

Haematology, Serum Analysis, Cholesterol Status, and Physico-chemical Evaluation of Rabbit Fed Africa Sunflower Leaf Meal in their Diet.

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ABSTRACT

Inclusion of forages as an alternative feed, to reduce cost of feed and improve rabbit health and production, is the focus. A ten weeks of feeding trials was conducted to investigate the effects of African Sunflower Leaf Meal (ASLM) inclusion on weaners rabbit on hematology, serum analysis, cholesterol status and physico-chemical evaluation. 48 (weaners) White New Zealand male rabbit at six weeks of 9.5 to 1kg were allotted to four dietary treatments. Treatment 1: *Tridax procumbens*, Treatment 2 :100% Concentrates, Treatment 3: 50% ASLM + 50% concentrates, Treatment 4: 100% ASLM with twelve rabbits per treatment in a completely randomize design (CRD). Hematological indices were significant highest ($P>0.05$) in T4 with PCV (32.01%), RBC (6.15×10^6 ul), WBC (8.950×10^3 UI), lymphocyte (71.33%), platelet (109.7) than followed by T1, T2 and T3. Serum metabolites had significant highest values in T4 for all parameters measured except for Alb (g/dl) than other treatments. When the dietary treatment on cholesterol was evaluated, T2 had the highest significant values for LDL (7.72 mg/dl) and HDL (60.43 mg/dl), followed by T1 with LDL (6.03 mg/dl) and HDL (59.12 mg/dl) while T4 had the least significant values of LDL (5.14 mg/dl) and (HDL 51.00 mg/dl). T4 had the highest ($P>0.05$) value for water holding capacity (80) followed by T1 (39) while T3 had the least (26). T3 and T4 were rated higher for color or appearance by the panelists than T1 and T2. Rabbits fed T4 100% ASLM in their diet, performed better than other treatments in all parameters measured.

Keywords: rabbits, haematology, serum, cholesterol, physico-chemical

1. Introduction

Malnutrition of animal protein is the major problem faced by majority of the ever increasing population of Nigeria. Production of animal protein from cattle, goat, sheep, swine and poultry requires much capital, time, space and their even their feeding competes with man for certain ingredients, which has led to increase in price of products (Belewu, 1986). Ojewole *et al.* (1999) reported that Animal protein is the best source of protein and often very expensive which it is not within the reach of an average Nigerian.

Rabbit play an important role in the supply of animal protein to Nigeria populace as reported by (Amaefule *et al.* 2005). Rabbits is close to modern broiler chicken in terms of growth rate, feed conversion efficiency and quality of meat produced (Anthony *et al.* 1990). It could feed on non - conventional feeds and it does not compete with man for certain ingredients. Meat of rabbits is tasty, suitable as their cholesterol content is low, sodium and fat are low with high protein contents, no religion and cultural taboo against the consumption of their meat (Biobaku *et al.* 1997).

With many advantages over others, availability and cost of conventional feed ingredients are some of the constraint to rabbit production and the use of non-conventional ingredients is an alternative to reduce cost of feed. The use of forage and agro-industrial by-products has become an area of interest to animal husbandry practitioners, especially, in production of good quality meat and challenges posed by conventional feedstuffs as observed by (Olayeni *et al.* 2006).

Rabbit majorly consumes *Tridax procumbens*, a forage crop known as rabbits weed for a very long time, incorporating other foliage or feeds, which could help to increase its production or the health status should be a welcome idea. Work have been going on, on African sunflower (*Tithonia diversifolia*) for a while now, since researchers has noticed that African sunflower is a good example of forage that contains essential amino acids

and minerals (Farinu *et al.* 1992), it crude protein has high concentration of methionine (FAO 2000), but currently, they are in a limited use, either due to lack of adequate nutritional information's.

Mahecha *et al.* (2005) noted that its protein content of Africa Sunflower could be compared favourably with other known conventional sources. It is rich in minerals and vitamins especially the B-complex vitamins, Day & Levin (1854). Odunsi *et al.* (1999), state that wild sunflower leaf meal contained 16.61% crude protein, 12% crude fibre, 5% ether extract, 14 % ash and 52.39 % Nitrogen free. Onifade (1993), Olayemi *et al.* (2006) also reported that, it has 11% crude fiber, 5.5 % ether extract, 18.2 % ash content and 18.9 % DM.

Most times during the raining seasons, rabbits often had serious problems with cold weather which has resulted in death and folding up of most rabbit farm in masses, this could be attributed to their genetic make-up as it usually affects most rabbit in respective of their breeds. And so the health of rabbit before, during and after the cold weather needs to be serious look into, one of the ways to easily identify rabbit health status is through the blood of rabbit. Onifade (1993), explained that blood examination is a good way of assessing the health status of animals as it played a vital role in physiological, nutritional and pathological status on the animal. Most times feed ingested create a lot of problem on the blood which could result on the health problems on the animal. Church *et al.* (1989) said ingested of numerous dietary component has measurable effect on blood parameters. Therefore, inclusion of African Sunflower (*Tithonia diversifolia*) in the diet of rabbit production could have a positive impact on rabbit blood. So this study will evaluate haematology, serum analysis, cholesterol status, and physico-chemical evaluation of rabbit fed Africa Sunflower Leaf Meal in their diet.

2. Materials & Methods

2.1 Experimental Location / Site

This study was carried out at the rabbitry unit of the Teaching and Research farm of Osun State University, Faculty of Agriculture, Ejigbo, Osun State. The Teaching and Research farms is situated in Ejigbo, Osun State, Southwestern region of Nigeria. The annual rainfall and temperature of the experimental site is 1,2000 mm and 26.6 °C respectively and located on latitude 4° 18 and an altitude of 426 above the sea level.

2.2 Experimental Animal and management

Forty-eight (48) (6 weeks olds) of weaner rabbits of male strain of New Zealand white were sourced from a reputable farm (Tao Rabbits Farm) in Osogbo. The cages were washed, disinfected and the animals were quarantined and acclimatized for a week and later allotted to four treatments and three replicate each. All routine management were carried out (feeding, clean water, observation of sick animal and mortality).

2.3 Experimental design and Treatment

The below dietary treatment was employed and the animals were allotted to the treatment in a Complete Randomized Design (CRD)

Treatment 1: Control 100% Tridax

Treatment 2: 100% Grower mash concentrate

Treatment 3: 50% Africa Sunflower Leaf Meal (ASLM) and 50% grower mash concentrate

Treatment 4: 100% Whole Africa Sunflower Leaf Meal

Table 1: Gross Composition of Grower's Mash

INGREDIENT	Percentage composition
Maize	47.7
Soya bean	20
Wheat offal	17
P.K.C	3.0
Bone meal	5.0
Oyster shell	6.5
Methionine	0.1
Lysine	0.1
Premix	0.5
Salt	0.1
	100
Calculated crude protein (%)	16.6
Metabolizable Energy (kcal/mg)	2600

2.4 Experimental Diets

Africa Sunflower Leaves were harvested at the vegetative stage around the school premises, chopped and air-dried on a concrete floor for three weeks and kept in an air-tight silo bag. Grower mash (concentrate) and *Tridax procumbens* were also fed to the animals.

2.5 Collection of blood samples and analysis

Blood samples were taken from the jugular veins and ear veins of the animals at the end of the feeding trial. The blood collected into sterilized plastic bottles one containing EDTA for haematological indices and the other bottles without EDTA for serum analysis. Packed cell volume was determined by centrifuging in a micro haematocrit capillary contained the blood for 5 mins at 3000rv/mins, RBC, WBC were determined by the use of Neubauer haemocytometer while haemoglobin was determined by cyanmethaemoglobin method and appropriate formula as outlined by Jain (1986), Aspartate amino transaminase (AST), Alanine amino transaminase (ALT), glucose, albumin, total protein were determined by kinetic method of Sampson *et al.* (1980) and kinetic method of Reichling and Kaplan (1988). Cholesterol contents were analyzed as reported by Wechelbaun, (2004), the low density lipoprotein (LDL) and high density lipoprotein were calculated by the use of Friedwald equation (Friedwald 1992).

2.6 Physico-chemical analysis and meat colour determination.

The parameter checked for the meat samples for physico-chemical analysis were; water holding capacity (WHC), determined by press method modified by Suzuki *et al.* (1991), cold shortening determined by Dun *et al.* (1995) method, thermal shortening determined by modified method of Mahendeakar *et al.* (1998), thaw rigor by mathematical formula. The colour of the meats were determined by the use of National Pork Colour Chart Unit of United State of America (NPB 2009).

2.7 Statistical analysis and Design

All data collected from the study was subjected to analysis of variance (ANOVA) and significant mean were separated by Duncan's multiple tests using procedure of SAS (1999). Completely randomized design was used for the study.

3. Results & Discussions

Table 2 shows the proximate composition of the experimental diets, T4 had the highest significant ($p < 0.05$) values in crude protein, crude fiber and ash content with lowest value in dry matter, ether extract and Nitrogen free extract than in other experimental diets. In this table, T4 had the highest nutrients. The crude protein values (22.35 %) was in line with the report of Nguyen *et al.* (2010) but greater than (20 % and 18.9 %) reported by (Patoummalangey 2010 & Olaniyi *et al.*, 2006) however, the values are lower than 28 % reported by (Katto and Salaza 1995). The crude fiber value is greater than the report of 11% reported by Olayemi *et al.* (2006) while the ether extract and the nitrogen free extract are lower than the report of 5.5 % for ether extract (Olayemi *et al.* 2006) and 38.4 % Nitrogen free extract 38.4% (Ngugen *et al.* 2010). The variation observed in the different authors could be due to the different types of species, soil type and environmental condition African Sunflower were subjected to.

Table 3 shows the effect of experimental diet on hematological parameters. T4 had the significant ($p < 0.05$) highest values in PCV (32.01%), RBC (6.15 g/dl), WBC (8390×10^2 /ul), Lymphocyte (71.33 %) and Platelet (109.7) than others experiment diets (T1 –T3). The values obtained were in agreement with the reported by Mitruka & Rawnsley (1997) who work on the clinical biochemical and haematological reference value in Normal Experimental animals. All values obtained were within the range established for healthy rabbits by Mitruka & Rawnsley (2007) The diets contained essential nutrients for normal functioning of the hematopoietic tissues (Esonu *et al.* 2006).

PCV value obtained fell within the range of the values reported by Iheukwumere *et al.* (2003), when tridax were given to rabbit at graded level and also Irekhore *et al.* (2016) with values of 28.0% - 38.0% when pigs were fed with L-carnitine supplemented diets. However, the value obtained was lower than (36.0 – 43.10%) reported by Duwa *et al.* (2015) when weaner rabbits were fed graded levels of sunflower seed meal. Haemoglobin obtained ranges from 8.28g/dl – 11.05g/dl, and the values fell within the values (9.30g/dl – 12.67g/dl) reported by Irekhore *et al.*, (2016). RBC values obtained were in line with that of (Sirdhar 2004 & Duwa *et al.* 2015) while

the value obtained for WBC ($6466.70 - 8950 \times 10^3$) agreed with Farinu (1999) when mango – seed kernel meal were fed to weaner rabbits and that of Iyayi (2001) when cassava leaves was supplemented for feeding of weaner swine.

Neutrophils results in this study fell within the report of Mitruka & Rawnsley (1997) for normal healthy rabbits and Ogbuewu (2015) when rabbits were fed with Neem leaf based diets. Lymphocyte values were within the range of Ogbuewu (2015) but higher than 53.50 % - 65.8 % reported by Mitruka & Rawnsley (1997) for clinically healthy rabbits. Platelet values in this study also fell within the range of (Olabanji *et al.* 2007). Ikewuchi (2009) concluded on haematological parameters that, the increase in monocytes counts and platelet numbers, increased haemoglobin concentration, neutrophil counts and the lymphocyte counts shows that both plant had improved haemoglobin concentration and had potential of anemia management. And Esonu *et al.*, (2001) reported that haematological constituents reflects the responsiveness of the animal to its internal and external environment which include feed and feeding, indicating good nutritional adequacy of all the diet used. In this study, it shows that T4 had the best choice of haematological study constituents a healthy rabbit could have.

Table 4 shows the effect of the treatment on the serum metabolites. T4 showed highest ($p < 0.05$) values for ALT (41.221ul), GLU (40.30 mg/dl), TP (7.37g/dl) and cholesterol (160.30mg/ul) than other treatment evaluated. T1 has the highest value of AST (41.561ul), T2 showed the highest value for albumin (4.07g/dl) and T3 as in T4 showed significant effect ($P < 0.05$) on glucose with the value (41.10 and 40.30mg/dl). The values obtained for ALT were in line with Fasuyi *et al.*, (2013) who fed growing pigs with varying levels of wild sunflower leaf meal. The value obtained for glucose were in line with Ogbuewu *et al.* (2015) when rabbits are fed with neem leaf meal based diets. TP values obtained agreed with the report of Irekhore *et al.* (2016) with (6.55g/dl - 7.55g/dl) values. While the albumin values obtained were not in agreement with the report of Fasuyi (2007), who worked on *Telfaria Occidentalist* leaf meal. Cholesterol values were contrary to what was observed by Irekhore *et al.* (2016) when pigs were fed with L-carnitine supplemented diets (134.3mg/dl – 184.0mg/dl). The highest value in T4 could be attributed to the high saponin content which has been shown to bind to serum lipids especially cholesterol thereby easing their excretion from circulation as reported by (Matawall 2009). LDL and HDL values were significantly different ($P < 0.05$) The values obtained were in line as reported by Ogbuewu (2008) of ASLM supplementation in pig increases serum HDL and lowers LDL. This is due to the secretion of Very Low Density Lipoproteins (VLDL).

Table 5 shows the physico-chemical parameters of rabbit meat fed African sunflower inclusion in their diet. T4 had the higher significant ($p < 0.05$) values for water holding capacity with 80.87 than T1 – T3. T2 was higher in thermal shortening and thaw rigor while T3 had the greater value in cold shortening. Cold shortening has been reported in recent years as resulting from a low temperature in the muscle before the onset of rigor mortis, which causes contraction in muscle resulting in reduction in the length of muscle from the initial length (Hedrick *et al.* 1994) T1 and T4 had the least cold shortening and thermal shortening, which is the reduction in length of meat under a higher temperature. Thaw rigor also follows the same trend as in T1 and T4, but the water holding capacity followed different ways as T4 had the highest value and T3 had the least value. Water holding capacity is the ability of meat to retain its water during application of external forces such as cutting, grinding and pressing, is also used as a good indication for quality good meat product. It could also be loss evaporation from meat surface, as exudates or when muscles are cut (Hedrick *et al.* 1994). The value obtained in this study are less than (44.1- 69.1%) reported by Fakolade (2008) and (42.2 - 67.0%) reported for scalded, singed and skinned dressed rabbit reported by Omojola & Adesheyinwa, (2006) but were far greater than (1.3 – 2.0%) reported by Babiker & Lawrie (1983) for water holding capacity of hot deboned beef. The parameters in Table 5 indicate that T4 muscle is a choice muscle since it has the high water holding capacity, low thaw rigor and lower cold and thermal shortenings. Figure 1. Also indicate that the colour of T3 appear best while T2 and T4 follows with bright pinkish red muscle colour.

4.0 Conclusion

Rabbit strived well with whole consumption of whole Africa Sunflower leaves meal in their diets, by affecting their health positively. Further studies on whole Africa Sunflower leaves meal on rabbit performance and meat quality could be considered.

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Table 2: Proximate Composition of Experimental Diet.

Constituents	T1 <i>Tridax</i>	T2 Grower's mash	T3 Grower's mash + <i>Tithonia diversifolia</i>	T4 <i>Tithonia diversifolia</i>	SEM
DRY MATTER(%)	90.10 ^{ab}	93.15 ^a	91.50 ^{ab}	88.12 ^b	0.249
CRUDE PROTEIN(%)	22.98 ^a	19.25 ^c	22.05 ^a	22.35 ^a	0.341
CRUDE FIBRE(%)	5.46 ^c	4.54 ^c	7.30 ^b	12.70 ^a	0.336
ASH(%)	3.10 ^d	5.50 ^c	10.30 ^b	15.70 ^a	0.190
ETHER EXTRACT(%)	5.10 ^a	5.50 ^a	3.50 ^b	2.11 ^c	0.275
NITROGEN FREE EXTRACT(%)	53.86 ^c	65.21 ^a	56.85 ^b	41.14 ^d	1.98

^{abcd} means on the same row with different superscripts are significantly different (P<0.05)

SEM-Standard error of mean

Table 3: Shows the effect of diet on the Haematological parameters of the experimental animal

Parameter	T1	T2	T3	T4
PCV (%)	29.01±3.79	33.78±2.32	26.88±3.57	32.01±0.57
H.b (g/dl)	9.53±1.37	11.05±0.51	8.28±1.01	9.41±0.40
RBC (10 ⁶ ul)	5.85±0.28 ^{ab}	5.36±0.11 ^b	5.30±0.06 ^b	6.15±0.08 ^a
WBC (10 ³ ul)	8950±862.17 ^a	8300±351.19 ^{ab}	6466.70±308.7 ^b	8390±195.19 ^a
Lymp (%)	70.80±0.92 ^a	65±0.003 ^b	65.68±2.33 ^b	71.33±0.33 ^a
Neut (%)	24.33±0.88 ^b	27.03±1.51 ^{ab}	40.82±8216.60 ^a	22.48±1.25 ^c
Mono (%)	2.33±0.67	2.67±0.67	2.00±0.58	3.67±0.67
Eos (%)	2.67±0.67	2.67±0.33	2.00±0.58	2.00±0.58
Platelet	104±3.61 ^{ab}	102.33±3.84 ^{ab}	93.00±4.73 ^b	109.7±3.90 ^a

^{a,b,c} Means within the same row with different superscripts differs significantly (P<0.05).

PCV- Parked cell volume, Hb- Haemoglobin, RBC-Red Blood Cell, WBC-White blood cell, Lymp-Lymphocyte, Neut-Neutrophil, Mono-Monocyte, Eos-Eosinophil, ±SEM-Standard Error of Mean

Table 4: Shows the effect of treatment on the serum metabolites

Parameters	T1	T2	T3	T4
AST(I.U/l)	41.56±2.61 ^a	39.41±0.47 ^{ab}	35.16±0.02 ^b	39.63±0.06 ^{ab}
ALT(I.U/l)	30.94±2.55 ^{ab}	30.25±5.77 ^{ab}	24.6±1.11 ^b	41.22±1.38 ^a
GLU(mg/dl)	24.67±2.70 ^b	39.97±1.52 ^a	41.10±1.67 ^a	40.30±0.20 ^a
Alb(g/dl)	3.83±0.14 ^{ab}	4.07±0.08 ^a	4.00±0.01 ^a	3.62±0.02 ^b
TP(g/dl)	6.15±0.04	7.26±0.14	6.27±0.82	7.37±0.54
Cholest.(mg/dl)	137.83±8.82 ^a	73.25±2.63 ^b	80.66±5.43 ^b	160.30±19.38 ^a
LDL(mg/dl)	6.03±0.02^b	7.72±0.14^a	5.72±0.04^c	5.14±0.08^d
HDL(mg/dl)	59.12±0.02 ^b	60.43±0.29 ^a	52.77±0.11 ^c	51.00±0.03 ^d

^{a,b} Means within the same row with different superscripts differs significantly (P<0.05)

AST-Aspartate amino transaminase, ALT-Alanine amino transaminase, GLU-Glucose level, Alb-Albumin, TP-Total protein, Cholest-Cholesterol content, HDL-High Density Lipoprotein, LDL-Low Density Lipoprotein. ±SEM-Standard Error of Mean

Table 5: Shows the effect of diets on the physio-chemical analysis of the animal

Parameters	T1	T2	T3	T4
Thermal shortening	13.33±0.27 ^d	19.95±0.72 ^a	17.43±0.40 ^b	10.18±1.02 ^c
Cold shortening	4.61±0.34 ^c	8.03±0.57 ^b	11.67±0.61 ^a	6.49±0.92 ^{bc}
Thaw rigor	18.70±0.91 ^d	39.12±2.14 ^a	26.58±0.32 ^b	22.66±0.73 ^c
Water Holding capacity	39.90±4.93 ^b	51.16±6.38 ^{bb}	25.55±7.19 ^c	80.87±8.06 ^a

^{a,b,c} Means within a row with different superscripts differs significantly (P<0.05)

Figure 1: Effect of dietary treatment on the colour of the animals

