NIGERIAN AGRICULTURAL JOURNAL
ISSN: 0300-368X
Volume 55 Number 1, April 2024 Pg. 237-241
Available online at: http://www.ajol.info/index.php/naj
https://www.naj.asn.org.ng

Growth and Haemato-Biochemical Response of Buck Rabbits Fed *Aspilia africana* (African Marigold) Supplemented with Zinc Gluconate

[*1]Ezea, J., [1]Ezike, J. C., [1]Nwaoko, C. G., [2]Ekwe, O. O. and [1]Oguike, M. A.

[1]Department of Animal Breeding and Physiology,
Michael Okpara University of Agriculture, Umudike, Nigeria
[2]Department of Animal Science, Ebonyi State University, Abakaliki, Nigeria
*Corresponding author: ezea.jonas@mouau.edu.ng

Abstract
Growth performance, haematological and serum chemistry indices were studied in 24 buck rabbits fed *Aspilia africana* supplemented with Zinc gluconate, in a 56-day experimental period. The bucks were divided into 4 treatment groups. The treatments were replicated three times in a completely randomized design. Treatment 1 was constituted by conventional forage mixture. Treatments 2, 3 and 4 were made up of *Aspilia africana*+0.00mgZG, *Aspilia africana*+100.00mgZG and *Aspilia africana*+150.00mgZG, respectively. Average daily feed intake was highest (P<0.05) in bucks fed Asp+150mgZG while average daily weight gain was similar (P>0.05) among bucks fed CFM, Asp+0.00mgZG and Asp+ 150mgZG and higher (P<0.05) than in bucks fed 100mgZG. The lowest/best feed conversion ratio (FCR) was obtained in bucks given Asp+0.00mgZG. Haemoglobin concentration, packed cell volume and red blood cell count were highest (P<0.05) in bucks fed Asp+0.00mgZG and CFM. Hb and PCV decreased (P<0.05) with increase in ZG. White blood cell count (WBC) was highest (P<0.05) at 100mgZG. Mean cell volume (MCV), mean cell haemoglobin(MCH) and mean cell haemoglobin concentration (MCHC) were highest (P<0.05) at Asp+0.00mgZG and Asp+100mgZG. Lymphocytes were highest (P<0.05) at 100mgZG. ZG resulted in significant increase in serum protein, while glucose was raised outside the reference range in bucks fed 150mgZG. It is concluded that 150mgZG stimulated feed intake and weight gain per buck per day. *Aspilia africana* without ZG enhanced best feed utilization, encouraged HB, PCV and RBC production while MCV, MCH and MCHC were increased at 100mg in the bucks. ZG supplementation at both levels decreased Hb and PCV but increased serum protein. Glucose was raised outside the reference range with CFM and 150mgZG. ZG evoked significant increase in lymphocytes at 100mg.
Keywords: Growth performance, buck rabbits, Aspilia africana, Zinc, haematology, and serum chemistry

Introduction

Intake of feed and feed supplements influences growth and blood parameters of farm animals. Herbivores such as rabbits have their concentrate diet supplemented with forages as a matter of principle, for them to develop and sustain a healthy gut and for optimum productivity. One of the forages relished by rabbits is *Aspilia africana* (Oko *et al*., 2016). The leaves of *Aspilia africana* have reportedly been administered to farm animals fresh, wilted or as aqueous, ethanolic or methanolic extracts. Fresh leaves of *A. africana* significantly stimulated growth in both gestational and lactating doe rabbits (Etim, 2015a). Adedeji *et al.* (2015) reporting enhanced litter weight of kits buttressed the growth-stimulatory effect of *A. africana* leaves fed to gestational doe rabbits. Earlier, significantly higher weight gain was reportedly induced in broilers fed 70g of *A. africana* leaf meal per Kg of diet (Adedeji *et al*., 2014). Growth was significantly promoted on administration of 125ml of *A. africana* extract per litre of water (Uchewa *et al*, 2018). Growing and laying Japanese Quails had improved growth on receiving 15% aqueous extract of the leaves of *A. africana*. (Agiang *et al*., 2011). On the contrary, there was no variation in weight of broilers fed 5% *A. africana* leaf meal per kg of diet (Oko *et al*., 2014a).

Wellbeing and optimum performance of animals have been attributed to a rich blood composition (Isaac *et al*., 2013). Diets impinge on blood constituents in a measurable way (Maxwell *et al*., 1990). Blood components and their dynamics can be pointers to renal and hepatic functions. *Aspilia africana* was reported as a stimulant of blood cell formation owing to a significantly higher red blood cell counts, haemoglobin concentration, volume of packed cells, serum protein, calcium and bicarbonate in lactating doe rabbits (Etim

and Oguike, 2011). Corroborating the erythropoietic potency of *A. africana* leaves, Ajeigbe *et al*. (2013) reported significantly improved red blood cells, haemoglobin concentration and packed cell volume following daily administration of 750mg/kg of aqueous extract of *A. africana* leaves to Wistar rats. It was later observed by Ajeigbe *et al*. (2016) that aqueous extracts of the same leaves improved osmotic stability and enhanced Na+K+ ATPase activities of red blood cells in Albino rats subjected to extracts of *A. africana* leaves. Significant increase in serum liver and kidney function markers, high-density lipoprotein, counts of white blood cells, but decreased counts of red blood cells and platelets were reported in female Wistar rats receiving up to 500mg/kg body weight of extracts of *A. Africana* in water and ethanol.

The toxicity of *Aspilia africana* leaves was highlighted by Taziebou *et al*. (2007), who classified it as a low-toxicity plant. However, Arunsi *et al*. (2020) expressed some reservations that the leaves might not be safe as a herb, citing reduced organ weights in rats given 500mg/kg body weight of ethanolic extracts of the leaves; as well as marked changes in hepatic and renal architecture of female rats of Wistar strain administered water and ethanol extracts of the leaves. Besides, hepatic and tubular necrosis as well as other degenerations of the kidneys were observed in WAD rams placed on the leaf extracts (Etim *et al*., 2020b).

The liver and kidneys play important roles in bio-functions. Whatever feed material that can affect the micro architecture of the organs may as well influence their proper functioning and metabolites.

Zinc plays three basic biological roles (Chasapis *et al*., 2011) in animal bodies, viz: as a catalyst of many reactions occurring in the body, as a structural constituent of proteins, growth factors, cytokines, receptors and enzymes; and as a regulator of several cellular processes where it acts as a co-factor for proteins, including hormones, nuclear factors and enzymes (Meyers *et al*., 2012). Promotion of resistance to apoptosis of epithelial cells has been credited to zinc. Zinc mediates cytoprotection, likely due to the antioxidant activity of its metallothioneins that are rich in cysteine (Lansdown *et al*., 2007). Lack of information growth performance and blood profile of buck rabbits fed fresh *Aspilia africana* leaves supplemented with Zinc gluconate necessitated this work.

Materials and Methods

Experimental Site

The study was carried out at the Rabbitry of Michael Okpara University of Agriculture, Umudike, Abia State. Umudike lies on co-ordinates 05^0 29' N and 07^0 33'E, and an altitude of about 122m above sea level. Average annual rainfall ranges from 1700 to 2100 mm. Minimum and maximum temperatures are in the ranges of 18-23^0 C and 26-36^0 C, respectively; while relative humidity is 57-91% (NRCRI, 2023).

Experimental Rabbits and Management

A total of twenty-four (24) grower buck rabbits) of New Zealand White breed were used for the study. The rabbits were sourced from a private farm; and were made to undergo acclimatization to the experimental site for three (3) weeks before commencing the study. Clean water, feed (concentrates and forages) were provided for the animals *ad lib*. They were randomly divided into four equal treatment groups. Each treatment group was replicated three times, in a completely randomized design (CRD). All the bucks were fed a basal concentrate diet. Treatment one (T_1) (control) was made up of conventional forage mixture of *Calopogonium mucunoides*, *Centrosema pubescens* and *Panicum maximum* with zero Zinc gluconate. Treatments two (T_2), three (T_3) and four (T_4) were constituted by fresh *Aspilia africana* leaves and *Panicum maximum* with Zinc gluconate supplemented at 0.00mg/kg, 100mg/kg and 150mg/kg of feed, respectively.

Experimental Design

Twenty-four rabbit bucks were randomly allotted into four equal treatment groups. The treatments were replicated three times in a completely randomized design.

Data Collection

Data were collected on growth performance indices such as average daily feed intake, average daily weight gain, and feed conversion ratio as well as on haematological and blood chemistry indices.

Growth performance:

Weight Gain*:* The bucks were weighed at the beginning of the experiment to get the initial live weight. They were weighed at weekly intervals susequently to get their weight gain, with a top-loading scale. At the end of the experiment (56 days), mean daily weight gain was computed as follows:

Total weight gain = Final live weight – Initial live weight

Mean weight gain = Total weight gain / Number of boars

Mean daily weight gain = Mean weight gain / 56 days

Feed Intake*:* Feed was weighed with a top-loading scale before feeding the bucks.

Mean daily feed intake was computed as follows:

$$\text{Mean daily feed intake } \frac{\frac{\text{Total Feed Intake}}{\text{Number of Boars}}}{56\text{days}}$$

Feed conversion ratio (FCR): FCR = Feed intake / Weight gain

Sampling and analyses of blood

Blood (2ml) was sampled from the vein of the boars into each of two separate bottles for the evaluation of haematological and blood chemistry indices, respectively. Samples for haematology were collected in bottles treated with an anticoagulant (ethylene diamine tetra-acetic acid (EDTA)), while those for serum chemistry were collected in bottles without EDTA to allow coagulation. Haematological markers were evaluated according to Jain (1986), while the serum chemistry parameters were determined using Randox kits.

Statistical analysis

Data collected on the different parameters were subjected to analysis of variance (ANOVA), according

to Steel and Torrie (1980). Mean separation was carried out using Duncan's Multiple Range Test (Duncan, 1955).

Results and Discussion

The growth performance of rabbit bucks fed *Aspilia africana* supplemented with Zinc gluconate is presented in Table 2. Bucks fed *Aspilia africana* with 150mgZG had the highest (P<0.05) average daily feed intake among all. This was followed significantly by the bucks fed *Aspilia africana* with 100mgZG and those fed CFM, which were the same (P>0.05). The lowest (P<0.05) average feed intake was obtained in bucks fed *Aspilia africana* leaves without ZG supplementation. Average daily weight gain did not vary among bucks fed CFM, *Aspilia africana* without ZG and *Aspilia africana* with 150mgZG, and were however, significantly superior to the value obtained in bucks fed *Aspilia africana* with 100mgZG. Feed conversion ratio (FCR) was highest/worst (P<0.05) in bucks fed *Aspilia africana* with 100mgZG. It was similar (P>0.05) in bucks fed *Aspilia africana* with 150mgZG and CFM and the lowest/best (P<0.05) in bucks fed *Aspilia africana* without ZG supplementation. The lower the FCR value the better the feed utilization by animals. Zinc as a mineral might have significantly stimulated appetite in the bucks at 150.00mgZG supplementation. Zinc administered orally stimulated feed intake in Sprague-Dawley rats (Ohinata *et al*., 2009; Komai *et al*., 2018). The authors explained that oral zinc administration resulted in increased messenger RNA expression of neuropeptide Y (NPY) and orexin, both of them being peptides of hypothalamic origin, after 3 hours. Further clarifications indicated that zinc stimulates feed intake through the afferent vagus nerve followed by activating the peptides of the hypothalamus associated with regulation of feed intake. Feeding rabbits conventional forage mixture has been recommended for general wellbeing and optimum productivity. It is not a surprise that the bucks placed on CFM had competitive feed intake and weight gain. Bucks fed *Aspilia africana* without ZG supplementation had the least feed intake and high weight gain. Rabbits relish *Aspilia africana.* Probably the bucks ate more of the fresh leaves instead of the concentrate diet. *Aspilia africana* has been variously reported as a stimulator of gain in body weight in herbivores. The haematological profile of rabbit bucks fed *Aspilia africana* supplemented with Zinc gluconate is presented in Table 3.

Haemoglobin concentration (Hb), packed cell volume (PCV) and red blood cell counts (RBC) were highest (P<0.05) in bucks fed *Aspilia africana* without ZG supplementation as well as bucks fed CFM. Hb concentration and PCV decreased significantly with increase in ZG, while RBC were the same (P>0.05) for the two doses of ZG. WBC were highest at 100mg ZG. Red blood cell indices (MCV, MCH and MCHC) were highest in bucks administered *Aspilia africana* without ZG and 100mg ZG. The platelets and lymphocytes were highest in bucks fed 100mgZG. Heterophils and monocytes were highest in bucks fed *Aspilia africana* without ZG. Eosinophils were higher in bucks fed CFM. The treatments did not have adverse effects on the haematological parameters studied in the rabbit bucks. The Hb concentration, PCV and RBC; and the red blood cell indices obtained in bucks fed *Aspilia africana* without supplementation could be another testament to the ability of *Aspilia africana* leaves to stimulate formation of red blood cells in herbivores (Etim and Oguike, 2011; Ajeigbe *et al*., 2013). The structural role of zinc in the red blood cells was pronounced at the dose of 100mgZG, considering the significant increase in MCV. The values of MCH and MCHC could suggest that zinc plays a positive role in production of haemoglobin. This corroborates the findings by Chen *et al.* (2017), who informed that Zn has both structural and catalytic functions in more than 200 metallo-enzymes. They found out that an appropriate amount of zinc stimulated red blood cell formation in anaemic rats. Thus, the presence of zinc in proper concentration in the diets of animals is of immense importance, not only for the well-being of the animals but also for optimizing the overall performance of the animals (Shinde *et al*., 2006).

Zn supplementation in buck diets sufficient to significantly stimulate and sustain erythropoiesis without concomitant intoxication is required. In rats, mice, and humans, it is known that zinc supplementation influences hemoglobin production (Chen *et al*., 2018). Group supplemented with 100mg ZG had significantly higher white blood cell counts and lymphocytes compared to the others. This suggests that 100mg supplementation of ZG in buck diet was sufficient to stimulate immune response as well as the activities of enzymes involved in antioxidant and anti-inflammatory response cascades, thus ameliorating the adverse effect of anti-nutritional factors in *Aspilia africana.* The serum chemistry profile of rabbit bucks fed *Aspilia africana* supplemented with Zinc is presented in Table 4. Serum total protein was significantly higher (P<0.05) in bucks fed *Aspilia africana* with 100mgZG and 150mgZG. This indicates a safe dose and the involvement of Zn in several reactions leading to protein synthesis. Serum glucose was significantly high in bucks fed CFM and *Aspilia africana* + 150mgZG. Blood glucose in these two groups were outside the normal range for rabbits. This could be due to induced liver damage owing to intoxication by anti-nutritional factors that interfere with glucose metabolism. Feeding with *Aspilia africana* leaves without ZG and dietary inclusion of ZG at 100mg restored blood glucose levels within the normal range. This could suggest that some active ingredients in *Aspilia africana,* on one hand, and ZG at 100mg might have played a role in activating enzymes that mediated detoxification of anti-nutritional factors or metabolism of glucose. Blood glucose concentration increased excessively again following inclusion of 150mg ZG. This could be the result of excessive Zn leading to alteration in glucose metabolism. Liver function markers (AST, ALT and alkaline phosphatase) and kidney function markers (urea and creatinine) were all within the normal ranges. This indicates that the treatments did not upset the hepatic and renal functions

of the bucks. However, they did not follow a well-defined or regular pattern to be attributed to the treatments.

Conclusion

It is concluded that 150mgZG stimulated daily feed intake and daily weight gain per buck, respectiely. *Aspilia africana* leaves without ZG enhanced best feed utilization, encouraged HB, PCV and RBC production, while MCV, MCH and MCHC were increased at 100mg in the bucks. ZG supplementation at both levels decreased Hb and PCV but increased serum protein. Glucose was raised outside the reference range with CFM and 150mgZG. ZG evoked significant increase in lymphocytes at 100mg. Rabbits fed fresh leaves of *Aspilia africana* may not need Zinc gluconate supplementation to achieve good finishing weight. Since the bucks had good feed utilization and competitive weight gain without supplementation, the weight gain achieved with 150mg ZG can be dispensed with. This is in consideration of the significantly reduced values of Hb and PCV in the bucks fed both levels of ZG.

References

Adedeji, O. A., Ajibade, M. O. and Folayan, J. A. (2015). Effect of *Aspilia africana* leaves on reproduction of rabbit. *Journal of Development and Agricultural Economics*, 1–236.

Adedeji, O. S., Amao, S. R., Ajayi, J. A. and Falade, O. F. (2014). Performance and quality assessment of broiler chickens fed different graded levels of *Aspilia africana* leaf meal. *Scholars Academic Journal of Biosciences*, *2*(1), 33–38.

Agiang, E. A., Oko, O. O. K. and Essien, G. E. (2011). Quails response to aqueous extract of bush marigold (*Aspilia africana*) leaf. *American Journal of Animal and Veterinary Sciences*, *6*(4), 130–134.

Ajeigbe, K. O., Enitan, S. S., Omotosho, D. R. and Oladokun, O. O. (2013). Acute effects of aqueous leaf extract of *Aspilia africana* CD Adams on some haematological parameters in rats. *African Journal of Traditional, Complementary and Alternative Medicines*, *10*(5), 236–243.

Clayden, E. C. (1967). Practical section, cutting and staining 4th edition. J. and E. Churchill Ltd, UK, p 89.

Duncan, D. B. (1955). Multiple range and multiple F-tests. Biomet. 11, 1-42.

Etim, N. N. (2015a). Growth stimulating effects of *Aspilia africana* fed to female pseudo-ruminant herbivores (rabbits) at different physiological states. *American Journal of Agricultural Science*, *2*(2), 59–62.

Ezea, J. and Ibe, R.C. (2021). Gonadal morphometry and semen characteristics of Buck rabbits fed African Marigold (*Aspilia africana*) leaves supplemented with zinc gluconate. *Nigerian Journal of Animal Science and Technology, 4 (3), 11-19.*

John, D. B. and Alan, S. (1977). Theory and Practice of histological techniques. Churchill Livingstone, Edinburgh, London. Pp 89, 108.

Komai, M., Goto, T. and Ohinata, K. (2018).Clarification of the mechanism involved in orexigenic action by oral zinc ingestion. Yakugaku Zasshi (Japanese) 138, 1011-1016

Lansdown, A. B., Mirastschijski, U., Stubbs, N., Scanlon, E. and Agren, M. S. (2007). Zinc in wound: theoretical, experimental and clinical aspects. *Wound Repair Regen, 15 (1).*

NRCRI (2023). Meteorological Data. National Root Crops Research Institute, Umudike, Nigeria.

Ohinata, K., Takemoto, M. and Kawanago, M. (2009). Orally administered zinc increases food intake via vagal stimulation in rats. *Journal of Nutrition* 139, 611-616.

Okwu, D. E., Josiah, C. (2006). Evaluation of the chemical composition of two Nigerian medicinal plants. *African Journal of Biotechnology. 5(4), 357-361.*

Steel, R. G. D. and Torrie, J. H. (1980). Principles and Procedures of Statistics: A biometric approach, New York, McGraw Hill, 1980,2nd ed.

Uchewa, E. N., Amaduruonye, W., Onunkwo, D. N. and Njoku, H. N. (2018). Performance of broiler chickens fed bush marigold (*Aspilia africana*) leaf extract. *Nigerian Journal of Animal Science*, *20*(3), 223–228.

Table 1: Ingredients composition (%) of grower rabbit diet

	Treatment			
Ingredient	**CFM**	**Asp+ 0.0mgZG**	**Asp+ 100mgZG**	**Asp+ 150mgZG**
Maize	44.94	44.94	44.94	44.94
Soya bean meal	17.31	17.31	17.31	17.31
Rice husk	32.00	32.00	32.00	32.00
Fish meal	2.00	2.00	2.00	2.00
Bone meal	3.00	3.00	3.00	3.00
Vitamin/mineral premix	0.25	0.25	0.25	0.25
Common Salt	0.50	0.50	0.50	0.50
Total	**100**	**100**	**100**	**100**
Zinc gluconate	0.00	0.00	100	150
Crude protein	17.00	17.00	17.00	17.00
Metabolizeable energy	2505.42	2505.42	2505.42	2505.42

CFM = Conventional forage mixture, Asp = *Aspilia africana* leaves, ZG = Zinc gluconate

Table 2: Growth performance of rabbit bucks fed *Aspilia africana* supplemented with Zinc gluconate

Parameters	CFM	Asp+ 0.0mgZG	Asp+ 100mgZG	Asp+ 150mgZG	SEM
Initial body weight (g)	1500.00	1500.00	1500.00	1500.00	0.00
Average feed intake(g)	568.50	466.13	570.50	600.00	153.07
Average daily feed intake(g)	10.15[b]	8.32[c]	10.18[b]	10.71[a]	0.51
Final body weight(g)	2043.33[a]	2053.33[a]	1943.33[b]	2060.50[a]	25.93
Average weight gain(g)	543.33[a]	553.33[a]	443.33[b]	560.00[a]	27.44
Average daily weight gain(g)	9.70[a]	9.88[a]	7.92[b]	10.00[a]	0.49
Feed conversion ratio	1.05[b]	0.84[c]	1.29[a]	1.07[b]	0.12

[a-c] Means with different superscripts in the same row are significantly different ($p<0.05$), S.E.M: Standard error of the mean, CFM = Conventional forage mixture, Asp = *Aspilia africana* leaves, ZG = Zinc gluconate

Table 3: Haematological profile of rabbit bucks fed *Aspilia africana* supplemented with Zinc

Parameters	CFM	Asp+ 0.00mgZG	Asp+ 100.00mgZG	Asp+ 150.00mg ZG	SEM
Haemoglobin (g/dl)	12.63[c]	14.90[a]	14.37[b]	11.20[d]	0.50
PCV (%)	26.33[c]	37.00[a]	33.67[b]	27.33[c]	1.63
RBC ($x10^6/mm^3$)	4.15[c]	4.99[a]	4.63[b]	4.64[b]	0.18
WBC ($x10^3/mm^3$)	7.09[b]	6.89[b]	8.08[a]	6.60[c]	0.21
MCV ($/mm^3$)	68.76[b]	71.16[a]	70.40[a]	64.90[c]	1.16
MCH (pg/cell)	23.68[c]	26.49[b]	29.15[a]	24.32[c]	0.97
MCHC (g/dl)	37.08[b]	40.71[a]	40.84[a]	37.65[b]	0.92
Platelets ($/mm^3$)	290.91[c]	345.22[b]	374.32[a]	264.28[d]	20.64
Lymphocyte (%/l)	51.67[b]	47.33[c]	54.33[a]	52.00[b]	1.10
Heterophils (%/l)	37.33[c]	41.00[a]	35.00[d]	39.00[b]	1.29
Monocytes(%/l)	2.67[c]	3.67[a]	3.33[b]	2.67[c]	0.23
Eosinophils(%/l)	2.00[a]	1.00[b]	1.00[b]	1.00[b]	0.13

[a-d] Means along the same row with different superscripts are significantly ($p<0.05$) different. CFM = Conventional forage mixture, SEM = Standard error of the mean, RBC = Red blood cell, WBC = White blood cell, PCV = Packed cell volume, MCV = Mean corpuscular volume, MCH = Mean corpuscular haemoglobin, MCHC = Mean corpuscular haemoglobin concentration, Asp = *Aspilia africana* leaves, ZG = Zinc gluconate

Table 4: Serum chemistry profile of rabbit bucks fed *Aspilia africana* supplemented with Zinc gluconate

Parameters	CFM	Asp+ 0.00mgZG	Asp+ 100mgZG	Asp+ 150mgZG	SEM
Total protein (g/dl)	4.56[c]	4.69[c]	5.76[a]	5.04[b]	0.24
Albumin (g/dl)	2.63[ab]	2.77[a]	2.77[a]	2.81[a]	0.15
Globulin (g/dl)	2.47[a]	2.58[a]	2.40[ad]	2.66[a]	0.94
Glucose (mg/dl)	177.67[b]	83.67[c]	97.33[c]	239.00[a]	19.41
AST (IU/l)	47.61[b]	53.80[a]	47.44[b]	52.77[a]	1.35
ALT(IU/l)	19.38[a]	16.36[b]	19.05[a]	15.06[b]	0.75
Alkaline phosphatase (IU/l)	13.53[ab]	10.93[b]	14.73[a]	14.09[ab]	0.62
Urea (mg/dl)	26.37[a]	23.41[b]	22.74[bc]	23.51[b]	1.58
Creatinine (mg/dl)	0.78[b]	0.63[c]	0.81[a]	0.63[c]	0.04

[a-c] Means along the same row with different superscripts are significantly ($p<0.05$) different. S.E.M = Standard error of mean. AST = Aspartate transaminase; ALT = Alanine transaminase, CFM = Conventional forage mixture, Asp = *Aspilia africana* leaves, ZG = Zinc gluconate