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SERUM BIOCHEMICAL CHANGES IN JAPANESE QUAIL (*COTURNIX COTURNIX JAPONICA*) EXPERIMENTALLY INFECTED WITH *SALMONELLA ENTERICA* SEROVAR GALLINARUM

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Abstract

Aim: To determine the serum biochemical changes in Japanese quail (Coturnix coturnix japonica) experimentally infected with Salmonella enterica serovar Gallinarum

Methods: A total of 160 (108 males and 52 females) Japanese quails (Coturnix coturnix japonica) were used for the experiment. Four weeks old quails were randomly selected and assigned into four groups (A, B, C and D) of forty quails each. Groups A, B and C were infected with Salmonella enterica serovar Gallinarum per os at the dose of 10^6,10^4 and 10^2, respectively, while group D served as the control. Sera were used for the determination of biochemical parameters.

Results: There were significant changes ($P<0.05$) in serum glucose, total protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and urea concentrations between infected groups and the control group. Significant changes ($P<0.05$) in these biochemical parameters were also observed post-infection compared to pre-infection within each infected group.

Conclusion: FT in quails results in severe multi organ dysfunction and damage as demonstrated by the serum biochemical changes noticed suggestive of immediate cause of mortality in FT infection.

Key words: Serum biochemistry, Quail, Salmonella, Nigeria

INTRODUCTION

The genus *Salmonella* is a member of the Family *Enterobacteriaceae* and consists of Gram-negative, non-spore forming bacilli (Popoff *et al*., 2003). *Salmonella enterica* serovar Gallinarum (*Salmonella* Gallinarum) is a non-motile host adaptive *Salmonella* that causes fowl typhoid (FT), a severe systemic disease responsible for heavy economic losses in commercial poultry industry through morbidity, mortality and pathological lesions (Parmer and Davies, 2007).With the continuous expansion of poultry farming in Nigeria, FT hasbecome a major endemic disease of poultry. (Agbaje *et al*., 2010).The impact of FT is difficult to evaluate in developing countries due to lack of systematic surveillance, but its importance is obvious, from both published and anecdotal evidence, with outbreaks in Mexico, Argentina, Nigeria and India(Barrow *et al*., 2012). Endemic FT is still found in many countries in both commercial production and backyard flocks including countries with expanding poultry industries such as Brazil and South Korea where there has been considerable research activity in recent years (Barrow *et al*., 2012).Quails are ideally suited for avian

research, because of their small size and require little cage space for rearing. They are easy to raise and are suitable for genetic studies since they attain sexual maturity rapidly (Haruna *et al*., 1997). Quails are hardy birds which thrive very well in cages and are relatively inexpensive to maintain. They are birds that every household can keep without stress(Huss *et al.*, 2008).The Japanese quail has the potential to complement the obvious gap in the protein needs of Nigerians, it is therefore important to investigate diseases that can interfere with quail production such as FT in order to control them(NVRI, 2008). Quail meat and eggs are known for high quality protein, high biological value and low caloric value, thus making them good choices for hypertension prone individuals (Chindo and Olowoniyan, 2006). Because its lifespan is relatively short and its physiology is comparable to that of humans, the adult quail is useful for studies of aging and diseases (Huss *et al*., 2008). An increasing number and variety of quails are being kept for food, experimental use, and preservation of endangered species, zoological displays and as companion birds (Khare *et al*., 1975). This study seeks to determine if any, the serum-biochemical changes in Japanese quail *(Coturnix coturnix japonica)* experimentally infected with *Salmonella enterica* serovar Gallinarum (*Salmonella* Gallinarum) reared in Zaria, Northern Nigeria.

MATHERIALS AND METHOD

Area of Study

The study was carried out in Zaria, Kaduna State, which is located within the Northern Guinea Savannah Zone of Nigeria, between latitude 7° and 11°N, and longitude 7° and 44°E; the average rainfall of this zone ranges from 1,000 to 1,250 mm annually, and the average temperature ranges from 17°C to 33°C (Saidu *et al*., 1994).

Experimental Birds

A total of 160, four-week old Japanese quails (108 males and 52 females) were obtained from the Poultry Division of National Veterinary Research Institute, Vom, Nigeria and used for the experiment. They were randomly selected and divided into four groups (A, B, C and D) of 40 birds each; with 27 males and 13 females in group A, 23 males and 17 females in group B, 29 males and 11 females in group C as well as group D.

Housing and Feeding

The quails were kept in deep litter but separated into different compartments using wire mesh measuring120 × 140 × 120 cm in size in an enclosed house with the litter changed every week throughout the experimental period. The quails were allowed to acclimatize for two weeks, during which they were observed for clinical signs of any infection. During the acclimation, they were fed on commercial chick mash, and thereafter switched to commercial layer mash throughout the120 days of the research. They had water *ad libitum* during this period.

Bacteriological Monitoring Before Infection

Before infection, cloacal swabs were collected from all the quails using sterile swabs in order to confirm if they were free from *Salmonella* organism or indeed any other bacterial pathogens. This was done by pre-enrichment of the swab samples in buffered peptone water, followed by plating on MacConkey agar (MCA) and blood agar (BA) using standard laboratory methods (Parmer and Davies, 2007).

Source of Organism and Infectivity Procedure

The bacterium, *Salmonella enterica* serovar Gallinarum, was obtained from the bacterial culture bank of the Central Diagnostic Laboratory, National Veterinary Research Institute, Vom, Nigeria. The isolate was from day old chick that died of natural *Salmonella* Gallinarum infection. The lyophilized bacterium from the culture bank was reactivated by sub-culturing on blood agar (BA) and MacConkey agar (MCA). The resulting colonies were examined for their features, colour and morphology and tested for Gram-reaction (Gram-negative). Three colonies were scooped and inoculated into 20ml of nutrient broth and this was incubated for 24 hours at 37°C after which a ten-fold dilution was carried out in test tubes. The colony counts from the test tubes were determined. To obtain the number of organisms that were inoculated into the quails, the number of organisms was multiplied by volume and by the dilution factor (CFU= No. of colony × Volume × Reciprocal of Dilution factor) (Miles and Misra, 1938). Challenge of the quails was done orally. Group A quails received a dose of 1×10^6 organisms/0.2ml of nutrient broth, group B quails received a dose of 1×10^4 organisms/0.2ml of nutrient broth, while group C quails received a dose of 1×10^2

organisms/0.2ml of nutrient broth. Group D quails served as control and were not challenged with the bacterium, but were given bacteria-free nutrient broth in accordance with the method of Miles and Misra (1938).

Determination of Serum Biochemical Parameters

The baseline values for serum-biochemical parameters were determined before the commencement of the experiment, according to standard procedures (Barham and Trinder, 1972; Patton and Crouch, 1977; Buttery *et al*., 1980; Grant, 1987). Blood samples were collected via the wing vein, using 25 gauge sterile hypodermic needles and syringes and then kept in a slanted position for serum to settle. These were collected 5 days before challenge and 5 days after challenge, so as to reduce stress pre-infection and to get the serum-biochemical picture within the incubation period of the disease. Two millilitres of serum from each quail sampled were used to measure the serum glucose, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), urea and total protein levels in the Clinical Chemistry Laboratory of the Central Diagnostic Laboratory, National Veterinary Research Institute (N.V.R.I), Vom, Nigeria.

Assay for Serum Albumin

Three small labeled tubes 'A (reagent)' containing 0.01ml of distilled water and 3.0 ml of bromocresol green (BCG) reagent were mixed thoroughly. In the second tube labeled 'A(standard)', 0.01ml of standard and 3.0ml of BCG reagent were mixed thoroughly in the third tube 'A(serum) 0.01ml of the quail serum and 3.0 ml of BCG reagent. The samples were mixed thoroughly and incubated at 20°C – 25°C for 5 minutes. The absorbance of each tube was measured using a spectrophotometer (model 6400/6405, Jenway) at 600 – 650 nm against the reagent blank as follows:

$$Albumin\ concentration\ (g/dl) = \frac{A(serum) \times concentration\ of\ standard}{A(standard)}$$

(Grant, 1987)

Assay for Serum Total Protein

Biuret method was used to determine total protein, as described by Benjamin (1985). A blank test tube, standard and the test sample tubes were labeled. 1,000 microlitre of biuret reagent was pipetted into each tube, and 200 microlitres of distilled water, standard and test samples were pipetted into the respective tubes. They were thoroughly mixed and incubated for 30 minutes at 25°C. The spectrophotometer (model 6400/6405 manufactured by Jenway, Barloworld Scientific, England) was set at 546 nm and zero using blank test tube, the absorbance was read, and the total protein calculated using the formula:

$$\frac{A_1 \times C}{A_2}$$

Where:

A_1= Absorbance of the test sample,

A_2= Absorbance of the standard,

C= Standard concentration

(Grant, 1987)

Assay for Serum Glucose

Ten microlitres (10µl) of serum sample and 1.0 ml of reagent were measured into a cuvette and labeled 'T(serum)' and were mixed thoroughly. A cuvette labeled 'T(standard)' containing 10 µl of standard and 1.0 ml of reagent were mixed thoroughly. The 10 µl of reagent pipetted into a cuvette was labeled 'T(reagent)'. The contents were mixed thoroughly and incubated at 20-25°C for 10 minutes. The absorbance of 'T(serum)' and 'T(standard)' was measured against reagent blank within 60 minutes in a spectrophotometer (model 6400/6405, Jenway) at 500nm wave length.

$$Glucose\ concentration\ (mg/dl) = \frac{T(serum) \times concentration\ of\ standard}{T(standard)}$$

(Barham and Trinder, 1972)

Assay for Serum Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT)

Pippeted into tubes was 100 microlitres of sample and 500 microlitres of buffer added, mixed and incubated for 30 minutes at 37°C. Then 500 microlitres of dye reagent was added and allowed to stand for 20 minutes at 25°C; after which 5 ml of sodium hydroxide was added, mixed and the absorbance of the sample against sample blank was read after 5 minutes. Calculation for both AST and ALT were

obtained from the activity table (Reitmans and Frankel, 1957).

Assay for Serum Alkaline Phosphatase (ALP)
This was done using the method of Buttery *et al.* (1980). 500µl of blank; standard and sample was pipette into test tube and incubated at 37°C for 10 minutes, after which 2500µl of alkaline phosphatase colour developer was added into each of the test tubes, mixed and then the absorbance was read using a spectrophotometer at wavelength of 590nm. The serum alkaline phosphatase was calculated using this formula:

$$\frac{Absorbance\ of\ test \times Concentration\ of\ standard}{Absorbance\ of\ standard}$$

Assay for Serum Urea
One ml of buffer was pippeted into 3 test tubes (blank, standard and sample) and 50 microlitres added to the 3 test tubes, mixed and incubated for 5 minutes at 37°C. Then 200 microlitres of hydrochloride was added and incubated for further 5 minutes at 37°C. The absorbance of the sample and standard was measured against reagent blank at 600 nm. The value was calculated using this formula:

$$\frac{Absorbance\ of\ test \times Concentration\ of\ standard}{Absorbance\ of\ standard}$$

(Patton and Crouch, 1977)

Data Analysis
Data obtained were expressed as mean ± S.E.M (standard error of the mean).They were subjected to repeated measure of one way analysis of variance (ANOVA) to determine the difference in the parameters before infection and post-infection between the groups. This was followed by Tukey's post-hoc multiple comparison tests using Graph-pad prism version 4.0 for windows. Values of $P < 0.05$ were considered significant.

RESULTS

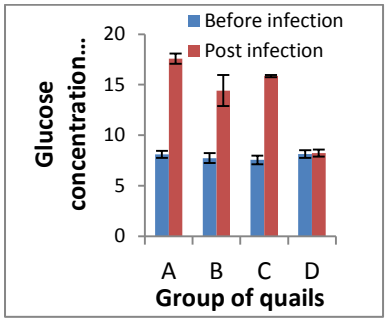

Fig 1: Mean serum glucose before and after infection of quails with *Salmonella enterica* serovar Gallinarum

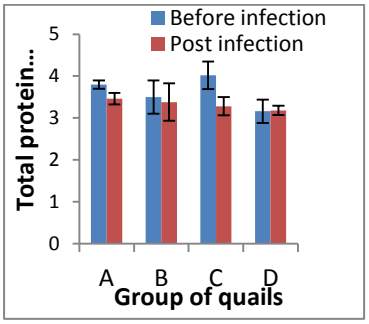

Fig 2: Mean serum total protein before and after infection of quails with *Salmonella enterica* serovar Gallinarum

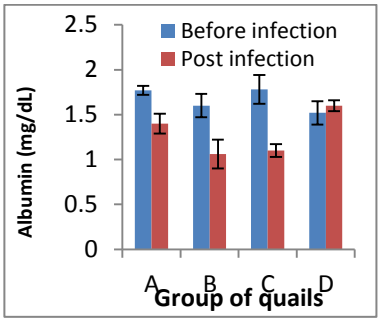

Fig 3: Mean serum albumin before and after infection of quails with *Salmonella enterica* serovar Gallinarum

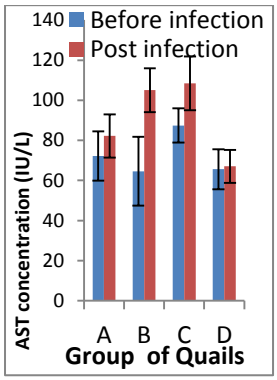

Fig 4: Mean serum AST before and after infection of quails with *Salmonella enterica* serovar Gallinarum

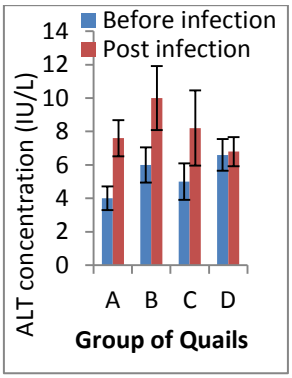

Fig 5: Mean serum ALT before and after infection of quails with *Salmonella enterica* serovar Gallinarum

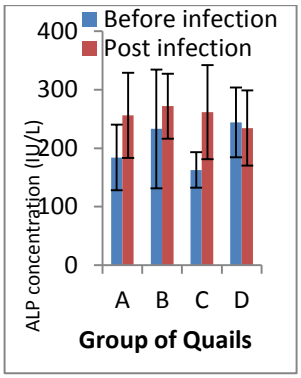

Fig 6: Mean serum ALP before and after infection of quails with *Salmonella enterica* serovar Gallinarum

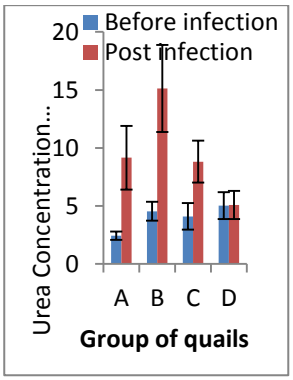

Fig 5: Mean serum urea before and after infection of quails with *Salmonella enterica* serovar Gallinarum

DISCUSSION

There was a marked increase (P<0.05) in the mean serum glucose after infection in all infected groups with values almost doubled what they were before infection and no change in the control group (Figure 1). There was significant decrease (P<0.05) in the mean serum total protein and albumin in all the infected groups post-infection, with no change in the control group (Figures 2 and 3). There was significant increase (P<0.05) in the mean aspartate transferase (AST), alanine transferase (ALT) and alkaline phosphatase (ALP), after infection in all the infected groups but no change (p>0.05) was noticed in the control group (Figures 4 to 6). There was a marked increase (P<0.05) in the mean serum urea values in all infected groups, post infection and no significant change (P>0.05) in the control group (Figure 7). Significantly elevated glucose levels are found most commonly in endocrine disorders, pancreatic, liver or kidney disease, while decrease levels may be due to fasting, drugs or toxins but can also be indicative of various physiological disorders, such as excess insulin production (Barham and Trinder, 1972). The elevation of the mean serum glucose level observed in this study could be due to lesions in the liver and kidney. Albumin is the most abundant serum protein in all species of animals, representing 55-65% of total protein. It is synthesized in the liver and the main biological function of albumin is to maintain water balance in serum and plasma and to transport and store substances with low plasma solubility such as fatty acids, calcium, bilirubin and hormones. Hypoalbuminaemia also reflected as ascites as seen in one of the infected groups, is associated with impaired albumin production in the liver (analbuminaemia) due to liver disease and over excretion in kidney disease and intestinal diseases (Grant *et al.*, 1987). The hypoalbuminaemia observed in this study could also be due to hepatic and renal lesions. Elevated levels of AST as seen in the infected groups can signal myocardial infarction, hepatic disease, muscular dystrophy and organ damage. The ALT levels are increased during various hepatic disease states including hepatitis. Elevation of alkaline phosphatase (ALP) may be attributed to liver or bone disease, congestive heart failure and abdominal bacterial infections (Reitmans and Frankel, 1957). The elevated levels in the mean AST, ALT and ALP observed in this study could be due to the lesions in the liver and heart. Elevated urea concentrations can be indicative of high protein diet, congested heart failure, dehydration and increased protein catabolism as a result of stress or renal disease (Patton and Crouch, 1977). In this study, stress due to infection and the kidney lesions could be the cause of the significant elevated urea concentrations observed. There was possibly a dehydration component due to diarrhea and loss of fluid into the abdominal cavity (ascites).

CONCLUSION

This study presents for the first time to the best of our knowledge that FT in quails results in severe multi organ dysfunction and damage which could be suggestive of immediate cause of mortality in FT infection. This study showed that *Salmonella* Gallinarum caused increase in serum glucose (65%), urea (50%), aspartate transferase (25%), alanine transferase (20%) and alkaline phosphatase (10%) above the values from control group; while a decrease was noticed in the mean serum albumin (30%) below the control group values in Japanese quails (*Coturnixcoturnix japonica*).

RECOMMENDATIONS

Further studies should be carried out to find out appropriate fowl typhoid vaccination schedule for the Japanese quails. A definite vaccination schedule should be developed for quails in Nigeria against FT and other diseases as other further research may indicate. Those keeping quails should adhere to strict biosecurity measures as means of prevention and control of fowl typhoid in the quail farm. Further studies should be carried out in the areas of pathogenesis and virulence of other organisms that were previously thought not to affect quails.

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