



Sexual Dimorphism on Haematological and Genetic Parameters of Japanese Quails

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The study evaluated effect of Sex on Haematological parameters and Correlation with body weight of Japanese quails at Teaching and Research Farm of University of Uyo, Uyo, Nigeria. Authors declared that "principle of laboratory animal care" (NIH publication 85-23 revised 1985) were followed as well as the University law. The experiment has been examined and approved by the University law. 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. 100 birds per sex were randomly selected and blood samples were collected for haematological analysis. The parameters include: White Blood Cell(WBC), Red Blood Cell(RBC), Haemoglobin(Hb), Packed Cell Volume(PCV), Mean Corpuscular Volume(MCV), Mean Corpuscular Haemoglobin(MCH), Mean Corpuscular Haemoglobin Concentration(MCHC) and Platelet. The results showed that sex significantly ($P < .05$) influenced some of the haematological parameters of Japanese quails at 7 weeks. Females had significantly ($P < .05$) higher mean values in WBC ($45.9 \pm 1.47 \times 10^9/L$), Hb (15.58 ± 0.23 g/l) and PCV ($47.75 \pm 0.72\%$) than males with WBC ($32.09 \pm 1.31 \times 10^9/L$), Hb

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(13.72±0.25 g/l) and PCV (41.50±0.66%). There were no significant ($P > .05$) difference in mean values for RBC, MCV, MCH, MCHC and Platelet in both sexes measured. Correlation between BW and haematological parameters for male quails ranged from -10% to 50% (low to medium trend) while that of Female counterparts was -19% to 60% (low to high trend). In conclusion, female quails had higher values in WBC, Hb and PVC than the Males counterparts. Hence, female quails were considered fit for studying quails' health status as this will help in establishing a breeding program of the quails than using Males. Body weight of female quails is a good selection tool to predict haematological parameters of quails.

Keywords: *Body weight; correlation; haematological parameters; Japanese quails.*

1. INTRODUCTION

Protein is very crucial for normal tissue growth and maintenance. Hence, 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 9.3 g/day as against the 34 g/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. It has been reported that eggs and poultry meat rank with cow's milk as the most economically produced animal protein [4]. Nevertheless, emphasis had been laid on chicken production but the nutritive and economic benefits can as well be obtained from quail production since quail is fast growing and resistant to many diseases than domestic fowls [5]. The term poultry is referred to all domesticated birds kept for egg or meat production which includes chickens, turkeys, ducks, geese, ostriches, quails [4]. Quail is a common name given to several genera of mid-sized birds classified in the order Galliformes. The Old world quail are in the family Phasianidae while new world quail are from Odontophoridae's family [6]. 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 [7,8,9,10,11]. The Japanese quail has several advantages which makes it to be utilized for biological and genetic studies. The advantages include 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 [12]. They have consumed less feed, an adult quail requires 20 to 25 g of feed per day in contrast to chicken of 120 to 130 g per day [13]. They have small body size which makes them easy to be handled. Day old or hatched quails weigh between 6-8 g [14] and grow faster in the first few days with full feathered body at about 4 weeks of age [15]. The female birds had higher body weights on days 35 and 42 of age, which is to be expected since female quail are normally heavier than males [16,17]. 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 [15,18]. A large number of birds can be kept in a limited space [12] and depending on the day length, some females start laying at thirty five (35) days of age and in full production by fifty (50) days. They also have short generation interval which confers the ability to produce three to four generations per year [19]. To ensure the production of fertile eggs, in their pen it should be placed in the ratio of 2:1 that is one male to two females [20]. 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. 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. [21,22] 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 [23]. The scale of the genetic parameters would show the amount of improvement by selection [24,25]. In addition, 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 among them [26].

Blood plays an important role in the transportation of nutrients, metabolic waste products and gases around the body. Moreover, 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 [27]. Haematology refers to the study of blood and an important part of clinical pathology as well as diagnostic process and also but also includes a study of the tissues that form, store and circulate blood cells [28]. Haematology refers to the study of the numbers and morphology of the cellular elements of the blood which include the red cells (erythrocytes), white cells (leucocytes), and the platelets (thrombocytes) and the use of these results in disease diagnosis and monitoring of physiological status of animals [29] as well as investigation of the extent of damage to blood [27] and in the selection of genetically resistant breed of animals. Serum includes all proteins not used in blood clotting and all the electrolytes, antibodies, antigens, hormones and any exogenous substances [30]. Haematology has been defined as the study of blood and an important part of clinical pathology as well as diagnostic process [31]. Haematology includes not only the examination of the cellular and fluid portions of blood, but also includes a study of the tissues that form, store and circulate blood cells. However, the serum is the component that is neither a blood cell nor a clotting factor. It is the part of blood that is like water and that contains substances (called antibodies) that fight disease. Serum includes all proteins not used in blood clotting and all the electrolytes, antibodies, antigens, hormones and any exogenous substances [30]. Haematological parameters are good indicators of the physiological status of animals [31]. Haematological parameters are those parameters that are related to the blood forming organs [32]. The blood profiles might serve as basic knowledge for studies in immunology and comparative avian pathology [33]. Haematological values also reflect the levels of stresses due to nutritional and environmental factors. [34,35] observed that factors which may affect haematological parameters include subclinical infections, age, species, sex, genotype, nutritional status, method of analysis, duration of storage of samples, health of animal, degree of physical

activity, haemostatic disorders such as bacterial and viral infections, and vitamin K deficiency and environmental factors affect blood values of animals.

Young quail had lower erythrocyte count, packed cell volume, haemoglobin content and percentage of lymphocytes than adult quail. Male birds showed higher erythrocyte count, packed cell volume, haemoglobin content and lower plasma protein than females [36]. [37] reported that sex has significant influence on Hb, RBC and MCHC with females having higher values than males ($P < 0.05$) while PCV, MCH and MCV show no significant sex difference. This observation is in consistent with previous studies [38]. [39] reported of no significant difference between sexes on haematology while [40] reported that males had significantly higher blood component values than females in Japanese quail. The same reporter mentioned that differences in value (females have higher values of these constituents than males) are attributable to the fact that at this age, there are physiological changes in metabolism of the female Japanese quail due to preparation for onset of laying. [41] observed that the male Japanese quail birds raised in Nsukka recorded significantly higher mean PCV, Hb, EC, MCHC, TLC, heterophil and lymphocyte values than the female birds. From the works of [42,43] male geese and budgerigars were shown to have higher values in most of their blood parameters relative to their female counterparts.[44] reported that Androgens have been associated with increase in number of erythrocytes in male quail birds.

The need for more and location specific research on sex [45] carcass and their underlying blood haematology in Japanese quail is crucial and has necessitated the need for this research aimed at evaluating effects of sex haematological parameters of Japanese quails and correlation with body weight at seven weeks.

2. MATERIALS AND METHODS

The experiment was conducted in the poultry unit of the Teaching and Research farm of University of Uyo, Nigeria. It lies within latitude $4^{\circ}32'N$ and $5^{\circ}33'N$, and longitudes $7^{\circ}25'E$ and $8^{\circ}25'E$ with annual rainfall of between 800 mm to 3200 mm from March to October. Annual temperature varies between $26^{\circ}C - 28^{\circ}C$. Its soil type is sandy loam soil with the soil pH of 4.5 - 6.5 [46].

The laboratory analysis was carried out at the Department of Biochemistry University of Uyo.

2.1 Management Procedures

The experimental site was cleaned by washing with detergent, 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 62 cm x 80 cm x 56 cm 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.

2.2 Procurement and Management of Experimental Birds

Five hundred *Coturnix coturnix japonica* eggs were purchased from National Veterinary Research Institute, Vom, Plateau State, Nigeria. The fertile eggs were sorted out and incubated four hundred and fifty fertile eggs of which four hundred and forty hatched. 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 sexed chicks were 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. Reason being that there was no commercial quail feed in the market, so, to avoid starvation of the birds, poultry commercial feed was used and the quails performed well with better 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 due to long transportation. 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 sensitive 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 and were immediately taken to the laboratory Department of Biochemistry, University of Uyo for haematological analysis for packed cell volume (PCV), haemoglobin concentration (Hb), and total red blood cell (RBC) count. Mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were obtained by calculation according to standard formulae [47].

Table 1. Nutrient composition (%) of the dietary requirement

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	2650

Source: Vital feed (Commercial feed), ME =Metabolizable Energy

2.5 Statistical Analysis

Data for body weight and haematological parameters collected were analyzed using General Linear Model (GLM) procedure of SAS. Means were compared using Duncan Multiple Range test at 5% probability [48]. Correlation between body weight and blood components were carried out using Pearson Correlation of SAS Software. The Experimental design used was Completely Randomize Design (CRD).

The statistical model was:

Model 1:

$$Y_{ij} = \mu + S_i + \Sigma_{ij}$$

where;

Y_{ij} = Observation of the j^{th} birds

μ = Overall mean

S_i = Fixed effect of the i^{th} sex

Σ_{ij} = Random residual error

3. RESULTS AND DISCUSSION

3.1 Effect of Sex on the Haematological Parameters of Japanese Quail

The results in Table 1 presents the values for the effect of sex on the least square means of haematological parameters of Japanese quails at seven weeks. Female Japanese quails recorded $45.9 \pm 1.47 \times 10^9/L$ for WBC, RBC ($3.24 \pm 1.22 \times 10^{12}/L$), Hb ($15.58 \pm 0.23 g/l$), PCV ($47.75 \pm 0.72\%$), MCV (148.00 ± 2.30 fl), MCH (49.50 ± 1.00 pg), MCHC (30.31 ± 0.91 g/l) and Platelet ($2.81 \pm 0.16 \times 10^9/L$) while Males recorded $32.09 \pm 1.31 \times 10^9/L$, $2.95 \pm 0.10 \times 10^{12}/L$, 13.72 ± 0.25 g/l, $41.50 \pm 0.66\%$, 152.94 ± 2.90 fl, 48.75 ± 0.82 pg, 27.69 ± 0.96 g/l and $2.63 \pm 0.35 \times 10^9/L$ for the same parameters, respectively. Females had significantly ($P < .05$) higher mean values in WBC ($45.9 \pm 1.47 \times 10^9/L$), Hb (15.58 ± 0.23 g/l) and PCV ($47.75 \pm 0.72\%$) than males with WBC ($32.09 \pm 1.31 \times 10^9/L$), Hb (13.72 ± 0.25 g/l) and PCV ($41.50 \pm 0.66\%$). The differences in value of females having higher mean values of these parameters than males are attributable to the fact that at this age, there are physiological changes in metabolism of the female Japanese quails due to preparation for onset of laying.

The results obtained in this study is consistent with previous studies [38,49] but disagrees with the study by [40,41,50,51] who reported that

males had significantly higher blood component values than females in Japanese quail. It also disagree with [42,43] which reported that male geese and budgerigars were shown to have higher values in most of their blood parameters relative to their female counterparts. Sex had significant ($P < .05$) difference on some of the haematological parameters which agrees with those reported previously by several authors [37,49,52,53,54,55] which reported that sex has significant influence on Hb, RBC and MCHC with females having higher significant values than Males. The results obtained in this study for Hb disagrees with the observation of [39,51] which observed no significant difference in both sexes of the quails. The results obtained in this study for PCV disagrees with the observation of [51] which observed male quails being significantly higher in values than the females counterpart. However, there was no significant ($P > .05$) difference for RBC, MCV, MCH, MCHC and Platelet in both sexes measured. The results obtained in this study for RBC, MCV is in agreement with the study of [37,51] which observed no significant difference for PCV and MCV in both sexes of Japanese quails. The results obtained in this study for MCH, MCHC agrees with the observation of [37] but disagreed with the observation of [51] which observed a significant higher values in Females quails than the Males counterpart.

3.2 Correlation between Body Weight and Haematological Parameters of Male Japanese Quails at Week 7

The Table 3 shows the phenotypic correlation of body weight and haematological parameters in Japanese quails at 7 weeks. The results of phenotypic correlation between body weight and haematological parameters of male Japanese quails ranged between -0.01 to 0.50. The trend indicated low to medium correlation. The result of this study is in agreement with the observation of [56] which observed that correlation coefficients estimate between BW and blood plasma GPX (enzyme) activity for Cobb, Ross and both strains showed considerable variations of the correlation values in the strain. The correlation between body weight and (MCHC (0.50), haemoglobin, (0.14), MCV (0.27), MCH (0.18), Platelet (0.06) were positive and significantly ($P < .05$) correlated while correlation between Body Weight and RBC (-0.15), PCV (-0.07) were negatively correlated and not significantly ($P > .05$) at 7 weeks. Positively correlated parameters implies that improvement of body weight will improve these

parameters [57,58]. Similar results of phenotypic correlation have been reported by [37,39]. The blood parameters in this study were phenotypically correlated with each other with low to high values while some were negatively correlated. The Correlation between WBC with RBC, HB and Platelet were positively correlated at the range of 0.01 to 0.02 indicating low range. Correlation between HB with PCV, MCV, MCH and MCHC and Platelet indicated very low to high correlation with values ranging from -0.19 to 0.55. Correlation between RBC and MCV, MCH and MCHC were positive but lowly correlated with 0.03 to 0.02. RBC was lowly correlated with HB, PCV and Platelet with 0.02 to 0.30. Haemoglobin positively correlated with PCV, MCV, MCH, MCHC but negatively correlated with platelet. Correlation between HB and MCHC recorded a high correlation of 55%. This implies that improvement of HB, leads to improvement of MCHC in the blood. Correlation between PCV, MCV, and Platelet ranged from 0.15 to 0.36. The trend indicated low correlation. Correlation between MCV with MCH, MCHC and Platelet indicated low correlation of -0.10 to 0.03. Correlation ranging from -0.45 to 0.60. The correlation between MCH and MCHC recorded high percentage of 60%, meaning that once the concentration of MCH is improved, the concentration of MCHC is also improved in males of the quails.

3.3 Correlation between Body Weight and Haematological Parameters of Female Japanese Quail at Seventh Week

Table 4 shows the phenotypic correlation of BW and haematological parameters of Japanese quails at 7 weeks. The results of phenotypic Correlation between BW and haematological parameters of female Japanese quail ranged between -0.19 to 0.60. The trend indicated low to high correlation. The correlation between BW with RBC, HB, PCV and Platelet were positively correlated with the values that range from 0.20 to 0.50, indicating that the improvement of BW will improve these haematological parameters. The result of this study is in accordance with the observation by [37] which had similar result in the correlation between BW and the blood parameters. Female Japanese quails should be improved for production. The highest correlation value was between BW and haemoglobin which was 60% indicating that once BW is improved, the HB standard is raised as well. Other haematological parameters measured were

phenotypically correlated with each other with low to high values while some were negatively correlated. Similar results of phenotypic correlation have been reported by [37] [39]. Correlation between WBC with RBC, HB, PCV, MCV, MCH, MCHC and Platelet indicated very low correlation. Correlation between WBC with HB and MCHC were positively correlated with the value that ranges from 0.13 to 0.44 respectively. Correlation between RBC with HB, PCV, MCV, MCH and Platelet, indicated very low correlation. The correlation between RBC with MCV, MCH and Platelet were positively correlated with the values that ranges from 0.06 – 0.46. Correlation between HB with PCV, MCV, MCH, MCHC, Platelet indicated very low correlation. The correlation ranges from -0.01 to 0.32. The correlation between PCV with MCV, MCH, MCHC and Platelet indicated very low to high correlation. The highest correlation value was between PCV and MCHC which was recorded 60%. Correlation between MCV with MCH, MCHC and Platelet ranged from -0.16 to 0.18. This showed low correlation. Correlation between MCH with MCHC and Platelet, indicated very low correlation. MCHC was positively correlated with Platelet at 3%.

On comparing both sexes, females had higher range than the males counterparts showing that female sex could be improved for body weight with those haematological parameters as they are positively correlated at the same time. The result of this study agrees with the observations of [57,58] who reported on sex differences with females having higher body weight and correlate with other parameters measures. Also, the high phenotypic correlation values obtained may be attributed to the pleiotropic effects. Hence, performing selection in any of the traits may lead to an improvement in the other trait. Correlation among these haematological parameters indicate that, estimate of one of these parameters could be used as a good indicator for the other parameters based on the high correlation values which were obtained in this study. [37,59,60] have reported that haematological parameters can act as indicators for the state of birds health and body data can be useful aids to the diagnose of diseases in birds and abnormalities due to diseases which can change blood parameters [35,61] it follows from the positive correlation that exists between body weight and blood constituents that selection for blood constituents can be done at early stages of life.

Table 2. Effect of sex on the haematological parameters of Japanese quail (LSM ± SE)

Sex	N	Parameters							
		WBC ($\times 10^9/L$)	RBC ($\times 10^{12}/L$)	HB (g/l)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (g/l)	PLATELET ($\times 10^9/L$)
F	100	45.9 ± 1.47 ^a	3.24 ± 1.22 ^a	15.58 ± 0.23 ^a	47.75 ± 0.72 ^a	148.00 ± 2.30 ^a	49.50 ± 1.00 ^a	30.31 ± 0.91 ^a	2.81 ± 0.16 ^a
M	100	32.09 ± 1.31 ^b	2.95 ± 0.10 ^a	13.72 ± 0.25 ^b	41.50 ± 0.66 ^b	152.94 ± 2.90 ^a	48.75 ± 0.82 ^a	27.69 ± 0.96 ^a	2.63 ± 0.35 ^a

a,b=Means with different superscript within the same column are significantly different (P<0.0001), ns= Not significant, N=Number of observations, WBC=White Blood Cell, HB= Haemoglobin, RBC= Red Blood Cell, PCV= Packed Cell Volume, MCHC=Mean Corpuscular Haemoglobin Concentration, MCH= Mean Corpuscular Haemoglobin, MCV= Mean Corpuscular Volume. F= Female, M= Male

Table 3. Correlation between body weight and haematological parameters on male Japanese quail at seventh week

	BWT	WBC	RBC	HB	PCV	MCV	MCH	MCHC	PLT
BWT									
WBC	-0.06								
RBC	-0.15	0.22							
HB	0.14*	0.01	-0.32						
PCV	-0.07	-0.28	-0.22	0.35*					
MCV	0.27*	-0.12	0.12	0.22	0.36*				
MCH	0.18*	-0.18	0.22	0.18	-0.08	-0.20			
MCHC	0.50*	-0.12	0.03	0.55	0.15	-0.10	0.60*		
PLT	0.06	0.06	-0.23	-0.19	-0.17	0.03	-0.45	-0.62	

WBC=White Blood Cell, HB= Haemoglobin, RBC= Red Blood Cell, PCV= Packed Cell Volume, MCHC=Mean Corpuscular Haemoglobin Concentration, MCH= Mean Corpuscular Haemoglobin, MCV= Mean Corpuscular Volume, PLT=Platelet

Table 4. Correlation between body weight and haematological parameters on female Japanese quail

	BWT	WBC	RBC	HB	PCV	MCV	MCH	MCHC	PLT
BWT									
WBC	0.18								
RBC	0.24	-0.33							
HB	0.50	0.13	-0.48						
PCV	0.20	-0.05	-0.13	0.32					
MCV	-0.02	-0.46	0.06	-0.01	0.28				
MCH	-0.13	-0.11	0.46	0.04	0.20	-0.16			
MCHC	-1.19	0.44	-0.09	-0.07	0.60	0.18	-0.03		
PLT	0.21	-0.04	0.20	0.01	-0.09	-0.26	-0.25	0.03	

BWT= Body Weight, WBC=White Blood Cell, PCV= Packed Cell Volume, MCHC=Mean Corpuscular Haemoglobin Concentration, MCH= Mean Corpuscular Haemoglobin, MCV= Mean Corpuscular Volume, PLT= Platelet

4. CONCLUSION

The results of the present findings showed that female quails had higher values in WBC, Hb and PVC than Male counterparts which implies that sex significantly influenced some haematological parameters of Japanese quails. High WBC in Female quails proof high tolerance to harsh environmental condition. This study showed that body weight as a parameter for selection has positive and significant relationship with some haematological parameters of Japanese quails. This can be a useful tool in developing breeding programmes in Japanese quails. Hence, Female quails are considered fit for the study of quails' health status as the clinical signs will be detected faster and earlier than in Males' counterpart. It is important to consider sex as a factor in studying the health status of quail birds.

DECLARATION

We write to inform the Editorial Board of British Journal of Applied Science and

Technology that, this article titled "Sex Effects on Haematological and Genetic Parameters of Japanese Quails" is a fresh paper article which is not in anywhere published. All contributing authors in this study have given consent for publication.

ETHICAL REPORT

All 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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