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EFFECT OF *Leptadinea reticulata* AND CHROMIUM YEAST (LAYPLUS^{R}) SUPPLEMENTATION ON THE GROWTH AND HAEMATOLOGICAL PARAMETERS OF JAPANESE QUAILS (*Coturnix coturnix* japonica)

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A 12-week study was conducted at the Teaching and Research Farm of the Obafemi Awolowo University, Ile-Ife to evaluate the effect of dietary inclusion of *Leptadinea reticulata* (LR) on hematological and growth parameters of Japanese quails, *Coturnix coturnix japonica*. Two hundred and ten birds were used for this study, and they were allocated randomly into seven treatment groups and replicated thrice. Group 1 served as control without inclusion of extract while groups 2, 3, 4 and 5 had 250 mg/kg, 500 mg/kg, 750mg/kg and 1000mg/kg inclusion of LR and group 6 had 250mg/kg of vitamin E, respectively and group 7 clomiphene citrate. The birds were weighed weekly to determine weight change. The blood parameters were analyzed at the completion of the experiment to determine packed cell volume (PCV), red blood cell (RBC) and white blood cell (WBC). The data obtained were analyzed using one-way ANOVA. There was significant increase ($p<0.05$) in the WBC and average daily weight gain of LR500mg/kg (1.45±0.12, 15.00±1.19, respectively) compared with other groups. It can be concluded from this experiment that supplementation of the feed with LR at 500mg/kg had beneficial effect on quail production.

Keywords: *Leptadinea reticulata, Coturnix coturnix japonica*, growth, haematology

Balochistan owes the credit to have been the The Japanese quail (*Coturnix coturnix japonica*) is the smallest avian species being farmed and has been considered as a source of animal protein and kept as a dual purpose breed for meat and egg production. Japanese quails are good model for research due to their small size, early sexual maturity (6-8 weeks), ability to produce three or four generations a year, and the relative ease of maintaining a colony (Wilson *et al.*, 1961; Vilchez *et al*., 1990). The testes are white and ovoid to elliptical in shape and are located within the body cavity, craniomedial to the cranial pole of the kidneys. They are attached to the dorsal body wall by the mesorchium and are encapsulated by a fibrous inner layer the tunica albuginea and a thin outer layer, the tunica vaginalis. The size of the testes increases during the breeding season (Fitzgerald, 1969).

Several studies have shown that vitamin E is used in the poultry diet because of its antioxidant properties (Sahin and Kucuk 2001, Sahin and Kucuk 2002, Ramnath*et al.* 2008). Chromium is essential for activating certain enzymes such as digestive enzyme trypsin and for stabilization of proteins and nucleic acids (Anderson, 1994).

Alternative measures now recommended for facilitating reproductive efficiency of animals is the application of organic extract of plants like leaves, seeds, stem and roots (Nikolova *et al;*2010, Kitanov *et al;*2003, Petkova *et al;*2008). These plant materials contains great amount of beneficial phytochemicals, anti-oxidants, vitamins and minerals which are known to increase growth and stimulate reproduction in humans and animals (Machebe *et al;* 2011, Nwangwa *et al;* 2007).

Antioxidants are generally defined as the substances that scavenge the formation of reactive oxygen species (ROS) or suppress their action (Sies, 1996). Different studies have demonstrated that antioxidants have widespread effects in improving male fertility, for example they prevent spermatozoa from ROS produced by abnormal spermatozoa, scavenges ROS generated by leucocytes, prevents DNA

fragmentation, reduces cryodamage to spermatozoa and blocks premature sperm maturation (Makker *et al*., 2009).

Extracts of plants have been used as natural feed additives and have generally been proven to be effective and non-toxic when consumed by humans and animals. *Leptadenia reticulata* is very valuable **medicinal plant** belonging to family Asclepiadaceae popularly known as Jivanti. Extract of roots and leaves of the plant act as antibacterial and anti-fungal agent (Patel and Dantwala, 1958). The plant possesses the potent lactogenic, anabolic and galactogogue effect (Anjaria *et al*., 1975; Ravishankar and Shukla, 2007; Anjaria *et al*., 1974). It is specially known for its stimulant and restorative properties, it possesses appetizer, aphrodisiac and antibacterial properties (Bawra *et al*., 2010). LAYPLUS® is a powdery plant product containing chromium yeast and *Leptadinea reticulata* formulated in the poultry industry to improve egg production and hatchability in layer. This product has not been evaluated in quail birds to establish its potential to improve growth and haematology; therefore this study was designed to assess the effects of varying level of the product to enhance the growth, haematological and histological characteristics of Japanese quails.

MATERIALS AND METHODS

The experiment was carried out at the Poultry Unit of the Teaching and Research Farm of Obafemi Awolowo University, Ile-Ife, Osun state, situated in the South-western part of Nigeria.

Housing and Management

A total of two hundred and ten 3weeks old Japanese quails (147 females and 63 males) were obtained from a reputable farm and were placed in a well-designed cage and adapted for two weeks. Feed and water were provided *ad-libitum* for consumption. Multi-vitamin drugs were given to the birds on arrival on the farm to serve as an anti-stress and during the period of their acclimatization to stabilize their condition.

Experimental design

After two weeks of adaptation the birds were weighed and randomly allotted into seven experimental treatments replicated three times. Each treatment contains 7 female birds with 3 male birds in each group. The experimental design was Randomized Complete Design. Treatment 1 served as the control, Treatment 2 comprised 250mg *Leptadenia reticulata* /kg diet, Treatment 3 comprised 500mg *Leptadenia reticulata*/kg diet, Treatment 4 comprised 750mg *Leptadenia reticulata*/kg diet, T5 comprised 1000mg *Leptadenia reticulata*/kg diet, T6 comprised 250mg/kg of vitamin E and T7 comprised 1.5mg/kg of clomiphene citrate.

Feeding

Birds were fed with grower mash compounded at feed mill mixed with different level of feed supplement. Feeds given to the birds were weighed with the remnants removed daily and weighed to determine the amount of feed consumed per birds for efficient calculation of the Feed Conversion Ratio and Average Daily Gain. The following formulae were used for the calculation:

Feed intake: Feed Supplied – Remnant of feed

Feed Conversion Ratio: $\frac{\text{Feed intake}}{\text{Average Daily Gain}}$

Average Daily Gain: $\frac{\text{Final weight – initial weight}}{\text{Number of days of the experiment}}$

Growth performance of Japanese quail

The performance of growing quail was evaluated in terms of Live Body Weight (LBW), Body Weight Gain (BWG), Feed Intake (FI) and Feed Conversion Ratio (FCR; g feed; g gain). Live body weights of quail were recorded at the beginning of the experiment and on a weekly basis thereafter. Weekly records on Feed intake of quail were also maintained on a replication basis; thus, Body Weight Gain and Feed Conversion Ratio were calculated.

Performance measurements

Body weight gain was determined by comparing individual measurements taken at the beginning and end of the study. Feed not consumed was weighed daily and average bird consumption was then calculated by dividing total feed consumed during 7 day

Table 1.Gross Composition of Quail Diets (%)

Ingredients	Growers	Layers
Fish meal	3.00	2.00
Wheat bran	12.13	-
Maize	45.12	51.89
Soybean meal	36.50	37.00
Bone meal	2.50	2.50
Limestone	-	6.00
Salt	0.25	0.25
DL-Methionine	0.15	0.11
L-Lysine	0.10	-
Vitamin-Mineral Premix	0.25	0.25
Crude protein	23.85	22.03
Metabolizable energy,Kcal/kg	2848	2838
Crude fibre	4.27	3.37
Calcium	0.84	3.09
Available phosphorus	0.38	0.36
TOTAL	100	100

by number of birds per cage. Feed conversion ratio was calculated as kg feed per kg egg.

Collection of samples

Blood sample

Blood samples were collected from nine birds per treatment at the end of the experiment for analysis of Total White Blood Cells, Total Red Blood Cells and Packed Cell Volume.

Procedure for blood collection used for hematological parameters

2ml of blood was collected from the jugular vein of the birds and was put directly into EDTA tube for hematological analysis.

Packed Cell Volume (PCV)

A microcapillary tube was filled with uncoagulated blood. An end of the tube was sealed with plasticine. The tube was centrifuged in a micro hematocrit centrifuge. The plasma at the top of the capillary tube could be used for determining plasma protein and fibrinogen and for assessing plasma colour and turbidity. The level of the settled blood cells was now read by placing the tube on the hematocrit graphic reader.

Erythrocyte counts

The red blood cell count per million was done using blood containing anticoagulants by the haemocytometer method. A red cell pipette was used to draw blood up to 0.5 mark and was dropped on a glass slide, smeared and was covered with a cover slip. This was viewed under the microscope. The erythrocytes in the five lower squares were counted. The volume counted was calculated the formula below:

$$RBC = \frac{\text{Total number of cell count}}{\text{Volume } 0.02mm^3} \times \text{dilution factor}$$

White blood cell count

The total white blood cells were counted under the microscope using the haemocytometer. A white blood cell pipette was used to draw blood to 0.5 mark and diluted to 1:2 mark with the aid of the white cell diluting fluid. The leucocytes in the four lower squares were counted. The number of cells counted was calculated using the formula below:

$$WBC = \frac{\text{Total number of cell count}}{\text{Volume } 0.4mm^3} \times \text{dilution factor}$$

RESULTS AND DISCUSSION

The performance of Japanese quails supplemented with *LAYPLUS* is presented in Table 2

Table 2: Effect of *Leptadenia reticulata* and Chromium yeast combination on the growth of Japanese quails

TREATMENTS	PARAMETERS				
	INITWT (g)	FINALWT (g)	ADFI(g)	FCR	AVGAIN (g)
T1 (CTR)	49.85±0.73	167.75±3.94^{bc}	24.24±1.83	2.47±0.34^{bc}	1.31±0.03^{bc}
T2 (VIT E)	51.00±1.47	168.16±2.95^{bc}	21.86±1.81	2.88±0.21^{bc}	1.31±0.03^{bc}
T3 (LRCY250)	50.13±1.80	159.22±3.17^{c}	22.85±4.09	3.12±0.59^{ab}	1.21±0.05^{c}
T4 (LRCY500)	50.66±0.95	181.71±11.14^{a}	25.01±1.01	2.18±0.41^{c}	1.45±0.12^{a}
T5 (LRCY750)	50.36±0.93	170.24±1.66^{b}	25.30±1.59	2.26±0.26^{c}	1.33±0.01^{b}
T6 (LRCY1000)	51.00±1.80	166.85±3.92^{bc}	24.36±2.47	2.80±0.40^{bc}	1.28±0.05^{bc}
T7 (CC)	50.68±0.67	162.10±3.97^{bc}	23.11±1.87	3.77±0.37^{a}	1.23±0.04^{bc}

^{a,b,c} *Means within each column with different superscripts are significantly different at (p< 0.05)*

INITWT- Initial weight
FINALWT- Final weight
ADFI- Average daily feed intake
FCR- Feed conversion ratio
AVGAIN- Average daily weight gain
CTR- Control (No feed supplement)
VIT E- Vitamin E 240mg/kg
LRCY- *Leptidenia reticulata* and Chromium yeast
CC- Clomiphene citrate 1.5mg/kg

Table 3: Effect of *Leptadenia reticulata* and Chromium yeast combination on the haematological parameters of Japanese quails

TREATMENTS	PARAMETERS		
	WBC (10^3/MM^3)	RBC (10^3/MM^3)	PVC (%)
T1 (CTR)	11.70±3.83^{ab}	3.38±0.264^{b}	43.56±2.72^{a}
T2 (VIT E)	9.80±2.65^{b}	3.430±0.226^{b}	39.22±2.49^{ab}
T3 (LRCY250)	9.20±2.88^{bc}	3.81±0.309^{ab}	39.33±1.67^{ab}
T4 (LRCY500)	15.00±1.19^{a}	3.38±0.220^{b}	34.11±1.07^{b}
T5 (LRCY750)	12.90±3.11^{ab}	4.50±0.596^{a}	39.00±1.83^{ab}
T6 (LRCY1000)	10.70±4.33^{bc}	3.14±0.345^{b}	34.78±2.49^{b}
T7 (CC)	8.722±1.29^{c}	3.39±0.135^{b}	39.33±2.02^{ab}

^{a,b,c} *Means within each column with different superscripts are significantly different (P< 0.05).*

CTR- Control (No feed supplement)
VIT E- Vitamin E 240mg/kg
LRCY- *Leptidenia reticulata* and Chromium yeast, CC- Clomiphene citrate 1.5mg/kg

There was significant increase (p< 0.05) in the final body weight and average daily weight gain of birds fed diet T4 (*Leptidenia reticulata* and Chromium yeast (LRCY) supplemented diet at 500mg/kg) (181.71±11.14g) compared to the control group (T1). Birds on diet T4 also had the lowest feed conversion ratio (2.18±0.41). Feed intake was similar (p< 0.05) across the treatment groups. Final body weight, average daily gain and feed conversion ratio indicated that *Leptidenia reticulata* and chromium yeast (LRCY) combination was effective in improving these parameters particularly in treatments T4 and T5. The growth performance of Japanese quail birds such as body weight gain, average daily gain and feed conversion was best in treatment T4. Similar results were reported by Guler *et al.* (2005) who stated that Coriander seeds supplementation at rate 2kg/ton improved body weight and feed conversion ratio in Japanese quail. This result was also in agreement with Singh *et al.* (2002) who observed significantly higher body weight, feed conversion ratio with the diet containing Livoliv fed to broiler chickens as compared to control diet. Lien *et al.* (1999) discovered that 1600 ppb or 3200 ppb chromium picolinate supplementation into broiler breeders' diet increased feed intake and improved weight gain. The

improvement in growth observed in birds supplemented with LAYPLUS® containing *Leptidenia reticulata* and chromium yeast combination could be as a result of the antimicrobial properties of plant extract (Ndip *et al,* 2007) and its anthelminthic activities (Adamu *et al.,* 2010). Aerial parts of *Leptidenia reticulata* is reported to contain tocopherol and possess several pharmacological activities such as galactogogue, antimicrobial and anti-inflammatory activity (Sathiyanarayanan *et al.,* 2007). The significantly increased average body weight in LRCY500 treated birds may be attributed to the presence of minerals (copper, iron, zinc, manganese, magnesium, calcium, phosphorus and potassium) which are required for regulation of body's metabolic functions. These results is in agreement with Jacob *et al.,* (1996) who found that mean body weights significantly increased by 12.1% for chicks fed sesame seed cake rations (antioxidant) for 8 weeks. Sands and Smith (1999) also reported that dietary chromium picolinate supplementation increased growth rate without affecting feed intake in broilers. Steele and Rosebrough (1981) similarly reported that addition of 20ppm chromium chloride as an inorganic Cr source increased weight gains of turkey poults.

There was no significant ($p<0.05$) difference in the packed cell volume among the groups fed *Leptadenia reticulata* and chromium yeast combination supplemented diets at level 250mg/kg, 500mg/kg, 1000mg/kg, 750mg/kg, vitamin E and 1.5mg/kg Clomiphine citrate (T7), whereas group supplemented with *Leptadenia reticulata* and chromium yeast combination at level 500mg/kg produced the lowest values for packed cell volume (34.11%). Birds on the control diet recorded similar ($p>0.05$) values with those on vitamin E (T2), LRCY250 (T3), LRCY750 (T5) and CC (T7). LRCY750 produced significantly ($p<0.05$) higher red blood cells (RBCs) values (3.14 – 4.5 x 106/1) than other groups except LRCY250 (T3). There was significant increase in the white blood cells of the group fed with *Leptadenia reticulata* and chromium yeast combination at level 500mg/kg, 750mg/kg, 1000mg/kg and 250mg/kg diet but the lowest result was obtained with clomiphene citrate of 1.5mg/kg diet.

The PCV of birds across all treatment groups were within the normal range of 30-40% (Aiello and Mays, 1998). Slightly higher values were obtained in the quails on the control (T1) group. All the values obtained from the treatments shows that the birds were not anaemic. The Red blood cells (RBCs) values obtained in this study for the quails were in range with those reported (3.59 – 4.71 x 106/1) by Akade *et al.,* (2012) for normal and healthy quails and higher than those reported (3.11 – 3.8 x 106/1) by Shehab (2012). The higher values obtained in the WBC of the birds in some of the groups (T4, T5, T6) is capable of generating antibodies in the process of phagocytosis. Phagocytes are cells that protect the body by ingesting harmful foreign particles, bacteria and dead or dying cells and have high degree resistance to diseases (Soetan *et al.,* 2013).

CONCLUSION

The effect of dietary levels of LAYPLUS® (combination of *Leptadenia reticulata* and Chromium yeast) on the growth performance and haematology of Japanese quails were studied. At the end of the experiment, it can be concluded that the group fed diet supplemented with LAYPLUS® at 500mg/kg had the best growth performance and had a positive effect on the haematological parameters of quails.

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