

Spermiogramic parameters of Japanese quails (*Coturnix coturnix japonica*) to aqueous administration of egg lime molasses mixture

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ABSTRACT

As demand for animal protein rises, raising Japanese quail holds much potentials in bridging the gap. Phyto-genics has been reported to have effect on spermiogramic parameters of animals. This research was done to ascertain the spermiogramic parameters of Japanese quails to administration of aqueous solution of egg lime molasses mixture (ELM). Fresh eggs were placed in a dish, followed by 500g of molasses and 1 liter of lime juice. It was then covered for 10 days and the entire mixture was blended. Two hundred day old Japanese quails were assigned to five treatments in a Completely Randomized Design and were subdivided into 4 replicates of 10 birds each. The control (T1) having no administration of ELM, T2 had an inclusion level of 10 mL, T3: 20 mL, T4: 30 mL and T5: 40 mL, all in 500 mL of water. Feed and water were provided ad libitum. The study was carried out for 49 days. Data were collected on genitalia morphometry and fertilizing potentials. The birds administered 20 mL of ELM had significantly ($p < 0.05$) higher left epididymis weight ($0.36 \pm 0.05g$) compared to the other groups. The inclusion of ELM in the water significantly influenced ($p < 0.05$) left epididymis volume, paired epididymis volume, left epididymis density, paired epididymis density and spermatozoa reserves in the right epididymis. Testosterone values significantly increased ($p < 0.05$) with increased ELM inclusion. It can be concluded that the administration of ELM did not alter growth parameters however birds that received 20 mL per 500 mL of water had the best reproductive parameters.

Keywords: epididymis, reproductive tract, spermatozoa, testes

INTRODUCTION

The Japanese quail have the benefit of being small, having a short life cycle, growing quickly, having good reproductive potential, having a high fecundity rate, and having shorter hatching

periods (Chimezie *et al.*, 2022). The high protein content, vital fatty acids, and minerals like sodium, potassium, and iron are known advantages of quail meat. The high metabolic activity of this animal increases the amount of glycogen stored in its muscles, resulting in meat

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of exceptional quality. Small game birds called quails are raised for their meat and eggs (DAFF, 2013). There are numerous quail breeds with a variety of traits, like other domesticated animals. According to the National Veterinary Research Institute (NVRI, 1994), Japanese quail were first brought to Nigeria in 1992. Through extensive quail farming, it was hoped to popularize the poultry subsector and supplement domestic chicken production. Japanese quail is a robust bird that does well in little cages and costs little to maintain.

One of the most promising methods for bridging the animal protein shortfall is quail farming. Due to modern applications like consumption of its goods and frequently its numerous by-products, the manufacturing of quail products is distinctive and has attracted particular attention. However, the potential of raising poultry has not been completely realized since most farmers have focused only on raising chickens, despite the fact that other birds have similar economic, social, and nutritional advantages. It is significant to note that numerous initiatives have been made to increase Nigeria's overall poultry production. Ndelekwute *et al.* (2010) conducted an experiment to study the effects of supplementing broiler chicken feed with molasses rather than combining it with the feed. The molasses-supplemented group consumed less feed during the finisher phase and had significantly larger body weight and conformation characteristics (breast width and keel length) at both stages. However, they came to the conclusion that molasses might be fed to broilers as a supplement by drinking water. Akintunde *et al.* (2022) reported that ELM mixture contained energy (1060.3 kcal/100 kg), moisture content (19.6%), crude protein (15.2%), lipids (5.5%), ash (14.6%), crude fiber (9.6%), carbs (35.2%),

and fatty acids (4.4%). Phytochemical analysis showed that ELM contained alkaloids (8.46 mg/100 g), flavonoids (2.3 mg/100 g), glycosides (0.08 mg/100 g), saponin (5.25 mg/100 g), steroids (0.22 mg/100 g), phenols (0.09 mg/100 g), terpenoides (0.56 mg/100 g), tannin (8.34 mg/100 g), and anthraquinones (1.6 mg/100 g) and Vitamin A (3.2 mg/100 g), Vitamin B1 (280 mg/100 g), Vitamin B2 (880 mg/100 g), Vitamin B3 (340 mg/100 g), Vitamin C (15.4 mg/100 g), and Vitamin E (0.015 mg/100 g) are the vitamins that make up each gram of food. Calcium (29.95%), magnesium (4.08%), potassium (23.2%), sodium (0.38%), phosphorus (6.9%), chlorine (0.3%), manganese (1.44 ppm), iron (3.6 ppm), aluminum (5.35%), titanium (2.1 ppm), and silicon (22.7 ppm) were all found to be present in the sample according to mineral analysis (Akintunde *et al.*, 2022).

The high values of vitamins C and E, phenols and steroids reveal its potentials as reproductive booster hence this necessitated the study. Numerous researchers have noted that unconventional feedstuffs have anti-fertility characteristics. However, studies have demonstrated that spermatogenesis and egg production in animal breeding may be delayed by phytochemical-rich diets (Akintunde *et al.*, 2020; Akintunde *et al.*, 2021; Akintunde and Toye, 2021). The importance of morphometrics in relation to the reproductive system was highlighted by Ogbuewu *et al.* (2009) since they are crucial for giving useful and predictive information in determining the capacity for animal reproduction. Additionally, Gage and Freckleton (2003) described the crucial roles that mammalian testes' size, length, and width play in spermatogenesis. They emphasized the significance of these sperm morphometric variables in predicting the reproductive potential of male animals as well as the preservation and fertilizing abilities of semen collected for artificial insemination.

Despite the enormous potentials of quails in

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bridging the animal protein gaps, there is dearth of information on the impacts of organic products on the spermiogramic parameters of quails. Hence, this study is aimed at studying the effect of aqueous administration of egg lime molasses mixture (ELM) mixture on the spermiogramic parameters of Japanese quails.

MATERIALS AND METHODS

Experimental site

This experimental was performed at a standard poultry unit (approved for research by the Department of Agriculture and Industrial Technology, Babcock University) at the farm house of Babcock University, Ilishan Remo Ogun State, Nigeria. Babcock university is located at latitude 6.8920° N and longitude 3.7181° E. It is covered predominately by rain forest and has wooden savanna in the northwest. with an annual rainfall of about 1500 mm, with a mean temperature range of 27-31 °C and a very high relative humidity (above 87%).

Preparation of ELM solution

The eggs were first checked to make sure they were incredibly fresh; this was done by submerging them in water. Following the placement of the fresh eggs in a bowl, 1 liter of lime juice and 500 g of molasses were then added. The bowl was then carefully covered and left for 10 days at a temperature of 27 °C and a relative humidity of 61%. The egg shells had broken down into solution after 10 days. The entire mixture was then combined.

Experimental birds

The Japanese quail's chicks were procured from a local farmer in Lagos State, Nigeria. Two weeks before the arrival of the two hundred Japanese quails, the pen was washed, disinfected and left to dry. The drinkers and feeders were also thoroughly washed and disinfected. One

hundred watt electric bulbs were installed in the cages to provide heat and illumination at night for continuous feed intake. On arrival, randomization method was used to allocate the birds to five treatments with four replicates of 10 birds per replicate in a completely randomized design.

Experimental treatments

Five oral treatments was formulated. The control T1 with no ELM solution in the water while, T2, T3, T4, T5 had 10, 20, 30 and 40 mL per 500 mL of drinking water respectively. The ELM solution was given to them 5 days in week one, 5 days in week 3, 5 days in week 5 and 5 days in week 7.

Daily water intake (DWI)

The volume of water consumed per day by bird was calculated as the total water supplied subtracted from the volume of water left over divided by the total number of birds.

Composition of experimental diet

Table 1 Gross composition for experimental starter and finisher diets (g/100 kg)

	starter (%)	finisher (%)
maize	48	59
soybean meal	33	30
wheat offal	6	5.64
fishmeal	4	-
palm oil	-	3
vegetable oil	4	-
meat – bone meal	2.5	-
limestone	1	-
dicalcium phosphate	0.5	1.56
oyster shell	-	1
salt	0.4	0.25
methionine	0.2	0.25
lysine	0.1	0.05
avatec	-	0.06
% CP	15.20	20.00

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The ingredient formulated for the starter phase of the experiment and was given to the Japanese quails from week one to week four, and the ingredient formulated for the finisher phase of the experiment and was given to the Japanese quails from week four to week seven when the experiment was terminated.

Spermiogramic parameters and morphometrics of male reproductive organs

The quails were weighed and slaughtered at day 49. Their testicles and epididymis were carefully excised and weighed using an analytical weighing balance. The measurements were made in grams. Thread was used to measure the testis and epididymis' lengths. After setting the measurement piece on a ruler, the reading was obtained and recorded in centimeters.

The relative testis and epididymis weight (left, right and paired) was calculated as testis and epididymis weight (left, right and paired) (wet weight, mg)/bw (g) × 100%; where, bw is body weight of the male Japanese quail (g). Testicular and epididymal volume was determined by pouring a known quantity of distilled water into a measuring cylinder and dropping the testis and epididymis into it. Using the Archimedes principles of water displacement, the quantity of water displaced in the cylinder was taken as the volume of the testis (Akinyemi *et al.*, 2014).

Testicular density was computed using the testis's volume and weight. Testicular and epididymal density was determined by dividing the mass of the testis and epididymis by their respective weights. Testicular circumference was measured by wrapping a length of thread around the testis, reading the measurement, and then folding the thread back around. For testicular diameter, one side of the testis was measured across with a piece of thread, which was then placed on a ruler and measured.

To determine testicular and epididymal sperm reserve, each testis and epididymis was homogenized with 1 ml of normal saline (0.154 M NaCl), then filtered through gauze. In order to determine the sperm count, the homogenate was diluted with normal saline at a ratio of 1:30. The diluted homogenate was then charged on the haemocytometer and inspected under a microscope at a magnification of x400. To determine daily sperm production (DSP), the testicular sperm reserves were used to estimate the daily sperm production. The nuclei of elongating spermatids are resistant to physical damage at some stage during spermatogenesis, which is the basis for the calculation of DSP from testicular homogenate. Therefore, the Clulow and Jones (1982) formula was used to compute the DSP of the Japanese quails by dividing the testicular sperm reserve by time divisor.

The formula stated by Akintunde (2018) and Akintunde *et al.* (2020) as the amount of motile sperms per the typical number of sperm cells necessary to fertilize hens' eggs was used to determine the number of female quails that could be mated per ejaculate.

Statistical analysis

Data collected were presented as mean ± standard error, the comparison was done using one-way analysis of variance and the treatment means were separated using Duncan Multiple Range Test (Steel and Torries, 1990), where p value <0.05 is considered to be statistically significant. Data were analyzed using Statistical Package for Social Sciences (SPSS) Version 22.

RESULTS

The result of the morphometric characteristics of male Japanese quail at different inclusion level is presented in Table 2. It was

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observed that there was no significant difference ($p > 0.05$) in the live weight of the birds. The male Japanese quails that received 20 mL of ELM had significantly ($p < 0.05$) higher left epididymis weight (0.36 ± 0.05 g) than other groups, however there was no significant difference ($p > 0.05$) observed in the testes weight. The inclusion of ELM in the water of the quails significantly influenced ($p < 0.05$) left

epididymis volume, paired epididymis volume, left epididymis density, paired epididymis density and spermatozoa reserves in the right epididymis. However, the birds administered 20 mL of ELM per 500ml of water had significantly highest ($p < 0.05$) values for left epididymal volume, left epididymal density, paired epididymal density and spermatozoa reserves in the right epididymis.

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Table 2 Spermiogramic parameters and fertilizing potentials of male Japanese quails to aqueous administration of egg lime molasses mixture

	T1	T2	T3	T4	T5
testicular weight (g)	5.520 ± 0.940	6.645 ± 1.145	6.190 ± 0.480	4.990 ± 1.190	5.030 ± 0.470
right testis weight (g)	2.575 ± 0.525	3.520 ± 0.680	2.745 ± 0.495	2.610 ± 0.450	2.570 ± 0.030
left testis weight (g)	2.945 ± 0.415	3.125 ± 0.465	3.445 ± 0.015	2.380 ± 0.740	2.460 ± 0.500
right testis volume (mL)	2.750 ± 0.750	3.000 ± 1.000	2.000 ± 0.000	1.900 ± 0.300	2.800 ± 0.100
left testis volume (mL)	2.250 ± 0.250	2.600 ± 0.600	3.500 ± 0.500	1.900 ± 0.100	2.500 ± 0.500
paired testes volume (mL)	5.000 ± 1.000	5.600 ± 1.600	5.500 ± 0.500	3.800 ± 0.200	5.300 ± 0.400
right testis length (mm)	2.000 ± 0.000	1.950 ± 0.050	2.000 ± 0.000	1.500 ± 0.800	2.250 ± 0.350
left testis length (mm)	2.350 ± 0.050	1.950 ± 0.050	2.300 ± 0.400	2.200 ± 0.200	2.200 ± 0.200
right testis circumference (mm)	1.600 ± 0.100	1.350 ± 0.150	1.450 ± 0.050	1.600 ± 0.300	1.500 ± 0.000
left testis circumference (mm)	1.050 ± 0.050	1.200 ± 0.200	1.700 ± 0.200	1.200 ± 0.200	1.250 ± 0.250
right testis diameter (mm)	0.509 ± 0.032	0.430 ± 0.048	0.461 ± 0.016	0.509 ± 0.095	0.477 ± 0.000
left testis diameter (mm)	0.334 ± 0.016	0.382 ± 0.064	0.541 ± 0.064	0.382 ± 0.064	0.398 ± 0.080
right epididymis weight (g)	0.365 ± 0.045	0.570 ± 0.310	0.490 ± 0.150	0.230 ± 0.090	0.115 ± 0.015
left epididymis weight (g)	0.300 ± 0.010 ^{bc}	0.230 ± 0.020 ^{abc}	0.360 ± 0.050 ^c	0.170 ± 0.070 ^{ab}	0.095 ± 0.005 ^a
paired epididymal weight (g)	0.665 ± 0.055	0.800 ± 0.330	0.850 ± 0.200	0.400 ± 0.160	0.210 ± 0.020
right epididymis volume (mL)	0.100 ± 0.000	0.150 ± 0.050	0.120 ± 0.030	0.100 ± 0.000	0.065 ± 0.005
left epididymis volume (mL)	0.100 ± 0.000 ^b	0.100 ± 0.000 ^b	0.090 ± 0.010 ^b	0.075 ± 0.025 ^{ab}	0.040 ± 0.000 ^a
paired epididymal vol. (mL)	0.200 ± 0.000 ^{ab}	0.250 ± 0.050 ^b	0.210 ± 0.040 ^{ab}	0.175 ± 0.025 ^{ab}	0.105 ± 0.005 ^a
right testis density (g/mL)	0.955 ± 0.070	1.235 ± 0.185	1.373 ± 0.248	1.370 ± 0.020	0.919 ± 0.022

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	T1	T2	T3	T4	T5
left testis density (g/mL)	1.305 ± 0.040	1.226 ± 0.104	1.004 ± 0.139	1.277 ± 0.457	0.983 ± 0.003
paired testis density (g/mL)	1.111 ± 0.034	1.228 ± 0.147	1.143 ± 0.191	1.3 ± 0.245	0.948 ± 0.017
right epididymis density (g/mL)	3.65 ± 0.45	3.5 ± 0.9	4.022 ± 0.244	2.3 ± 0.9	1.762 ± 0.095
left epididymis density (g/mL)	3.000 ± 0.100 ^b	2.300 ± 0.200 ^a	3.988 ± 0.113 ^c	2.200 ± 0.200 ^a	2.375 ± 0.125 ^a
paired epididymal density (g/mL)	3.325 ± 0.275 ^{ab}	3.058 ± 0.708 ^{ab}	4.012 ± 0.188 ^b	2.200 ± 0.600 ^a	1.995 ± 0.095 ^a
spermatozoa reserves-RT	0.987 ± 0.333	1.125 ± 0.370	0.801 ± 0.057	1.535 ± 0.065	0.781 ± 0.747
spermatozoa reserves-LT	0.612 ± 0.030	0.529 ± 0.342	0.918 ± 0.672	0.565 ± 0.056	0.762 ± 0.116
spermatozoa reserves-RE	0.312 ± 0.068 ^a	0.858 ± 0.057 ^{bc}	1.236 ± 0.214 ^c	0.771 ± 0.024 ^b	0.486 ± 0.144 ^{ab}
spermatozoa reserves-LE	1.110 ± 0.097	0.543 ± 0.051	0.925 ± 0.487	0.981 ± 0.114	0.462 ± 0.329
spermatozoa reserves-PT	1.600 ± 0.364	1.654 ± 0.028	1.719 ± 0.729	2.100 ± 0.121	1.543 ± 0.862
spermatozoa reserves-PE	1.422 ± 0.166	1.402 ± 0.006	2.162 ± 0.701	1.752 ± 0.138	0.948 ± 0.185
DSP-TESTES	1.600 ± 0.364	1.654 ± 0.028	1.719 ± 0.729	2.100 ± 0.121	1.543 ± 0.862
hen inseminated/day	31.990 ± 7.279	33.079 ± 0.552	34.387 ± 14.582	41.991 ± 2.429	30.861 ± 17.246
relative testes weight (g)	4.046 ± 0.602	4.937 ± 0.154	4.219 ± 0.413	3.850 ± 0.904	3.719 ± 0.100
relative epididymis weight (g)	0.488 ± 0.030	0.574 ± 0.165	0.581 ± 0.148	0.308 ± 0.122	0.155 ± 0.004
testosterone (U/I)	2.250 ± 0.250 ^a	3.100 ± 0.100 ^b	3.050 ± 0.050 ^b	3.000 ± 0.000 ^b	3.650 ± 0.150 ^c
live weight (g)	136.000 ± 3.000	134.000 ± 19.000	147.000 ± 3.000	129.500 ± 0.500	135.000 ± 9.000
water intake (mL)	6177.75 ± 1023.587	61740 ± 686.617	6517.250 ± 739.776	6091.333 ± 1076.256	6379.500 ± 545.757

^{a,b,c} means within a row with difference superscripts are significantly different ($p < 0.05$); T1: control with no egg lime molasses (ELM) mixture in the drinking water; T2, T3, T4, T5: had an inclusion level of 10, 20, 30 and 40 mL ELM per 500 mL of drinking water respectively; the ELM solution was given 5 days in week one, 5 days in week 3, 5 days in week 5 and 5 days in week 7; RT: right testis; LT: left testis; RE: right epididymis; LE: left epididymis; PT: paired testes; PE: paired epididymis; DSP: daily sperm production.

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There was however no significant difference ($p > 0.05$) in testicular weights, testicular volumes, testicular lengths, testicular circumference, testicular diameter, testicular density and spermatozoa reserves in the testes. Testosterone values increased significantly ($p < 0.05$) with increased ELM inclusion across groups.

DISCUSSION

Understanding the genitalia morphometry of male and female species is crucial since it can be used to produce these species more abundantly and with greater output. In order to assess and estimate quantitative changes in testicular components and spermatogenic function resulting from factors like age, season, temperature, and diseases, morphometric analysis on the testis of any species or breed is required (Egbunike *et al.*, 1976). Additionally, nutrition has some impact on gonadal sperm stores and testicular morphometric parameters in Corriedale rams (Bielli *et al.*, 1997). Mammalian testes were characterized as perfect predictors of spermatozoa production (Gage and Freckleton, 2003).

The basic morphometric properties of the reproductive organs must be understood in order to measure and anticipate not only sperm production but also the breeder male's capacity for fertilization and sperm storage. Significant connections between paired testes weight and body weight, sperm output, and reserve potentials in boars have been found in mammalian species (Gbore and Egbunike, 2008; Akintunde *et al.*, 2021). Seasonal differences in testicular morphology and sperm storage have been seen in domestic cats and camels (Al-Qarawi *et al.*, 2001; Franca and Godinho, 2003). There is a correlation between avian testicular growth and body weight, according to a few studies (Kumaran and Turner, 1949). However,

in order to determine the sexual maturity of fowls, Marvan (1969), Tingari *et al.* (1980), and Aire (1982) also made an effort to make a relationship between the age of birds, testicular growth, and testicular weight. Testicular microanatomy and morphometry in quail (*Coturnix coturnix japonica*) were described by Artoni (1993), who also established the annual testicular cycle in this species. In addition to describing the microanatomy of the epididymal area and the ductus deferens in the turkey, Hess *et al.* (1976) also characterized the ductus succession from the seminiferous tubule to the ductus deferens papilla (*Meleagris gallopavo*). On the other hand, Reviere (1971) reported the ponderal expansion of the testis using organ weight and histological investigations through measurement of the width of the seminiferous tubules. Reviere was examining the testis development of hybrid Rhode x Wyandotte, the testes, epididymis, and ductus deferens are the most crucial functional parts, but the complete avian reproductive system is required for breeding. The testes, epididymis, ductus deferens, ejaculatory area, and mating organ make up a male bird's reproductive system. Because that birds are a fantastic source of nutrients, experts have recently considered studying them. Many traditional accounts of the male reproductive system exist, all of which attempt to draw a connection between shape, testicular size, age, and sexual maturity (Bull *et al.*, 2007; Ewuola *et al.*, 2015). It has been demonstrated that the sperm production efficiency and sperm stores are significantly correlated with the testicular weight (Osinowo *et al.*, 1981). Seminiferous tubule development may be responsible for this. This could be the cause of the non-significant difference in testicular weights and spermatozoa reserves in the testes that was found in this study. The testicular measurements observed in this study indicated that the male quail's reproductive

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organs were exposed to an aqueous solution of ELM mixture and were actively functioning. As a result, they were able to perform spermatogenic activities at their best level because there was no significant difference between the treated group and the control group.

The findings of this study are consistent with those made by Akintunde *et al.* (2020), who found that left epididymal weights of Yoruba Ecotype Nigeria local chickens and Isa Brown chooks fed varying amounts of *Moringa oleifera* seed meal showed a significant difference. According to Oyeyemi and Okediran (2007), a higher concentration of spermatozoa may indicate a high fertility rate due to the quantity of spermatozoa present during service or insemination. The study's results showed that the right epididymis of male quails given 20 mL of ELM/500 mL of water had the largest spermatozoa reserves. This implies that aqueous administration of 20 mL of ELM in 500 mL of water could increase the male quail's ability to reproduce.

According to Ahemen *et al.* (2016), there was no discernible difference between the spermatozoa reserves in the left testis of chickens with frizzle feathers, a naked neck, and regular feathered birds ($p > 0.05$). The research employed sperm morphometrics to assess the internal reproductive systems of chickens with genotypes of chickens indigenous to Nigeria's Southern Guinea Savanna. The aqueous delivery of ELM in the current investigation had no discernible impact on these parameters either. According to Ezekwe (1998) and Perry and Petterson (2001), the size, length, and width of the testes can be utilized to gauge and assess a livestock's capacity to produce sperm.

According to Aviagen (2004), a large percentage of guys were infertile when their testicles were underweight. Yet, it was noted that an increase in testis weight generally led to an improvement in fertility. However, Aviagen

(2004) came to the conclusion that testicles under 5 g would be small and non-functional, those between 6 g and 10 g would be borderline, and testicles beyond 10 g would be functioning. Given that quails were employed in this study, the changes seen there could be due to differences in the species. Additionally, because there is a known association between body weight and testicular parameters, the non-significance of body weights could explain the non-significance of testicular parameters (Daramola *et al.*, 2010; Akintunde *et al.*, 2021).

A strong association between testicular weights and sperm reserves in the testes or epididymis has also been noted, and this is a clear indicator of the health of the testicles in terms of sperm production (Osinowo *et al.*, 1981; Adeyemo *et al.*, 2007). This does support the significant differences in weight, volume, and density of the left and the right epididymis' spermatozoa reserves. These variables are essential for spermatogenesis (Steinberger *et al.*, 1973).

The study's findings are consistent with those of prior studies, which have shown that administering onions and their extracts raises testosterone levels (Banihani, 2019). According to Banihani (2019), the main ways onions increased testosterone production in males were increased luteinizing hormone production, neutralizing the negative effects of free radicals formed, primarily in the testes, enhancing the antioxidant defense mechanism (e.g., antioxidant enzymes, glutathione) in the testis, improving insulin resistance, promoting nitric oxide production in Leydig cells, and altering 5' AMP-dependent protein kinase activity. The anti-oxidant potentials and significant concentrations of flavonoids, steroids, vitamins A, C, and E in ELM, as described by Akintunde *et al.* (2022), may account for the study's findings that the levels of testosterone increased with increasing levels of the aqueous

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administration of ELM.

These results are further supported by evidence from this study, which have demonstrated that ELM can increase fertility in males and lead to reproductive health benefits. Therefore, the results of this study suggest that aqueous administration of ELM is beneficial for male quails' reproductive health.

CONCLUSION

It can be concluded from this study that the administration of aqueous solution of ELM mixture in drinking water of Japanese quails did not alter the growth parameters of Japanese quails however birds that received 20 mL per 500 mL of water had the best reproductive parameters hence administration of 20 mL per 500 mL is recommended for optimum reproductive performance of Japanese quails.

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AOA, LCN, MML and OAO: Management of experimental animals, data collection, data management and data analysis. AOA: Conceptualization, design of the experiments,

manuscript writing and data analysis, OAO2, SOO, BAS and OEA: Visualization, manuscript review and final approval of manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests

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