



## Productive Performance of Laying Tortoise Genotypes in Rainforest Mid-Western, Nigeria

Kperegbeji JI<sup>1\*</sup>, Meye JA<sup>1</sup>, Nwadiolu R<sup>2</sup>, Ewododhe ACA<sup>2</sup>,  
Onwumere-Idolor SO<sup>1</sup> and Okhale OE<sup>1</sup>

<sup>1</sup>Department of Animal Production, Faculty of Agriculture, Southern Delta University, P. M. B. 5, Ozoro, Nigeria

<sup>2</sup>Department of Agricultural Economics, Faculty of Agriculture, Southern Delta University, P. M. B. 5, Ozoro, Nigeria

\*Corresponding Author: Kperegbeji JI, Department of Animal Production, Faculty of Agriculture, Southern Delta University, P. M. B. 5, Ozoro, Nigeria.

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### Abstract

In the humid rainforest belt of Mid-Western Nigeria, the over-exploitation of wild tortoise populations for “bushmeat” has led to a significant decline in biodiversity. The study was conducted to evaluate the productive performance of laying tortoise genotypes in the rainforest zone. A total of 150 sexually mature tortoises were used, comprising 30 individuals each of Marginated tortoises (MT), African Spurred tortoises (AST), Pancake tortoises (PCT), Greek tortoises (GT) and West African Mud tortoises (WAMT). Egg production traits evaluated included age at first egg (AFE), body weight at first egg (BWFE), weight of first egg (WFE), egg number (EN), and clutch number. Genotype had a significant ( $P < 0.05$ ) effect on all egg production traits. The highest AFE was observed in WAMT (4380 days), followed by GT (4088 days), PCT (3650 days), AST (3431 days) and MT (3103 days) respectively. WAMT recorded the highest BWFE (1427.36 g), whereas MT had the lowest (1254.08 g). Similarly, WAMT produced the heaviest first eggs (38.35 g), while MT had the lowest WFE (19.54 g). Mean EN per tortoise per clutch was highest in WAMT, although this genotype showed lower egg weight (EWT) per tortoise per clutch. The WAMT genotype exhibited the highest annual EWT gain (101.26 g) compared with the other genotypes. AST recorded the highest clutch number 5 while PCT and GT produced four clutches each. It was suggested that the genetic potential of WAMT breed of tortoise can be effectively exploited by practicing crossbreeding to improve or increased laying performance.

**Keywords:** Egg Production; Egg Weight; Genotype; Egg Number; Crossbreeding; Weight of First Egg

### Introduction

A critical gap exists in understanding the precise causes of hatching failures, especially distinguishing between fertilization failure and early embryo death in wild populations. A significant proportion of undeveloped eggs are often misclassified using traditional methods, which can misdirect conservation efforts [1-3]. More data is needed on: Frequency of nesting; longevity

and generation time; and impact of climate change on incubation temperatures and potential population sex biases. These gaps hinder effective reproduction, conservation and management efforts [4-8]. Addressing these gaps is crucial, as over half of all tortoise species are currently threatened with extinction. Foundational data is required to form the basis for successful and cost-effective conservation strategies.

[9] emphasized that tortoise reproduction involves internal fertilization, where females lay eggs in a dug-out nest after a variable gestation period. Productive performance of laying in tortoises varies significantly by species, with factors like clutch size (number of eggs), clutch frequency, egg size, and annual reproductive potential differing between species and even populations [10-12]. Generally, larger tortoises lay large eggs, and there is an inverse relationship between egg size and clutch size, with larger eggs leading to fewer eggs. For example, Russian (Horsfield) tortoises lay fewer clutches (2-3 per year), but each clutch contains 2-5 larger eggs than Hermann's (Boettgeri) tortoises, which lay more clutches per year, but these contain more eggs (5-8, up to 12), which are generally smaller [13-15]. [16] also noted that tortoises that lay fewer, larger eggs may be following a different parental strategy compared to those that lay more numerous, smaller eggs. In environmental conditions, factors like diet and climate can influence a tortoise's ability to lay eggs.

Tortoise eggs and meat are products among the most valuable sources of animal protein available for human consumption in rural communities. These products offer means of meeting the animal protein deficiencies in many African countries [17-19]. In most of these developing countries, demand for tortoise eggs and meat far outstrip supply to urban areas for consumption, as evidenced of alternative to poultry egg by steep rises in prices in recent times.

Tortoise population has a preponderance of survival genes to the detriment of productive genes. These may be partly for the reason that tortoises have not been subjected to adequate genetic selection for increased productivity [20-22], but more to natural selection by the adverse environmental conditions. Several researches have been conducted towards the effective genetic improvement of tortoise species by few Nigerian researchers across the different ecological zones of the country [23-25]. All improvement methods make use of information on phenotypic and genetic performance of the tortoises. Limited information is available on the egg production traits of Nigerian tortoises compared to exotic breeds. Thus, the objective of this study was to evaluate productive performance of laying tortoises of different species in rainforest ecological zone.

## Materials and Methods

### Experimental site

The research was conducted at the Department of Animal Production Research Farm (DAPRF), Southern Delta University

(SDU), Ozoro situated between Latitude 5° 32' N and Longitude 6° 15'E of Greenwich meridian in mid-western Nigeria's rainforest. The mean annual rainfall in the area ranges between 2500 and 3000 mm while the mean temperature and Relative Humidity are 27.4°C and 85 % respectively [26].

### Experimental animal and design

A total of one hundred and fifty (150) sexually matured tortoises were screened after balancing for weight, the tortoises were randomly assigned into five (5) laying treatments with three (3) replicates of ten (10) tortoises per replicate. Feed and water were provided *ad-libitum*. The experimental design used was Complete Randomized Design (CRD).

### Experimental diets

The experimental animals were fed on a grower's mash that supplied 30% crude protein and 2560 kcal/kg ME. Thereafter, a high-quality diet containing 24% crude protein and 3350 kcal/kg ME were fed at the laying phase. Provision of source of calcium, such as limestone flour sprinkled on their diet. Fresh, clean water was also provided.

### Management of experimental animals

The experiment was carried out with sexually matured adult tortoises comprised of 30 each of Marginated tortoises (*Testudo marginata*), African Spurred tortoises (*Centrochlys sulcata*), Pancake tortoises (*Malacochersus tornieri*), Greek tortoises (*Testudo graeca*) and West African mud tortoises (*Pelusios niger*) respectively. Sexually matured tortoises from each genotype group were properly identified by shell pattern, shape of the shell, and carapace was tag as T1, T2, T3, T4 and so on. Only healthy sexually matured adults ranging between 8 and 12 years of age were selected based on size and weight. The female tortoises were transferred into previously disinfected laying nesting area with deep, loose, well-drained substrate like a mix of soil and sand, adequate sun exposure was provided. The mating system was polygyny in 1 male: 10 females' ratio. Tortoises in each treatment group were maintained in a water bath at a depth of 5 cm. To ensure hygiene, the water was replaced at regular intervals throughout the 52 - week experimental period.

Intensive housing system was used for this study. The dimension of the area is 8ft x 8ft per treatment. Tortoises were kept in wet environments of concrete floor with a temperature gradient with

a basking area of 30-32°C and a cooler side of 20-24°C. Lighting of 12 to 14 hours of light from specialized UVB bulb were provided.

**Data collection**

Data were collected on the laying performance parameters (AFE, BWFE, WFE, number of clutches and egg weight gain) of individual tortoises.

**Statistical analysis**

The effect of genotype on the laying performance traits was analyzed using the model below:

$$Y_{ij} = \mu + G_i + e_{ij}$$

Where:

$Y_{ij}$  = Observation on the  $j^{th}$  tortoise in the  $i^{th}$  genetic group

$\mu$  = Overall mean;

$G_i$  = Effect of the  $i^{th}$  genotype (Marginata, African Spurred, Pancake, Greek tortoise and West African mud)

$e_{ij}$  = random residual error normally distributed with zero mean and variance ( $\delta^2 e$ )

Significant treatment means were subjected to analysis of variance (ANOVA) using statistical analysis system package [27].

Means were separated using Duncan multiple range test [28] at 5% level of significance.

**Ethical considerations**

All procedures involving animals were conducted in accordance with institutional and national guidelines for the care and use of laboratory animals. The study protocol was reviewed and performed under the oversight of the Department of animal Production. Tortoises were housed and managed to minimize stress (controlled thermal gradient, UVB lighting, appropriate substrate and *ad libitum* access to feed and water), and end-points were predefined to avoid unnecessary suffering. Where applicable, permits for animal use and handling were obtained from the relevant authorities. Detailed welfare measures and husbandry practices are reported in the materials and methods.

**Results**

Table 1 showed the productive performance on age among the five tortoises’ genotypes studied. The result on AFE, BWFE, WFE and mortality show that there were significantly ( $P < 0.05$ ) difference across the genotype groups, but WAMT had the highest mean values except for mortality. This is followed by PCT, AST, GT and MT had the least AFE with corresponding mean value of 3103 days, approximately 8 year and 5 months and weight of first egg value 19.54g. Mean BWFE was also significantly ( $P < 0.05$ ) different except for PCT and GT.

Parameters	MT	AST	PCT	GT	WAMT	SEM
AFE (days)	3103 <sup>e</sup>	3431 <sup>d</sup>	3650 <sup>c</sup>	4088 <sup>b</sup>	4380 <sup>a</sup>	72.16
BWFE (g)	1254.08 <sup>d</sup>	1306.43 <sup>c</sup>	1385.21 <sup>b</sup>	1390.34 <sup>b</sup>	1427.36 <sup>a</sup>	30.42
WFE (g)	19.54 <sup>e</sup>	22.87 <sup>d</sup>	24.60 <sup>c</sup>	28.21 <sup>b</sup>	38.35 <sup>a</sup>	1.46
Mortality (%)	0.00	0.00	0.00	0.00	0.00	0.00

**Table 1:** Least squares mean ± SEM of productive performance as affected by genotypes at different laying ages.

<sup>abcde</sup> Means in the same row with different superscripts are significantly different ( $P < 0.05$ ).

AFE = Age at first egg, BWFE = Body weight at first egg, WFE = Weight of first egg, MT = Marginata tortoise, AST = African Spurred tortoise, PCT = Pancake tortoise, GT = Greek tortoise, WAMT = West African Mud tortoise and SEM = Standard Error of Means.

The results also revealed that MT had the lowest BWFE value (1254.08g). The superior weights of egg were recorded for WAMT, GT, PCT, AST, and MT in increasing order (38.35g, 28.21g, 24.60g, 22.87g and 19.54g) respectively (Table 1).

The egg number laid per tortoise per clutch is shown on Table 2. There were significant ( $P < 0.05$ ) difference on clutch number among the genotypes. The highest overall mean was recorded for WAMT with a mean value of 6.35 eggs/tortoise/clutch with a range

between 4.68 and 6.35 eggs/tortoise/clutch, while the WAMT and MT had the lowest clutch number of 2 and 3. The AST had the highest clutch number of 5 while PCT and GT had clutch number of 4 each.

Clutch No.	Genotypes					
	MT	AST	PCT	GT	WAMT	SEM
1	3.16 <sup>bc</sup>	4.05 <sup>b</sup>	3.64 <sup>b</sup>	3.32 <sup>b</sup>	5.76 <sup>a</sup>	0.38
2	4.63 <sup>c</sup>	4.24 <sup>c</sup>	5.52 <sup>ab</sup>	5.04 <sup>b</sup>	6.94 <sup>a</sup>	0.40
3	6.24 <sup>a</sup>	5.42 <sup>b</sup>	6.36 <sup>a</sup>	6.63 <sup>a</sup>	-	1.23
4	-	5.75 <sup>b</sup>	8.58 <sup>a</sup>	6.28 <sup>a</sup>	-	1.07
5	-	4.50	-	-	-	0.36
Overall mean	4.68 <sup>d</sup>	4.79 <sup>c</sup>	6.02 <sup>b</sup>	5.58 <sup>b</sup>	6.35 <sup>a</sup>	1.42

**Table 2:** Least squares mean ± SEM of egg number per tortoise per clutch as variable by genotype.

<sup>abc</sup> Means in the same row with different superscripts are significantly different (P < 0.05).

The result of egg weight gains per clutch by genotypes at different laying ages is presented in Table 3. The result revealed significant differences (P < 0.05) on egg weight gains in all the genotype group. The overall mean of WAMT had the highest value

weight of 102.26g/tortoise/clutch. This was closely followed by AST, PCT, MT and GT had the least clutch overall mean egg weight gain with the values of 98.59g, 95.93g, 95.56g and 94.38g respectively.

Clutch No.	Genotypes					
	MT	AST	PCT	GT	WAMT	SEM
1	92.80 <sup>e</sup>	96.00 <sup>b</sup>	94.73 <sup>c</sup>	93.16 <sup>d</sup>	99.53 <sup>a</sup>	7.54
2	98.36 <sup>b</sup>	102.36 <sup>a</sup>	97.46 <sup>c</sup>	95.49 <sup>d</sup>	103.00 <sup>a</sup>	9.31
3	95.52 <sup>c</sup>	101.76 <sup>a</sup>	98.64 <sup>b</sup>	94.60 <sup>c</sup>	-	6.42
4	-	90.51 <sup>c</sup>	92.90 <sup>b</sup>	94.28 <sup>a</sup>	-	7.18
5	-	104.30	-	-	-	3.55
Overall mean	95.56 <sup>c</sup>	98.59 <sup>b</sup>	95.93 <sup>c</sup>	94.38 <sup>d</sup>	102.26 <sup>a</sup>	6.22

**Table 3:** Least squares mean ± SEM of egg weight gains per tortoise per clutch as affected by genetic variation.

<sup>a,b,c,d</sup> Means in the same row with different superscripts are significantly different P(<0.05).

### Discussion

The variation observed in age at first egg (AFE) among the genotype groups may be attributed to differences in their genetic make-up. The relatively lower AFE and body weight at first egg (BWFE) recorded in *Testudo marginata* (MT) could be due to selection for a faster rate of sexual maturity and earlier onset of egg laying [29]. The lower BWFE observed in MT may further be

explained by the fact that this tortoise is a light-bodied breed, specifically selected for egg production efficiency and reduced feed consumption.

[30] reported that genetic factors significantly influence the rate of egg production and the age at which tortoises commence laying. In contrast, the higher AFE and BWFE observed in *Pelusios niger*

(WAMT) compared with the other genotypes may be because this species is considered a dual-purpose breed, selected for both egg and meat production. This observation agrees with the findings of [31,32], who reported higher values in heavier or wild type tortoise populations [33].

The variation in the weight of first egg observed in different genetic groups studied indicated that heavier tortoises had heavier weights of first egg. This variation is subject to both genetic as well as environmental effect, as observed by [34,35]. [36] also reported that species differences in size and appetite lead to variation in egg size. The genetic implication of this is that the weight of first egg seems to depend to a large extent upon body weight at sexual maturity.

The superior performance of WAMT relative to other genotypes since tortoise is a light breed selected for high laying performance. [37] found a negative correlation among egg number and age at sexual maturity, body weight at sexual maturity but positive correlation between egg number and rate of lay. The lowest egg number observed in MT could be attributed to its hibernation nature during hot season as reported by [38]. The variation observed in EWT among the genetic groups is similar to the findings of [39,40]. The mean clutch weights of individual tortoises in a genetic group throughout the period of lay were a function of total number of eggs laid, which to a large extent is influenced by the genetic make-up of the breed and the environment inter-play [41]. Better productive performance of egg weight gain observed in WAMT could probably be due to clutch number as reflected in the weights of first egg per year. According to [42], the first egg laid by an adult is almost her smallest egg and it affects the size of eggs that a tortoise lays in future. If the first egg is large, the tortoise usually continues to lay large eggs. The lower egg number per tortoise per clutch observed in MT could be due to genotype and environmental interaction effect as reported by [43] that lowly heritable traits of three clutch number per year affected egg production, as traits are greatly affected by genetic make-up, environmental conditions and non-additive gene action.

## Conclusion

Egg production characteristics varied among the genotypes owing to the differences in their genetic make-up. The better laying performance observed in WAMT, PCT, and AST revealed that the

captive tortoises have been artificially selected for the productive traits.

The high BWFE, EWG exhibited by WAMT is due to the dual-purpose function of the tortoise. The productive performance of WAMT in terms of clutch number advantage exhibited over the other genotypes (AST, PCT and MT) showed that our captive tortoises can easily be improved upon and inculcated in artificial insemination (AI) programme.

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