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Haematological Parameters of Japanese Quails (*Coturnix coturnix japonica*) Subjected to High Stocking Density

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Abstract **-** This study evaluated high stocking density and its effect on haematological parameters of 296 day-old apparently healthy Japanese quails for a period of eight weeks. They were divided into four stocking densities of 252.20 cm^2/bird (11birds); 173.43 cm^2/bird (16 birds), 132.10 cm^2/bird (21birds), and 106.73 cm^2/bird (26 birds) that represented Treatments I-IV respectively. The treatments were replicated four times adopting a completely randomized (CRD) design. Blood samples were drawn through their jugular veins into Ethylene di-amine tetra acetic acid (EDTA) and plain bottles for haematology and serum biochemistry respectively. Analyses of the blood samples were done using standard laboratory protocols. The haematological and serum biochemistry results differed significantly ($p < 0.05$) among the treatments. The haemogram revealed a corresponding ($p < 0.05$) rise in erythrocyte sedimentation rate (ESR), mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) as the stocking density increased while haemoglobin, red blood corpuscles (RBC), packed cell volume and mean corpuscular haemoglobin concentration (MCHC) decreased ($p < 0.05$) correspondingly The leuckogram showed a significant increase in the lymphocytes while heterophils decreased significantly relative to the stocking density. The minimum and maximum osmotic fragility for quails in this study was estabilished at 0.30 % and 0.10 % saline concentration respectively. Serum proteins, cholesterol, glucose, urea and alanine amino transferase were not affected by high stocking density but the treatment effects lowered the triglyceride component and concomitantly increased the high density lipoprotein components of the total cholesterol. Conclusively, most of the haematological parameters including the osmotic fragility of RBCs were negatively affected by high stocking density of 106.73 cm^2/bird (TIV). Therefore, the stock densities of 173.43 cm^2/bird (TII) and 132.10 cm^2/bird (TIII) could be used for profitable quail production where there are constraints on land for this rapidly expanding backyard poultry venture.

Keywords**:** Haematology, leuckogram, osmotic fragility, serum biochemistry, stocking densities, Japanese quails

I. INTRODUCTION

One of the major characteristics of underdeveloped and developing economy is high degree of unemployment [1] of which Nigeria is a good example. Unemployment in a way could also be attributed to high birth rate, massive annual turnover of school graduates, and low level of technology. Apart from the great challenge of massive unemployment, Nigeria has not succeeded to meet up with FAO recommendation of daily animal protein intake of 35 g/day. The average per caput consumption of animal protein consumption of an average Nigerian is about 10 g/day, which is a far cry from FAO recommendation as reported by [2]. Livestock production [3] in which quail rearing has been singled out could provides the ground by which the above twin-problem of unemployment and animal protein consumption deficit could be mitigated and by extension help in alleviation of poverty [4]. Quail is a small sized poultry, highly prolific, and less susceptible to some common diseases of poultry [5]. According to [6], [7], [8], daily feed consumption of quail is very low (20 – 25 g/day), less floor space requirement, and early maturity of coming to lay at 5- 6 weeks old. Quail rearing can easily be embarked upon by the unemployed youths and graduates as a means of self help venture and panacea to unemployment challenge. Haematological parameters are indispensable in ascertaining the health and physiological status of animals [9], [10], [11]. These parameters are quintessential in livestock production, can be quantified by manual and electronic means [12]. Haematological indices are useful diagnostics of the health of animals, various conditions of negative impacts on blood cells, especially haemostatic disorder [13], [14], [15]. The increased pressure on land has necessitated keeping livestock especially poultry under high stocking density in order to maximize the available land space. High stocking densities is one of the factors that can lead to heat stress and even a management challenge to livestock production in the tropics. Quails are susceptible to changes in environmental temperature and thus cannot effectively dissipate the heat produced. Heat stress affects rectal temperature, increased respiration rate, thermal imbalance or disrupted homeostasis among others [16], [17], [18]. On the basis of the need to have important or relevant information on the consequence(s) of high stocking densities on the haematological indices of Japanese quails (*Coturnix*

coturnix japonica), this study was carried out during the rainy season of the hot, wet equatorial climate of south-west Nigeria.

II. METHODOLOGY

Experimental Site

The study was conducted from late May to early September, 2019 at the Poultry Section of Teaching and Research Farm, Federal University of Technology, Akure, Ondo State, Nigeria.

Management

Two hundred and ninety-six day-old sexed Japanese quails, purchased from a reliable source were reared under the same managerial and hygienic conditions. They were brooded for three weeks in a deep litter system and thereafter randomly allotted in a completely randomized design (CRD) into four stocking densities (representing the treatments) and replicated four times. T I comprises 11 birds at 252.20 cm^2/bird, T II, 16 birds at 173.43 cm^2/bird, T III, 21birds at 132.10 cm^2/bird, and T IV, 26 birds at 106.73 cm^2/bird. The birds were housed male to female in the ratio 1:1 in a battery cage system and fed to satiation. Blood samples were taken from 2 females and 2 males from each replicate of the treatments. The birds were fasted prior to collection of blood from the jugular vein using sterile needles and syringes into EDTA bottle for haematological evaluation while that of serum biochemistry were collected into plain bottles. The haematological and serum parameters were analyzed following standard laboratory protocols and manual calculations were done where necessary [19], [20]. Erythrocyte osmotic stability determination test was carried out with red blood cell collected into heparinized bottle, test tubes, distilled water and sodium chloride (0.00 g to 0.09 g) as described by [21], [22], [23].

Statistical analysis

Generated data were analyzed using linear procedure for one-way analysis of variance (ANOVA). Means with significant difference were separated using Duncan Multiple Range Test [24] set at 0.05 % level of significance.

III. RESULTS AND DISCUSSION

The results from the analyses viz; haematological parameters, leuckogram, osmotic stability of the erythrocytes, and serum biochemical parameters of japanese quails subjected to different stocking densities are presented in Tables 1 to 4 respectively.

Haematological results (Table 1) showed ESR for Treatment IV to be the poorest owing to the highest value obtained from the treatment when compared with other treatments. The red blood cells normally should fall slowly, and a rapid fall may be an indication of inflammation, infection among others [25]. Probably the very high stocking density in this treatment predisposed the quails to infection which the blood cells were trying to overcome through inflammatory response. This fact could also be supported by the highest value of lymphocyte obtained in the leuckogram data from this same treatment (Table 2). The highest values were obtained for PCV, RBC and Hb for quails in the control treatment (T1) at 252.20cm^2/bird, and the lowest in treatment IV (106.73cm^2/bird) Apart from the control, PCV values did not differ significantly ($p > 0.05$) among TII, TIII and TIII. The ESR, MCV, MCH and MCHC values did not follow a regular trend across the treatments. However, the PCV and Hb values agreed with [26] who reported the range of 30 % – 41 % and 10 % - 13% for PCV and Hb of Japanese quails respectively. Similar results were obtained by [27] - [31].

The lymphocytes (Table 2) increased in number following a corresponding increase in stocking density in this study. The result is in harmony with [32] but in contrast to [29], [30], [31] who all reported a significant decrease in LYM with increasing environmental temperature. The heterophils as observed decreased significantly as the stock density increased. Eosinophils did not follow a particular pattern but the monocytes and basophils did not differ significantly across the treatments. The values of basophils and eosinophils fell within the range of the observation of [33] in normal examination of Japanese quails' blood, but the values for the heterophils, lymphocytes and monocytes were higher when compared with the results from this same author but normal according to [34] who reported 16 % as average normal value for monocytes. The differences may be due to different ecological zones and environmental conditions.

Osmotic fragility test of the red blood cells presents the avenue whereby they can be evaluated for their ability to withstand osmotic pressure which according to reports increases during low oxygen tension, red blood cell membrane abnormality, and during oxidative stress [35] - [37]. The minimum and maximum osmotic fragility for quails in this study was estabilished at 0.30 % and 0.10 % saline concentration respectively which corroborates the report of [26]. In addition, a progressive decrease in the stability of red blood cells was noticed as the concentration of the salt decreased from 0.09 % to 0.00 %. This result corroborates the findings of [38] on chicken RBC's. The stability of T2 and T3 compared with the control, while TIV appeared to be the most fragile in all the concentrations. Thus, this might suggest that the stocking density in this Treatment (106.73cm^2/bird) was too high, as it had negative effect on the osmotic stability of erythrocytes of the quails.

Table 1: Haematological Parameters of Japanese quails subjected to different stocking densities

Treatments	Treatment 1	Treatment II	Treatment III	Treatment IV
ESR (mm/hr)	0.63±0.06[c]	0.94±0.14[b]	1.03±0.13[a]	1.28±0.23[a]
PCV (%)	39.94±1.26[a]	36.56±1.57[b]	35.25±1.59[b]	34.13±1.59[b]
RBC (x10^6/mm^3)	6.01±0.36[a]	5.10±0.44[b]	4.80±0.46[c]	4.55±0.40[d]
Hb (g/dL)	13.25±0.41[a]	12.17±0.52[a]	11.78±0.55[b]	11.36±0.53[b]
MCV (fL)	68.59±2.43[b]	76.16±3.55[ab]	78.55±3.72[ab]	80.76±4.43[a]
MCH (pg)	22.77±0.83[b]	25.36±1.19[a]	26.22±1.23[a]	26.87±1.47[a]
MCHC (g/dL)	33.29±0.02	33.18±0.07	33.40±0.09	33.28±0.02

Treatment I= 252.20cm^2/bird (11 birds); Treatment II = 173.43cm^2/bird (16 birds); Treatment III = 132.10cm^2/bird (21 birds); Treatment IV = 106.73cm^2/bird (26 birds); ESR = Erythrocyte Sedimentation Rate; PCV = Packed Cell Volume; RBC = Red Blood Cell; Hb = Haemoglobin; MCV = Mean Corpuscular Volume; MCH = Mean Corpuscular Haemoglobin; MCHC = Mean Corpuscular Haemoglobin Concentration

Table 2: Leuckogram of Japanese quails subjected to different stocking densities

Treatments	Treatment I	Treatment II	Treatment III	Treatment IV
LYM (%)	51.13±0.55[c]	52.88±0.75[b]	53.44±0.77[a]	53.75±0.79[a]
HET (%)	33.75±0.84[a]	32.00±0.82[b]	30.75±0.83[c]	31.00±0.89[c]
MON (%)	12.19±0.51	12.25±0.60	12.63±0.58	12.13±0.58
EOS (%)	2.25±0.11[b]	2.25±0.11[b]	2.56±0.13[a]	2.44±0.13[a]
BAS (%)	0.69±0.12	0.63±0.13	0.63±0.13	0.69±0.12

LYM = Lymphocytes; HET= Heterophils; MON = Monocytes; BAS = Basophils; EOS = Eosinophils

Table 3: Osmotic Stability of the erythrocytes of Japanese quails subjected to different stocking densities

Saline Concentration	I	II	III	IV
0.00%	2.57±0.22[a]	2.30±0.23[ab]	2..22±0.24[ab]	1.89±0.16[b]
0.10%	2.82±0.24[a]	2.49±0.24[ab]	2.44±0.24[ab]	2.11±0.18[b]
0.20%	3.14±0.26[a]	2.75±0.26[ab]	2.70±0.27[ab]	2.36±0.20[b]
0.30%	3.52±0.27[a]	3.03±0.27[ab]	2.96±0.29[b]	2.61±0.23[c]
0.40%	3.93±0.28[a]	3.35±0.28[ab]	3.26±0.33[b]	2.92±0.25[c]
0.50%	4.46±0.32[a]	3.67±0.31[b]	3.59±0.34[b]	3.25±0.28[c]
0.60%	4.94±0.34[a]	4.01±0.32[b]	3.96±0.37[b]	3.58±0.32[c]
0.70%	5.35±0.36[a]	4.44±0.36[b]	4.35±0.42[b]	3.91±0.37[c]
0.80%	5.76±0.38[a]	5.04±0.44[b]	4.78±0.46[c]	4.27±0.41[d]
0.90%	5.81±0.38[a]	5.08±0.44[b]	4.83±0.45[bc]	4.33±0.42[c]

Table 4: Serum Biochemical Parameters of Japanese quails subjected to different stocking densities

Parameters	I	II	III	IV
Total Protein (mg/dl)	35.27±3.86[a]	29.86±1.30[c]	33.88±2.48[b]	33.06±2.49[b]
Albumin (mg/dl)	20.07±3.16[a]	17.94±1.25[b]	19.27±1.00[a]	19.67±1.70[a]
Globulin (mg/dl)	15.20±1.43[a]	11.92±1.10[b]	14.61±1.96[a]	13.39±0.95[ab]
Cholesterol (mg/dl)	82.95±8.49[b]	86.93±23.68[a]	87.43±22.92[a]	88.3±13.43[a]
Glucose (mg/dl)	203.75±29.67[b]	192.75±18.17[b]	243.25±26.78[a]	208.25±22.44[b]
Urea (mg/dl)	4.69±0.68[c]	7.44±2.29[a]	6.24±1.27[a]	5.05±0.72[b]
AST (mg/dl)	148.50±12.94[b]	158.00±12.76[a]	144.44±11.22[b]	158.38±7.26[a]
ALT (mg/dl)	12.73±1.91[b]	22.10±6.22[a]	12.55±1.57[b]	12.90±2.06[b]
Creatinine (mg/dl)	4.18±0.69[a]	3.58±0.72[b]	2.93±0.59[d]	3.33±0.26[c]
Trig (mg/dl)	5.90±0.69[a]	5.51±0.98[a]	5.09±0.89[a]	3.79±0.78[b]
HDL (mg/dl)	22.90±6.75[b]	38.61±5.11[a]	41.04±16.72[a]	29.99±6.62[a]
LDL (mg/dl)	58.87±10.80[a]	47.22±25.31[b]	45.37±11.71[b]	57.55±15.44[a]
Phosphorous (mg/dl)	4.31±0.35[a]	3.15±0.52[b]	2.11±0.34[d]	2.86±0.58[c]
Calcium (mg/dl)	0.93±0.08[b]	1.10±0.08[a]	1.22±0.15[a]	1.15±0.10[a]

[a, ab, b, c, d] = Means in the same row but with different superscripts are statistically ($p < 0.05$) significant.

AST (Aspartate Aminotransferase); ALT (Alanine Aminotransferase); Trig (Triglycerides); HDL (High Density Lipoprotein); LDL (Low Density Lipoprotein

The effect of the treatments on the serum biochemistry of experimented quails is as presented in Table 4. Serum biochemical parameters in this study differ significantly across the treatments. Total protein, albumin, globulin, cholesterol, creatinine, Trig, and LDL were highest in treatment I. Chol, urea, Trig, and HDL were statistically the same in treatments II and III. The total protein, Albumin, Cholesterol, Urea, and creatinine values in this study were higher than the report of [39] on Japanese quails while the calcium, phosphorus, glucose, AST, and Trig values were less. The reason could be due to experimental design as the authors' experiment was based on dietary crude protein, but the values obtained provided basis for comparison. The lower AST and ALT in this study showed that the birds were not predisposed to hepato-toxicity as reported by [40] that low serum enzymes is an indication of the absence of hepatoxicity. The total protein, and albumin values were higher than the ealier report by [39] and this could be a matter of better nutrition profile of the diets of the quails in the present study. When a diet is rich in nutrients with well balanced amino acid, there would be no problem of muscle degeneration of birds and appreciable level of protein and albumin will be available in the serum [41]. However, the trend of the total protein in this study disagreed with [42] who reported that birds stocked at low stocking density had the lowest value. The blood cholesterol level increased vis a vis an increase in the stocking density in this study. Low Density Lipoprotein (LDL) and Aspartate Trans-Aminase (AST) according to the report of [42] were not significantly affected by high stocking density which actually disagrees with the result of the current investigation. The serum phosphorous decreased while the calcium increased with increase in stocking density. The result on serum phosphorus harmonizes that of [42], but is at variance with that of calcium. Adduced reason for the lowered serum phosphorus at higher stocking densities could be as a result of altered phosphorus absorption at higher stocking densities

IV. CONCLUSION AND FUTURE SCOPE

The erythrocyte sedimentation rate, red blood cells, and the lymphocytes values for treatment IV (106.73 cm^2/bird) were negatively affected by the stocking density while T II (173.43 cm^2/bird) and T III (132.10 cm^2/bird) compared with the control (252.20 cm^2/bird). The serum biochemistry parameters values differed significantly but fell within the recommended range for avians species. Osmotic stability of TII and TIII compared with the control, while TIV appeared to be the most fragile at all saline concentrations. The osmotic stability of the red blood cells decreased progressively from TI to TIV at all saline concentrations but hypotonicity especially below 0.40 % saline concentration seemed to confer better osmotic stability on the birds' erythrocytes across the four treatments. It can be concluded that most of the evaluated haematological parameters including the osmotic fragility of RBCs were negatively affected by high stocking density of 106.73 cm^2/bird (TIV).

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