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Effects of *Moringa Oleifera* Leaf Extracts and Lipozyme on The Hematological Profile and Leukocytes Population of Japanese Quail, *Coturnix Japonica*

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***Abstract*:- The study evaluated effects of *Moringa oleifera* leaf extracts and Lipozyme® on the hematological profile and leukocytes population of Japanese quail (*Coturnix japonica*). A 3week old quail birds (mean weight of 110.00±1.00g) were randomly stocked at 5 birds per unit and administered with 1g/L aqueous leaf extracts of Moringa and Lipozyme® as treatments 1 and 2. The third treatment was without any additive and served as the control experiment. These prepared solutions were administered to birds 6hours in every 48 hours of fresh water administration. The study lasted for 56days. At the end of this period, blood samples were taken and examined for Red Blood Cells (RBC), Packed Cell Volume (PCV), White Blood Cells (WBC), Erythrocyte Sedimentation Rate (ESR), Mean Corpuscular Volume (MCV), Hemoglobin (HB), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Heterophil, Basophil, Lymphocyte, Monocyte and Thrombocyte. The analyses revealed that quail birds in group treated with Moringa had significantly higher ($p<0.05$) values of HB (9.80g/dl), RBC (3.10ml/cm3), PCV (29.50%), MCV (85.17Fl/red cell) and MCH (31.60) than other groups. Basophil (2%) and Thrombocyte 32% of the Moringa group were also significantly higher ($p<0.05$) than those of control and group administered with Lipozyme®. The results also showed that Moringa leaf extracts did not affect ($p>0.05$) the number of Monocyte in the quail blood. Monocyte in this study ranged from 1.5%-2.0% for all treatments. The data from this study indicated that the treatment with Moringa leaf extracts does not negatively affect the health status of growing Japanese quail. It is therefore concluded that treatment with Moringa leaf extracts improved the hematological parameters and leucocyte population of growing quail.**

Keywords: Moringa oleifera, leaf extract, Lipozyme®, Japanese quail, haematological profile

INTRODUCTION

The demand for animal protein has gone far beyond supply as a result of the rapid growth of human population in many countries of the world, especially in the developing countries. An urgent need is therefore necessary to increase the production of protein sources (Ufele and Ebenebe, 2017). Nigerian economic statistics reveal annual economic growth rate that averaged over 7% in recent decades, making Nigeria one of the fastest growing economies in the world (Byerlee *et al*., 2013). Nonetheless, this growth has not reduced poverty or created much-needed jobs. Unemployment is still very high, and more than 60% of the population lives below the poverty line (AON, 2012). The popularity of poultry production can be explained by the fact that poultry birds are good converters of feed into useable protein in meat and eggs. The production cost per unit remains relatively low, and the return on investment is high. (Heinke, 2015). Quail belongs to the family Phasianidae. Japanese scientist first tamed the wild quails and revealed the ways to raise them as domestic birds. Now, people throughout the world are performing quail farming business commercially for the purpose of meat and eggs production. Quail farming is very profitable like other farming ventures, such as chicken, turkey or duck farming (Ufele and Ebenebe, 2017). The advantages of quail farming include minimum floor space, low investment, comparatively sturdy birds, early market age and sexuality, high rate of egg production and less fed requirement (EFSA, 2004). Teixeira *et al*. (2014) reported that quail eggs are more nutritious than other poultry eggs because of its comparatively more protein, phosphorus, iron, vitamin A, B and B2 and can play a vital role to meet up the demand of food and nutrition. Quail requires protein, minerals, vitamins and other growth factors and energy sources for growth, reproduction and other normal physiological functions like other animals (Lovell, 1980; Ayoola, 2011). However, high-quality poultry diet should contain not only high quality and levels of dietary nutrients but also additive to keep birds healthy and improve growth. The demand for cheap and quality food is continuously increasing due to the growing world population and this highlights the importance of maximizing the efficiency of poultry production in a cost effective manner, through the application of growth promoters, which are non-nutrients aimed to maximize utilization of the nutrients present in feed (Patterson and Burkholder, 2003; Kocher, 2006; Akinleye *et al.*, 2008; Kuldeep *et al*., 2014). Growth promoters are the substance that are added to a nutritionally balanced diet which provoke response towards the exploitation of maximum genetic potential of the host (Kuldeep *et al.*, 2014). There are different types of growth promoters, which are used to exploit the broiler industry. These include antibiotics, probiotics and exogenous enzymes (Allen, 1999; Dhama *et al*., 2007; Kuldeep *et al*., 2014). However, the use of synthetically produced growth promoters had been found to have objectionable side

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effects (Faluyi and Agbede, 2008). On this note, the use of all kinds of growth promoter was banned in animal feed in Europe (Nkukwana *et al*., 2014). Revolutions in animal feed production gave rise to the idea of phylogenic feed additives (Grashorn, 2010). Plant and their metabolites known as bioactive compounds, play a key role because of their feed additive attributes, which helps to maintain animal health and production. Newcomb (1999) affirmed that feed manufacturers are adopting new forms of natural feed additive that are products of modern sciences. Moringa is a great supplement to human nutrition because it helps with digestive processes. Moringa oils and fibers are excellent antioxidants which help to scrub toxins and food remnants from the intestine. By cleaning them the body is more capable of fully absorbing the nutrient from food. Detoxification is an important process in human body strength and overall health (Ted, 2011). Leaves of Moringa tree contains seven times of vitamin of orange, four times the calcium of milk, four times the vitamin A of carrot, three times the potassium of bananas and two times the protein of yogurt. They are also rich in iron, zinc and B vitamin (Sanchez-Machado *et al*., 2010). *Moringa oleifera* plants are laden with bioactive ingredients and those also found in Lipozyme and are very locally available. They are common growing plant found almost in every backyard within the locality. Blood is a good indicator to determine the health of an organism (Joshi *et al*., 2002a; Ayoola 2011). It also acts as pathological reflector of the whole body; hence hematological parameters are important in diagnosing the functional status of exposed animal to toxicants (Joshi *et al*., 2002b, Ayoola, 2011). Meanwhile little work has been done on the effect of exogenous enzyme on quail bird. Thus this study aimed at investigating effects of *Moringa oleifera* leaf extract and Lipozyme® on the hematological profile and leukocytes population of Japanese quail.

MATERIALS AND METHODS

Experimental Site and Materials

The research was carried out at the production farm of the Department of Agricultural and Bioenvironmental Engineering Technology, Federal Polytechnic Oko, Anambra State. A total of 45, 3-week old quail birds (mean weight of 110.00±1.00g) were procured from the College of Animal Science and Animal Health, Michael Okpara University of Agriculture, Umudike, Abia State. The birds were allowed to acclimate for 7 days before commencement of the experiment. Fresh Moringa oleifera leaves were obtained within Oko locality. The leaves were air dried separately in the laboratory for 5 days and then ground into fine particles using a simple hammer mill as describe by Julia (2008). Liptozyme® (a digestive enzyme supplement) was procured from a veterinary dispensary outlet in Oko metropolis.

Experimental Design

A nine (9) pen unit, with an area of half square meter each that could accommodate 5 quail birds was constructed. Each unit of the pen were disinfected with germicide (IZAL®) after washing with detergent and water. The feeders and drinking cans were cleaned and disinfected and set in a place accessible to the birds. Each pen unit was properly labeled according to treatments. The birds were randomly allotted at 5 birds per treatments. Three treatments designated as T0, T1andT2 with three replicates each were designed. T0 was the control experiment with no additive. T1 was treated with 1g/liter of aqueous preparation of *Moringa oleifera* leaf extracts while T2 was treated with 1g/liter of aqueous preparation of Lipozyme®.

Aqueous Extract formulation and preparation

A 1g of the ground Moringa particles was soaked in one liter of water for 24hours. The preparation was then filtered using muslin cloth to separate the debris from the filtrate (extracts) and the extracts placed in a clean container and diluted using distilled water (v/v) 1g/1000ml water each for treatment 1 (Julia, 2008). This procedure was carried out daily and filtrate served to the experimental birds as drinking water. Also, 1g of Lipozyme® was dissolved in one liter of distilled water and placed in a clean container and diluted using distilled water (v/v) 1g/1000ml water as described by Julia (2008).

Rearing of Experimental Birds

The pens were randomly stocked with quail birds (mean weight of 110.00±1.00g) at 5birds per unit. The experimental solution was administered to birds 6 hours in every 48 hours of administering fresh water. Commercial poultry feed (Top feed mash) was used to feed the birds *ad libitum* till the end of the experiment. The experiment lasted for 56 days.

Hematological Profile and Leukocyte Population Determination

At the end of the trial, birds were allowed to starve for 12 hours before sample collections. Samples were randomly collected in triplicate from each treatment by cutting the jugular vein of each bird with a sharp knife and they were left hanging until bleeding stopped. Concurrently, about 2mL of blood was collected using 2ml disposable syringes and needle and transferred immediately into anti-coagulant sterilized bottles. All hematological parameters were determined using an automated IDEXX Laser-Cyte Hemo-analyzer (IDEXX Laboratories, Inc.). Among the parameters examined were: Erythrocyte Sedimentation Rate (ESR), Packed Cell Volume (PCV), Red Blood Cell Count (RBC), Hemoglobin concentration (HB) and white blood cell differentials, Mean corpuscular hemoglobin concentration (MCHC) mean corpuscular hemoglobin (MCH) and the mean corpuscular volume (MCV). Blood analysis was done within 2hours of blood collection.

Statistical Analysis

Data collected were subjected to analysis of variance (ANOVA) implemented in SPSS Software Package Version 21.0.

RESULTS AND DISCUSSION

The results obtained for hematology and differential leukocytes population of Japanese quail administered aqueous extracts of *Moringa oleifera* leaf and Lipozyme alongside ordinary water are hereby presented. In Table 1, results of weekly water intake revealed than the birds consumed more water as they were growing bigger. Similar observation was seen in all treated groups.

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Table 1: The weekly mean average water intake (ml) of Japanese quail

samples	Week1	Week2	Week3	Week4	Week5	Week6	Week7	Week8
Control	32.820	38.500	35.200	33.690	39.167	33.470	39.700	41.110
Moringa	29.734	37.266	27.000	29.380	36.700	29.930	31.500	32.154
Lipozyme®	32.966	43.400	36.434	31.660	40.930	32.120	38.040	36.440

Hematological values are influenced by various factors including breed, sex, age, and reproductive status handling procedure and nutrition status of animal (Minurani *et al*., 2015). The results of haematological profile are presented in Table 2. From the results, the mean counts of RBC were 4.60±0.10, 3.10±0.10 and 1.86±0.60 for Control, Moringa and Lypozyme groups respectively. The number of RBC in the Moringa group was significantly higher ($p<0.05$) than the Lipozyme group but slightly lower ($p>0.05$) than the value obtained in the Control. The haemoglobin concentration of the birds among the groups were 12.67±0.35, 9.80±0.20 and 4.17±0.15 for Control, Moringa and Lipozyme groups respectively. The values obtained in the Lipozyme group were significantly lower ($p<0.05$) compared to other groups. Moringa treated group recorded higher ESR and PCV values than the Lipozyme group, almost twice the values. The MCV and MCH of Moringa group were observed to be 85.17±8.55 and 31.60±0.40 whereas for Lipozyme group, the values were 73.07±4.65 and 24.07±1.45 respectively. In other words, PCV, MCV, MCH, HB, ESR and RBC showed higher ($p<0.05$) values in Moringa group. The levels of MCV, MCH, MCHC and HB of Japanese quails obtained in this study were within the normal range reported for Japanese quail (Agina *et al*., 2017). Prahsanth *et al*., 2012 reported that increase or decrease in values of PCV depends on metabolic rate of organisms. This may explain the variations in the PCV values observed in this study.

Table 2: Means values of hematological indices of Japanese quail treated with Moringa leaf extract and Lipozyme®

	Treatments		
Parameters	Control	Moringa	Lipozyme®
RBC (ml/cm^3)	4.60 ± 0.100^{a}	3.10 ± 0.10^{b}	1.86 ± 0.60^{c}
PCV(%)	38.00 ± 1.00^{a}	29.50 ± 0.05^{b}	13.50 ± 0.05^{c}
ESR(mm/hr)	2.50 ± 1.50^{ab}	3.00 ± 0.00^{a}	1.00 ± 0.00^{b}
MCV(fL/redcell)	87.70 ± 4.80^{a}	85.17 ± 8.55^{ab}	73.07 ± 4.65^{b}
HB level(g/dl)	12.67 ± 0.35^{a}	9.80 ± 0.20^{b}	4.17 ± 0.15^{c}
MCH (pg)	27.47 ± 0.15^{b}	31.60 ± 0.40^{a}	24.07 ± 1.45^{c}
MCHC(g/dl)	33.23 ± 0.06^{ns}	33.20 ± 0.10^{ns}	32.90 ± 0.10^{ns}

Values are mean± standard error; Means with different superscript within a row are significantly different ($p<0.05$).

The present study revealed an increased value for total Leucocyte counts of the Moringa group ($p<0.05$) when compared with that of Lipozyme. From the results, Hetrophil, Basophil and Lymphocyte revealed significant differences ($p<0.05$) among the groups, whereas the percentages of Monocyte and Thrombocyte showed no significant difference ($p>0.05$) (Table 3).

Table 3: Mean values of leukocytes indices of Japanese quail treated with Moringa leaf extracts and Lipozyme®

	Treatments		
Parameters(%)	Control	Moringa	Lipozyme®
Mean Heterophil±SD	40.50 ± 0.50^{c}	50.50 ± 0.50^{b}	62.00 ± 0.00^{a}
Mean Basophil±SD	1.00 ± 0.00^{b}	2.00 ± 0.00^{a}	2.50 ± 0.50^{a}
Mean Lymphocyte±SD	26.50 ± 0.50^{a}	13.50 ± 0.50^{b}	13.50 ± 0.50^{b}
Mean monocyte±SD	2.00 ± 0.00^{ns}	2.00 ± 0.00^{ns}	1.50 ± 0.50^{ns}
Mean Thrombocyte ±SD	30.00 ± 0.00^{b}	32.00 ± 1.00^{a}	20.50 ± 0.50^{c}

Values are mean± standard error; Means with different superscript within a row are significantly different ($p<0.05$).

The results on the hematological parameters revealed that growing Japanese quails given extracts of Moringa had higher HB, RBC, PCV, MCV, MCH and MCHC than those treated with Lipozyme and the Control group. This higher value may be attributed to the nutritional contents of *Moringa oleifera* as similar report was given by Gous and Morris (2005). Packed Cell Volume and Mean Corpuscular Volume counts increases depending on environmental temperature and storage duration of samples (Hadzimusic, *et al.*, 2010). MCV, MCH and MCHC were calculated on the basis of PCV, RBC and HB (Campbell, 1995). MCV is known to determine the average volume of red blood cell in femtoliters (fl) or cubic microns (µm3). In this study MCV was observed to be higher in group given the extracts of *M. oleifera* than quails in other groups. The results of leucocytes population showed that treatment with *M. oleifera* extracts did not affect the number of monocytes in the quail blood. Monocytes are an essential components of the innate immunity of an organism. Monocytes in this study ranged 1.5-2.0% for all the treatments. These values were within the acceptable range of between 0-8.1% reported by Sturkie and Griminger (1976) who stated that the percentages of quail monocytes should fall within this range. This indicated that there was no acute infection in quail under the different treatments. This situation indicated that extracts of *M. oleifera* would not negatively affect the health status of growing Japanese quail.

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Lymphocytes have very important roles in the immune system of animals (Melvin *et al*., 1993). Lymphocyte values of quails given *M. oleifera* compared well with those of Lipozyme. However, in all the treatments lymphocyte values recorded were lower than the submission of Sturkie and Griminger (1976) who stated that the percentages of quail lymphocytes normally ranged between 30 - 66%. The variances may have arisen from possible environmental and other extrinsic factors.

CONCLUSION

Comparative study on the effects of *M. oleifera* leaf extracts and Lipozyme on the haematology and leucocytes population of growing Japanese quail was carried out. The results revealed that treatment of young growing quails with *Moringa oleifera* leaf extracts and and Lipozyme® improved their hematological parameters.

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