (Tasheen and Mishra, 2013), anticancer (Isidorov *et al*., 2008) and antidiuretic (Zhang, 2014).

Previous studies have shown that administration of *Phyllantus amarus* at 8 % in the diet of rabbit significantly improved the growth of rabbits (Omokore and Alagbe, 2019).Similarly, administration of *Piliostigma thonningii* leaf extract at 0.4g/kg significantly (P>0.05) reduce blood cholesterol level in wister rats (Akinpelu and Obutor, 2000). Nutrients are known to influence the responses of livestock to a disease challenge (Gary and Richard, 2002) and blood plays an important role in the transportation of nutrients, metabolic waste product and gases around the body (Tavares *et al*., 2004). Both *Piliostigma thonningii* and *Phyllantus amarus* leaves are loaded with various bioactive chemicals when could synergistically work together to improve performance and blood parameters of animals.

Therefore, this study was designed to evaluate the performance, haematology and serum biochemical parameters of growing grasscutters (*Thrynomys swinderianus*) fed *Phyllantus amarus* and *Pilogstigma thonngii* leaf meal mixture as partial replacement for soya bean meal

MATERIALS AND METHODS

Site of the experiment

The experiment was carried out at Division of Animal Nutrition, Sumitra Research Institute, Gujarat, India during the month of December to February, 2019.

Source and processing of test materials

Fresh and mature leaves of *Piliostigma thonningii* and *Phyllantus amarus* leaves were was purchased from a local market in Gujarat and authenticated at biological science department of the research farm. It was air dried under shade to reduce moisture content until they were crispy to torch. The leaves thereafter were crushed separately with hammer mill to form *Piliostigma thonningii* leaf meal (PTM) and *Phyllantus amarus* leaf meal (PAM) respectively. The samples were later mixed in ratio of 1:1 to form PATML. The samples were kept in an air tight container for further analysis.

Pre-experimental operations

Prior to the commencement of the experiment the hutches were disinfected two weeks before the arrival of the animals. Cement feeders, drinkers, foot deep at the entrance of the pens were properly cleaned and kept in the store. A separate cage was also prepared to accommodate sick or culled animals.

Experimental animals and management

Thirty five (35) weaned grasscutters of mixed sex between 5-6 weeks with an average weight of 436.1 and 437.0 grams were used for the experiment. The animals were randomly divided into five groups of seven grasscutter per replicate and each animal served as a replicate in a completely randomized design. Grass cutters were allowed two weeks adjustment period during which they were fed with basal diet (morning and evening), housed in an all wired cage measuring 45×53×40 cm and given prophylactic treatment with Oxytetracycline administered intramuscularly and Ivermectin given subcutaneously adhering strictly to the package insert. Animals were fed twice daily between 7:30 am and 3:30 pm (110 – 130 g each). Fresh feed and water were provided ad libitum and all other management practices were strictly observed.

Diet formulation and chemical analysis

The basal diet was formulated to meet the nutrient requirements of weaner grasscutters according to Adeniji (2009).

Treatment 1 (Control): Basal diet + 0 % PATML
Treatment 2: Basal diet + 10.0 % PATML
Treatment 3: Basal diet + 20.0 % PATML

Treatment 4: Basal diet + 30.0 % PATML
Treatment 5: Basal diet + 40.0 % PATML

Proximate analysis of experimental diet was determined using methods described by AOAC (2000). Phytochemical screening of PTM and PAM were analyzed according to methods outlined by Harbone (1973); Trease and Evans (1983). Mineral analyses were carried out using Atomic Absorption Spectrophotometer (AAS) model 12-0TA.

Measurements

✓ **Performance parameters**

Feed intake (g) was determined by subtracting feed left over from feed served, it was estimated for each of the replicate daily.

Weight gain (g) was calculated by finding the difference between initial weight and final weight at the end of the experiment.

Feed: gain ratio was determined from the average feed consumed by the average weight gained in each treatment.

Mortality rate was recorded as it occurs daily.

✓ **Blood collection and analysis**

At the end of the 12th week of the experiment, blood samples were collected by cardiac puncture from four randomly selected grass cutters per treatment. The operation is done very early in the morning to reduce stress on the animals, 6ml each was collected from each animal. 3 ml of blood sample was transferred into a sterile EDTA bottle for hematological assay. The parameters (Pack cell volume, haemoglobin concentration, red blood cell, mean corpuscular volume, mean corpuscular haemoglobin concentration, mean corpuscular haemoglobin concentration, white blood cell and its differentials) were analyzed using Diasis Diagnostic Systems ASI-671N, India (an automated digital analyzer).

The remaining 3 ml was put into another bottle without anticoagulant (EDTA) for serum biochemical analysis. Albumin, globulin, cholesterol and total glucose, creatinine, calcium, sodium and chlorine ions were analyzed using Randox ® commercial kits, USA (Model 2R-TR4). Activities of Alanine transaminase (ALT), Aspartate transaminase (AST) and Alkaline phosphatase (ALP) were recorded according to (Doumas and Briggs, 1972).

Statistical analysis

All data were subjected to one -way analysis of variance (ANOVA) using SPSS (23.0) and significant means were separated using Duncan multiple range tests (Duncan, 1955). Significant was declared if $P \leq 0.05$.

Table 1. Chemical composition of experimental diets

Ingredients	T1	T2	T3	T4	T5
Maize	40.00	40.00	40.00	40.00	40.00
Wheat offal	25.00	25.00	25.00	25.00	25.00
PKC	11.10	11.10	11.10	11.10	11.10
Soya meal	20.00	18.00	16.00	14.00	12.00
PATML	**0.00**	**2.00**	**4.00**	**6.00**	**8.00**
Bone meal	2.00	2.00	2.00	2.00	2.00
Limestone	1.00	1.00	1.00	1.00	1.00
Lysine	0.20	0.20	0.20	0.20	0.20
Methionine	0.20	0.20	0.20	0.20	0.20
Premix	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00
Cal analysis (%)					
Dry matter	86.87	87.10	88.61	88.92	88.95
Crude protein	19.02	18.51	18.38	18.24	18.10
Crude fibre	7.62	7.78	7.94	8.10	8.26
Ether extract	3.89	3.78	3.67	3.56	3.46
Calcium	0.99	1.09	1.19	1.29	1.39
Phosphorus	0.41	0.47	0.47	0.58	0.63
Energy (Kcal/kg)	2754.0	2750.2	2755.0	2759.0	2761.0

* Premix supplied per kg diet :- Vit A, 10,000 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 2 Proximate composition of PATML

Parameters (%)	PATML
Dry matter	89.73
Crude protein	37.21
Crude fibre	16.22
Ether extract	1.31
Ash	10.22
Energy	2510.5

PATML: *Piliostigma thonningii* and *Phyllantus amarus* leaf meal mixture

Table 3 Mineral composition of PATML

Parameters (mg/100g)	PATML
Calcium	100.04
Phosphorus	78.02
Potassium	30.56
Sodium	11.31
Magnesium	4.07
Zinc	5.02
Copper	2.93
Iron	1.81
Selenium	2.00
Manganese	1.88

Table 4 Phytochemical analysis of PATML

Parameters	PATML	*Permissible range (%)
Flavonoids	29.67	36.11
Phenol	15.08	20.01
Alkaloids	3.05	3.50
Tannins	3.38	11.50
Saponin	4.88	7.02
Glycosides	1.33	-
Steroids	1.02	1.30

*Alagbe, J.O (2019)

Table 5 Performance characteristics of weaner grasscutters fed different levels of PATML

Parameters	T1	T2	T3	T4	T5	SEM
Initial weight (g)	437.0	436.1	436.9	436.5	436.1	0.29
Final weight (g)	1000.4^{c}	1193.0^{c}	1200.4^{b}	1291.5^{b}	1300.1^{a}	3.11
Weight gain (g)	563.0^{c}	756.9^{b}	763.5^{b}	855.0^{a}	864.0^{a}	2.93
Feed intake (g)	3500.2^{a}	3300.6^{b}	3300.5^{b}	3300.1^{b}	3300.0^{b}	2.04
A.F.I (g)	50.15^{a}	47.15^{b}	47.10^{b}	47.05^{b}	47.02^{b}	0.06
Feed: gain	5.86^{a}	4.36^{b}	4.32^{b}	3.86^{c}	3.82^{c}	0.01
Mortality	1	-	-	-	-	-

Means in the same row with different superscripts are significantly different ($P<0.05$)

A.F.I = Average feed intake ; SEM= standard error of mean

Table 6 Haematological parameters of weaner grasscutters fed different levels of PATML

Parameters	T1	T2	T3	T4	T5	SEM
PCV (%)	36.71^{b}	41.04^{a}	43.12^{a}	43.57^{a}	44.10^{a}	0.42
Hb (g/dl)	9.88^{c}	11.44^{b}	12.33^{b}	14.08^{a}	14.53^{a}	1.10
RBC $\times 10^{6}$µl	4.94^{c}	5.03^{b}	5.18^{b}	5.63^{b}	6.72^{a}	0.10
MCV (fl)	71.22^{b}	90.21^{a}	92.56^{a}	92.67^{a}	93.41^{a}	2.33
MCH (pg)	24.51^{b}	25.43^{b}	26.04^{b}	27.11^{a}	27.83^{a}	1.26
MCHC (g/dl)	28.34^{b}	28.67^{b}	29.60^{b}	30.18^{a}	31.60^{a}	0.51
WBC $\times 10^{6}$µl	2.41^{b}	2.62^{b}	2.74^{b}	2.88^{b}	3.04^{a}	0.31
Lymphocytes (%)	29.45^{c}	31.44^{b}	33.60^{b}	33.71^{b}	34.04^{a}	5.32
Monocytes (%)	0.06^{b}	1.01^{a}	1.44^{a}	1.56^{a}	1.82^{a}	0.03
Neutrophils (%)	41.23^{b}	52.06^{a}	53.44^{a}	55.89^{a}	56.02^{a}	4.41
Eosinophils (%)	0.74^{b}	1.22^{a}	1.45^{a}	1.61^{a}	1.87^{a}	0.01

Means in the same row with different superscript are significantly different (P<0.05)
RBC, red blood cell; WBC, white blood cell; MCV, mean corpuscular volume; MCHC, mean corpuscular haemoglobin concentration; MCH, mean corpuscular haemoglobin

Table 7 Serum biochemical parameters of weaner grasscutters fed different levels of PATML

Parameters	T1	T2	T3	T4	T5	SEM
Albumin(g/dl)	1.74^{b}	2.09^{a}	2.11^{a}	2.17^{a}	2.30^{a}	0.17
Globulin (g/dl)	3.11^{b}	3.78^{b}	3.96^{b}	4.00^{a}	4.02^{a}	0.06
T.PRO (g/dl)	4.85^{c}	5.87^{b}	6.07^{a}	6.17^{a}	6.32^{a}	1.01
CHOL. (mg/dl)	109.2^{a}	100.2^{a}	99.61^{b}	90.88^{b}	88.45^{c}	6.71
GLU (mg/dl)	63.10^{c}	73.21^{b}	81.44^{a}	87.80^{a}	89.34^{a}	4.02
Creatinine (mg/dl)	0.09^{b}	1.82^{a}	1.31^{a}	1.24^{a}	1.05^{a}	0.07
Sodium (mmol/l)	109.4^{c}	121.5^{b}	128.9^{b}	134.8^{a}	135.1^{a}	10.02
P (mmol/l)	2.01^{b}	3.03^{a}	3.18^{a}	3.03^{a}	3.00^{a}	1.14
Chlorine (mmol/l)	89.81^{b}	93.10^{a}	97.83^{a}	98.44^{a}	98.09^{a}	5.41
ALP (u/l)	73.44^{a}	69.44^{b}	65.32^{b}	60.15^{b}	56.30^{c}	3.56
AST (u/l)	63.91^{a}	57.10^{b}	51.50^{b}	44.72^{c}	41.29^{c}	2.19
ALT (u/l)	40.34^{a}	40.01^{a}	33.40^{b}	29.67^{c}	28.12^{c}	1.23

Means in the same row with different superscripts differ significantly (P<0.05)
ALP, alanine phosphatase; AST, alanine serum transaminase; ALT, alanine transaminase; CHOL, cholesterol; P, phosphorus; T.PRO, total protein; GLU, glucose.

RESULTS AND DISCUSSION

Table 2 shows the chemical composition of PATML. The chemical components of *Piliostigma thonningii* and *Phyllantus amarus* leaf meal mixture (PATML) used for this study were 90.73 %, 37.21 %, 16.22 %, 1.31 %, 10.22 % and 2510.5 (Kcal/kg) for dry matter (DM), CP, CF, EE, ash and energy respectively. This crude protein and crude fibre value obtained in this study is higher than those of Telfaria occidentalis 31.49 % and 9.80 % respectively (Olanipekun et al., 2016). PATML is high in protein above 20%; it can therefore be used as a protein supplement in grass cutters (NRC, 1994).The ether extract and ash content is similar to those of *Momordica charantia* and *Enantia chlorantha* leaves reported by Olanipekun *et al.* (2016). Higher fibre in PATML can increase digestion in animals, lower the serum cholesterol level and risk of coronary heart disease (Alinnor *et al.*, 2011). The energy level of PATLM is lower than the value reported by Obikaonu et al. (2011) for neem leaves.

The mineral composition of PATML is presented in Table 3. The leaf meal contained calcium, potassium, phosphorus, sodium, zinc, iron, magnesium, manganese, selenium and copper at 100.04, 30.56, 78.02, 11.31, 5.02, 1.81, 4.07, 2.88, 2.00 and 2.93 (mg /100 g). Ca > P> K> Mg> Zn> Cu> Fe> Na> Se> Mn, The leaves are abundant in calcium which plays a key role in constitution of biological systems; it also provides strong bones to the animal (Ibrahim et al., 2011). Phosphorus provides energy for the breakdown of carbohydrates, protein and fats; also needed for growth, maintenance and repair of tissues and cells and for the production of DNA and RNA (Akpanyung, 2005). Alagbe (2020) reported a higher calcium level of 101.17 mg/100g and potassium level (71.62 mg/100g) in dried *Lagenaria brevlifora* fruit.

According to Ojewuyi *et al*. (2015) potassium is responsible for the regulation of water and electrolyte in the body. Ajibade and Fagbohun (2010) reported that magnesium is necessary for major biological processes, including the production of cellular energy and the synthesis of nucleic acids and proteins. Iron combines with myoglobin to transport nutrients within the body and enhance the normal functioning of the central nervous system (Moyo *et al*., 2011). Sodium is needed for proper fluid balance, nerve transmission, and muscle contraction; zinc is a component of many enzymes; needed for making protein and genetic material (Ross, 2005).

The phytochemical constituent of PATML is presented in Table 4. The leaf meal contained the following phytochemical components 3.05 mg/100 g alkaloids, 29.67 mg/100g flavonoids, 15.08 mg/100 g phenols, 3.38 mg/100 g tannins, 4.88mg/100 g saponin, 1.02 mg/100 g steroids and 1.33 mg /100 g glycosides. Flavonoids had the highest value while steroids had the least value. Phytochemicals are secondary metabolites or a bioactive chemical which performs multiple biological functions such as antimicrobial, antiviral, anti-inflammatory, anti-helminthic, antidiuretic and antioxidant effect (Liu *et al*., 2012). The concentration of phytochemicals in plants are not the same for all species, it depends on the extraction method, storage condition, environmental factors, storage conditions and geographical origin (Lillehoj and Lee, 2012; Alagbe and Oluwafemi, 2019). Flavonoids are used as adjuvants in vaccine production and have the ability to scavenge free radicals (Allan and Miller, 1996). Saponin inhibits the growth of gram +ve and gram –ve bacteria (Min *et al*., 2005) and also performs antiprotozoal role (Wallace et al., 1994). A phenol act as antioxidant and prevents the risk of diseases (Hollman, 2001). Tannins have been shown to possess antimicrobial, antiviral and antibacterial activity (Redondo *et al*., 2014). Alkaloid exhibits cytotoxic effect and growth inhibition against a variety of all making them have anti-inflammatory and anti-plasmodic properties (Faizi *et al*., 2008). However, all the phytochemical components were within the safety level recommended by Alagbe and Oluwafemi (2019).

The performance characteristics of weaner grasscutters fed PATML as partial replacement for soyameal is shown in Table 5. Final live weight (LW) and weight gain (WG) range between 1000.4 – 1300.1 g and 563.0 – 864.0 g respectively. They were highest in T4 and T5, intermediate in T2 and T3 and lowest in T1 ($P<0.05$). The result obtained is in agreement with the findings of Omer et al. (2012) who observed a positive effect on the growth of rabbits fed diet mixed with 0.5 % oregano leaves but contrary to the reports of Alagbe (2013) when weaner grasscutters were fed wild sunflower (Tithonia diversifolia) at 20 %. Total feed intake (TFI), average feed intake (AFI) and feed: gain range between 3300 – 3500.2 g, 47.02 – 50.15 g and 3.82 – 5.86 respectively. There is a significant (P<0.05) difference among the treatments in terms of the feed intake and feed:gain. According to Frankič et al. (2009) plants have the ability to regulate feed intake and stimulate digestive secretions due to the presence of phytochemicals in PATML. This agrees with the report of Srinivasan (2005) who concluded in his experiment that supplementation of spices such as black pepper, turmeric and clove at 0.5% in feed of rabbit is capable of stimulating the secretion of pancreatic enzymes and also increase the digestive enzymes of gastric mucosa. One (1) mortality was recorded in T1 and none was recorded in other treatments, this could be attributed to the presence of bioactive chemicals or phytochemicals in PATML. According to Kim *et al*. (2015), presence of phytochemicals in diets stabilizes the intestinal microbiota and reduces microbial toxic metabolites in the gut preventing intestinal challenge and immune stress.

Hematological parameters of weaner grasscutters fed PATML is presented in Table 6. PCV values ranged between (36.71 – 44.10 %), Hb (9.88 – 14.53 g/dl), RBC 4.94 – 6.72 (10^6/µl), MCV (71.22 – 93.41 fl), MCH (24.51-27.83 pg), MCHC (28.34– 31.60 %), WBC 2.41 – 3.04 (10^6/µl), lymphocytes (29.45 -34.04 %), monocytes (0.06 – 1.82 %), neutrophils (41.23 – 56.02 %) and neutrophils and eosinophils (0.74 – 1.87 %) respectively. The treatments were significantly ($P<0.05$) influenced by the dietary inclusion of PATML, the values follow similar pattern as they increased from treatment 1 to 5. However, all the results are within the physiologic range established for healthy grasscutters by Opara *et al*. (2006); Ogunsanmi et al. (2002); Byanet et al. (2008). Similar observation was made by Gboshe *et al*. (2020) who reported a PCV, Hb, RBC and WBC range of (41.70 – 41.90 %), (12.07 – 12.55), 4.95 – 5.35 (10^6/µl) and 2.50 – 2.55 (10^6/µl) respectively. The normal range in the values could be an indication that the test material (PATML) is safe and that the animals were well nourished (Alagbe, 2018; Gboshe *et al*., 2020).

According to Ovuru and Ekweozor (2004) Haematological studies are of ecological and physiological interest in helping to understand the relationship of blood characteristics to the environment. Fluctuations in haematological parameters could be attributed to age and sex (Azeez *et al*., 2009), nutritional deficiencies and feed replacement (Adeyemo *et al*., 2010), breed (Elagib and Ahmed, 2011; Oloyede *et al*., 2010). The increase in the RBC level from treatment 1 to 5 is an indication of enough oxygen in the blood, which also has the ability to carry nutrients round the body and keep the animal healthy. PCV, Hb and RBC are indices used to ascertain anaemia (Isaac *et al*., 2013). Reductions in red and white blood cell indicate haemolytic anaemia and expose the animals to high risk of infection (Akporhuaho, 2011).

White blood cells play a vital role in the prevention of disease or infection, thus animals with low WBC level stand a risk of disease infections. Thus, animals in T_2, T_3 T_4 and T_5 with high WBC are capable of generating antibodies and have a high degree of resistance to diseases (Isaac *et al*., 2013). Leucocyte counts have also been reported to increase during stress, breed, age and unfavorable conditions (Gotoh *et al*., 2001). Butterworth (1999) described basophils and eosinophils as important effector cells in allergy and host defense responses particularly against parasitic infections.

The serum biochemical indices of the experimental animals are presented in Table 7. Whereas total protein (4.85 – 6.32 g/dl), globulin (3.11 – 4.02 g/dl), albumin (1.74 – 2.30 g/dl), glucose (63.10 – 89.34 mg/dl), creatinine (0.09 – 1.82 mg/dl), sodium (109.4 – 135.1 mmol/l), phosphorus (2.01 – 3.18 mmol/l) and chlorine (89.81 – 98.09 mmol/l) were lowest (P<0.05) for T1. Cholesterol (88.45 – 109.2 mg/dl) were highest (P<0.05) for T1 relative to other treatments. ALP (56.30 – 98.09 u/l), AST (41.29 - 63.91 u/l) and ALT (28.12 – 40.34 u/l) were lowest (P<0.05) for T5 relative to other treatments.

Serum biochemical analysis is basically used to ascertain the chemical constituents in the blood (Hrubec *et al*., 2002). The serum total protein, albumin and globulin increases with increasing inclusion of PATLM (P<0.05). According to Alikwe *et al*. (2010), serum protein may be used as an indirect measurement of dietary protein quality. Globulin play a significant role in fighting infections, hormone carrier as well as blood clotting process because of the presence of antibodies and enzymes in them (Vivian *et al*., 2015). However, all within the recommended range according to Opara *et al*. (2006). Cholesterol level decrease with increase in PATML, this clearly removes the risk of cardiovascular disease and ensures that the meat of the animal is safe for consumption. This result is in agreement with the reports of Obikaonu *et al*. (2011) but contrary to the findings of Shittu *et al*. (2019) when rabbits were fed different levels of Ipomoea batatas leaf meal. According to Jenkins (2008), glucose levels in animal's can be elevated due to environmental stress, nutritional stress and improper handling during blood collection. The serum glucose levels determined in this experiment were higher than in some other studies. For instance Gboshe *et al*. (2020) reported a range of 69.35 – 87.73 (mg/dl). Sodium, creatinine, phosphorus and chloride levels were significantly (P<0.05) influenced by the inclusion of PATML, the values obtained in this study is in consonance with the reports of Byanet et al. (2008). The creatinine and sodium level is an indication that the integrity of the kidney is maintained. ALT, AST, ALP levels were depressed as the level of PATML increased, indicating that PATML is non-toxic and thus, the risk of liver damage is prevented. This result is consistent with the report of Iyayi (1994) on the supplemental feeding of high and low cyanide cassava to growing pigs.

Conclusion

It can be concluded that PATML contains it contains several secondary metabolites or bioactive chemicals such as tannins, saponins, alkaloids, flavonoids, oxalate etc. which offers wide range of activities including animal performance and increasing nutrient availability which makes them useful as digestive stimulants, antioxidants as well as treatment and prevention of diseases. The inclusion of PATML at 40% resulted in a significant weight gain and does not have any deleterious effect on the general performance of the animal.

Funding:

This research received no external funding.

Conflicts of Interest:

The authors declare no conflict of interest.

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6/2/2021