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HEMATOLOGICAL AND CLINICAL CHEMISTRY OF WEANER RABBITS FED GRADED LEVELS OF AFRICAN STAR APPLE *CHRYSOPHYLLUM ALBIDUM* SEED MEAL

*AMAO, Emmauel. Ayodele, **SOKUNBI, Adedamola Olajide and ***AGBAYE, Folorunso Peter
*Department of Animal Production Technology, The Oke Ogun Polytechnic, Saki.
**Department of Animal Science, University of Ibadan, Ibadan.
***Department of Animal Production Technology, Lagos State Polytechnic, Lagos.

Corresponding author E-mail: amaoemmanuelayodeleamao@gmail.com

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Abstract
This study was carried out to determine the haematological profile and serum biochemical indices of rabbits fed graded level of African star apple (*Chrysophyllum albidum) seed meal*. The study involved forty eight (48) mixed breed weaned rabbits of seven - eight weeks old, with average weight of 186.87±0.03g.The rabbits were allotted into four dietary treatments comprising twelve (12) rabbits per treatment. The dietary treatments were 0%, 5%, 10% and 15% ASASM inclusion levels. The experimental diet contained 15% crude protein and 2488.68 Kcal/Kg metabolizable energy. The feeding trial lasted eight weeks, during which period feed and water were provided *ad – libitum*. Two blood samples each were collected per rabbit; one set of the blood samples was collected into plastic bottles containing Ethylene Diamine Tetra acetic acid (EDTA) for haematological studies while the other sample was collected into plain sterile bottles for the serum biochemical indices determination. Data generated were subjected to analysis of variance. Results obtained from this study revealed that the haematological profile and serum biochemical indices of rabbits recorded no significant ($P > 0.05$) differences among dietary treatments except cholesterol, AST and ALT ($P < 0.05$). MCV, MCH, MCHC and other hematological values were within the normal range ($p>0.05$), the platelet counts recorded in this study ranged from115 to 146 $x10^9$/L which is however lower than the normal range (250.00 – 600.00 $x10^9$/L) for rabbits. The study concludes that inclusion of graded levels of *C albidum seed meal* up to 15% will not pose any adverse effects on the blood characteristics of rabbits except the lower value of platelets across the treatment.

Introduction
Feed scarcity has remained a major limitation to livestock production and productivity (Agbede and Aletor, 2003). The high cost of animal feeds precipitated by the ever increasing cost of feed ingredients has resulted in poor nutrition leading to declining productivity of animals in developing nations like Nigeria (Nodu *et al*., 2014). According to Fetuga (1997) there is a disappointing rate and low level of performance in the Nigerian livestock industry, due to high cost of feeds, poor quality feeds and inefficiency in production and distribution in the feed industry. The low level of livestock production has affected adequate protein intake. However, intensive rabbit production and domestication can help bridge the meat supply gap in Nigeria and ensure animal protein adequacy. Increase in meat production can be achieved through proper nutrition, inclusion of feed ingredients at normal or required levels (Etim and Oguike, 2010).

The domestic rabbit (*Oryctolagus cunniculus*) is an important non-ruminant herbivore for meat production. Rabbit meat is a source of healthful food as it is low in cholesterol, but high in protein, 22g/100g (Aduku, and Olukosi 1990). Rabbits can also utilize the available proteins in cellulose rich plants, whereas it is not economical to feed these to chicken and turkeys. Since there is high demand for additional source of food worldwide the exploitation of plants of low economic importance would be a step towards better resource utilization (Telek, and Martin 1983) which is in line with the strategy to achieve sustainable animal production systems by matching them with locally available feed resources (Preston and Sansoucy 1987).

African star apple (*Chrysophyllum albidum)* is a dominant canopy tree of lowland and mixed rainforests, sometimes, riverine (Madubuike and Ogbonnaya, 2003). The plant is propagated by seedlings, wildings and direct sowing. It is a seasonal fruits-bearing tree. The fruit is fleshy and juicy, producing whitish gummy exudates (Adewusi, 1997). It is widely eaten in South Western Nigeria, popular among women and children (Amusa *et al.*, 2003).

The seeds of *C. albidum* are about 1-1.5 x 2 cm, beanlike, shiny when ripe, compressed, with one sharp edge and a star-shaped arrangement in the fruits (Orwa *et al.*, 2009). The seed coat is hard, bony, shiny, and dark brown, and when broken reveal white-colored cotyledons (Amusa *et al.*, 2003).

The examination of blood provides the opportunity to clinically investigate the presence of several metabolites and other constituents in the body and it plays a vital role in the physiological, nutritional and pathological status of the animal (Aderemi, 2004; Doyle, 2006). It also helps to distinguish normal state from state of stress which can be nutritional (Aderemi, 2004). Haematological parameters are good indicators of the physiological status of animals (Hawkey and Dennett, 1989; Adenkola and Durotoye, 2004; Khan and Zafar, 2005). They are also excellent medium for the measurement of potential biomarkers, because its collection is relatively non-invasive and it encompasses an enormous range of physiological process in the body at any given time (Ginsbury and Haga, 2006). Haematological constituents reflect the physiological responsiveness of the animal to its internal and external environments which include feed and feeding (Esopnu *et al.*, 2001). According to Daramola *et al.* (2005), haematological values could serve as baseline information for comparisons of nutrient deficiency. Dietary content affect the blood profile of healthy animals (Odunsi *et al.*, 1999; Kortuglu *et al.*, 2005). Isaac *et al.* (2013) stated that haematological components, which consists of red blood cells, white blood cells or leucocytes, Mean Corpuscular Haemoglobin and Mean Corpuscular Haemoglobin Concentration are valuable in monitoring feed toxicity, especially, with feed constituents that affect the blood as well as the health status of farm animals. Many feed products are fed to rabbits usually without recourse to their health and physiological implications on the animals. The commonest parameter for measuring these implications is through the haematology of the animals (Aro *et al.*, 2013). Moreover, the comparison of blood profile with nutrient intake might indicate the need for adjustment of certain nutrients upward or downward for rabbits (Rafiu *et al.*, 2013).
However, there is no information on the effect of seeds of *C. albidum* on haematology and serum biochemical parameters of rabbits. Therefore, this study was carried out to investigate the effect of feeding graded levels of seeds of *C. albidum* on the haematological and serum biochemical parameters of rabbits.

Materials and Methods

The study was carried out at the rabbitary unit of the Oke-Ogun Polytechnic, Saki. Oyo State, Nigeria.

African Star Apple seeds were collected from primary and secondary schools in Saki, Oyo State, Nigeria. The seeds were sun dried and further broken to obtain the cotyledons. The cotyledons were sun dried and milled for inclusion in diet mixture of growing rabbits.

Experimental Animals and Management

A total of Forty-eight (48) growing rabbits (7 – 8 weeks of age with average weight 186.87 ±0.03g) of mixed sexes were obtained from a reputable farm in Oyo State, Nigeria for the purpose this study. The rabbits were randomly assigned to the four (4) treatments with six (12) rabbits per treatment. At the end of the feeding period which lasted for 8 weeks, the animals (six per treatment) were starved of feed for 24hour and thereafter bled. The animals were housed in wooden cages raised from the ground level. Before the animals were brought into the rabbitary, the cages were thoroughly washed and disinfected and allowed to dry. The floor of each cage was covered with wire mesh to allow for the passage of waste materials. Feed and water troughs were provided in each cage, while feed and water were provided *ad – libitum.* The rabbits were caged individually in clearly marked cells. The feeding trial lasted eight weeks.

3.3 EXPERIMENTAL DIET

Four experimental diets were formulated with the inclusion of 0%, 5%, 10% and 15% African Star Apple seed meal (ASASM) as substitute for maize in rabbit diet.

Parameters	0% (ASASM)	5% (ASASM)	10% (ASASM)	15% (ASASM)
Maize	40.00	38.00	36.00	34.00
ASAS	0.00	2.00	4.00	6.00
Maize offal	25.00	25.00	25.00	25.00
Rice offal	18.00	18.00	18.00	18.00
Soybean meal	2.00	2.00	2.00	2.00
Fish meal	1.20	1.20	1.20	1.20
Groundnut cake	10.00	10.00	10.00	10.00
Limestone	1.00	1.00	1.00	1.00
Bone meal	2.00	2.00	2.00	2.00
Salt	0.20	0.20	0.20	0.20
Premix	0.30	0.30	0.30	0.30
Methionine	0.20	0.20	0.20	0.20
Lysine	0.10	0.10	0.10	0.10
Total	100kg	100kg	100kg	100kg

Blood collection:
Blood collection was done at end of the 8th week of feeding trial from six rabbits per treatment. Blood samples were obtained from the prominent ear vein with the aid of a hypodermic needle and syringe. Two blood samples each were collected per rabbit; one set of the blood samples was collected into plastic bottles containing Ethylene Diamine Tetra acetic acid (EDTA) for haematological studies while the other sample was collected into plain sterile bottles for the serum biochemical indices determination. All haematological parameters were determined by conventional laboratory methods of Baker and Silverton (1982); Bitto and Gemade (2001), while serum biochemical indices were determined by methods described by Ochei and Kolhathar (2007).

STATISTICAL ANALYSIS

All data collected were subjected to analysis of variance (ANOVA) technique using statistical analysis software. Significant differences between treatment means were separated using Duncan's Multiple Range Test

Results and Discussion

Table 2: Haematological indices of rabbit fed African Star Apple seed meal.

Parameters	0%ASASM	5%ASASM	10%ASASM	15%ASASM	SEM	Normal range
PCV (%)	39.00	40.33	40.66	39.33	3.16	33.00 – 50.00
Haemoglobin(g/dl)	12.96	13.60	13.60	12.76	1.08	9.40 – 17.40
RBC($x10^{12}$/l)	6.33	6.60	6.64	6.44	0.53	3.80 – 7.90
MCV (fl)	61.93	61.47	63.30	66.17	0.80	58.50 – 66.50
MCH (pg)	20.48	20.92	20.47	19.69	1.42	18.00 – 24.00
MCHC (g/dl)	33.44	33.98	33.93	32.63	1.80	27.00 -34.00
WBC($x10^9$/l)	5.93	5.86	5.96	5.77	3.25	5.00 – 13.00
Platelet ($x10^9$/l)	128.33	146.00	125.66	115.00	0.83	200.00 – 650.00
Lymphocyte (%)	68.33	67.66	69.33	69.33	0.83	43.00 – 80.00
Neutrophils (%)	28.66	28.33	28.33	28.00	2.62	34.00 – 70.00
Monocyte (%)	2.00	2.00	1.00	2.00	0.43	0.00 – 4.00
Eosinphils (%)	1.00	2.00	1.33	0.66	0.41	0.00 – 2.00

KEY: Packed cell volume (PCV), White blood cell (WBC), Red blood cell (RBC), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC), African Star Apple Seed Meal (ASASM).

Table 3: B**iochemical indices of rabbit fed African Star Apple Seed Meal.**

Parameters	0%ASASM	5%ASASM	10%ASASM	15%ASASM	SEM	Reference range
Glucose(mg/dl)	78.39	65.33	66.66	64.18	3.87	75.00 – 140.00
Cholesterol(mg/dl)	75.23^b	113.33^a	70.83^b	87.59^b	6.57	10.00 – 80.00
AST (IU/L)	51.66^b	97.90^a	55.69^b	43.40^b	4.47	10.00 – 98.00
ALT (IU/L)	41.59^a	38.23^a	28.07^b	30.08^a	1.58	55.00 – 260.00
ALP (IU/L)	56.09	58.26	76.49	68.84	6.40	10.00 – 96.00
Total protein (g/L)	7.14	5.93	5.97	5.28	0.50	50.00 – 75.00
Albumin (g/L)	3.14	3.42	3.89	3.49	0.60	25.00 – 40.00
Calcium ion (mg/dl)	7.44^a	6.99^b	5.84^b	10.18^a	0.55	5.50 – 12.50
Sodium ion (mmol/L)	126.95^a	59.69^b	106.39^{ab}	103.30^{ab}	12.36	130.00 – 155.00
Chloride ion (mmol/L)	97.58^a	90.88^{ab}	88.60^{ab}	78.35^b	3.60	92.00 – 120.00
Creatinine (mg/dl)	1.40^a	1.13^{ab}	1.06^b	1.26^{ab}	0.00	0.50 – 2.60
Urea (mmol/L)	6.68	6.35	5.97	5.55	0.38	9.10 – 25.50

Key: Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline Phosphatase (ALP).
Reference ranges obtained from Medirabbit.com (http://www.medirabbit.com/EN/haematology/bloodchemistry.htm)

Results and discussion

It is well established that changes in haematological and biochemical parameters reflect the physiological status of animal. Packed cell volume (PCV) values obtained in this study were not significantly ($p>0.05$) influenced by dietary treatments. The values (39 – 40.66%) were within the reference range of 33 to 50% reported by MediRabbit (2007). The results showed that inclusion of ASASM does not pose anemic condition on rabbits fed up to 15%. The red blood cell (RBC) range of 6.33 – $6.64x10^6/mm^3$ and haemoglobin (Hb) range of 12.76 to 13.60 were within standard range of 4.86 to 7.9 x $10^6/mm^3$ for RBC and 10 - 15g/dl for Hb respectively (RAR, 2009). This result indicates that *C. albidum* seed support red blood cell synthesis and effective oxygen transport to the animals' tissues for oxidation of ingested feed to release energy for other body function as well as transport of CO_2 out of the body. MCV, MCH and MCHC values were within the normal range as shown in table 2. MCV of rabbits fed ASASM indicates normocytosis, MCH showed no deficiency in iron and MCHC reflects normochromic status of the animals. The platelet counts recorded in this study ranged from115 to 146 $x10^9$/L for rabbits fed graded

levels of *C. albidum* seed. The values were however lower than the normal range (250.00 – 600.00 $x10^9$/L) for rabbits reported by Medirabbit (2011). This low platelet concentration suggests prolonged blood cloting which can result in excessive blood loss in case of injury. The white blood cell (WBC) values obtained in this study which were not significant among treatments were within the normal range of 4.5x 10^9 /L to 11 x 10^9 /L reported by (RAR, 2009). This suggests normal production of defensive mechanism to combat infection. The white blood cell differentials namely lymphocytes, neutrophils, monocytes and esonophils were not significantly influenced by dietary treatments. Inclusion of ASASM in rabbits' diet had no negative influence on the production and release of antibiotics to fight infection and defense against bacterial invader.

Biochemical response of rabbits fed *C. albidum* seed was presented in table 3. No significant difference ($p>0.05$) was observed in the values of glucose, total protein and albumin. Values for glucose range from 64.18 to 78.39mg/dl, only animals on control diet falls within the standard range of 75 – 140mg/dl (Medirabbit, 2011). Animals placed 5%, 10% and 15% had values below the standard range and this implies hypoglycemia in the rabbits. Values of total protein (5.28 – 7.14g/l) and values of albumin (3.14 – 3.89g/l) were within the standard reference of 5.0 – 7.5g/l and 2.7 – 5.0 g/l respectively (Medirabbit, 2011). This suggests that normal protein metabolism occurred in rabbits, since protein synthesis is related to the amount of protein available in the diet (Iyayi and Tewe, 1998). Cholesterol and Aspartate aminotransferase (AST) followed the same trend with animals placed on 5% having highest values, cholesterol ranged from 70.83 – 113.33 while AST value ranged from 43.40 – 97.90 values for the two parameters falls within the standard range of 10 to 80mg/dl and 10 to 98mg/dl respectively (Medirabbit, 2011). This implies no deleterious influence of ASASM on rabbit biochemical response.

Conclusion and Recommendation

Results obtained in this study have shown that *C. albidum* seed can be included in weaner rabbits' feed without adverse effects on their haematological profile and serum biochemical indices except for lower platelet values which can lead to excessive lost of blood in case of injury.

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