Reproductive performance of *Archachatina marginata* fed graded levels of dry okra fruit meal as phytogenic feed additive

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Abstract

Diversification of livestock production with micro-livestock, such as snails, through the use of phytogenic feed additives can contribute to increase in production and alleviating the problem of deficiency of animal protein in the diets of Cameroonians. The objective of this study was to evaluate the effect of dietary inclusion of graded levels of dry okra fruit meal on the reproductive performance of *Archachatina marginata* snails. A 10 month study was conducted using 360 one month old hatchlings of mean weight 1.11g ± 0.01, shell length 15.90mm ± 0.11 and shell diameter 12.80mm ± 0.15. They were allotted to 4 groups (T_0, $T_{2.5,}$ $T_{5.0}$ and $T_{7.5}$) of 15 snails each and 6 replicates in a completely randomised design. The control group received the basal diet and the other 3 groups had in addition to the basal diet dry okra fruit meal supplemented at 2.5, 5.0 and 7.5g/kg feed respectively. Subsequently the reproductive indices were evaluated and data generated were analysed using SPSS. Results showed that dietary inclusion of dry okra fruit improved spawning characteristics of snails. There was significant increase in egg weight and egg morphometric characteristics with 7.5g/kg okra supplementation. Hatch rate and gonadosomatic index were significantly increased with 5.0g/kg okra supplementation. A 7.5g/kg supplementation resulted in significantly shorter incubation period and increased gonad weight in addition to improved spat weight, spat morphometric characteristics and mortality rate. It was therefore concluded that okra could be included in the diet of growing snails as phytogenic feed additive for improved reproductive performance.

Keywords: Egg Morphometric characteristics, Fertility, Snails, Spawning characteristics, Supplementation
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Introduction

The Giant African Land Snails are pulmonate, nocturnal, hermaphroditic gastropods of the Family Achatinidae (Ejidike and Adewuyi, 2018). The Achatinidae account for about15-30% of the global snail market and constitute the African species of snails referred to as *Achatina achatina, Archachatina marginata and Achatina fulica* (Manet *et al*., 2022). These species are indigenous to Africa and dwell mostly in humid areas. Ngenwi *et al*. (2010) revealed that they are collected during the rainy season from their natural habitat which are tropical forests, savannahs, farms, and often gardens where they have unlimited vegetation to feed on hence they can then be consumed or used for other purposes. However, if kept intensively, rearing processes are mainly traditional and they are fed with green leaves (leaves from paw-paw, sweet potatoes, plantain, cassava, etc.), fruits (banana, avocado, mangoes, etc.) and tubers (Ndah *et al*., 2017).

Considering the food insecurity spike in most regions of Africa from 2019 to 2020, which showed that 281.6 million people (over one-fifth of the population) faced hunger in 2020, this increased by 46.3 million compared to 2019, while 37.4% of global severely food insecure people and 36.7% of global malnourished people lived in Africa in the same period as reported by FAO (2021). The same authors opined that in Cameroon, 16% of households were food insecure (3.9 million people). Several reasons attributed to malnutrition are centred on low consumption of animal protein (Mpupu, 2012) and insufficient agro-pastoral production and a surge in the price of basic foodstuffs (FAO, 2019), which exacerbates the already precarious global food insecurity. Defang *et al*. (2014) in a holistic approach, attributed the low protein intake to low level of animal production and high cost of animal products and suggested the intensification and diversification of livestock production with emphasis on micro-livestock production as means to find sustainable solutions to the acute shortage of animal protein in the diets of average Cameroonians.

The snail is one of such micro-livestock species and meat from snails is often viewed as tasty, tender, highly nutritious and healthy. Indeed Kehinde (2009) and Omole *et al*. (2011) reported on the high amino acid profile of lysine, methionine, as well as vitamin content. Its rich calcium content (185.70mg/100g), phosphorus (61.24mg/100g) have also been highlighted (Ademolu *et al*., 2004). Bayode (2009) elucidated on the iron content of 45-50mg/kg, low fat content (0.05-0.08%), and low cholesterol levels (20.28mg/100g). In addition, snails are cheap to produce, efficient in nutrient recycling, easy to manage, their droppings contribute less to greenhouse gas emissions and are indeed an alternative substitute with great zoo-technical potential to conventional animal protein sources. Indeed Oyeagu *et al*. (2018) opined that they are one of the micro livestock species that could serve as an alternative cheap source of meat for human populations. However among the various challenges facing intensive snail farming is the slow growth rate which could be attributed to unfavourable weather condition, genetic makeup, stocking density and nutrition (Ejidike, 2001). If nutrition is manipulated snail growth and reproduction performance can be enhanced especially under intensive production system (Abiona, 2013).

Phytogenic feed additives contain phyto-chemicals and supplementation in the nutrition of swine and poultry has resulted in improved animal performance and reproductive efficiency (Steiner & Syed, 2015; Mahfuz *et al.,* 2021 & Swelum *et al*., 2021).They have been proven to have positive effect on meat quality, intestinal morphology and intestinal micro biota, zoo-technical parameters, anti-inflammatory and antioxidant activity and improve digestibility and flavouring effects (Steiner & Syed, 2015). The authors further opined that phytogenic feed additives could therefore influence feed intake, feed conversion, growth rate, egg production, liveability, carcass and meat characteristics.

Okra has been reported to contain various nutrients and phyto chemicals whose abundance in phenolic compounds has been elucidated (Arapitsas, 2008; Gemede *et al*., 2015; Hafeez *et al*., 2020 & Nikpayam *et al*., 2022). These phenolic compounds are rich in bioactive properties like catechin oligomers, quercetin derivatives and hydroxyl cinnamic derivatives abound in their seeds, skins and pods. In addition, 100 g of dried okra pods contains 2.44 g crude protein, 2.11 g deoxidized sugar, 0.682 g carotene, 1.06 g cellulose, 10.2 mg vitamin B, 1.25 mg vitamin A, 26.5 mg vitamin C, and several minerals, which are slightly higher than the proportions found in common fruits and vegetables (Liu *et al*., 2007). Evidence from research findings has shown improvement in growth performance with okra fruit supplementation in broiler diets as reported by Zhang *et al*. (2017); Ashour *et al*. (2020) & Abbas (2021). The use of okra seed and fruit extracts have equally improved reproductive performance in turkey hens (Machebe *et al.,* 2013) and in Wistar rats (Obeten *et al*., 2022), respectively. There is however paucity of information on the use of okra as a phytogenic feed additive in the production of snails. Hence this study was aimed at assessing the role of okra (*Abelmoschus esculentus*) fruit meal as a phytogenic feed additive on the reproductive performance of the Giant African Land Snail (*Archachatina marginata*).

Materials and Methods

Ethical statement

Ethical clearance for this research was granted by the University of Buea – Institutional Animal Care and Use Committee (UB-IACUC) of ethical clearance number *UB- IACUC N^{o} 14/2021.*

Period and Study Area

This study was carried out from October 2021 to August 2022 at the Snail Unit of the Faculty of Agriculture and Veterinary Medicine Teaching and Research Farm (FAVM-TRF) of the University of Buea (Figure 1), South West Region (LN: 4^{o}12′−4^{o} 25′and LE: 9^{o}19 -9^{o} 20′), at an altitude of 870 - 4095m). The prevailing climate is equatorial, characterized by tropical rainforest vegetation with a short dry season (mid-November to mid-March) and a long rainy season (mid-March to mid-November). Rainfall ranges from 2,000 to 4,000mm per year and relative humidity 85 to 95%. The annual average temperatures oscillate between 20 and 29°C.

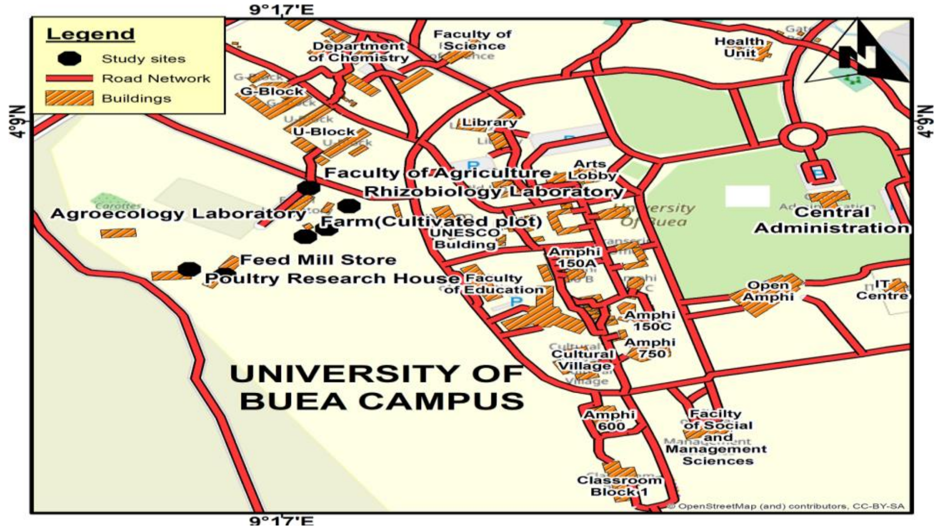

Figure 1 Study area Faculty of Agriculture and Veterinary Medicine Teaching and Research Farm, University of Buea, Cameroon

Animal, housing and equipment

A total of 360 one-month-old *Archachatina marginata* hatchlings (Figure 2a) bred at the Snail Unit of mean weight 1.11g ± 0.01, mean shell length 15.90mm ± 0.11 and mean shell diameter 12.80mm ± 0.15 free from wounds, cuts and other shell defects were used. The hatchlings were previously selected from a multitude of breeder snail hatchlings monitored, fed a common diet (Figure 2b) for 1 month before being used for the experiment. The animals were housed in raised trench pens of dimension 100cm x 100cm x 50cm, covered with wire mesh and filled with loamy soil to a depth of about 10cm to serve as bedding material and soft substrate for the snails. The substrate was disinfected with Virunet® (broad spectrum disinfectant) using a knapsack sprayer at 0.5g/L/Substrate two weeks before the animals were introduced. The soil was sprinkled with water as required and was changed every 3 months to prevent over fouling by snail droppings, mucus secretions and reduce the risk of spread of infections. The pens were equipped with plastic feeders and drinkers and dry banana leaves were also included to serve as mulch.

a b

Figure 2 Hatchlings 1month old (a) Hatchlings at birth fed a common diet (b)

The plant source and processing

Okra *(Abelmoschus esculentus L.*(Moench) purchased from the local market (Figure 3a) was identified by the Department of Agronomy and Applied Molecular Sciences of the Faculty of Agriculture and Veterinary Medicine in the University of Buea. The okra was washed, sliced, sundried (Figure 3b) for a period of about 2 weeks to constant weight and crispy to touch and then ground to obtain the product Dry Okra Fruit Meal (DOFM) Figure 3c.

a b c

Figure 3 Fresh okra (a), Dry okra fruit (b) and Dry okra fruit meal (c)

Nutritional value of DOFM

The DOFM was taken to the laboratory for proximate analysis and the nutritional composition of its various constituents is presented in Table 1.

Table 1 Proximate analysis of DOFM

Constituents	Composition
Dry matter (%)	90.69
Ash (% DM)	9.88
Organic matter (% DM)	90.12
Fat (% DM)	0.45
Crude protein (% DM)	13.43
Crude Cellulose (% DM)	8.96
Gross energy (Kcal/kg DM)	3997.91
Metabolisable energy (Kcal/kg DM)	2777.66

Experimental diet

Diets were formulated to be iso-caloric and iso-nitrogenous (Table 2). The control group received the basal diet and the other groups had the basal diet and in addition, DOFM supplemented at 2.5, 5.0 and 7.5g/kg of feed respectively.

Table 2 Composition and nutritional value of snail basal diet

Feed Ingredients	Composition (Kg)
Corn	35.5
Soy beans (49)	35.0
Fishmeal	7.0
Shells	17.5
Palm oil	5.0
Total	**100.00**
Calculated Nutritional value	
Crude protein (%)	24.05
Metabolizable energy (kcal/kg)	2607.2
Crude fibre (%)	2.05
Calcium (%)	7.17
Phosphorus (%)	0.43
Lysine (%)	1.59
Methionine (%)	0.42

Experimental procedure and data collection

The *Archachatina marginata* hatchlings were randomly allotted to 4 treatments (T_0, $T_{2.5}$, $T_{5.0}$ and $T_{7.5}$) of 15 comparable hatchlings (weight and size) in 6 replicates. Each replicate was further assigned to one of the experimental diets offered *ad libitum*in addition to DOFM corresponding to 0, 2.5, 5.0 and 7.5g/kg of feed respectively. The substrate was watered (0.5L/substrate) as required, the animals monitored till the onset of lay and the experiment lasted for 10 months.

From the 6th month, the substrate was stirred entirely on a daily bases for presence of eggs. They were collected, taken to the laboratory and morphometric characteristics carefully taken and recorded (egg weight; Digital weighing balance KERN model sensitivity 0.01g and shell morphometric characteristics; Digital Caliper TMT322001 TOTAL Model). Spawning characteristics were evaluated and eggs were incubated based on spawning cluster to a soil depth of 4cm. From the 26th day of incubation, they were gently checked for hatchlings, spat were weighed and shell morphometric characteristics measured and recorded. Characteristics of fertility were also evaluated and recorded. Un-hatched eggs after about 40-45 days were cracked and the state of embryonic development was observed (Dafem *et al.,* 2008). Adult mortality was monitored and recorded.

At the end of the breeding trial, 3 snails per replicate were randomly selected for organ assessment. The animals were sacrificed, dissected, gonads harvested and weighed with an electronic balance of 0.01g sensitivity and gonadosomatic index evaluated as expressed in Jimoh & Akinola, (2020).

Parameters evaluated

Spawning characteristics

- Age at first egg = Age when the first eggs were laid
- Total clutches per treatment = All clutches in a treatment evaluated by counting
- Total eggs per treatment = All eggs laid per treatment evaluated by counting

Morphometric characteristics

- Egg weight (g) = Weight taken with an electronic balance of 0.01g sensitivity
- Egg length (mm) = longest distance round the egg from one pointed end to the blunt end
- Egg diameter (mm) = Shortest distance round the egg

Fertility characteristics

- Incubation period = Time taken (days) for eggs to hatch
  = Interval in days between date of incubation and date of hatch
- Hatch rate $= \frac{\text{Number of eggs hatched}}{\text{Number of eggs laid}} \text{x}100$
- Fertilization rate $= \frac{\text{Number of embryonated eggs}}{\text{Number of eggs laid}} \text{x}100$
  Where Embryonated eggs = Number of eggs that hatched + Number of dead − in − shell.
- Weight of gonad (ovotestis) = Weight taken with an electronic balance of 0.01g sensitivity
- Gonadosomatic index $= \frac{\text{Gonad weight}}{\text{Live weight}} \text{x}100$(Jimoh & Akinola, 2020)

Body weight and shell morphometric characteristics of spat

- Spat Weight (g) = Weight at hatch taken with an electronic balance of 0.01g sensitivity
- Spat Shell length (mm) =
  length at hatch measured along shell axis through the apex to the bottom
- Spat Shell diameter (mm) =
  Maximum width measured at hatch along shell axis through the apex to the bottom

Embryonic mortality rate and mortality rate of laying snails

- Early embryonic mortality $= \frac{\text{Number of eggs with dead embryo without shell}}{\text{Number of eggs laid}} \text{x}100$
- Late embryonic mortality $= \frac{\text{Number of eggs with dead embryo with shell}}{\text{Number of eggs laid}} \text{x}100$
- Adult mortality rate $= \frac{\text{Number ofdead adult snails during the breeding period}}{\text{Total number of adult snails}} \text{x}100$

Statistical analysis

Data were analysed using Statistical Package for Social Sciences (SPSS) version 20.0 (IBM Corp, 2011).One-way analysis of variance (ANOVA) was used to compare the means and when the differences were significant, Duncan's test was used to separate them at the 5% level.

Results

Effect of graded levels of DOFM on the reproductive performance of *Archachatina marginata*

The effect of graded levels of DOFM on the reproductive characteristics of snails is presented in Table 3, 4, 5, 6, 7 and Figure 4, 5 and 6.

The influence of graded levels of DOFM on spawning characteristics

The influence of dietary inclusion of graded levels of DOFM on spawning characteristics is shown in Table 3 and Figure 4, 5 and 6. Though dietary inclusion of graded levels of DOFM did not have any significant effect on all parameters evaluated, there was however an increase in values recorded with the use of DOFM in snails fed diets T_2 and T_3over the control diet. Age at sexual maturity was 10.09 ± 0.19 months in snails that received the control diet and 9.78 ± 0.25 in snails that received (7.5g/kg) DOFM in the diet. Mean eggs per clutch recorded 5.17 ± 0.19 eggs in the control and the values though not significant increased to 5.91 ± 0.29 eggs in snails fed diet T_3(7.5g/kg) DOFM. The same trend was observed in mean clutches per treatment and total eggs per treatment for the control group 1.21 ± 0.09 clutches and 6.39 ± 0.58 eggs and 1.33 ± 0.12 clutches and 7.85 ± 0.84 eggs respectively in snails that received diet T_3 (7.5g/kg) DOFM.

Table 3 Effect of graded levels of Dry Okra Fruit Meal on spawning characteristics (mean±SE)

Reproductive indices	Treatments (DOFM g/kg of feed)				ANOVA
	T_0(0)	T_1 (2.5)	T_2(5.0)	T_3 (7.5)	
Age at sexual maturity (months)	10.09 ± 0.19	10.00 ± 0.22	9.70 ± 0.21	9.78 ± 0.25	P=0.58
Mean eggs per clutch	5.17 ± 0.19	5.27 ± 0.17	5.37 ± 0.20	5.91 ± 0.29	P=0.08
Mean clutches per treatment	1.21 ± 0.09	1.10 ± 0.06	1.30 ± 0.09	1.33 ± 0.12	P=0.24
Mean total eggs per treatment	6.39 ± 0.58	5.80 ± 0.35	6.85 ± 0.86	7.85 ± 0.84	P=0.08

Duncan: Means within a row with no superscripts are statistically similar (P>0.05)

Age at first egg

Effect of dietary inclusion of graded levels of DOFM on age at first egg (AFE) in *Archachatina marginata* is presented in Figure 4. Though not significantly different, the value for AFE was 7 months in animals that received T_3 (7.5g/kg) DOFM, which is lower than 8 months recorded in the other snails that received T_0 (0), T_1 (2.5g/kg), and T_2 (5.0g/kg) DOFM.

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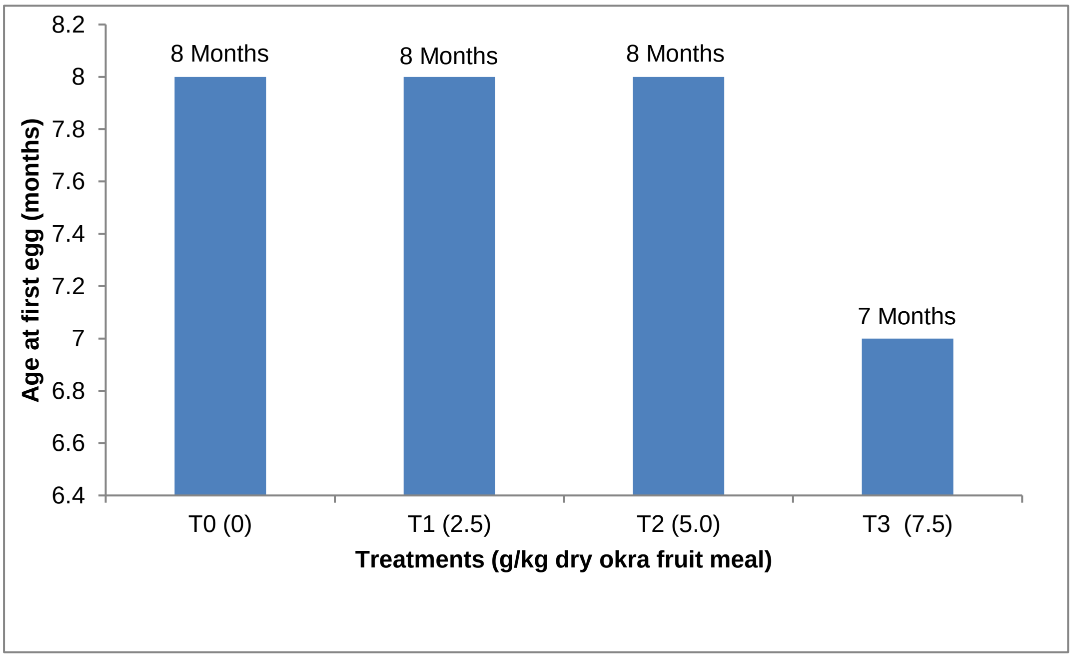

Figure 4 Effect of dry okra fruit meal on age at first egg

Total clutches per treatment

The effect of graded levels of DOFM on total clutches per treatment in *A. Marginata* is presented in Figure 5. There was no significant increase in total clutches per treatment across the groups. However Snails fed diet T_3 (7.5g/kg) DOFM had 36 clutches of eggs and the least number of clutches (28) was recorded in snails that received the (control diet) T_0 (0g/kg) DOFM.

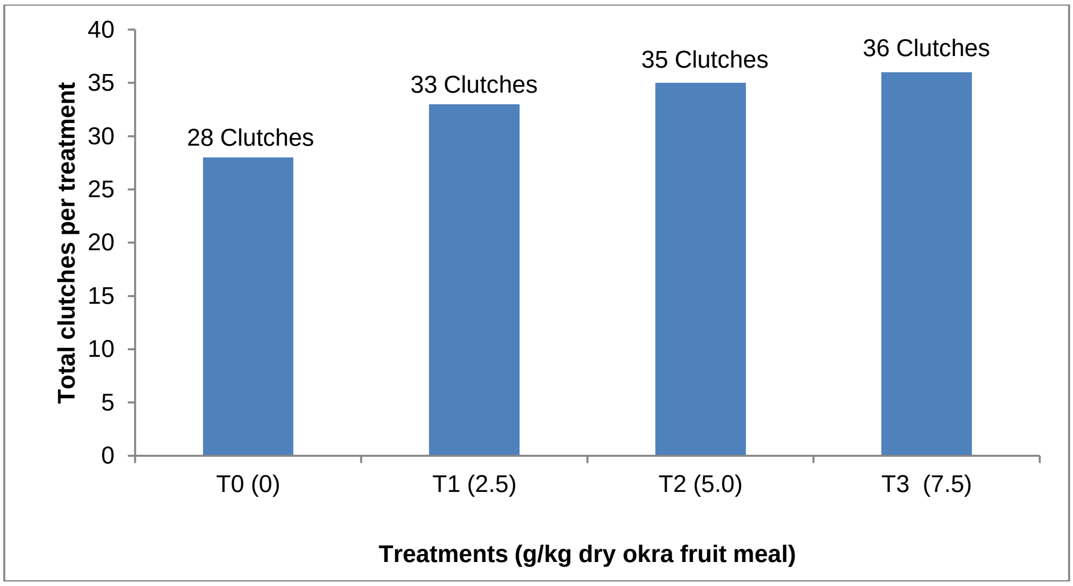

Figure 5 Effect of dry okra fruit meal on total clutches per treatment

Total eggs per treatment

The effect of graded levels of DOFM on total eggs laid per treatment in *A. marginata* is presented in Figure 6. Though dietary inclusion of DOFM did not lead to a significant increase in total eggs laid per treatment, there was an increase in value from 147 eggs recorded in snails that received T_0 (0g/kg) DOFM to 212 eggs in snails fed diet T_3 (7.5g/kg) DOFM.

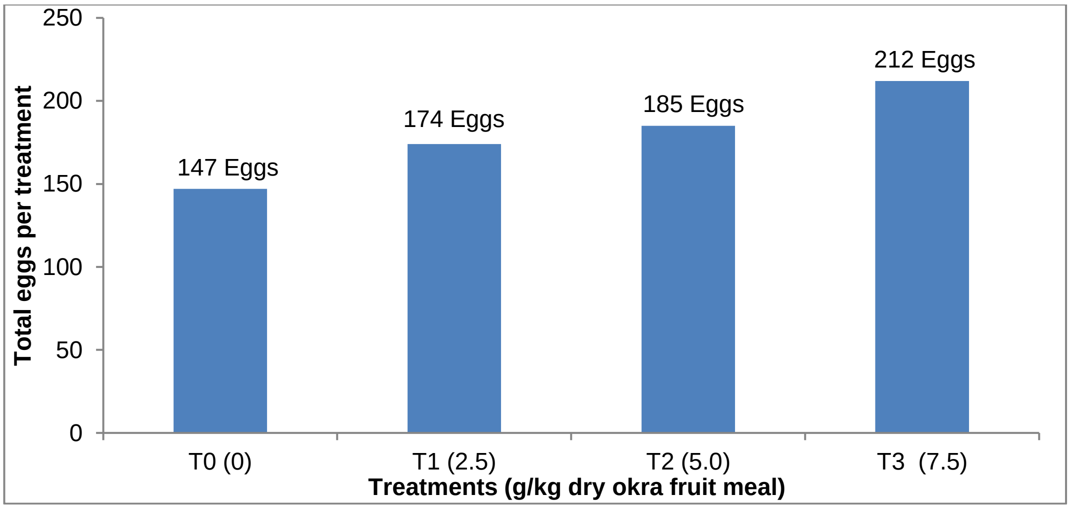

Figure 6 Effect of dry okra fruit meal on total eggs laid per treatment

The effect of graded levels of DOFM on egg morphometric characteristics

The effect of DOFM on egg morphometric characteristics is shown in Table 4. Inclusion of graded levels of DOFM led to a significant increase in the egg weight, egg shell length and egg shell diameter. The snails in the treatment that received diet T_3 (7.5g/kg) DOFM showed significantly higher egg weight, shell length and shell diameter from the treatments that received T_0 (0g/kg) and T_1 (2.5g/kg) DOFM ($p<0.05$).

Table 4 Effects of graded levels of dry okra fruit meal on egg morphometric characteristics (mean±SE)

Reproductive indices	Treatments (DOFM g/kg of diet)				ANOVA
	T_0(0)	**T_1 (2.5)**	**T_2(5.0)**	**T_3 (7.5)**	
Egg weight (g)	0.93 ± 0.01^c	1.02 ± 0.01^b	1.08 ± 0.01^a	1.07 ± 0.01^a	P=0.00
Egg shell length (mm)	13.17 ± 0.09^c	13.46 ± 0.04^b	13.58 ± 0.07^{ab}	13.68 ± 0.04^a	P=0.00
Egg shell diameter (mm)	10.38 ± 0.04^c	10.55 ± 0.04^b	10.95 ± 0.07^a	10.97 ± 0.05^a	P=0.00

^{a,b,c}Duncan: Means within a row with different superscripts are statistically different (P<0.05)

The Effect of graded levels of DOFM on fertility characteristics

The effect of dietary inclusion of graded levels of DOFM on the fertility of snails is presented in Table 5.Dietary inclusion of DOFM led to a significant increase in hatch rate, gonad weight and gonadosomatic index (p<0.05). The incubation period was significantly reduced from 31.96 ± 0.36 in the control group to 29.89 ± 0.28in the 7.5g/kg DOFM supplemented group. The highest values for Hatch rate, gonad weight and gonadosomatic index were recorded in snails fed 5.0g/kg and 7.5g/kg DOFM while the least values were recorded in snails that received the control diet T_0 (0g/kg) DOFM respectively.

Table 5 Effect of graded levels of dry okra fruit meal on fertility characteristics (mean±SE)

Reproductive Indices	Treatments (DOFM g/kg)				ANOVA
	T_0 (0)	**T_1 (2.5)**	**T_2 (5.0)**	**T_3 (7.5)**	
Incubation period (days)	31.96 ± 0.36^a	31.03 ± 0.31^b	31.26 ± 0.26^{ab}	29.89 ± 0.28^c	P=0.00
Hatch rate (%)	57.6 ± 4.09^b	70.42 ± 2.30^a	73.48 ± 2.82^a	72.11 ± 2.82^a	P=0.00
Fertilization rate (%)	78.52 ± 4.14	86.5 ± 2.12	87.05 ± 2.08	86.14 ± 2.53	P=0.12
Gonad weight (g)	0.43 ± 0.03^b	0.55 ± 0.06^{ab}	0.58 ± 0.03^{ab}	0.60 ± 0.05^a	P=0.04
Gonadosomatic index (%)	1.08 ± 0.05^b	1.37 ± 0.10^a	1.43 ± 0.05^a	1.29 ± 0.08^{ab}	P=0.01

^{a,b,c}Duncan: Means within a row with different superscripts are statistically different (P<0.05)

The effect of graded levels of DOFM on b**ody weight and shell morphometric characteristics of spat**

The effect of DOFM on body weight and shell morphometric characteristics of spat is shown in Table 6. Body weight and shell morphometric characteristics of spat in snails fed T_3 (7.5g/kg) DOFM was significantly different from those fed the control diet T_0 (0g/kg) DOFM (p<0.05). The values in all parameters measured were significantly higher in the group of snails that received the DOFM compared to the control group.

Table 6 Effect of graded levels of dry okra fruit meal on body weight and shell morphometric characteristics (mean±SE)

Morphometric characteristics of spat	Treatments (DOFM g/kg)				ANOVA
	T_0(0)	**T_1 (2.5)**	**T_2(5.0)**	**T_3 (7.5)**	
Hatchling weight (g)	0.79 ± 0.01^c	0.87 ± 0.01^b	0.91 ± 0.01^a	0.92 ± 0.01^a	P=0.00
Hatchling shell length (mm)	12.31 ± 0.10^c	12.80 ± 0.05^b	12.96 ± 0.05^b	13.25 ± 0.04^a	P=0.00
Hatchling shell diameter (mm)	10.13 ± 0.10^c	10.47 ± 0.08^b	10.66 ± 0.05^{ab}	10.76 ± 0.04^a	P=0.00

^{a,b,c}Duncan: Means within a row with different superscripts are statistically different (P<0.05)

The effect of graded levels of DOFM on embryonic mortality rate and mortality rate of laying snails

The effect of graded levels of DOFM on the mortality rate is represented in Table 7. There was treatment difference effect (p<0.05) in the mortality rate of adult laying snails with dietary inclusion of graded levels of DOFM. The snails in the control group recorded the highest mortality and the least was observed in snails that received T_2 (5.0g/kg) DOFM. The values for early and late embryonic mortality were highest in the control group (21.48% and 20.93% and least in group T_2 (12.95% and 13.57%) respectively, but these values were however not significantly different from each other.

Table 7 Effects of graded levels of dry okra fruit meal on embryonic mortality and mortality rate of laying snails (%±SE)

Reproductive indices (%)	Treatments (DOFM g/kg)				ANOVA
	T_0 (0)	T_1(2.5)	T_2(5.0)	T_3 (7.5)	
Early embryonic mortality rate	21.48 ± 4.14	14.17 ± 2.08	12.95 ± 2.08	13.86 ± 2.53	P=0.13
Late embryonic mortality rate	20.93 ± 3.88	16.08 ± 2.51	13.57 ± 1.88	14.03 ± 2.93	P=0.28
Laying snails mortality rate	6.52 ± 1.02^a	1.00 ± 0.56^b	1.11 ± 0.62^b	0.37 ± 0.37^b	P=0.00

^{a,b}Duncan: Means within a row with different superscripts are statistically different (P<0.05)

Discussion

The influence of graded levels of DOFM on spawning characteristics of snails

Dietary inclusion of DOFM did not have any significant effect on age at sexual maturity, mean eggs per clutch, mean clutch per treatment and total eggs per treatment. However there was an increase in values in AFE (age at first egg), total clutches and total eggs per treatment. There is however a dearth in the communication of information on the use of DOFM as phytogenic feed additive on the reproductive performance of snails and more specifically on the spawning characteristics. AFE of 7 months for snails in treatment (T_3) over the control treatment (T_0) could be attributed to the fact that okra is known to contain vitamins and minerals as well as bioactive components that could influence fertility and other reproductive functions in animals. Similar observations have been made by Hafeez *et al*. (2020) who reported that the tender green pods of Okra (*Abelmoschus esculentus)* are rich sources of vitamins (A, B1, B3, B6, C and K), magnesium, potassium, calcium and folic acid. Indeed their role in stimulating sperm cells and enhancing reproductive potential of animals has been reported by Machebe *et al.*, (2013). In addition, phenolic compounds like cathecins have been shown to possess the ability to scavenge free radicals and terminate oxidative reactions thereby improving the reproductive capacity of the snails.

The increase in total clutch and total eggs per treatment with increasing levels of DOFM in the diet complement results obtained by Machebe *et al.* (2013) who reported an increase in number of eggs laid by turkey hens with inclusion of 50mls okra seed extract/litre of water and 50g pumpkin seed supplement/kg of feed. Phenolic compounds like quercetin supplemented at 0.2g/kg of diet has been reported to improve laying rate in birds (Liu *et al.,* 2014). The role of phytogenic feed additives in enhancing egg production especially in birds has been fully elucidated (Liu *et al* 2014; Steiner & Syed 2015; Chen *et al*. (2018). Indeed Kitanov *et al*. (2003) & Semerdjie *et al*. (2008) reported that plants can produce phyto-chemicals with sex-enhancing potency and the ability to stimulate high reproductive potential in animals. Our results can again be attributed to the nutrients and bioactive components present in the okra fruit as previously highlighted.

The effect of graded levels of DOFM on egg morphometric characteristics of snails

Dietary inclusion of DOFM had a significant effect on egg weight, length and diameter. Our values were lower than 2.0 g (mean egg weight), 15.20 mm (egg length) and 11.40 mm (egg width) reported by Okon *et al.* (2013) for the giant African land snail, *Archachatina marginata.* The lesser values reported in this study could be attributed to the fact that the first clutches of eggs from first cycling growing snails and maiden eggs were among the eggs evaluated. Egg weight and weight of hatchlings are expected to increase with age and with subsequent clutches as suggested by Ejidike *et al*. (2002). Our results seem to complement reports on the role of bioactive components in plant extracts and their effect on egg morphometric characteristics. Indeed Abdel-Wareth & Lohakare (2020) reported that essential oils (peppermint) from dry leaf extract (*Mentha piperita*) administered to laying hens led to higher egg production, egg weight and egg shell thickness.

Our results are attributive to the antimicrobial, anthelmintic and antioxidant property of okra fruit that help to improve gut health and feed utilization and subsequently growth. Indeed its bioactive components (quercetin, cathecins) have been shown to improve the growth and development of animals which could have contributed to the increase in the weight of the snail eggs. In addition, Okra (*Abelmoschus esculentus*), has been reported to contain high amounts of calcium, magnesium, potassium and folic acid (Hafeez *et al.,* 2020) the increase in egg shell length and egg shell diameter with increase in DOFM could be tied to the high calcium and phosphorus present in Okra.

The effect of graded levels of DOFM on fertility characteristics of snails

Dietary inclusion of graded levels of DOFM led to significant improvement in incubation period, hatch rate, gonad weight and gonadosomatic index. The increase in hatch rate from 57.6% in the control group to 73.48% in the T_2 (5.0 g/kg DOFM) group and in the values for fertilization rate from 78.52% in the control to 87.05% in the T_2 group with dietary inclusion of graded levels of DOFM in the diet of snails corroborates the works of Machebe *et al.* (2013). The ability of *Abelmoschus esculentus* to influence hatch rate and fertility can be attributed to the phyto-chemicals and minerals contained in okra fruit hence responsible for sustaining the egg embryo during incubation and influencing hatchability. Poor hatchability and low fertility in poultry eggs have been attributed to insufficient nutrients such as vitamins, folic acids, zinc and selenium (Machebe *et al.*, 2013) which on the contrary abound in Okra. In addition the higher values in fertility rate could purport better production of ovum and viable sperm hence improving reproductive potential of the snails.

The significant increase in gonad weight and gonadosomatic index with dietary inclusion of DOFM is contrary to the report of Uchenna *et al.* (2014) who administered methanolic extract of okra fruit to male Albino Wistar rats and reported a reduction in testicular weight. However Jimoh & Akinola (2020) studied the effect of roughages and different concentrate mixes on the reproductive performance of laying *Archachatina marginata* snails and reported high gonadosomatic index in leaf meal-based diets and significantly higher gonadosomatic index for snails fed *leucaena leucocephala*-inclusive diet. The authors further opined that the reproductive ability of snails is predicted by the gonadosomatic index (GSI); the higher the gonadosomatic index, the better the reproductive performance. The enhanced reproductive organ development of the laying snails observed in our study by higher gonad weight and subsequently higher gonadosomatic index with dietary inclusion of DOFM could be attributed to the robust nature, dietary fibres' and distinct seed protein contained in okra as well as its balanced levels of lysine and tryptophan amino acids. Its high vitamin and mineral content may significantly influence reproductive functions by assisting the rate of activity in the gonads.

The influence of graded levels of DOFM on body weight and shell morphometric characteristics of spat

There was significant increase in body weight, shell length and shell diameter of spat with increase in levels of DOFM in the diet. Increase in body weight in boiler chickens with dietary inclusion of okra or its extract has been reported by (Zhang *et al.,* 2017; Ashour *et al.,* 2020 & Abbas 2021) and in Wistar rats (Uchenna *et al*., 2014). Body weight has been reported to correlate with shell traits (Ibom *et al.,* 2012) this is however reflected in the trend experienced in this study for body weight of spat and shell morphometrics. This could also be attributed to the same reasons as for egg weight, egg shell length and egg shell diameter as highlighted above.

The effect of graded levels of DOFM on embryonic mortality rate and mortality rate of laying snails

Dietary inclusion of DOFM significantly reduced mortality rate of laying snails. Though there was no significant decrease in early or late embryonic mortality rates, the values 12.95 ± 2.08 and 13.57 ± 1.88 were least in T_2 (5.0 g/kg DOFM) and highest in the control group. This corroborates the findings of Machebe e*t al*. (2013) who reported improved EEM rate of 11 ± 1.41 with okra seed extract in breeder turkey hens. Our results are suggestive of the presence of bioactive agents inherent in okra fruit which are responsible for its role as a phytogenic additive, these could have exerted some level of protection to snail embryos during incubation hence offer survivability to other treatments (T_1,T_2 and T_3) compared to the control treatment T_0 (0g/kg) DOFM.

The decrease in mortality rate with increase in levels of DOFM in the diet can be attributed to the health benefit of okra owing to its antimicrobial, immunological and antifungal properties, thus maintaining and improving the health condition of the snails by its decreased bacterial count and pathogen load throughout the breeding period. Okra contains quercetin as one of its active ingredients and Hafeez *et al*. (2020) reported that quercetin is a phenolic flavonoid capable of ameliorating stress, and ultimately improving health by exerting its strong antioxidant activity of scavenging free radicals. Phenolic compounds have been reported as a potential tool for improving the growth performance and reduce mortality in farm animals (Christaki *et al.*, 2020) and more specifically, they have been reported to offer an immunological effect when incorporated in the diet of laying hens especially owing to their long production cycle (Mahfuz *et al*., 2021). In addition, the role of okra in fetal development especially at the early stage of pregnancy has been elucidated by Hafeez *et al*. (2020). The authors attributed this to the high content of folic acid, vitamin A and vitamin C.

We can therefore infer that the phyto-chemicals in Okra fruit might have exerted these effects as well as some level of protection to the developing embryos and laying snails throughout the growing and breeding period. It further indicates that okra is safe and well tolerated by *Archachatina marginata* snails as phytogenic feed additive.

Conclusions

The results suggest that dietary inclusion of DOFM Increased the values for AFE, total number of clutches per treatment and total number of eggs laid per treatment indicative of improved spawning characteristics. There was significant increase in egg weight and egg morphometric characteristics with 7.5g/kg DOFM supplementation. Hatch rate and gonadosomatic index were significantly increased with 5.0g/kg DOFM supplementation. A 7.5g/kg DOFM dietary supplementation resulted in significantly shorter incubation period and increased gonad weight in addition to higher spat weight, spat morphometric characteristics and reduced mortality rate. It was therefore concluded that DOFM could be included in the diet of growing snails as phytogenic feed additive for improved reproductive performance.

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Authors' contributions

Salome Mokabe Itoe (Conceived the study, wrote the first draft and carried out data collection and analysis). Tchowan Guy Merlin (participated in the design and supervision of the field work), Ewane Divine (participated in the design, supervision of the field work, reading and correction of the manuscript). Shu Raisa Lum (participated in data collection). Ndam Lawrence Monah (Reviewed and edited the manuscript). Ambeno F.N (data analysis, interpretation of results and reading of manuscript) Pius Mbu Oben (conceptualized the study, supervised and validated the field work). Henry Defang (conceptualized the study, supervised and validated the field work).

Conflict of interest declaration

The authors declare no potential conflict of interest among them.

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