Evaluation of Sex Effects on Serum Biochemical and Genetic Parameters of Japanese Quails

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Authors’ contributions

This work was carried out in collaboration among all authors. Authors UHU and AAA did the vetting of the manuscript while author JEU did the developments of the work and manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The study evaluated effect of Sex on serum biochemical parameters and Correlation with body weight of Japanese quails. A total of 400 birds (200 birds per sex) were reared in a Completely Randomized Design under standard management practices for 7 weeks with feed and water given ad libitum. Sex were considered treatments. Weekly body weight (BW) was measured. The three authors have declared that, “principle of laboratory animal care” (NIH publication 85 - 23 revised 1985) were followed as well as the University law. All the experiment have been examined and approved by the University law.100 birds per sex were randomly selected and blood samples were collected for serum biochemical parameters: blood Glucose (GLU), Total Cholesterol (CHOL), Triglycerides (TG), Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), Total Protein (TP), Albumin (ALB) and globulin (GLB). The results indicated that sex significantly (P < 0.05) influenced all the serum biochemical parameters of Japanese quail measured in this study at week 7. All data collected were analyzed with GLM of SAS ver. 9.2 and
INTRODUCTION

Animal protein consumption is very essential for meeting the protein requirement of man, which could be supplied by livestock such as cattle, sheep, goat, rabbits, fish and poultry [1]. Low intake of this protein has been identified as the principal cause of malnourishment in many developing countries of Africa and Asia, thus negatively impacting on the physical and health conditions of their people [2]. For instance, Nigeria is deficient in animal protein intake per capita consumption of 9.3g/day as against the 34g/day recommended by the FAO as the minimum requirement for the growth and development of the body [3]. However, Poultry production has been identified as one major means of solving the problem of low animal protein intake. Poultry can be managed easily with little technical knowledge. Poultry industry is one of the largest animal agriculture sectors in South Africa and other developing countries. Poultry industry continues to grow large with the introduction of new species of poultry, such as the Japanese quail (Coturnix coturnix japonica), to complement the existing species. Japanese quail farming is rapidly gaining popularity for its commercial exploitation and in near future may acquire an important segment in rapidly expanding poultry industry [4]. It has been growing rapidly over the past 100 years from backyard household production to larger commercial production units. According to [5] who reported that eggs and poultry meat rank with cow’s milk as the most economically produced animal protein. The report of [6] emphasized that even though there has been some laid down facts on chicken production, nutritive and economic benefits can be derived from quail production since quails are fast growing and resistant to various diseases than domestic fowls. Farming quails is economically viable and technically feasible because quails are sexually matured at 6 weeks of age and easily adapt to various rearing conditions [7]. According to [8] which reported that quail is a common name given to collective genera of mid-sized birds classed in the order Galliformes; Old world quails are in the family Phasianidae while new world quail are from Odontophoridae’s family. Japanese quail (Coturnix coturnix japonica) is the smallest avian species farmed for egg and meat production and it has assumed worldwide importance as a laboratory animal [9,10,11,12]. Japanese quail has several advantages which makes it to be utilized for biological and genetic studies. They includes: hardy birds that thrive in small cages and require less floor space, of which 8-10 adult quails can be reared in a space required for one adult chicken and are inexpensive to produce than chicken as reported by [13]. The observation by [14] reported of quails consuming less feed, an adult quail requiring 20 to 25 g of feed per day in contrast to chicken of 120 to 130 g per day. They have small body size which makes them easy to be handled; Day old or hatched quails weigh between 6-8 g [15]. They grow faster in the first few days with full feathered body at about 4 weeks of age [16]. The female quails had higher body weights on days 35 and 42 of age, which is to be expected since female quail are normally heavier than males [17] [18]. The young male get matured and crow at 5-6 weeks of age and weighed between 100 to 130 g while the adult female quail weighed between 120-160 g which is slightly heavier than the male weighing from 120-160 grams [16,19]. According to [20] which reported of high rate of egg production for female quails...
production while high quality of protein, high biological value and low caloric content of the meat and eggs was reported by [13,21].

In order to establish a breeding programme, it is essential to estimate genetic parameters which will be used for improving the traits of economic importance. The methods that can be used to assess genetic relationships are obtained through estimations such as heritability, repeatability and correlation between traits. Heritability of traits is the surest method used to predict the genetic progress obtained [22]. The higher the heritability of the trait under selection, the faster the genetic progress is. In practice, however, it is common to use correlation simply as a measure of strength and direction of a relationship, whether or not it is a cause and effect relationship. [23,24] reported that correlation’s relationship are reported in many papers as a part of regression analysis but this in general is not how correlation should be applied for the simple reason that it measures different phenomena and between different types of variables than regression analysis [25]. The scale of the genetic parameters would show the amount of improvement by selection [26,27]. In addition according to [28] who reported that if there is a genetic correlation between characters under selection, the overall response will change according to the heritability of the traits examined and the strength and sign of the genetic correlation amongst them.

Blood represents a means of assessing clinical and nutritional health status of animals as well as investigation of the extent of damage to blood and in the selection of genetically resistant breed of animals [29]. Selection, breeding and genetic improvement have a marked effect on characteristics of the blood biochemistry; as researchers utilize the biochemical parameters of the blood as markers in livestock species to enhance productivity and reproductive performance, [30,31]. Genetic resources such as serum enzymes and serum proteins have been established as genetic markers in farm animals, [32]. Several researches have shown the relationship between poultry birds’ performance and some blood parameters, these includes: plasma Alkaline Phosphatase activity in Rhode Island birds [33] the relationship between growth and blood constituents in Japanese quail by [34] and variation of plasma constituents at different ages of female Japanese quail [35]. In biochemical studies, common parameters like calcium (Ca), glucose, and cholesterol are assayed while serological tests, however, albumen, globulin and total protein are usually estimated [36]. AST and ALT are enzymes that are useful in detecting injury to liver parenchymal cells. They are also present in other tissues, including erythrocytes and triated muscles. In birds, elevated AST and ALT activity are indicative of damage to hepatocytes and intestinal mucosal cells [37,38] reported that males had significantly higher blood component values than females in Japanese quail. The plasma cholesterol level (150.72 mg/dl) was found to vary significantly between sexes in quails [39], [40] reported that cholesterol values in both the sexes of turkeys were ascending with age from the day of hatch to 12-18 weeks and it fluctuated from group to group before reaching the lowest value in above 50 weeks age group. The cholesterol level was found higher (P<0.01) in adult female Japanese quails than males [37]. Sex related differences were observed in females having higher albumin, globulin, total protein, and total cholesterol concentrations than males [41]. These differences could be explained by the physiological changes in the female quail due to egg laying [41]. During the laying period, hepatic synthesis of triglycerides, phospholipids and cholesterol is increased [42]. These lipids make up the lipoproteins, which are circulated in the blood and incorporated into the oocytes of the ovaries. Thus, laying birds have higher circulating concentrations of triglycerides and cholesterol in contrast to male birds. In addition, females of ovisporous species demonstrate a marked increase in total protein concentration during egg production [42]. This is as a result of increased secretion of oestrogen which causes an increase in production of egg yolk precursor’s vitellogenin and lipoproteins [43] thus leading to an increase in the total protein concentration in blood and serum of these birds. Glucose and cholesterol produced energy while total proteins indicated the albumin and globulin of the blood [44].Hence, the objective of this study was to evaluate the effect of sex on serum biochemical and genetic parameters of Japanese quails.

2. MATERIALS AND METHODS

The experiment was conducted in the poultry unit of Teaching and Research farm of University of Uyo, Nigeria. It lies within latitude 4°32’N and 5°33’N, and longitudes 7°25’E and 8°25’E with annual rainfall of between 800 mm to 3200 mm from March to October. Annual temperature varies between 26°C – 28°C [45]. The laboratory analysis was carried out at the Department of Biochemistry University of Uyo, Nigeria.
2.1 Management Procedures

The experimental site was cleaned by washed, disinfected and fumigated a week before the arrival of the birds to avoid spread of diseases or incidence of pest attack on the experimental birds. A rearing pen containing hutch with 20 cells of 62cm x 80cm x 56cm each was constructed for the research. Beddings (wood shavings) were laid on the floor of each cell of the hutch. This served as a source of heat to the birds. Adequate sanitary measures were kept; wood shavings were changed twice a week. Feeding troughs, drinkers and other needed equipment for successful rearing of the quail birds were kept clean and also heat source was provided. The birds were kept under intensive management system [4].

2.2 Procurement and Management of Experimental Birds

Four hundred day old chicks were weighed then kept in the brooder for three weeks. Commercial feed (starter mash) were fed to the chicks from 1st to 3rd week and fresh water was given ad libitum. A forth-nightly body weights was taken on these birds till the 7th week. On the 3rd week, 400 chicks were sexed and randomly selected and placed in five replicates, 200 birds per sex and were transferred to the rearing pen made of wooden battery cage system (hutch). Water and grower mash were fed to the birds from 4th to 5th week and layer mash from the 6th to 7th week, Table 1. In all the dietary feed, fed to the birds, lysine and methionine were used to fortify the feed to meet the quail nutrients requirement. This upgraded the quails feed to its nutrients requirement. Reason being that there was no commercial quail feed in the market, so, to avoid inconsistency in feeding of the birds, poultry commercial feed was fed to the quails and they performed well with better egg production. The research was terminated at 7th week.

2.3 Vaccination

Glucose and vitamins were added to their drinking water on the first day to cushion the effect of stress of transporting them from hatchery unit to the brooder. Feed and water were supplied ad libitum. Medications were administered too to prevent/cure diseases. They were vaccinated against Newcastle disease and Infectious Bursal disease (gumboro).

2.4 Data Collection

Throughout the experimental period of seven weeks, body weight for the birds was taken forth-nightly using digital weighing balance. Two hundred mature quails of 100 males and 100 females were randomly selected from the experimental unit and blood samples were collected from these quails on the 7th week. The blood samples for serum biochemical analysis were collected in plain sample bottles without anticoagulant. Before the blood was collected, the area for blood collection was cleaned and sterilized and maintained using cotton wool and spirit. Then 2ml syringes of (22 to 26) gauze and 1 inch of needle were used for every 5ml blood sample. The blood collected were immediately taken to the laboratory Department of Biochemistry, University of Uyo for serum biochemistry. 5 ml of blood collected in sterile test tubes from wing vein of each of the 100 randomly selected quails from each sex. The blood samples were allowed to clot and serum was separated within 2 h of collection after centrifugation at 5000 rpm for 30 min in a table top centrifuge machine, then stored at -20°C for determining blood Glucose, Total Cholesterol, Triglycerides, Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline

| Composition      | Starter mash | Grower mash | Layers mash |
|------------------|--------------|-------------|-------------|
| Crude Protein (%)| 23.00        | 15.00       | 16.00       |
| Fat (%)          | 5.10         | 3.60        | 3.60        |
| Crude Fibre (%)  | 4.30         | 8.60        | 4.20        |
| Calcium(mg)      | 1.20         | 1.10        | 4.20        |
| Available Phosphorus (mg) | 0.45 | 0.40 | 0.50 |
| Methionine (mg)  | 0.56         | 0.37        | 0.45        |
| Lysine (mg)      | 1.20         | 0.70        | 0.850       |
| ME (Kcal/kg)     | 3000         | 2500        | 2850        |

Source: vital feed (commercial feed), ME =Metabolizable Energy [55]
Phosphatase (ALP), Total Protein, Albumin and globulin by Spectrophotometry using appropriate reagents kits by Randox.

2.5 Statistical Analysis

Data for body weight and serum biochemical parameters collected were analyzed using one-way analysis of variance of SAS ver.9.2 [46]. Means were separated with Duncan Multiple Range test at 5% probability [47]. Correlation between body weight and blood components were carried out using Pearson Correlation. The Experimental design used was Completely Randomize Design (CRD). The statistical model was:

\[
Y_{ij} = \mu + S_i + \Sigma_{ij}
\]

Where;

- \(Y_{ij}\): Observation of the \(j^{th}\) birds
- \(\mu\): Overall mean
- \(S_i\): Fixed effect of the \(i^{th}\) sex
- \(\Sigma_{ij}\): Random residual error

3. RESULTS AND DISCUSSION

The results obtained in Table 2 showed that sex significantly (\(P < 0.05\)) influenced all the serum biochemical parameters of Japanese quail measured in this study at week 7.

Females of Japanese quail had the highest least square means in the serum biochemical parameters measured than the Males counterpart. Females recorded higher means in TP (49.79 ±9.82 g/dl), ALB (3.39 ± 0.30 g/dl), GLB (44.40 ± 9.66 g/dl), TG (3.55 ± 0.00mmol/L), CHOL (6.42 ± 0.06 mmol/L), AST (5.00 ± 1.15 \(\mu\)l), ALT (9.00 ± 0.5 \(\mu\)l) and GLU (391.05 ±23.6 mg/dl) significantly (\(P<0.05\)) higher than males with 13.11± 1.73 g/dl, 2.09±0.24 g/dl, 11.05 ±1.83 g/dl, 1.21 ±0.01 mmol/L, 5.41± 0.09 mmol/L, 3.73 ± 2.02 \(\mu\)l, 5.00 ±0.00 \(\mu\)l, 276.67 ±25.01 mg/dl for the same parameters, respectively. However, males showed higher significant (\(P<0.05\)) difference in ALP with 683.56 ±142.3 \(\mu\)l than females with 145.78 ± 5.07 \(\mu\)l measured.

The results obtained in this study for Total Protein is higher than that observed by Lumeji [48] which reported values of 3.2 g/dl and 3.6 g/dl for total protein in male and female of 4-month-old red-legged partridges, respectively. The elevated concentration of total protein in egg-laying birds is accompanied by significant increase in levels of serum albumin and globulin [49].

This study agrees with a relative study of Mary and Gomathy [40] and Elizabeth et al [50] on serum biochemical parameters of female bronze turkey, who observed that female bronze turkey performed better than males on serum biochemical parameters. The result of this study is in accordance with the work of Ojedapo [51] who also observed that total protein, albumin, globulin, AST, ALT were highly significant \(P<0.05\) in female quails than males. Meanwhile, the glucose mean value obtained in this study was in agreement with those reported for female Japanese quails by Scholtz et al [37] who had similar observations in their study. The reason could be because of several changes which may occur in the metabolism of female birds due to egg production [50]. The results obtained for glucose and globulin in this study disagrees with the observation of Kabir [36] who had 193 ± 4.42 and 14915.50 ± 128.29 mg/dl for glucose and globulin respectively in his studies. The result obtained in this study for globulin is higher than the range of 1.94 g/dl [1.00 – 3.11] as reported by Agina et al [42]. The result obtained for ALB in this study is in agreement with the result reported by Agina et al [42] who reported the overall mean value for ALB, with the minimum and maximum value as 3.25 g/dl (1.08 to 5.47). However, the result obtained in this study for ALB is not consistent with the result of Kabir [36] who reported that the amount of Albumin observed at 5th week was 1.29±0.12 g/dl and the level increased to 2.59±0.17 g/dl at 7th week of age significantly.

The results obtained in this study for Total Protein disagrees with the observation of Kabir [36] who had 30464.50 ± 238.50 mg/dl for Total Protein in his quail studies. The results of this study for T.CHOL is not in consistent with the report of Krupakaran [52] of 105±4.45 mg/dl and also disagrees with the observation of Kabir [36] who had 144 ± 2.58 mg/dl for Total Cholesterol in his quail studies. It also disagrees with the range of 146.69 mg/dl [33.33 – 266.67] observed by Agina et al [53]. The result for ALP obtained in this study is not in agreement with the report of Nazifi and Anasi [54] who reported ALP value of (52.72l \(\mu\)/l) for Japanese quail of 5 weeks. Furthermore, the report obtained in this study falls within the range of mean values reported by Agina et al [42] who had overall mean value for ALP, with the minimum and maximum values as: ALP
107.54 µ/L (86.21 ± 113.29 µ/L). Also, this study observed inconsistence result from that of Nazifi and Anasi [54] who had AST value of (122.14µ/l) for Japanese quail of 5 weeks. The results for AST in this study is not in agreement with the range observed by Agina et al [42] as 59.99 IU/L [23.85 – 90.70]. The result obtained in this study for ALT is slightly lower than the range and observation of Agina et al [42] which was 20.85 IU/L [10.17 – 38.51] for ALT.

3.1 Correlation between Body Weight and Blood Biochemical Parameters on Male Japanese quail

Correlation between body weight and blood biochemical parameters ranged from -0.15 to 0.99. The trend indicated low to high correlation. Correlation between body weight with (TG, AST, ALP and GLU) indicated low correlation with values ranging from -0.15 to 15. This implies that improvement of any of the parameters has no or little effect on body weight for male Japanese quails. Correlation between body weight and (Triglycerides (0.15), cholesterol (0.15), aspartate aminotransferase (0.15), alkaline phosphatase (0.15) and glucose (-0.15) There was no correlation between body weight and ALT recoded in this study. Triglyceride was positively correlated with glucose (0.99) but indicated no correlation with Alanine aminotransferase and highly negative correlation with CHOL, AST, ALP with similar values of (-0.99) (Table 4). The positive and high correlation between TG and T.CHOL means that increment of TG will also increase CHOL while the negatively correlated parameters indicates that increment of one parameter will have no effect on the other.

Correlation between cholesterol, aspartate aminotransferase, alkaline phosphatase and glucose recorded high negative correlation with values -0.99 but showed no correlation with Alanine aminotransferase.

Correlation between aspartate aminotransferase with alkaline phosphatase and glucose, indicated negative correlation. All the parameters that showed negative correlation implied that improvement of one parameter will not affect the other parameter. In Japanese quails, information in correlations between body weight and serum biochemical profiles is scarce. More researches are yet to be done.

3.2 Correlation between Body Weight and Blood Biochemical Parameters on Female Japanese quail

Correlation between body weight and blood biochemical parameters on females Japanese quail ranged from -0.73 to 0.99. The trend indicated low to high correlation (Table 5).

Table 2. Effect of sex on serum biochemical parameters of Japanese quail (LSM ± SE)

| Sex    | N  | Parameter       | TP (g/dl)  | ALB (g/dl) | GLOB (g/dl) | TG (mmol/L) |
|--------|----|----------------|-----------|------------|-------------|-------------|
| Male   | 100| TP (g/dl)      | 13.11 ± 1.73<sup>a</sup> | 2.06 ± 0.24<sup>a</sup> | 11.05 ± 1.83<sup>a</sup> | 1.21 ± 0.01<sup>a</sup> |
| Female | 100| TP (g/dl)      | 47.79 ± 9.82<sup>b</sup> | 3.39 ± 0.30<sup>b</sup> | 44.40 ± 9.66<sup>b</sup> | 3.55 ± 0.00<sup>b</sup> |

<sup>a,b</sup>=Means with different superscript within the same column are significantly different (P<0.0001), ns= not significant, N=Number of observations, TP= Total Protein, ALB= Albumin, GLB= Globulin, TG= Triglyceride, CHOL=, AST= Aspartate aminotransferase, ALT= Alanine aminotransferase, ALP= Alkaline Phosphatase, GLU= Glucose

Table 3. Contd. effect of sex on serum biochemical parameters of Japanese quail (LSM ± SE)

| Sex    | N  | Parameters        | CHOL (mmol/L) | AST (µl) | ALT (µl) | ALP (µl) | GLU (mg/dl) |
|--------|----|-------------------|---------------|----------|----------|----------|-------------|
| Male   | 100| CHOL (mmol/L)    | 5.41 ± 0.09<sup>a</sup> | 3.73 ± 2.02<sup>a</sup> | 5.00± 0.00<sup>a</sup> | 683.56 ±142.3<sup>a</sup> | 276.67 ± 25.01<sup>b</sup> |
| Female | 100| CHOL (mmol/L)    | 6.42 ± 0.06<sup>a</sup> | 5.00 ± 1.15<sup>a</sup> | 9.00± 0.56<sup>a</sup> | 145.78 ± 5.07<sup>a</sup> | 391.05 ± 23.6<sup>a</sup> |

<sup>a,b</sup>=Means with different superscript within the same column are significantly different (P<0.0001), ns= not significant, N=Number of observations, TP= Total Protein, ALB= Albumin, GLB= Globulin, TG= Triglyceride, CHOL=, AST= Aspartate aminotransferase, ALT= Alanine aminotransferase, ALP= Alkaline Phosphatase, GLU= Glucose
Table 4. Correlation between body weight and blood biochemical parameters on male of Japanese quail

|       | BWT | TG   | CHOL  | AST   | ALT   | ALP   | GLU   |
|-------|-----|------|-------|-------|-------|-------|-------|
| BWT  | -0.15 |      |       |       |       |       |       |
| TG   | -0.15 |     | 0.99*** |       |       |       |       |
| CHOL | 0.15  | 0.99*** |       |       |       |       |       |
| AST  | 0.15  | -0.99*** | -0.99*** |       |       |       |       |
| ALT  | -     | -     | -     | -     | -     |       |       |
| ALP  | 0.15  | -0.99*** | -0.99*** | -0.99*** |       |       |       |
| GLU  | -0.15 | 0.99*** | -0.99*** | -0.99*** | -0.99*** |       |       |

Correlation between body weight with (TG, CHOL, AST, ALP and GLU) indicated positive correlation with values ranging from -0.73 to 0.73. Body weight was highly correlated with TG and CHOL with similar values at 73%. The positive and high correlation between body weight (TG and CHOL) means that increment of TG and CHOL will increase BW. The negatively correlated parameters indicated that increment of one parameter has no effect on the other.

Correlation between TG with CHOL and glucose indicated a positive correlation at 99% but high negative correlation with AST and ALP with similar values -0.99.

CHOL correlated positively with ALP and glucose with values 0.99 but negatively correlated with AST with value -0.99.

AST correlated positively with ALP up to 99% but negatively correlated with GLU at -99%.

ALT positively correlated with ALP with 0.99 but negatively correlated with GLU with value of -0.99. ALP correlated negatively with glucose at -99%. All positive correlation showed the relationship that the improvement of one parameter also increased the other while the negative correlation showed that improvement of one parameter had no effect on the other.

Table 5. Correlation between body weight and blood biochemical parameters on female of Japanese quail

|       | BW  | TG   | CHOL  | AST   | ALT   | ALP   | GLU   |
|-------|-----|------|-------|-------|-------|-------|-------|
| BW   | 0.73 |      |       |       |       |       |       |
| TG   | 0.73 | 0.99*** |       |       |       |       |       |
| CHOL | -0.73 | -0.99*** | -0.99*** |       |       |       |       |
| AST  | -0.73 | -0.99*** | -0.99*** | 0.99*** |       |       |       |
| ALT  | -0.73 | -0.99*** | 0.99*** | 0.99*** | 0.99*** |       |       |
| ALP  | -0.73 | -0.99*** | 0.99*** | -0.99*** | -0.99*** | -0.99*** |       |
| GLU  | 0.73 | 0.99*** | 0.99*** | -0.99*** | -0.99*** | -0.99*** | -0.99*** |

BW = Body weight, TG= Triglyceride, CHOL= Cholesterol, AST= Aspartate aminotransferase, ALT= Alanine, Aminotransferase, ALP= Alkaline Phosphatase, GLU= Glucose

Correlation between body weight with (TG, CHOL, AST, ALP and GLU) indicated positive correlation with values ranging from -0.73 to 0.73. Body weight was highly correlated with TG and CHOL with similar values at 73%. The positive and high correlation between body weight (TG and CHOL) means that increment of TG and CHOL will increase BW. The negatively correlated parameters indicated that increment of one parameter has no effect on the other. On comparing the two sexes, female sex recorded higher correlated value of 73% between body weight and TG and CHOL than the males. This indicates that improvement of one trait will improve the other traits.

4. CONCLUSION

In conclusion, female quails had higher values in TP, ALB, GLB, TG, CHOL, AST, ALT and GLU than the Males counterparts’ exception of ALP. Hence, female quails were considered fit for studying quails’ health status as early clinical signs will be observed for quick responds to arrest the situation that will lead to disease outbreak and a breeding program will be established for quails. Body weight of female quails is a good selection tool to predict serum biochemical parameters of quails. The correlation coefficients between body weight and serum biochemical parameters was high in favour of female Japanese quails. A positive and low to high trend was observed in female quails. Body weight of female quails is a good selection tool to predict serum biochemical parameters of quails.

ETHICAL APPROVAL

The three authors have declared that, “principle of laboratory animal care” (NIH publication 85 -
23 revised 1985) were followed as well as the University law. All the experiment have been examined and approved by the University law.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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