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Hematology and SerumBiochemistry of Rabbits fed gradeddietary levelsof Rice offal and Palm Kernel Cake

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Target Audience: Animal Scientists, Feed millers, Farmers

Abstract
The effects of feeding graded levels of Rice offal (RO) and Palm kernel cake (PKC) mixtures in a ratio of 1:1 on hematological and serum biochemical parameters of rabbit were studied. Thirty six (36) unsexed, mixed breed grower rabbits aged between 5-7 weeks were randomly divided after weight balancing into four treatment groups each of which was further divided into three replicates which were each housed in a separate cage in a Completely Randomized Design. All replicates contained three rabbits each. Four diets were formulated, diet1of which contained zero RO/PKC served as control, while diets 2, 3 and 4 incorporated 12, 24, and 36% RO/PKC mixture respectively. Before commencement of the experiment, the animals were stabilized with long acting Terramycine injection and a uniform concentrate diet containing 15% crude protein for one week, after which they were fed with experimental diets for 8 weeks. Throughout the experimental period, feed and water were offered to the animals atadlibitum. Results of the experiment showed that hematological parameters including the Red blood cell(RBC), lymphocytes, monocytes, neutrophils, packed cell volume(PCV), hemoglobin(Hgb), mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC) were not significantly ($p>0.05$) affected by the treatments, whereas the white blood cell (WBC), showed significant ($p<0.05$) treatment effect. The serum biochemical characteristics of the rabbits indicated that serum total protein (TP), albumin, globulin, aspartate aminotransaminase(AST), alanine aminotransaminase(ALT) were not significantly ($p>0.05$) affected, while Alkaline phosphatase (ALP) was significantly affected ($p<0.05$) by the treatments. The results suggests that inclusion of Rice offal/palm kernel cake mixture in a ratio of 1:1 at levels up to 36% in rabbits diets can provide healthy nutrition in rabbits feeding programmes.
Key words*: Rabbits, hematology, serum biochemistry, rice offal, palm kernel cake*

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I. Introduction

In most developing countries where animal protein supply required to meet the protein need of the populace is grossly inadequate, rabbit production can be harnessed to bridge this gap. According to [1], the rabbit appears to be the most sustainable means of producing high quality animal protein for the expanding populations of the lesser developed countries like Nigeria. The rabbit is small-bodied (2.5 – 5.4Kg) animal,and has a short generation interval (6 months). Other desirable attributes of this animal include rapid growth rate, genetic diversity, large litter size, ability to utilize forage and agricultural by-products; and adaptation over a wide range of ecological environments. Rabbits do not competewith humans for grains as strongly as chickens [2, 3]. Unlike bigger animals such as cattle, rabbits can be tended by women, children or old men, as they do not need force to be restrained [4]. The small body of a rabbit provides a small carcass that can be consumed by a family in one meal, thus eliminating the need for meat storage and refrigeration.In addition, it is affordable and its management requirements are low-cost. Rabbit production can provide the impoverished urban population and the resource-poor rural dwellers the opportunity to meet part of their total protein needs, as well as earn additional income.

Nutritionally, the rabbit meat is high in protein, and low in fat content, thus providing quality meat for human consumption.

To make rabbit rearing more viable as a small-scale business, [5] advocated the development of alternative feed with materials that will be relatively cheap when compared to conventional commercial feeds.

This calls for research into alternative source`s of energy and protein yielding ingredients to replace the expensive conventional cereal grains and legumes, such as maize, rice, sorghum, groundnut and soybean, the prices of which have soared in recent times such that it is becoming uneconomical to use them in rabbit feeds[1].

Rice offal (RO), palm kernel cake (PKC), and oil palm slurry are potential feed resources which can be used in formulating balanced rations for rabbits.

Rice mill waste (rice offal), is a mixture of all the by-products obtained in the milling of rice, it contains approximately 60% hulls; 35% bran and 5% polishing. According to [6] the proximate composition of rice offal thus: 94.42% dry matter, 5.09% crude protein, 30.39% crude fibre, 3.40% ether extract, 16.67% ash and 46.10% nitrogen free-extract.

Rice offal(RO) is not consumed by humans and the rice milling industries are searching for means of its disposal in the developing countries.

Palm kernel cake(PKC), is a by-product of the palm kernel oil industry. It is an interesting feed ingredient due to its availability and low cost. [7] reported that PKC contains 19% CP, 2.0% EE, 16.0% CF, 58.8% NFE, 8.2% Ash, 0.34% Ca, 0.96% P and 48.04 ppm Cu; with low soluble fibre, starch and sugar concentrations[8]. This feedstuff is suitable to meet nutrient requirement of high productive rabbits [9].

Oil palm slurry (OPS) is another untapped non-conventional feedstuff in developing countries. Due to the cost and competitive demand for Palm oil as energy source, there is need to fall back to the inclusion of OPS in rabbit diets. The dry slurry contains 90.7% DM, 13% CP, 12.31% Crude fat, 32.07% Crude fibre and a metabolizable energy of 1105.87 kcal/kg[10]. Feeding trials suggest that up to 10% of oil palm mill effluent can replace corn offal in diets without deleterious effects [11]. The absence of major side effects such as diarrhea suggests that the pressed residue did not contain toxic compounds which could interfere with nutrients utilization in rabbits [11]

It is important to note that the nutrient absorbed after digestion of feed/food are carried by blood to different parts of the body.If the nutrient absorbed are toxic to the body, the physiology of the animal will be affected. Therefore hematological and serum biochemical studies were vital in this research. Many feed stuffs and ingredients are fed to rabbits usually without recourse to their health and physiological implications on the animals. Findings of [12] posited that blood parameters can be used to assess the health as well as the physiological status of farm animals/human subjects.[13] and [12] reported that hematological parameters like hematocrit value, hemoglobin concentration, WBC count, RBC count, among others are used in routine screening for health and physiological status of livestock and even humans; while [14] maintained that PCV is involved in transport of oxygen and absorbed nutrients.Indeed, hematological and serum biochemical components of the blood of an animal have been found to be influenced by the quantity and quality of its feed [15].Agreeably therefore, blood analysis provides a clue to the health status of exposed animals to toxicants and other stress factors [16]. According to [17], investigations on the nutrition of animals need to go beyond their growth response, nitrogen balance, and efficiency of feed utilization. The researcher opined that it is also important to monitor the influence of nutrition on the metabolism of the cells in the various tissues. It has been established [18, 14, 19] that hematological parameters are useful in monitoring feed toxicity, especially with feed constituents that affect the blood. On the other hand, serum biochemical analysis has been reported to provide information on internal organs (liver and kidney) integrity, serum proteins and electrolyte balance, as well as the nutritional or metabolic functions of the organism [20, 21].

This work studied the hematological and serum biochemical characteristics of grower rabbits fed with PKC and RO based diets.

II. Materials and methods

Experimental site

This study was conducted at the Animal Science Unit of the Teaching and Research Farm, Faculty of Agriculture and Forestry, Cross River University of Technology, Obubra Campus, Cross River State.The geographical location of the site lies along Longitude 8 - 9°E and Latitude 6 - 7°Nof the equator, with a warm weather and ambient temperature of about 21 - 30°c and has an annual rainfall of 500 - 1070mm [22].

Sources of feed materials

Rice offal was packed from Rice milling plant in Ofodua, while Oil palm slurry (OPS) was sourced from an oil palm mill close to the University campus, all in Obubra Local Government Area of Cross River State. Palm kernel cake and other feed ingredients were purchased from certified feed merchant in Uyo, Akwa Ibom State, Nigeria.

Processing of feed Stuffs

For test diets, OPS was aggregated with rice offal at the rate of 4% inclusion. Similarly, OPS meant for incorporation in the control diet (diet 1) was mixed with wheat offal, also at the rate of 4%. The two combos were sun-dried for 3 – 5 days, this was to reduce the moisture introduced by OPS to a safe level while at the same time taking advantage of the ultraviolet-radiation of the sun to deal with pathogenic organisms in the aggregates during the process. After the materials were dried to a moisture content of 5 – 7%, they were stored in air-tight plastic bags prior to their use in ration formulation.

Experimental Treatments

Thirty six unsexed mix-breed grower rabbits (hybrid of Dutch and Chinchilla) between the ages of 5 – 7 weeks were sourced from the Nigerian Veterinary Research Institute(NVRI), Vom, in Jos, Plateau State. They were divided into four similar groups on weight equalization basis after which they were randomly assigned to four treatments of nine rabbits, each of which was further divided into three replicates of three rabbits per replicate in a completely randomized design. The replicates were housed in separate wooden/wire mesh cages.

Experimental Diets

Four experimental diets containing 0, 12, 24, and 36 percent of rice offal and palm kernel cake mixture in a 1:1 ratio were formulated for treatments 1 – 4, respectively.

The experimental diets and the feed ingredients are as shown in Table 1.

Management of experimental Rabbits

Before the commencement of study, the rabbits were put through a stabilization period of one week, during which they were administered long acting Terramycine anti-biotic injection, and placed on concentrate feed containing 15% crude protein.

Throughout the period of study which lasted for fifty-six (56) days, fresh experimentalfeed and clean drinking water were offered to the animals, *adlibitum.* Otherprophylactic precautions were also observed during the study.

Table 1: Composition of Experimental Grower Rabbits Diets (g/100g)

Ingredients	T_1 0% RO/PKC	T_2 12% RO/PKC	T_3 24% RO/PKC	T_4 36% RO/PKC
Maize	45.0	33.0	21.0	9.00
Rice offal	——	6.00	12.0	18.0
Palm kernel cake	——	6.00	12.0	18.0
Soybean	12.2	10.6	8.9	7.30
Wheat offal	32.5	34.1	35.8	37.4
Blood meal	2.00	2.00	2.00	2.00
Bone meal	3.50	3.50	3.50	3.50
Oil palm slurry	4.00	4.00	4.00	4.00
Salt	0.30	0.30	0.30	0.30
Vitamin premix	0.50	0.50	0.50	0.50
Total	100	100	100	100
Calculated composition				
CP	16.0	16.0	16.0	16.0
CF	8.30	8.50	11.1	14.2
ME(Kcal/kg)	2666.45	2473.29	2279.3	2086.14
Proximate composition				
CF	12.27	12.83	13.81	14.67
CP	15.25	15.26	14.81	14.82
NFE	72.13	72.67	71.10	70.59
DM	95.77	96.37	96.18	96.63
EE	2.28	3.43	2.99	2.46
Ash	3.84	2.18	3.47	3.89
ME(kcal/kg)	3309.55	3342.24	3314.21	3253.55

CP=crude protein; CF=crude fibre; EE=ether extract; DM=dry matter; ME=metabolizable energy; NFE=nitrogen free extract

Data collection

At the end of the feeding trial period of eight weeks, twelve rabbits were bled between 9.00am and 10.30am from a punctured ear vein. Animals for bleeding were randomly selected, and triplicate blood samples were collected from three rabbits per experimental group. Twelve millilitres (12ml) of blood was aspirated from each of them. Two millilitres (2ml) of each blood sample was discarded into ethylene di-amine tetra acetic acid (EDTA) treated bijou bottles for hematological assay. The remaining ten milliliters (10ml) of each blood sample was allowed to coagulate to produce sera for blood chemistry measurements.

Hematology

Blood samples were analyzed within three hours (3hours) of their collection for total erythrocyte (RBC) and leukocyte (WBC) counts, hematocrit (PCV), and hemoglobin concentration (HC).

Other hematological indices (Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin Concentration (MCHC)) were calculated from results of the indices measured.

The concentration of blood hemoglobin in the test samples were estimated according to the cyanomethaemoglobin method of [23]. All hematological analysis were carried out in accordance with standard methods described by [23]

Serum Biochemistry

The bottles of coagulated blood were centrifuged at 3000 rpm for ten minutes for serum separation.

Thereafter, the harvested sera were used for evaluation of total serum protein (TSP), serum albumen (SA) and globulin.

Other serum parameters monitored included aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP). The activities of these enzymes were determined using spectrophotometric methods. All serum biochemical analyses were done according to standardmethods described by [17].

Statistical Analyses

Data on hematology and serum biochemistry were subjected to one-way analysis of variance (ANOVA) as outlined by [24]. Where ANOVA detected significant treatment effects, means were separated using the Duncan's New Multiple Range Test (DNMRT) as outlined by[25].

III. Results

The hematological and serum biochemical indices of rabbits fed different dietarylevels of RO/PKC mixture (1:1) are summarized in table 2.

Hematological indices

The results showed that Red blood cell (RBC), Neutrophils, Lymphocytes, and Monocytes, were not significantly ($p>0.05$) affected by the treatments. Other hematological indices that indicated none significant treatment effects were, packed cell volume (PCV), Hemoglobin (Hb), Mean cell volume (MCV), and Mean cell hemoglobin concentration (MCHC). However, White blood cell (WBC) differed significantly ($p<0.05$) among the treatment means.

Serum biochemical analysis

Results on serum biochemical assay indicated that total protein, albumin, globulin, AST, and ALT, were statistically ($p>0.05$) similar among the treatment means, While ALP was significantly ($p<0.05$) affected by the dietary treatments.

Table 2: Hematological and serum biochemical indices of grower rabbits fed graded dietary levels of PKC/Rice offal based diets

Parameters	T1 (0%) RO/PKC	T2 (12%) RO/PKC	T3 (24%) RO/PKC	T4 (36%) RO/PKC	SEM
Hematological parameters					
WBC(10^3/ul	20.30^{ab}	20.33 ^{ab}	24.40^{a}	24.70^{a}	1.04
RBC(10^6/ul)	4.88	5.03	5.10	5.50	0.40
Neutrophils (%)	36.01	37.50	37.61	38.92	1.09
Lymphocytes (%)	78.00	79.30	79.50	80.30	2.59
Monocytes (%)	0.76	0.90	1.30	1.40	0.06
PCV (%)	49.52	50.21	50.21	50.43	1.80
Hemoglobin (g/l)	17.01	17.11	17.41	17.43	0.98
MCV	49.77	51.33	51.36	51.47	1.83
MCHC (g/dl)	29.30	31.30	31.50	31.70	1.09
Serum biochemical indices					
Tot. protein(g/dl)	51.86	52.68	52.74	53.13	1.84
Albumin (g/dl)	26.31	26.67	26.78	26.89	1.07
Globulin (g/dl)	24.75	25.01	25.58	25.98	1.04
AST (u/l)	44.64	45.15	45.58	45.67	1.67
ALT (u/l)	21.49	23.15	23.70	25.03	1.46
ALP (u/l)	41.59 ^{a}	35.92 ^{ab}	35.66 ^{ab}	31.39 ^{b}	2.65

^{a,b}Means along the same row with different superscripts are significantly different ($P < 0.05$)

Table 3: Normal Hematological values of Rabbits

PARAMETERS	RANGE
PCV (%)	33 – 50
Hgb(g/dl)	9.4 - 17.4
Lymphocytes (%)	43 –80
MCHC(g/dl)	27 –34
WBC(10^3/ul)	5 –13
RBC(10^6/ul)	3.8 - 7.9
MCV(fl)	50 –75
Monocytes (%)	0 – 4
Neutrophils (%)	34 –70

Adapted from [26]

Table 4: Normal values for some biochemical parameters in rabbit serum

PARAMETERS	RANGE
TP (g/l)	50-75
ALT (U/L)	55-260
AST (U/L)	10-98
ALP (U/L)	10-96
Albumin (g/l)	25-40
Glubulin (g/l)	25-40

Adapted from [26]

IV. Discussion

Hematological indices

All values of blood cell indices obtained in the study indicated a healthy state of experimental animals. Indeed allparameters in question except RBC were in agreement with normal values reported for rabbits by [26].It was observed that apart from the control treatment, values obtained for RBC, neutrophils, monocytes, PCV, hemoglobin, MCV and MCHC showed a dose-related numerical increase. The foregoing indicate that the test factors did not exert deleterious effects on the blood building physiology of the experimental animals, up to 36% dietary inclusion. Agreeably, blood plays a vital role in the physiological integrity of an animal and changes in its constituents is a function of the nutritional and pathological state of the animal. Some researchers [13,12] have posited that hematological parameters such as hematocrit value, hemoglobin concentration, WBC count, among others are used in routine screening for health and physiological status of livestock and even humans, while [14] added that PCV is involved in transport of oxygen and absorbed nutrients. [27] also posited that dietary protein may affect the physiological process involved in erythropoietin production. This is corroborated by the reports of [28] that hemoglobin falls gradually in animals on a low protein intake and/or liver damage or anemia. The foregoing suggest that the test factor is safe and nutritionally suitable for incorporation in compounded diets for grower rabbits.

White blood cells (leucocytes) increased significantly ($p<0.05$) in the experimental animals with increasing dietary levels of RO/PKC. This could have been caused by the introduction of pathogenic organisms by the test feed stuff, hence the dose-related increase in the production of leucocytes of animals fed the different dietary levels of RO/PKC. On the other hand, it might be that the test feed stuff enhanced the ability of treated groups to produce anti-bodies to fight against a local infection challenge in experimental animals. Whichever might have been the case, the important thing is that immune system of animals in the treated groups were not compromised. Major functions of leucocytes and its differentials include, fighting infections, defense of the animal body against invasion by pathogenic organisms, by the process of phagocytosis; production, transport and distribution of antibodies by immune response. That is why animals with low white blood cells are more vulnerable to disease infection, while those with higher white blood cell status have a relatively greater degree of resistance against the same [29]. Animals with higher white blood cells count are also capable of enhanced adaptability to local environmental disease and disease prevalent conditions [30, 31].

There was no significant ($p>0.05$) treatment effect among the treatment means in the value of lymphocytes. However, there was a dose-related numerical increase in its values. The lowest value was recorded in the control treatment, while the highest value was in treatment 4. All values of lymphocytes in the experiment were within the normal range for rabbits reported by [26]. Lymphocytes are important in forming barriers

against local disease conditions and may be involvo1ed in antibody formation [32]. Many authors have reported that a large variation in lymphocytes is due to age and nutritional condition of animals [33, 34]. This implies that the test ingredient had a positive effect on the lymphocytes count, thereby producing a normal health status in animals in the treated group comparable to those in the contro

Serum biochemical parameters

Serum total protein (TP), albumin, globulin, Aspartate amino transaminase (AST), and Alanine amino transaminase (ALT) were not significantly ($p>0.05$) affected by the treatments. However, Alkaline phosphatase (ALP), indicated significant ($p<0.05$) effects.

The values of total serum protein were within the normal range reported by [Medirabbits.com] for rabbits. The TP assay showed a dose-related increase in the parameter. The result implies that the experimental animalsdid not suffer challenges of protein malnutrition; it also indicates that there were no toxic factors in the diets which could have compromised the hepatocellular integrity of the animals. It has been observed that the value of serum total protein is a function of both the quality and quantity of dietary protein [20]. According to [35], a specific amount of protein is required by the body to maintain protein homeostasis. The amount of serum total protein can give an insight into the general health status of the animal: Low level of total protein is attributable to malnutrition, liver disease or even digestive disorder, in which case the system has an impairment in absorption of dietary protein. On the other hand, increase in serum total protein reflects the ability of the animal to store reserve protein when they have reached the maximum capacity for protein intake [36, 37]. However, excessively high serum total protein could be a sign of chronic infection or inflammation,it can also be an early sign of bone marrow disorder.The value of serum total protein obtained here suggests that the test feed stuff supplied quality protein in amounts required to meet the nutritional protein needs of the experimental animals.

The values obtained for Albumin was within the normal range reported by [26].The result trend showed a narrow dose-related increase in the values of the parameter. Albumin is one of the proteins produced by the liver, implying that if hepatocellular integrity is compromised, serum level of this protein will be low.It was reported by [38] that a case of severe malnutrition decreases albumen fraction in the blood. Albumen also holds water in the blood and decreases in liver and kidney diseases. The result of this test indicated that the test diets have no adverse effects on renal and hepatocellular function of the experimental rabbits.

The serum globulin levels from the different treatments were statistically ($p>0.05$) similar. Globulin is a set of proteins in the blood, some of which are made by the liver, while others are made by the immune system. They function in the fight against infections, and in the transport of nutrients. Serum globulin level is a function of dietary protein quality, and in combination with albumin, promotes normal water retention in the blood [17].The level of serum globulin recorded in the study is within the normal range reported by [26]. A healthy albumin/globulin (A/G) ratio was observed in the study. Healthy animals have a little more albumin than globulin, the reverse would be the case when they are sick.

Although values obtained for ALP showed significant ($p<0.05$) treatment effect, they were within the normal range reported by [Medirabbits.com]. AST, ALT and ALP are essentially liver enzymes, and may leak out into the blood when hepatocellular integrity is compromised.The serum activities of these enzymes are therefore indicators of hepatocellular integrity and function [17]. Functions of the liver include, filtering blood from the digestive tract, processing of waste products, and bioremediation. Others are, production of proteins, such as albumin, globulin and prothrombin (a factor that functions in blood clotting), production glucose (blood sugar), and lots more. These underscore the need for healthy hepatocytes.

Reports by[39] coroborates that AST and ALT are enzymes commonly found in the liver and leak out into the general circulation when liver cells are injured. It has also been observed that a high value of ALP suggests increased activity of the liver due to presence of toxic substance [40, 41, 42]. On the other hand,low levels of ALP (below the normal range), can be a sign of protein deficiency arising from malnutrition or advanced liver disease.

The findings of the study indicated that up to 36% dietary inclusion of RO/PKC would not be hepatotoxic to grower rabbits.

V. Conclusion

The results of this investigation suggests that up to 36% mixture of rice offal and palm kernel cake (in a ratio of 1:1), can be included in the diets of grower rabbits, without any deleterious effects on their hematological and serum biochemical parameters. This could ameliorate feeding constraints in rabbit production in developing countries, where conventional energy feed stuffs like maize, sorghum and millet, among others needed for formulating diets for formulating diets for rabbits are in competitive demand for human feeding and industrial use.

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