ANIMAL SCIENCE AND GENETICS
Published by the Polish Society of Animal Production
vol. 19 (2023), no 4, 3-16
DOI: 10.5604/01.3001.0053.9649

Research Article ***Open Access***

Improving the reproduction and gonadosomatic index of *Archachatina marginata* using a medicinal plant (*Thymus vulgaris*) in the Equatorial Zone of Cameroon

Guy Merlin Tchowan[1#], Jude Jua Jam[1], Ferdinand Ngoula[2], Joseph Tchoumboue[2]

[1]University of Buea, Faculty of Agriculture and Veterinary Medicine, Department of Animal Science, P.O. Box 63, Buea, Cameroon
[2]University of Dschang, Faculty of Agronomy and Agricultural Science, Department of Animal Science, P.O. Box 222, Dschang, Cameroon

SUMMARY

A study was carried out to assess the reproduction and gonadosomatic index of *Archachatina marginata* fed with a medicinal plant (*Thymus vulgaris*). Three hundred adult snails were randomly allocated to four treatments and five replicates of 15 comparable snails. Treatment T_0 (control) received only the basal diet. The other treatments ($T_{0.25}$, $T_{0.5}$, and $T_{0.75}$) received, in addition to the basal diet, *Thymus vulgaris* powder at 0.25%, 0.5% and 0.75%, respectively. The results showed the lowest egg weight (1.33 ± 0.66) in animals receiving 0.5% *Thymus vulgaris* in the diet compared to the other treatments, while the lowest egg length (14.68 ± 1.14) was recorded in the control group. The number of clutches per treatment was lowest (16.25 ± 0.00) in animals that received 0.5% *Thymus vulgaris* in the diet compared to the control group and other treatments. The hatching and fertilization rates increased with the share of *Thymus vulgaris* in the diet, while the reverse was observed for the mortality rate. The highest gonadosomatic index (0.02±0.00) was recorded in animals receiving the highest level of *Thymus vulgaris* ($T_{0.75}$), followed by animals in the control group (T_0). Total protein and triglycerides increased significantly ($p < 0.05$) with increasing *Thymus vulgaris*. In contrast, total cholesterol decreased significantly ($p < 0.05$) as the level of *Thymus vulgaris* in the diet was increased. In conclusion, *Thymus vulgaris* at 0.75% can be used in the diet of breeding snails.

KEY WORDS: ***Archachatina marginata*, gonadosomatic index, *Thymus vulgaris*, Cameroon**

#Corresponding author e-mail: tchowanguymerlin@yahoo.fr
Received: 09.09.2023 Received in revised form: 02.10.2023
Accepted: 28.09.2023 Published online: 15.10.2023

INTRODUCTION

For many decades, studies have focused on using medicinal plants to improve the reproductive performance of animals. The goal is to stop the use of antibiotics and hormones as growth promoters in animal feed due to their harmful effects on human health, resulting from the accumulation of residues in animal products (Castanon, 2007). Phytobiotics represent a wide range of bioactive compounds that can be extracted from a variety of plant sources (Vidanarachchi et al., 2005), having various beneficial effects on animals, such as antioxidant, antimicrobial, anti-carcinogenic, analgesic, insecticidal, and anti-parasitic effects, promotion of growth and fertility, appetite enhancement, stimulation of bile secretion and digestive enzyme activity, and hepatoprotection (Kasiri et al., 2011). The advantages of phytobiotics include their effectiveness, availability, accessibility, low toxicity, and low cost (Molla et al., 2012). The Cameroonian pharmacopoeia includes a wide variety of medicinal plants. One of these is thyme (*Thymus vulgaris*) (Ngene et al., 2015), which belongs to the family Lamiaceae (Dorman and Deans, 2000). Thyme has potent antimicrobial and antioxidant activity due to its very high contents of thymol (40%), carvacrol (15%), cymene, eugenol and 4-allylphenol (Rota et al., 2008), owing to which it can be used instead of commercial antibiotics (Dorman and Deans, 2000; Dorojan et al., 2015). It is used in the pharmaceutical and cosmetic industries (Jordán et al., 2006) and in traditional medicine (ADWAN et al., 2006). Infusions of thyme leaves can treat lung infections and improve digestibility, weight gain, feed conversion (Wade et al., 2018), growth performance and carcass characteristics in broilers (Navid and Mahmoud, 2011; Vakili and Heravi, 2016). *Thymus* species are also reported to have beneficial effects on reproductive parameters. Shanoon and Jassim (Shanoon and Jassim, 2012) showed that adding aqueous extracts of thyme significantly increased semen ejaculate volume, the number of sperm in the ejaculate, and mass and individual sperm motility in male broiler breeders. Likewise, Shanon and Jassim (2012) demonstrated that ethanolic extracts of thyme have protective effects on testosterone secretion and sperm quality in mice infected with *Toxoplasma gondii*. In addition, thyme essential oil is reported to increase total serum protein levels (Zhu et al., 2014) and to reduce serum triglyceride, glucose and total cholesterol levels (Khaksar et al., 2012). However, few studies have investigated the effect of including thyme powder in the diet of snails on their reproductive parameters.

The general objective of the study is to contribute to knowledge of *Thymus vulgaris*, and specifically to evaluate the effect of the inclusion of *Thymus vulgaris* powder in the diet of the Giant African land snail *Archachatina marginata* on the reproductive parameters of these snails.

MATERIALS AND METHODS

Study area

The study was conducted between July 2022 and October 2022 at the snail farm of the University of Buea, Southwest Region (N: 4°12'–4°25', E: 9°19'–9°20') at an altitude of 870–4,095 m. The climate is equatorial, with a short dry season and a long rainy season. Rainfall ranges from 2,000 to 4,000 mm per year, relative humidity from 85% to 95%, and temperatures between 20 and 29°C.

Animal material, housing, plant material and experimental diet

Animal material and housing

A total of 300 adult snails (Fig. 1) purchased in various markets of the city of Buea in Cameroon were used in the study, weighing between 60 and 80 g, with shell length and diameter of 2.80–3 cm and 0.5–1 cm, respectively, and free of injury or breakage. The snails were placed in 20 cages made of cement blocks (1x1x1 m) at a density of 15 snails per square metre, each equipped with a feeder and a plastic drinker 5 cm in diameter. The bottom of each cage was covered with 10 cm of loose soil substrate previously disinfected with Virunet (0.1 g/0.5 l/substrate) two weeks before the animals were placed in the cages. The cages were covered with mosquito netting (1 mm mesh) as an anti-leak device, then placed in a cinder block building (4 m long by 3.5 m wide with a cement floor) covered with thatch mats, at room temperature (25°C) and natural lighting (12 hours of light and 12 hours of darkness). The acclimatization period for the snails was one month.

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

Figure 1. Adult snail

Plant material

The plant material (Fig. 2) consisted of *Thymus vulgaris* powder. The root of *Thymus vulgaris* was harvested from Buea and then cleaned and dried in the Laboratory at room temperature for seven days. Then it was ground, and the resulting powder was packaged in previously labelled plastic bags and stored in a tightly closed plastic jar.

Figure 2. *Thymus vulgaris*

Experimental diet

The snails were given *ad libitum* water and an experimental diet whose percentage composition and calculated nutritional characteristics are shown in Table 1. The feed was stored in a tightly closed plastic bucket.

Table 1.
Percentage composition and nutritional value of the basal diet

Ingredient and nutritional value	Quantity (kg)
Ingredient	
Maize flour	15.50
Wheat offal	8.50
Cotton meal	16.00
Soybean meal	16.50
Fish meal	10.50
Shell	23.50
Palm oil	9.50
Total (%)	100.00
Calculated nutritional value	
Crude protein (%)	24.32
Metabolizable energy (kcal/kg)	2812.45
Energy-to-protein ratio	115.64
Fat (%)	13.04
Calcium (%)	10.07
Phosphorus (%)	0.89
Phosphocalcic ratio (%)	0.08
Lysine (%)	1.32
Methionine (%)	0.52

Experiment and data collection

A total of 300 adult snails (*Archachatina marginata*) were randomly allocated to four treatments (T_1–T_4) and five replicates of 15 snails (comparable in weight and size). Treatment T_1 (controls) received only the basal diet (Table 1). The other treatments (T_2–T_4) received, in addition to the basal diet, *Thymus vulgaris* powder at 0.25%, 0.5% and 0.75%, respectively. Snails in each treatment received fresh pawpaw leaves (60 g), withered for 24 hours, as a staple diet. The breeding substrate was watered with 0.5 L of water every day, and the animals were monitored for three months.

Every morning, the substrate of each breeding cage was stirred thoroughly and carefully to collect the eggs for determination of laying characteristics and the morphometric characteristics of the eggs. The eggs were then placed 4 cm deep in a soft soil substrate (5 cm thick in plastic jars) until hatching to assess fertility parameters. Unhatched eggs were broken, and the state of embryonic development (Dafem et al., 2008) was observed to determine the early and late embryonic mortality rates. At the end of the experiment, the animals were sacrificed, and the haemolymph was removed by cardiac puncture (Naresh et al., 2013) to determine biochemical characteristics. The gonad was collected to determine the gonadosomatic index.

Parameters and characteristics analysed

Reproductive parameters and characteristics

The following reproductive characteristics were considered:

- Average number of eggs per clutch
- Number of clutches per treatment
- Egg weight (g)
- Egg length (mm)
- Egg diameter (mm)
- Gonad (ovotestis) weight
- Incubation period = time taken (days) for eggs to hatch
- Fertilization rate = (number of embryonated eggs / number of eggs laid) x 100 (Dafem et al., 2008).
- Hatching rate = (number of eggs hatched / number of eggs laid) x 100
- Average newly hatched rate = (mean number of newly hatched / mean number of hatched eggs) x 100
- Early embryonic mortality rate = (number of eggs with a dead embryo without shell/number of eggs laid) x 100
- Late embryonic mortality rate = (number of eggs with a dead embryo with shell / number of eggs laid) x 100

Gonadosomatic index (GSI)

GSI = Weight of gonad / weight of animal

Biochemical characteristics

- The total cholesterol level in the haemolymph was measured by the CHOD-POD enzymatic colorimetric method according to the instructions for the SGM Italia commercial kit (Ref: 10028 - 4 x 100 ml).
- The Biuret colorimetric method was used to determine the total protein level of the haemolymph according to the instructions for the CHRONOLAB commercial kit (Ref: 101-0240).
- The triglyceride level in the haemolymph was determined by the GPO-POD colorimetric method (Ref: 101-0241).

Statistical analysis

Analysis of variance (ANOVA) was used to compare means when the differences were significant; Duncan's test was used to separate them at the 5% level. The statistical model was as follows:

$$y_{ik} = \mu + \alpha_i + \varepsilon_{ik}$$

where:

y_{ik} = observation
μ = mean effect
α_i = effect of treatment i
ε_{ik} = residual effect

RESULTS

Effect of *Thymus vulgaris* on the reproduction of the Giant African land snail

The effect of *Thymus vulgaris* on the reproduction of Giant African land snails is presented in Table 2.

The egg weights and egg lengths were comparable among the treatments. The lowest egg weight was registered in animals receiving 0.5% *Thymus vulgaris* in the diet compared to other treatments, while the lowest egg length was recorded in the control group. No significant difference was observed among the treatments.

The egg diameter increased significantly in the treatments that received *Thymus vulgaris* in the diet compared to the control group. The highest value was registered in animals receiving 0.5% *Thymus vulgaris* in the diet.

The significantly ($p < 0.05$) lowest number of eggs per clutch was recorded in the treatment that received the highest percentage of *Thymus vulgaris* ($T_{0.75}$) in the diet compared to other treatments.

The lowest number of clutches per treatment was registered in animals that received 0.5% *Thymus vulgaris* in the diet compared to the control group and other treatments.

The highest hatching and fertilization rates were observed in the treatment receiving the highest level ($T_{0.75}$) of *Thymus vulgaris* in the diet. The lowest values were noted in the control group. However, no significant difference was observed among the treatments.

Table 2.
Effect of *Thymus vulgaris* on the reproduction of snails

Reproductive characteristics	**Treatment (%)**			
	T_0	**$T_{0.25}$**	**$T_{0.5}$**	**$T_{0.75}$**
Egg weight (g)	1.38 ± 0.87^a	1.35 ± 0.68^a	1.33 ± 0.66^a	1.39 ± 0.71^a
Egg length (mm)	14.68 ± 1.14^a	15.06 ± 6.01^a	14.73 ± 0.96^a	14.89 ± 1.05^a
Egg diameter (mm)	11.30 ± 1.14^a	11.42 ± 0.83^{ab}	11.62 ± 3.62^b	11.52 ± 0.82^b
Number of eggs per clutch	6.95 ± 1.52^a	7.66 ± 2.81^a	7.14 ± 2.15^a	6.87 ± 0.82^b
Number of clutches	18.00 ± 0.00^a	17.50 ± 0.00^a	16.25 ± 0.00^a	16.50 ± 0.00^a
Incubation period	25.00 ± 0.00^a	23.00 ± 0.00^a	23.00 ± 0.00^a	23.00 ± 0.00^a
Hatching rate	92.81 ± 15.5^a	95.84 ± 33.12^a	96.15 ± 7.41^a	96.79 ± 66.48^a
Fertilization rate	93.77 ± 5.50^a	96.76 ± 32.78^a	97.02 ± 6.59^a	98.79 ± 66.19^a
Newly hatched rate	100.00 ± 0.00^a	100.00 ± 0.00^a	100.00 ± 0.00^a	100.00 ± 0.00^a
Early embryonic mortality	8.84 ± 2.41^a	4.41 ± 0.97^b	4.32 ± 1.2^b	2.73 ± 0.88^b
Late embryonic mortality	1.88 ± 0.65^a	0.95 ± 0.40^a	0.90 ± 0.39^a	0.84 ± 0.36^a

[a,b] Means in the same row with different letters are significantly different ($P < 0.05$)

The newly hatched rates were comparable among the treatments.

The significantly highest early and late embryonic mortality rates were registered in the control treatment (T_0).

Effect of *Thymus vulgaris* on the gonadosomatic index of *Archachatina marginata*

The effect of *Thymus vulgaris* on the gonadosomatic index of *Archachatina marginata* is illustrated in Figure 3. The gonadosomatic index can be seen to increase with the level of *Thymus vulgaris* in the diet.

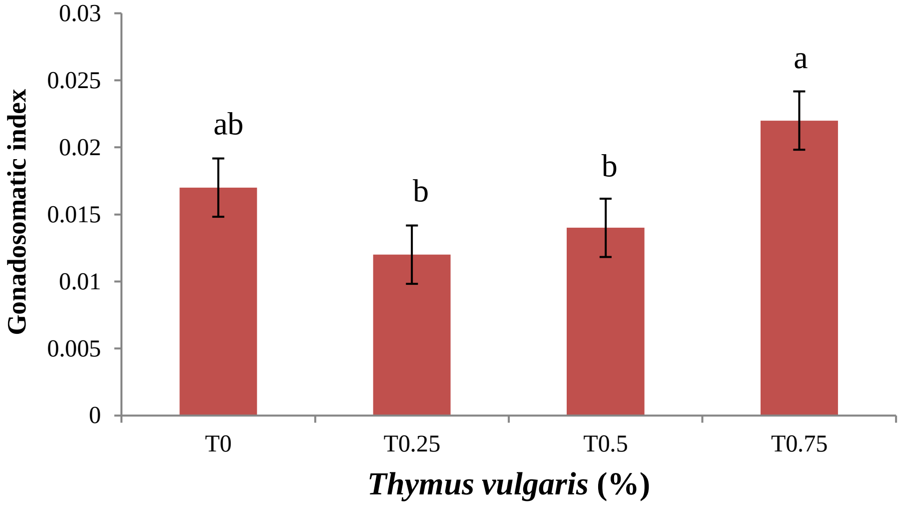

Figure 3. Effect of *Thymus vulgaris* on the gonadosomatic index

The highest gonadosomatic index was recorded in snails receiving the highest level of *Thymus vulgaris* ($T_{0.75}$) in the diet, followed by the animals in treatment T_0. The lowest value was registered in the treatment that received 0.25% *Thymus vulgaris* ($T_{0.25}$) in the diet.

Effect of *Thymus vulgaris* on triglyceride and total protein content in the haemolymph

The effect of *Thymus vulgaris* on the triglyceride (Fig. 4a) and total protein (Fig. 4b) content in the haemolymph is illustrated in Figure 4.

The triglyceride (Fig. 4a) and total protein (Fig. 4b) content can be seen to increase with the level of *Thymus vulgaris* in the diet. The highest value ($p < 0.05$) was registered in animals that received 0.75% *Thymus vulgaris*, followed by $T_{0.5}$. The lowest value was recorded in the control group.

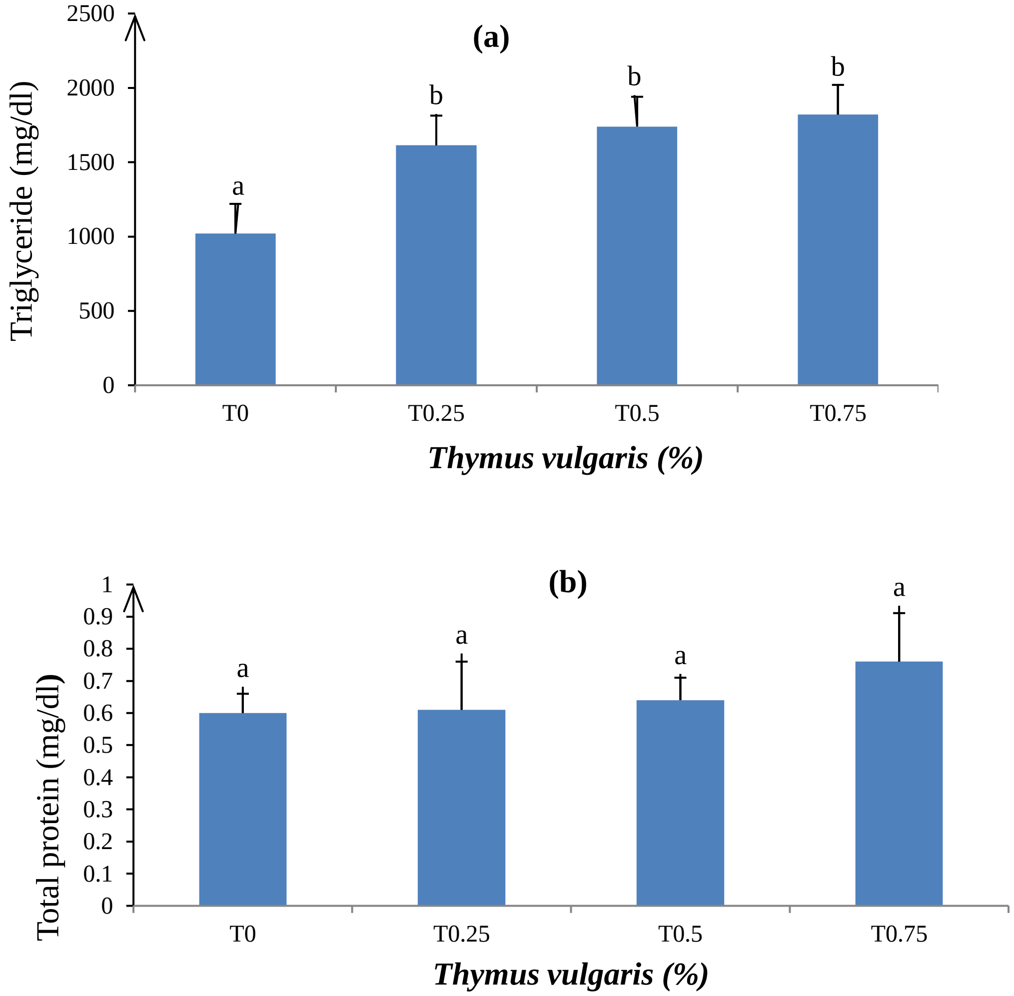

Figure 4. Effect of *Thymus vulgaris* on triglyceride (a) and total protein (b) content in the haemolymph

Effect of *Thymus vulgaris* on total cholesterol in the haemolymph

The effect of *Thymus vulgaris* on the total cholesterol level in the haemolymph is presented in Figure 5. The total cholesterol level can be seen to decrease as the level of *Thymus vulgaris* in the diet decreased.

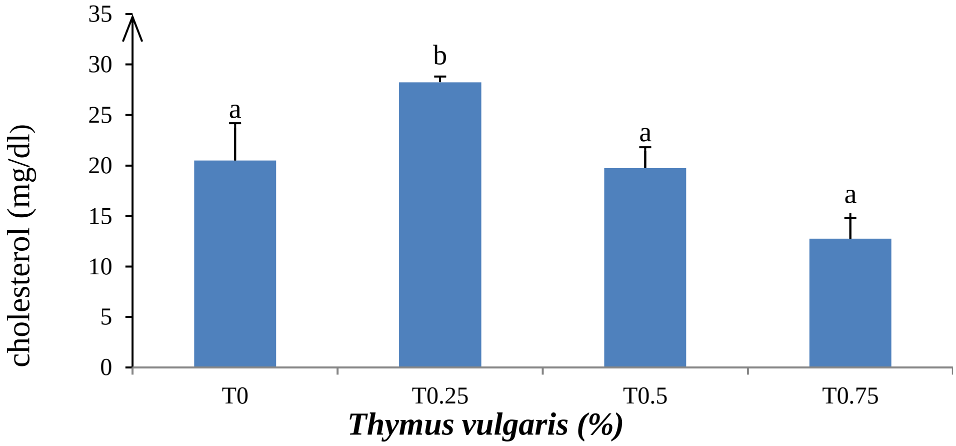

Figure 5. Effect of *Thymus vulgaris* on total cholesterol in the haemolymph

Significantly the highest ($p < 0.05$) cholesterol value was registered in the treatment that received 0.25%, followed by T_0 and $T_{0.5}$. The lowest value was obtained in the treatment that received 0.75% *Thymus vulgaris* in the diet.

DISCUSSION

Egg weight increased in the treatments that received *Thymus vulgaris* in the diet compared to the control, which did not receive the plant. The highest value (1.39 ± 0.71 g) was recorded in the treatment with the highest proportion of *Thymus vulgaris* ($T_{0.75}$). Our results corroborate those reported by Yalcin et al. (2020) and Park et al. (2012), who demonstrated that increasing *Thymus vulgaris* in the diet of laying hens increases egg weight. The value obtained in the present study was significantly higher than those obtained reported by Tchowan et al. (2022a), who evaluated the reproductive performance of *Achatina achatina*, but lower than those recorded by Tchowan et al. (2022b) in *Archachatina marginata*. Still, it remains within the range of egg weight values for land snails given by Cobbinah et al. (2008). Our result also showed the highest number of eggs per clutch in animals receiving 0.25% *Thymus vulgaris* in the diet. The result aligns with those obtained by Bölükbaşı et al. (2010) and Manafi et al. (2016), who indicated that dietary supplementation with mixed essential oils and thyme oil in laying hens increased egg production. Similarly, Ali et al. (2007) observed that thyme supplementation improved egg production and the feed conversion ratio. In contrast, other authors (Arpášová et al., 2015; Cufadar, 2018) reported that egg production, egg weight and feed conversion ratio were not affected when the diets of laying hens were supplemented with thyme leaves and essential thyme oil. Our results could be attributed to improvement in digestion and nutrient absorption and the overall health of the digestive system. The active ingredient in thyme (thymol) may have enhanced the digestive enzymes, resulting in better nutrient digestibility and enhanced egg production, and thereby improving egg weight, length and diameter in snails receiving *Thymus vulgaris* in the diet. Research carried out by Mohammed et al. (2022) supports these hypotheses. In contrast, Ding et al. (2017) reported no significant difference in egg production or egg weight in hens fed thyme. The differences in results observed by various authors could be due to the type and variety of thyme, the composition of the basal diet, environmental conditions, and the utilization of thyme in snails.

The highest hatching and fertilization rates were recorded in the treatment that received the highest percentage ($T_{0.75}$) of *Thymus vulgaris* in the diet compared to other treatments. These results are similar to those observed by Tchowan et al. (2022a) in *Achatina achatina* and Radwan Nadia et al. (2008), who found that fertility was improved in hens fed a diet containing 0.5 % and 1% thyme. Ali et al. (2007) also reported that adding 0.25% thyme to diets for hens improved their fertility. Similar observations were made by Toukala et al. (2020) in laying Kabir chickens in Cameroon. The highest hatching rate in the treatment receiving 0.75% *Thymus vulgaris* could be explained by the high egg weight. Indeed, Iqbal et al. (2016) demonstrated a relationship between egg size and hatchability; as egg size increases, the ratio between its surface area and volume decreases, which makes gas and heat exchange less efficient. Codjia and Noumonvi (Codjia and Noumonvi, 2002) reported that small-sized eggs hatch earlier than large ones but with a lower hatching rate. Our result could also be explained by the fact that thyme contains antioxidant compounds, which may reduce oxidative stress by inhibiting lipid oxidation, thus decreasing the sources of radicals passing to eggs (Radwan Nadia et al., 2008). The highest fertilization rate observed in this treatment could also be due to the ability of antioxidant activity to improve semen characteristics, as reported by several authors (Toukala et al., 2020).

The mortality rate decreased as the level of *Thymus vulgaris* in the diet increased. The lowest value was registered in animals receiving 0.75%. This result could be attributed to various properties of the plant.

The gonadosomatic index increased with the level of *Thymus vulgaris* in the diet. This result could be explained by the excellent utilization of plants in the diet, which may have increased the body weight of the snails and thus the weight of organs. Results reported by Tchowan et al. (2022b) for *Archachatina marginata* support this hypothesis.

The cholesterol level in the haemolymph decreased with increasing levels of *Thymus vulgaris* in the diet. The results are in agreement with Mohammed et al. (2022). Our result could be explained by the presence of thymol and carvacrol, the active components of thyme. These components may have reduced the liver activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase, a key enzyme in cholesterol synthesis, as reported by Abdulkarimi (2011) and by Vakili and Heravi (2016), thereby reducing the cholesterol level in the haemolymph.

The protein level also increases with the level of *Thymus vulgaris* ($T_{0.75}$) in the diet. The results are in agreement with those reported by Yalcin et al. (2020). The result could be explained by the presence of an active component in the plant. Thyme possesses some hypocholesterolemic and antilipidemic properties (Abdulkarimi et al., 2011) due to the action of its active component carvacrol on hydroxymethylglutaryl-CoA reductase, which could reduce the absorption of fat from the gut or lipid catabolism for gluconeogenesis. The same observations have been made by some authors (Khan et al., 2012) in poultry, who showed that thyme decreases total cholesterol and triglycerides in poultry. In contrast, the triglyceride level in the haemolymph increased with increasing proportions of *Thymus vulgaris* in the diet. The differences in the results could be due to the differences in the animal species used. Our result could also be due to the fact that the increase in lipid digestibility due to higher secretion of bile and digestive enzymes might have resulted in low haemolymph cholesterol, thus improving nutrient supply and transport (Yalcin et al., 2020).

CONCLUSION

The study showed that *Thymus vulgaris* positively affected the reproduction characteristics of snails *Archachatina marginata*. In addition, the highest gonadosomatic index was recorded in animals receiving the highest proportion of *Thymus vulgaris* in the diet. *Thymus vulgaris* at 0.75% can be used in the diet of breeding snails.

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