IMPACT OF INCUBATION TEMPERATURE ALTERATION DURING LATE EMBRYOGENESIS ON HAEMATOLOGICAL AND SERUM BIOCHEMICAL INDICES OF JAPANESE QUAILS (*Coturnix japonica*)

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ABSTRACT

Evidence abound that incubation temperature manipulation, during embryogenesis often results in improved thermo-tolerance, meat quality and body weight but, there is little or no information on the effects on haematological and serum biochemical indices hence, the present study. A total of 615 Japanese quail eggs were set in 5 artificial incubators, representing the treatments (36°C, 37°C (control), 38°C, 39°C and 40°C) and paused on incubation days 11, 12 and 13, to improve post-hatch performance. The hatched chicks were brooded for 3 weeks and transferred to the grower's unit and at 8 weeks of age, blood samples were collected and processed for haematological and serum biochemical indices. There were no significant differences (P>0.05) in all the haematological indices evaluated except in packed cell volume, red blood cells and neutrophil. Packed cell volume (45.21%) and white blood cells (9.33 x 10^9/L) were superior in T_4. On the other hand, there were statistical variations (P<0.05) in all the serum biochemical indices determined except, albumin whose value ranged from 13.89g/L (T_4) – 17.53g/L (T_1), alanine aminotransferase [6.00U/L (T_1) – 26.56U/L (T_5)], total protein (26.67g/L – 53.42g/L), creatinine (39.30µmol/L – 46.57µmol/L) and cholesterol was lowest (162.42mg/dL) in T_5 with the highest (200.12mg/dL) recorded in T_1. Since the haematological and serum biochemical indices were within the values reported in healthy birds, incubation temperature alteration to improve post-hatch performance in Japanese quails, may not compromise organ quality vis-à-vis function. Therefore, incubation temperature range of 36 – 40°C, may be adopted with or without pausing during Japanese quail eggs incubation.

Keywords: Blood profiles, Chicken quality, Japanese quails, Incubation, Post-hatch performance

INTRODUCTION

Based on FAO (2013) report, poultry products are the best sources of high-quality proteins, vitamins and minerals needed by many people who live in abject poverty. It was emphasized that poultry meat is the healthiest and cheapest of all livestock meat with no major taboos on the consumption. Hence, increased commercial poultry production becomes imperative, in order to boost the livestock subsector's contributions to national and economic growth. Poultry species include fowl, ducks, guinea fowl, geese and turkey. Others are pheasants, swan, ostriches, emu, pigeons and quails.

Among these species, the Japanese quail has been singled out due to its short generation interval with the ability of the hen to start laying at about 40 days old, capability of producing about 250 – 320 eggs per year, while the cock matures at about 30 to 60 days old (Deveau, 2009). With these economic traits, Woodard *et al*. (1973) stated that three to four generations annually were possible in Japanese quail. Other attributes include requirements of little starter-pack, land area, labour and medical attention as well as high resistance to diseases. Essentially, Japanese quail has been reported to be a veritable source of nutrients (Randall, 2008) and the products especially egg has been speculated to be rich in minerals and vitamins but low in cholesterol (Fah, 2009). Hence, commercial Japanese quail production is likely feasible in making the meat and eggs available at an affordable rate.

Unfortunately, Japanese quail hens in captivity hardly incubate their eggs. Therefore, artificial incubators are used to simulate and mimic the role of a broody hen, in providing optimum environmental conditions that will stimulate embryonic development and maintain the growth until hatching. Yet, hatchery failures, low chick yield, late hatching that often led to high chick mortality rate and poor post-hatch chick performance have been reported in the use of artificial incubators (Hill *et al*., 2001; Lourens *et al*., 2005). Consequently, alteration of incubation temperature, during late embryogenesis to enhance post-hatch chick performance becomes necessary. Thus, this study aimed at evaluating the effect of incubation temperature set at 36°C, 37°C, 38°C, 39°C and 40°C and paused for 5 hours on incubation days 11, 12 and 13 on haematological and serum biochemical indices of Japanese quails.

MATERIALS AND METHODS

Experimental site

The study was conducted at the National Veterinary Research Institute, Vom, Jos North, Plateau State, Nigeria, located on latitude 9° 44' 0"N, longitude 8° 47' 0"E.

Experimental design and procedure

Five different electric incubators representing the treatments: 36ºC, 37ºC (control), 38ºC, 39ºC and 40ºC, were used. A total of six hundred and fifteen Japanese quail (*Coturnix japonica*) eggs, were collected from layers reared in a deep litter system, for 4 days and were cooled at room temperature before setting on the 5th day. In each of the incubators, 123 eggs were distributed in a completely randomized design, based on weight such that each of the 3 egg trays in the incubators held 41 eggs. On incubation days 11, 12 and 13, the temperature was paused for 5 hours by switching off the main to improve post-hatch performance. The hatched chicks were allowed to dry fluffy in the incubators, brooded for 3 weeks and transferred to the grower's unit where they were fed standard feed and offered clean drinking water *ad libitum*.

Blood samples collection

At 8 weeks old, the only survival bird in T_1, 8 in T_2, 8 (T_3), 6 (T_4) and 8 in T_5 were taken for blood samples collection through the jugular veins in EDTA bottles and processed according to standard operating procedures in haematology (Campbell and Ellis, 2007). Another blood samples were collected in bottles without EDTA, left on the racks in a slant position for 1 hour and were carefully decanted and processed following Randox procedures as given by Fudge (2000).

Data collection and statistical analysis

Haematological data obtained were white blood cells, neutrophils, lymphocytes, monocytes, eosinophil, red blood cells, haemoglobin and packed cell volume while those of the serum biochemical indices were alanine aminotransferase, total protein, albumin, creatinine and cholesterol. The values were subjected to statistical analysis procedure of SPSS (2010) and the means were separated using Duncan Multiple Range Test of the same software package.

RESULTS

Haematological indices of Japanese quail chicks hatched at 36ºC, 37ºC, 38ºC, 39ºC and 40ºC is given in table 1. There were no significant differences ($P>0.05$) in all the parameters evaluated except in packed cell volume, red blood cells and neutrophil. Packed cell volume was superior ($P<0.05$) in T_4 (45.21%), slightly followed by T_5 (44.79%), T_3 (40.48%), T_2 (40.00%) and T_1 (35.04%). While a similar trend was observed in red blood cells, T_2 value (28.52%) was highest in neutrophil, slightly followed by 28.00% in T_1 with the least value (17.51%) recorded in T_4. White blood cells were more (9.33 x 109/L) in T_4, slightly followed by T_5 (9.03 x 10^9/L), T_2 (8.36 x 10^9/L), T_1 (5.44 x 109/L) and T_3 (4.95 x 109/L). Even when monocytes were not detected in birds in T_1 (absolutely 0.00%), it was as high as 2.50% in T_4, lymphocytes value ranged between 68.67% and 81.02% and eosin was only detected in T_2 (0.63%) and T_4 (0.67%).

Table 1: Haematological indices of Japanese quail chicks hatched at 36 – 40ºC

	Treatments					Statistics			
	T_1	T_2	T_3	T_4	T_5			Overall	
Parameters	36°C	37°C	38°C	39°C	40°C	Min	Max	mean	SEM
NCH	44	69	72	67	61	-	-	-	-
NC	1	8	8	6	8	1	8	-	-
PCV (%)	35.04^{b}	40.00^{ab}	40.48^{ab}	45.21^{a}	44.79^{a}	28	50	41.42	0.86
RBC (x10^{12}/L)	0.82^{c}	1.43^{bc}	1.84^{abc}	2.77^{a}	2.09^{ab}	0.8	4.0	1.92	0.15
WBC (x10^{9}/L)	5.44	8.36	4.95	9.33	9.03	2.3	15.6	7.37	0.53
HB (g/dL)	8.44	9.75	9.57	9.43	8.78	6.0	13.8	9.34	0.28
Neut (%)	28.00^{a}	28.52^{a}	21.12^{ab}	17.51^{b}	22.02^{ab}	4.0	43.0	21.22	1.38
Lym (%)	72.00	68.67	76.55	81.02	76.89	49	96	76.6	1.81
Mono (%)	0.00	2.13	2.38	2.50	1.13	0.0	19.0	2.08	0.69
Eosin (%)	0.00	0.63	0.00	0.67	0.00	0.0	4.0	0.31	0.13

abc: Mean values on the same row with different superscript differ statistically at 5% probability test; Min: Minimum value; Max: Maximum value; SEM: Standard error of means; PVC: Packed cell volume; RBC: Red blood cells; WBC: White blood cells; HB: Haemoglobin; Neut: Neutrophils; Lym: Lymphocytes; Mono: Monocytes; Eosin: Eosinophil; NCH: Number of chicks hatched; NC: Number of birds at 8 weeks of age.

Table 2 provides the serum biochemical indices of Japanese quail chicks hatched at 36°C, 37°C, 38°C, 39°C and 40°C. There were statistical variations (P<0.05) in all the parameters determined except, in albumin whose value ranged from 13.89g/L (T_4) to 17.53g/L (T_1). Alanine aminotransferase value varied from 6.00U/L (T_1) to 26.56U/L (T_5), total protein (26.67g/L to 53.42g/L), creatinine (39.30µmol/L to 46.57µmol/L) and cholesterol value was lowest (162.42mg/dL) in T_5 with the highest value (200.12mg/dL) recorded in T_1.

Table 2: Serum biochemical indices of Japanese quail chicks hatched at 36 – 40°C

	Treatments					Statistics			
	T_1	T_2	T_3	T_4	T_5				
Parameters	36°C	37°C	38°C	39°C	40°C	Min	Max	Mean	SEM
NCH	44	69	72	67	61	-	-	-	-
NC	1	8	8	6	8	-	-	-	-
ALT (U/L)	6.00^{b}	12.56^{ab}	15.14^{ab}	14.00^{ab}	26.56^{a}	4.0	52.0	15.67	1.36
TP (g/L)	53.42^{a}	30.10^{b}	32.78^{b}	26.67^{b}	31.02^{b}	20.4	53.4	31.33	1.36
ALB (g/L)	17.53	16.58	14.10	13.89	15.58	9.9	30.5	15.59	0.77
CREAT (µmol/L)	39.30^{b}	45.11^{ab}	42.89^{ab}	45.44^{ab}	46.57^{ab}	32.2	84.4	46.78	1.61
CHOL (mg/dL)	200.12^{a}	183.89^{ab}	168.78^{ab}	177.54^{ab}	162.42^{ab}	99.90	219.90	169.73	4.85

ab: Mean values on the same row with different superscript differ statistically at 5% probability test; Min: Minimum value; Max: Maximum value; SEM: Standard error of means; ALT: Alanine aminotransferase; TP: Total protein; ALB: Albumin; CREAT: Creatinine; CHOL: Cholesterol; NCH: Number of chicks hatched; NC: Number of birds at 8 weeks of age.

DISCUSSION

According to Samour (2006), avian blood analysis often expresses the health status of the birds. The observed value of packed cell volume was somewhat higher than a range of 29.42 – 37% reported to be normal in avian species (Anggraeni *et al*., 2016) but close to 40.9 – 55.00% reported by El-Kholy *et al*. (2019) in Japanese quails at 6 weeks of age. The disparities could be due to the age of the birds, environmental conditions of where the birds were raised and probably due to the laboratory protocol adopted (Givens *et al*., 2000). The red blood cells were similar to a normal range of 2.30 – 3.86 x 10^6/mm3 reported in healthy birds of similar age (Anggraeni *et al*., 2016; El-Kholy *et al*., 2019). Also, white blood cells, haemoglobin, neutrophils, lymphocytes, monocytes and eosinophil were within the normal ranges reported (Anggraeni *et al*., 2016).

This probably indicated that the treated chicks were not stressed, as evidently provided by Tamzil *et al*. (2014) that there was no depression in the haematological values, when chickens were subjected to acute heat stress. Meanwhile, the consistent least values recorded in T_1 (36°C), in all the parameters evaluated except in neutrophils, could be largely due to single sample (no replicate), late hatching of the chicks and partly due to the incubation temperature (36°C) of the eggs that probably led to facultative hypothermia resulting in high mortality leaving only one survivor. Essentially, avian neutrophils have been reported to be normally higher than white blood cells unlike in mammals. More so, it was speculated that avian neutrophils were equivalent to heterophil in mammals (Samour, 2006).

The organ function test as revealed by serum alanine aminotransferase, total protein, albumin, creatinine and cholesterol values compared favourably well with 6.5 – 9.6U/L, 31.6 – 36.5g/L, 13.3 – 15.3g/L, 4.0 – 4.5µmol/L and 235 – 259mmol/L respectively reported by Scholtz *et al*. (2009) as reference values in adult Japanese quails. Both values were similar to those given more recently, as normal range in avian species (Mnisi and Mlambo, 2017). Nevertheless, there were little discrepancies in some of the parameters, probably as influenced by the age, strains, environmental conditions, unit of measurements and the trial the birds were subjected to in the respective studies. This probably showed that the birds' physiological systems were not compromised by the low or high incubation temperatures. Consequently, Japanese quails could build thermos-tolerant traits during embryogenesis in order to resist, survive and thrive well in high ambient temperature at post-hatch growth phases. This observation corroborated the report of Piestun *et al*. (2008) that avian species could acquire thermos-tolerant traits during embryogenesis.

CONCLUSION

The haematological and biochemical indices of the Japanese quails hatched at incubation temperature of 36°C, 37°C, 38°C, 39°C and 40°C and paused for 5 hours on incubation days 11, 12 and 13, were somewhat within the normal range reported in healthy birds. It could be concluded that Japanese quails may build thermos-tolerant traits during embryogenesis in order to resist, survive and thrive well in high ambient temperature at post-hatch growth

phases. Therefore, incubation temperature range of 36 - 40°C may be adopted with or without pausing during the incubation period.

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