https://doi.org/10.52973/rcfcv-e34393 Revista Científica, FCV-LUZ / Vol. XXXIV, rcfcv-e34393

Comparative reproduction performance of Giant African Land Snail (*Archachatina marginata*) fed with the leguminous plant *Leucaena leucocephala*

Rendimiento reproductivo comparativo del caracol terrestre gigante africano (*Archachatina marginata*) alimentado con *Leucaena leucocephala*

Guy Merlin Tchowan[1]*, Ndum Gorreti Tufoin[1], Ferdinand Ngoula[2], Joseph Tchoumboue[2]

[1]*University of Buea, Faculty of Agriculture and Veterinary Medicine, Department of Animal Science. Buea, Cameroon.*
[2]*University of Dschang, Faculty of Agronomy and Agricultural Science, Department of Animal Science. Dschang, Cameroon.*
**Corresponding author: tchowanguymerlin@yahoo.fr*

ABSTRACT

The study was carried out between July and October 2022 at the sub-station of the Faculty of Agriculture Research and Teaching farm to assess the reproductive performance of *Archachatina marginata* fed the leguminous *Leucaena leucocephala* leaf meal as feed additive. 208 adult snails were randomly partitioned into 4 treatments and 4 repetitions of 13 comparable snails (weight and size). The treatment T_0 (control) received only the experimental diet as described while three other treatments received not only diet but, *L. leucocephala* leaf meal at 0.25%, 0.5% and 0.75% for a period of 12 weeks. In each treatment, every morning, the substrate of each breeding bucket was stirred entirely and minutely to collect the eggs to determine the laying and morphometric characteristics of the eggs. The eggs were then placed 10 cm deep in loose soil substrates until hatching to evaluate the characteristics of the fecundity. The unhatched eggs were opened, and the state of embryonic development observed to determine early embryonic mortality rate and late embryonic mortality. At the end of the experiment, animals of each replicate were sacrificed; the hemolymph was collected determine the total cholesterol and protein. The results showed that the characteristics of reproduction were affected with graded level of *L. leucocephala*. The highest number of eggs laid (17.29 ± 13.44) and the number of clutches (268.91) was recorded in animals receiving 0.5% and 0.75% *L. leucocephala* in the diet respectively while the highest value of fecundation rate (95.50 ± 8.80), hatch rate (94.55 ± 9.36), and hatchability (98.99 ± 3.15) was registered in the treatment that received 0.75%. The highest value of total protein and cholesterol was recorded in the same treatment. In conclusion 0.75% of *L. leucocephala* can be added in the diet of adult snail to increase its reproductive performance.

Key words: *Archachatina marginata*; cholesterol; hemolymph; protein; reproduction

RESUMEN

El estudio fue realizado en la subestación de la granja de la Facultad de Investigación y Docencia Agrícola para evaluar el rendimiento reproductivo del caracol terrestre gigante africano (*Archachatina marginata)* alimentados con hojas de la leguminosa *Leucaena leucocephala* en polvo como aditivo alimentario. 208 caracoles adultos fueron divididos aleatoriamente en 4 tratamientos y 4 repeticiones de 13 caracoles comparables (en peso y tamaño). El tratamiento T_0 (control) recibió solamente la dieta experimental según lo descrito mientras que los otros tres tratamientos recibieron no solamente la dieta experimental sino con hojas de *L. leucocephala* en polvo a el 0,25 %, el 0,5 % y el 0,75 % por un período de 12 semanas. En cada tratamiento, cada mañana, el sustrato de cada cubo de cría se removió entera y minuciosamente para recoger los huevos y determinar el desove y las características morfométricas de los huevos. A continuación, los huevos se colocaron a 10 cm de profundidad en sustratos de tierra suelta hasta la eclosión, para evaluar las características de la fecundidad. Los huevos no eclosionados se abrieron y se observó el estado de desarrollo embrionario para determinar la tasa de mortalidad embrionaria temprana y la tardía. Al final del experimento, se sacrificaron los animales de cada réplica; se colectó la hemolinfa para determinar colesterol total y proteínas. Los resultados mostraron que las características de la reproducción se afectaron con el nivel graduado de *L. leucocephala* El mayor número de huevos puestos (17,29 ± 13,44) y de puestas (268,91) se registraron en los animales que recibieron 0,5% de *L. leucocephala* en la dieta, mientras que el valor más alto de tasa de fecundación (95,50 ± 8,80), tasa de eclosión (94,55 ±9,36) e incubabilidad (98,99 ± 3,15) se registraron en el tratamiento que recibió 0,75%. Los más altos valores de proteína total y colesterol total se registraron en el mismo tratamiento. En conclusión 0.75% de *L. leucocephala.*

Palabras clave: *Archachatina marginata*; colesterol; hemolinfa; proteína; reproducción

Received: 19/02/2024 Accepted: 01/04/2024 Published: 28/06/2024

INTRODUCTION

Leucaena leucocephala (Fabaceae) is a vigorous drought resistant tree that contains proteins, minerals, xanthophyl's and carotene [1]. According to Agricultural and Research [2], this plant is rich in nutrients and roughage. De Angelis *et al*. [1] also noted that among leguminous vegetable sources, *L. leucocephala* plant is suitable to fill the deficiencies of other legume sources particularly the protein and amino acid point of view. *L. leucocephala* leaves have also been reported to contain secondary metabolites like flavonoid glycosides and phenols which are antioxidants and anti-inflammatory [3]. However, Leucaena leaf powder has been tested in different species and different proportions as feed supplement to determine its effect in the animal performance. Okonkwo *et al*. [4] noted that the inclusion of 15% oven dried Leucaena meal improved growth in broiler chicken ration without causing deleterious effects in animal performance. In snails, Jimoh *et al*. [5] reported that the supplementation of *Archachatina marginata* snails with Leucaena leaf meal, *Gliricidia* meal and *Moringa* meal at 16.5 g·100 g^{-1} each as protein source resulted in a standout performance of snails fed *Leucaena*-inclusive diet in terms of egg production, fecundity and hatchability compared to other leaves. But few study seeks to explore the effect of *Leucaena* leaf potential as feed additive on the reproductive performance of *A. marginata* snails.

The main objective is to contribute to assess the inclusion of graded levels of *L. leucocephala* leaf meal as natural phytogenic feed additive on the reproductive performance of the African land snail (*A. marginata*) and specifically to evaluate the effect of *L. leucocephala* on the characteristic of reproduction, gonadosomatic index and biochemical characteristics of hemolymph.

MATERIALS AND METHODS

Period and study area

This study was carried at the sub-station of the Faculty of Agriculture Research and Teaching farm, located in Molyko, Buea (Cameroon), between July and October 2022 South-West Region. Buea is located within 9°14'27.60" to 9°19'27.60" LE and 4°09' 9.72" to 4°12' 9.72" LN and on the altitude of 970 – 409 m.a.s.l. The prevailing climate in Buea is equatorial characterized by short dry season (Mid-November to mid-March) and a long rainy season (mid-March-mid November). Rainfall ranges from 2,000 to 4,000 mm per year and average relative humidity ranges from 85% to 95%. Annual average temperatures oscillate between 20°C and 29°C.

Animal Material, Housing, Plant and Experimental Diet

Animal Material and housing

A total of matured 208 *A. marginata* snails (FIG. 1) aged at least four months, weighing between 70–80 g, free of injury or breakage were purchased in Muea Market of the Buea Municipality. The snails were placed in perforated plastic buckets (30 cm in diameter and 40 cm deep each) equipped with a plastic feeder and a drinker of 5 cm in diameter. The bottom of each bucket consisted of 10 cm thick loose soil substrate previously disinfected with Virunet™, two weeks before the animals were introduced. The buckets were covered with mosquito-type netting (1 mm mesh) constituting an antileak device and then placed in a block building FIG. 2, (7 m long by 6 m wide with floor made of cement) covered with a metal sheet at room temperature and natural lightening.

FIGURE 1. African giant land adult snail *Archachatina marginata*

FIGURE 2. Snail housing of adult *Archachatina marginata*

Engine oil was sprinkled around the pens and structure to offer protection from ants and other predators. The temperature within the pens was monitored between the range 25–29°C while the relative humidity within each replicate of pens was between 80–90%.

Plant material

The leaves of *L. leucocephala* were harvested from St Anthony of Paduea Parish compound Buea Town. The leaves were dried in a hot air circulating oven (60°C for 24 h) in the Laboratory of Biochemical Sciences in the University of Buea. The dried leaves were then ground into powder using a milling machine and the power obtained packaged in a Ziploc bag and stored in an airtight tied closed plastic container.

Experimental diet

The composition and proximate characteristics of the snail diets are summarized in TABLE I. The feed was formulated weekly to avoid rancidity. The feed was stored in labeled plastic containers and classified according to the treatments. All the diets were prepared and stored at room temperature (37°C).

Experimental procedures and data collection

A total of 208 adult snails were randomly partitioned into 4 treatments (n=13 each one) and 4 repetitions of 13 comparable snails (weight and size). The Treatment T_0 (control) received only the experimental diet as described above while three other treatments received not only diet but, *L. leucocephala* leaf meal at 0.25, 0.5 and 0.75%. Diet and fresh pawpaw leaves were measured and served daily, and the left over were quantified using an electronic weighing (Standard ME Analytical Lab Balance, Mettler Toledo™ 30029077; ME54E, England)

TABLE I
Ingredients and Nutritional Values of Snail Experimental Diet

Ingredients	Value (%)
Corn	224
Wheat offal	160
Soy Beans	106
Cotton meal	134
Shell	225.30
Fishmeal	97
Palm oil	45
Vitamin Premix (2%)	8.7
Total (kg)	1,000
Nutritional values (g·DM^{-1})	
Crude protein (%)	22.00
Metabolizable Energy (kcal·kg^{-1})	2,601.77
Fat (%)	8.42
Calcium (%)	9.62
Phosphorus (%)	0.87
Lysine (%)	1.18
Methionine (%)	0.49

scale (0.05 g precision) from each treatment to determine feed and leave consumption. Every day, the rearing substrates were watered (0.5 L·substrate^{-1}) and the animals were monitored for a period of 12 weeks.

In each treatment, every morning, the substrate of each breeding bucket was stirred entirely and minutely to collect the eggs to determine the laying and morphometric characteristics of the eggs. The eggs were then placed 10 cm deep in loose soil substrates (10 cm thick in plastic jars) until hatching in order to assess the characteristics of the fecundity. The unhatched eggs were opened, and the state of embryonic development observed to determine early embryonic mortality rate and late embryonic mortality [6].

At the end of the experiment, three animals of each replicate were sacrificed; the hemolymph was removed by cardiac puncture according to the method proposed by Naresh *et al.* [7] to determine the total cholesterol and protein in the hemolymph.

Statistical Analysis

Data from the reproductive parameters and characteristics were analyzed using the Statistical Package for Social Sciences (SPSS) version 20.0 [8]. One–way analysis of variance (ANOVA) was used to compare the means and when the differences were significant, Waller's Duncan test was used to separate them at 5% level.

RESULTS AND DISCUSSION

The effect of *Leucaena leucocephala* as additive on the leave intake of African land snails

The effects of *L. leucocephala* on leave intake is illustrated in FIG. 3. We can observe that, the trend, profile and shape of the curve were comparable among treatments.

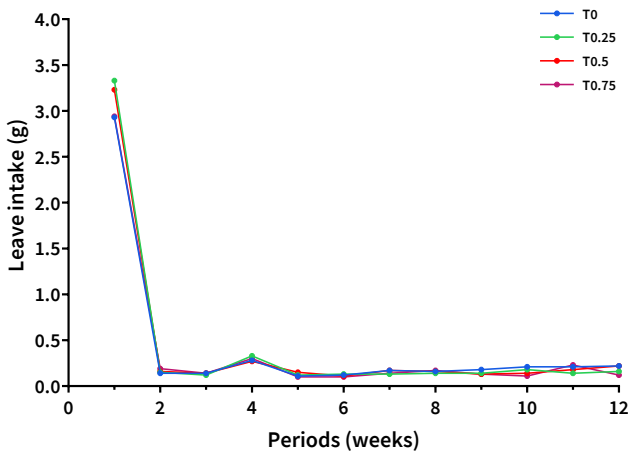

FIGURE 3. Effect of *Leucaena leucocephala* on the evolution of leaf intake of *Archachatina marginata*

When considering the period of the experiment, during the first and second period of the experiment, the leave intake decreased. However, there was no significant difference (P<0.05) among the treatments.

In contrast, the leave intake increased from the third to the fifth period of the experiment. The highest value was recorded in animals receiving 0.25% *L. leucocephala* ($T_{0.25}$) in the diet followed by animals that received 0.75% *L. leucocephala* in the diet ($T_{0.75}$). The lowest value was recorded in the control group (T_0).

At the end of the experiment, the highest value of leave intake was recorded in the treatment that received 0% and 0.5% *L. leucocephala* in the diet compared to other treatments.

The effect of *Leucaena leucocephala* on feed additive on the Feed intake of African land snails

The evolution of feed intake in relation to the proportion of *L. leucocephala* is presented in FIG. 4. Irrespective of the period of the experiment, the trend of feed intake was comparable among treatments during the period of trials.

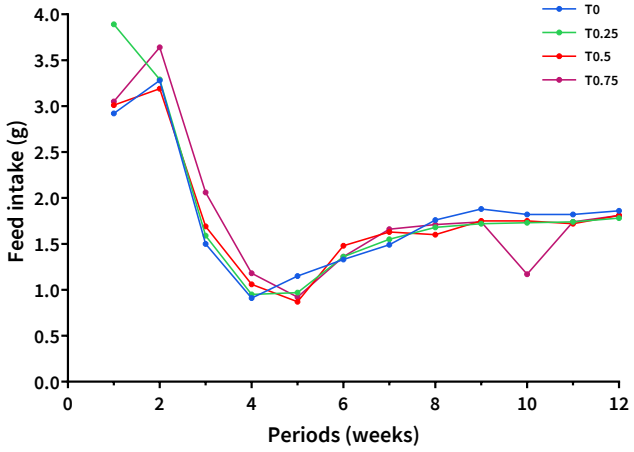

FIGURE 4. Effect of *Leucaena leucocephala* on the evolution of feed intake of *Archachatina marginata*

When was consider the period of the experiment; during the first period, the feed intake increased in treatments that received 0.5, 0.75% *L. leucocephala* in the diet and the control group (T_0) compared to the other treatments. From the second to the fourth period of the experiment, the feed intake is decreasing and comparable among the treatments. Conversely in the fifth period of the experiment, the lowest value of feed intake was recorded in treatments that received 0.75% *L. leucocephala* ($T_{0.75}$) in diet and the highest value in the control group (T_0). At the end of the experiment, the highest value of feed intake was registered in the control group (T_0) followed by animals that received 0.25% *L. leucocephala* ($T_{0.25}$).

Effects of *Leucaena leucocephala* on the characteristics of reproduction

The effect of *L. leucocephala* on the characteristics of reproduction is presented in TABLE II. We can observe that: The characteristic of the reproduction was affected by *L. leucocephala* during the period of trials.

The significantly (P<0.05) highest number of eggs laid was observed in treatments that received 0.5% *L. leucocephala* ($T_{0.5}$) in the diet, whereas, the lowest value obtained in the control group (T_0).

The highest number of eggs per clutch was registered in the control group (T_0) and the lowest in animals that received 0.25, 0.5% *L. leucocephala* ($T_{0.25}$, $T_{0.50}$). Contrary, the highest number of clutches was observed in treatments that received 0.5% *L. leucocephala* ($T_{0.5}$) in the diet and lowest in animals that received 0.25% *L. leucocephala* ($T_{0.25}$).

The highest value of egg length and egg width was obtained in the treatments that received 0.25% *L. leucocephala* ($T_{0.25}$) and the lowest value was recorded in the control group (T_0) and the treatments that received 0.75% *L. leucocephala* ($T_{0.75}$) in the diet respectively.

The incubation period was comparable among the treatments while the fecundation rate increased with increasing proportion of *L. leucocephala* in the diet compared to the control group (T_0).

In contrast, the significantly (P<0.05) highest value of hatch rates and hatchability was registered in the treatments that received 0.75% *L. leucocephala* ($T_{0.75}$) in the diet and the animals that received 0.5% *L. leucocephala* ($T_{0.5}$).

The highest value of early embryonic mortalities was obtained in the treatments that received 0.25% *L. leucocephala* ($T_{0.25}$) and the lowest value was observed in treatments that received 0.5% *L. leucocephala* ($T_{0.5}$). In contrast, the significantly (P<0.05) highest value of late embryonic mortality was obtained in the control group (T_0).

However, the significantly (P<0.05) highest breeding mortality was obtained in animals that received 0.5% *L. leucocephala* ($T_{0.5}$) in snail diet. The lowest values were recorded in animals receiving 0.25% *L. leucocephala* ($T_{0.25}$) in the diet.

Effects of *Leucaena leucocephala* on the gonadosomatic index

The effect of *L. leucocephala* on gonadosomatic index is presented in FIG. 5. We can notice that the gonadosomatic index was influenced by *L. leucocephala* during the period of feed trials.

The highest value was recorded in animals that received 0.75% *L. leucocephala* in the diet ($T_{0.75}$) and animals that received 0.25% *L. leucocephala* ($T_{0.25}$). The lowest value was recorded in the control group (T_0). However, there was no significant difference among the treatments.

Effects of *Leucaena leucocephala* in the diet on total cholesterol and total protein in the hemolymph of *Archachatina marginata*

The effect of *L. leucocephala* on the total cholesterol and protein in the hemolymph of *A. marginata* is presented in FIGS. 6 and 7.

Total cholesterol

The effects of *L. leucocephala* on the total cholesterol in the snail hemolymph is presented in FIG 6.

TABLE II
Effect of *Leucaena Leucocephala* on the Characteristics of Reproduction of Snail Eggs

Characteristics of reproduction	Treatments (% Leucaena leucocephala)			
	T_0	$T_{0.25}$	$T_{0.5}$	$T_{0.75}$
Number of eggs laid	10.10±4.18[a]	11.13±5.13[a]	17.29±13.44[b]	12.93±7.83[ab]
Number of eggs per clutch	7.18±0.31[a]	6.29±0.22[a]	6.29±0.24[a]	6.34±0.32[a]
Number of clutches	215.50	195.00	268.91	201.37
Weight eggs	1.33±1.15[a]	1.32±0.64[a]	1.29±0.62[a]	1.27±0.66[a]
Length eggs	14.21±0.95[a]	14.53±1.21[b]	14.39±1.17[ab]	14.35±1.14[ab]
Width eggs	11.39±1.22[a]	11.52±1.43[a]	11.41±1.40[a]	11.38±1.43[a]
Incubation period	25.00±0.00[a]	25.00±0.00[a]	25.00±0.00[a]	25.00±0.00[a]
Fecundation rate (%)	93.74±13.12[a]	92.66±10.01[a]	94.21±8.59[a]	95.50±8.80[a]
Hatch rate (%)	80.64±18.81[a]	82.02±16.48[a]	90.90±11.42[b]	94.55±9.36[b]
Hatchability (%)	85.70±14.93[a]	88.22±13.22[a]	96.31±6.44[b]	98.99±3.15[b]
Early embryonic	6.25±2.39	7.58±1.78	5.78±1.47	6.99±2.75
Late embryonic	13.10±2.56[a]	10.63±2.13[a]	3.308±0.97[b]	0.941±0.46b[a]
Breeding mortality	8.79±2.97[a]	8.46±2.43[a]	13.18±81.00[b]	9.89±1.41[ab]

[a, b]: mean along the same row having different superscript are significantly different (P<0.05)

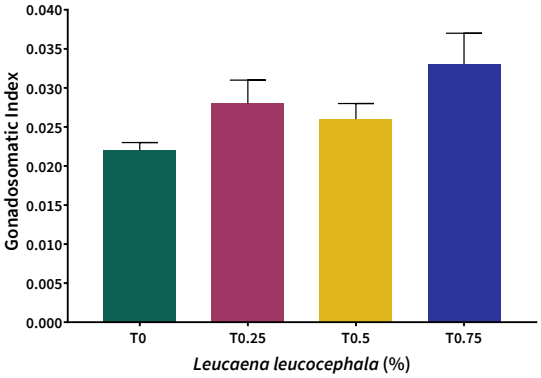

FIGURE 5. Effect of *Leucaena leucocephala* on the gonadosomatic index of *Archachatina marginata*

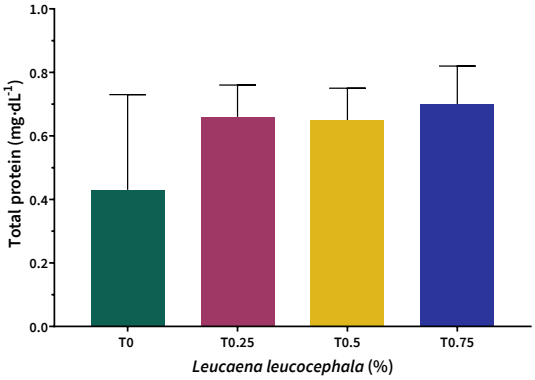

FIGURE 7. Effects of *Leucaena leucocephala* on total protein in the hemolymph of african land snails

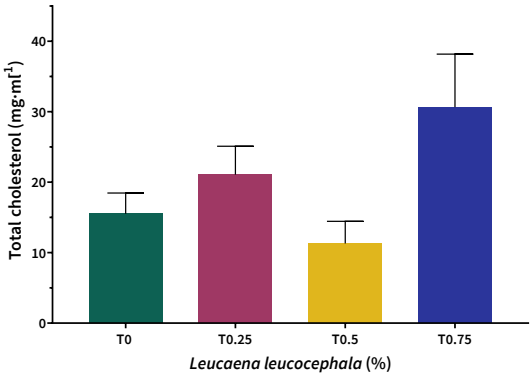

FIGURE 6. Effects of *leucaena leucocephala* on total cholesterol in the hemolymph of african land snails

The level of total cholesterol was affected by *L. leucocephala* in the diet. The significantly (P<0.05) highest value of total cholesterol was obtained in animals receiving 0.75% *L. leucocephala* ($T_{0.75}$) and animals that received 0.25% *L. leucocephala* ($T_{0.25}$) in snail diet. The lowest value was registered in animals that received 0.5% *L. leucocephala* ($T_{0.5}$).

Total protein

The effects of *L. leucocephala* on the total protein in the snail hemolymph is presented in FIG.7.

The total protein increased with increasing proportion of *L. leucocephala* in the diet. The highest value of total protein was recorded in animals that received 0.75% *L. leucocephala* ($T_{0.75}$) in the diet and the lowest value was registered in the control group (T_0).

This study was aimed at investigating the potential of *L. leucocephala* leaf meal as feed additive on the reproductive performance of the giant African land snail (*A. marginata*).

The highest value of leave intake (5.07 ± 0.22) was recorded in animals that received 0.5% Leucaena in the diet compared to other treatments. The results are similar to those of De Angelis *et al.* [1] who concluded that the inclusions of leaf-based diets in snail's (*A. marginata*) have a positive influence in terms of feed intake and survival rates. *Leucaena* leaves are legumes endowed with amino acid pattern that compares with soya beans and fish meal; minerals (Ca, K, P and vitamin K) and other digestible nutrients xanthophyl's and carotene [1]. These nutrients might have helped enhanced digestive function by stimulating digestive enzymes for nutrient transport and absorption, thereby increasing appetite [9] in the snails.

The effect of *L. leucocephala* on feed intake in relation to different proportion was highest in animals that received 0.75% *Leucaena* in the diet. These results also consistent with the reports of Abawi *et* Diambra [10] who tested the effect of *Leucaena* meal and found out that the addition of 3% *Leucaena* meal and crushed fossil coral to the diet increased feed consumption in the diet of laying hens. According to Okonkwo *et al.* [4], the inclusion of 15% roasted *Leucaena* meal improved growth in broiler chicken. Indeed, *Leucaena* leaves contain phenols which possess antioxidants properties that improve digestive function and appetite in livestock [9]. Moreover, *L. leucocephala* leaves when compared to pure grass pasture significantly increases feed intake and live-weight gain when used as a supplement during the dry seasons [11].

Regarding reproductive characteristics, the hatch rate (94.55 ± 9.36), hatchability (98.99 ± 3.15) and fecundation rate (95.50 ± 8.80) was obtained in animals that received 0.75% *L. leucocephala* in the diet. These results are like those of Jimoh *et al.* [5] in achatines who reported that the supplementation of *A. marginata* snails with *Leucaena* leaf meal, *Gliricidia* meal, *Moringa* meal at 16.5 g·100 g^{-1} as protein source resulted in a standout performance of snails fed *Leucaena*–inclusive diet in terms of egg production, fertility, hatchability, and juvenile compared to other leaves. This result suggests the beneficial ability of *Leucaena* to influence reproduction positively. Lamidi and Akilapa [12] studied different supplementary levels of *Leucaena* in the diet of rabbits and noted that 10% *L. leucocephala* inclusion in the diet enhanced reproductive performance and fecundity. *Leucaena* leaves and seed oils have been reported to contain flavonoids and phenolic compounds such as, quercetin, myricetin, kaempferol, catechin, caffeic acid which possess antimicrobial, anti-inflammatory, anthelmitic (worm repellant) and antibacterial activities against gram positive-*Bacillus subtilus* and gram negative-*Escherichia coli* [9]. These phytochemical compounds might have improved fecundity attributes by inhibiting these bacterial and fungal pathogens thereby creating a favorable environment in the soil substrate during egg incubation, hence improved hatch rates and survival rates. Highest number of eggs per clutch recorded highest values in the control group. This result could also be justified by

exogenous factors such as temperature, or water quality, may have contributed to the differences in reproductive success observed between the treatment groups.

The early (5.78 ± 1.47) and late embryo mortalities (0.941 ± 0.46) were significantly lowest at 0.5% and 0.75% *L. leucocephala* in the diet respectively. The results are also like those of Christopher *et al.* [13] who investigated the performance of adult laying *A. marginata* snails supplemented with phyto-seed meal cola and revealed a reduction in embryo mortalities with better hatching rates in the diet. The phenolic and flavonoid bioactive compounds found in *L. leucocephala* leaves possess antimicrobial mechanisms involve in the disruption of protozoal membranes, have antibacterial activities against *E. coli* and anti-inflammatory activities that might have improved animal health and reduced mortalities at these inclusion levels in the diet [14, 15]. The highest late and early embryo mortalities values were recorded in the control group (T_0) and those that received 0.25% *Leucaena* in the diet respectively. This might suggest nutritional inadequacies; there might have been limited nutrients required to sustain egg viability leading to increased embryo mortalities at these inclusion levels. The absence of *L. leucocephala* supplements in the control group (T_0) may have led to increased embryo mortality rates compared to the other treatments since diet quality remains the most important factor that influences animal performance under captivity [2].

It is also observed that, the highest values of breeding mortality (13.18 ± 81) were recorded at 0.5% *Leucaena* in the diet. This can be justified by the presence of mimosine, a non-protein amino acid in *L. leucocephala* leaves linked to deleterious effects in the animal like loss of appetite and reduced feed intake leading to stunted growth and eventual mortalities.

The animals that received highest 0.75% *L. leucocephala* in the diet recorded the highest values of gonadosomatic index. These results corroborate Jimoh and Akinola [5] who reported that *A. marginata* snails fed *L. leucocephala* inclusive diet had higher gonadosomatic index across all treatment accompanied with better reproductive output in laying snails. These suggest the immune-stimulating potentials of the plant on this organ with increased leaf proportion in the diet due to its high herb's concentration of phenolic compounds.

Regarding egg morphometrics, egg length (14.53 ± 1.21) and width (11.52 ± 1.43) was significantly (P<0.05) highest in animals receiving 0.25% *L. leucocephala* in the diet. These results are similar to those of Ahmed and Abdelati [16] in laying hens. These can be justified by the immune-stimulating potentials of the leaf's phenolic compounds on egg width and length at this supplementary level. The results could also be explained by the genetic makeup of the parent, the age of that parent and before food availability for that parent [17].

The significantly (P<0.05) highest value of total cholesterol (30.65 ± 7.53) was obtained in animals receiving 0.75% *L. leucocephala* in snail diet compared to the control group (T_0). Tchowan *et al.* [18] also noted that increased energy level in the diet resulted in a decrease in the total cholesterol level in the hemolymph of snails. This is justified by the fact that *L. leucocephala* leaves are legumes known to be high in crude protein (21.30%) and fiber (11.20%) which helped trap-bind with triglycerides and lipids thereby preventing its absorption [19]. Moreover, triglycerides and cholesterol have been reported to be the least concentrated hemolymph organic substance and hence, snails have less fat content and are good diet for patients of heart related ailments [20].

The animals that received 0.75% *L. leucocephala* recorded highest values of protein (0.70 ± 0.12) in the hemolymph. This can be explained by the fact that *L. leucocephala* leaves contains polyphenolic substances like tannins which helps decrease dry matter digestibility thereby increasing by-pass proteins that are present in the snail hemolymph. Also, among leguminous vegetable sources, *L. leucocephala* plant is suitable to fill the deficiencies of other legume sources particularly the protein and amino acid point of view for livestock which justifies its abundance in the snail hemolymph [1]. Moreover, these results can be justified by the fact that *L. leucocephala* leaves are in high crude protein of about 21.30% [19]. Saleh *et al.* [21] noted that the dietary supplementation of *Thyme* leaf in broiler chicken significantly increase total protein and globulin while Imevbore and Ademosun [22] reported that snails are good source of protein which is the most abundant substance in the gastropods' hemolymph. The snails used in study were in their adult stage which is the highest stage with highest concentration of proteins in the hemolymph than young animals.

CONCLUSION

It can be concluded from this study that feeding giant African land adult snail *A. marginata* with 0.75% inclusion level of the leguminous plant *L. leucocephala* leaf meal increased the fecundation rate, hatch rate, hatchability, and total protein in the hemolymph.

Ethics approval and consent to participate

The study was approved by the Ethical committee of the Department of Animal Science of the University of Dschang (ECDAS-UD_S 20/03/2017/UD_S/FASA/DSAES) and was in conformity with the internationally accepted standard ethical guidelines for Laboratory animal use and care as described in the European community guidelines; EEC Directive 86/609/EEC, of the 24th of November 1986.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Funding

There was no funding for this study.

Authors' contributions

Tchowan Guy Merlin designed the study and carried out the experimental protocol. He wrote the first draft. Ndum Gorreti Tufoin participates for data collection. Ngoula Ferdinand analyzed and interpreted the results. Tchoumboué Joseph conceptualized the study and supervised the field. All authors read and approved the final manuscript.

Conflict of interest statement

The authors declare that they have no conflict of interest

BIBLIOGRAPHIC REFERENCES

[1] De Angelis A, Gasco L, Parisi G, Danieli PP. A multipurpose leguminous plant for the Mediterranean countries: *Leucaena leucocephala* as an alternative protein source: a review. Animals. [Internet]. 2021; 11(8):2230. doi: https://doi.org/gmtw6f

[2] National Research Council. Leucaena: Promising Forage and Tree Crop for the Tropics. [Internet]. Washington, DC: The National Academies Press; 1977. 126 p. doi: https://doi.org/m5rp

[3] Lin L-Z, Mukhopadhyay S, Robbins RJ, Harnly JM. Identification and quantification of flavonoids of Mexican oregano (*Lippia graveolens*) by LC-DAD-ESI/MS analysis. J. Food Compos. Anal. [Internet]. 2007; 20(5):361-369. doi: https://doi.org/fqgqvw

[4] Okonkwuo AL, Isaac LJ, Ebereso AJ, Umoh BI, Usoro OO. Effects of roasted *leucaena leucocaphala* leaf metal on the performance of broiler chickens. Global J. Pure Appl. Sci. [Internet]. 2002; 8(4):477-482. doi: https://doi.org/dxqcp2

[5] Jimoh OA, Akinola MO. Reproductive performance of laying snails (*Archachatina marginata*) fed on roughages and different concentrate mixes. Bull. Natl. Res. Cent. [Internet]. 2020; 44(118). doi: https://doi.org/m5rq

[6] Dafem R, Ngoula F, Teguia A, Kenfack A, Tchoumboué J. Performances de reproduction de l'escargot géant africain (*Archachatina marginata*) en captivité au Cameroun. Tropicultura. [Internet]. 2008 [cited 12 Jan 2024]; 26(3):155-158. Available in: https://goo.su/2YahzX

[7] Naresh K, Krupanidhi S, Rajan S. Purification, spectroscopic characterization and *o*-diphenoloxidase activity of hemocyanin from a freshwater gastropod: *Pila globosa*. Protein J. [Internet]. 2013; 32(5):327-336. doi: https://doi.org/m5rr

[8] Mayers A. Introduction to statistic and SPSS in Psychology. London: Pearson; 2013. 611 p.

[9] Deivasigamani R. Phytochemical analysis of *Leucaena leucocephala* on various extracts. J. Phytopharmacol. [Internet]. 2018 [cited 17 Jan 2024]; 7(6):480-482. Available in: https://goo.su/FxaEKQF

[10] Abawi F, Diambra O. *Leucaena* leaf meal and local limestone in the diet of laying hens. Micronesica [Internet]. 1993 [cited 17 Jan 2024]; 26(2):147-153. Available in: https://goo.su/hIzpWYP

[11] Jones RJ, Lefeuvre RP, Playne MJ. Loss of dry matter, nitrogen, mineral and fiber fractions from nylon bags containing *Leucaena leucocephala* and *Calliandra* species in the rumen. Animal Feed Science and Technology. [Internet]. 1992; 37(3-4):297-307. doi: https://doi.org/dn3x73

[12] Ladimi WA, Akilapa TP. Performance Characteristics of Rabbits fed with Leucaena leaf meal based diet in Natural Housing System. Agricultural Science Research Journal. [Internet]. 2013 [cited 17 Jan 2024]; 3(5):144-151. Available in: https://goo.su/hUzRb

[13] Christopher GI, Ogundu EC, Umoren UE, Udofe MJ. Performance of Growing African Giant Snails (*Archachatina marginata*) Fed Forage Mixtures and Maize-based Concentrate Supplemented with Monkey Cola (*Cola rostrata*) Seed Meal. Asian J. Adv. Agric. Res. [Internet]. 2019; 8(4):1-7. doi: https://doi.org/m5rt

[14] Rashid MRS, Hanafy MA, Youssef MSH, Archimède H, Sallam SMA, Soltan YA, Ghoneem WMA. Chemical and *in vitro* evaluation of Leucaena (*Leucaena leucocephala*) Leaves as a Substitute of Alfalfa (*Medicago sativa* L.) with/without Rejected Green Banana Fruits (*Musa paradisiaca*). World Vet. J. [Internet]. 2021; 11(4):685-697. doi: https://doi.org/m5rv

[15] Xu Y, Tao Z, Jin Y, Yuan Y, Dong TTX, Tsim KWK, Zhou Z. Flavonoids, a potential new insight of *Leucaena leucocephala* foliage in ruminant health. J. Agric. Food Chem. [Internet]. 2018; 66(29):7616-7626. doi: https://doi.org/gd3jzk

[16] Ahmed ME, Abdelati KA. Effect of dietary graded levels of *Leucaena leucocephala* seeds on layers performance, egg quality and blood parameters. Int. J. Poult. Sci. [Internet]. 2009; 8(5):475-479. doi: https://doi.org/bgdfjx

[18] Tchowan GM, Toukala JP, Ngoula F, Tchoumboué J. Evaluation of the Reproductive Performance, Body Proportions, Nutritional Value, and Biochemical Parameters of *Achatina achatina*. Int. J. Zool. [Internet]. 2022; 2022(3945163):1-11. doi: https://doi.org/m5rx

[19] Eniolorunda OO. Evaluation of biscuit waste meal and *Leucaena leucocephala* leaf hay as sources of protein and energy for fattening "yankassa" rams. African Journal of Food Science. [Internet]. 2011 [cited 17 Jan 2024]; 5(2):57-62. Available in: https://goo.su/qL3Z9f

[20] Ademolu KO, Idowu AB Mafiana CF, Osinowo OA. Performance, proximate and mineral analyses of African giant land snail (*Archachatina marginata*) fed different nitrogen sources. Afr. J. Biotechnol. [Internet]. 2004; 3(8):412-417. doi: https://doi.org/m5rz

[21] Saleh N, Allam T, El-Latif AA, Ghazy E. The effects of dietary supplementation of different levels of thyme (*Thymus vulgaris*) and ginger (*Zingiber officinale*) essential oils on performance, hematological, biochemical and immunological parameters of broiler chickens. Glob. Vet. [Internet]. 2014 [cited 18 Jan 2024]; 12(6):736-744. Available in: https://goo.su/Ph0r

[22] Imevbore EA, Ademosun AA. The nutritive value of the African giant land snail, *Archachatina marginata*. J. Anim. Prod. Res. [Internet]. 1988 [cited 17 Jan 2024]; 8(2):76-87. Available in: https://goo.su/HzcH6f