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## Enhancing Giant African Land Snail (Archachatina marginata) production with Thymus vulgaris (Lamiaceae) in the south-west region of Cameroon

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**Abstract.** This study was designed to determine the effect of *Thymus vulgaris* leaf meal on the production of *Archachatina marginata*. Two hundred and twenty-four adult snails were divided into four treatments of 14 snails and four replicates. Treatment  $T_0$  received only the experimental diet while three other treatments received diets with different inclusion levels of *T. vulgaris* leaf meal at 0.25%, 0.50%, and 0.75%. Feed and leftovers were quantified daily using an electronic weighing scale. The results obtained show that feed intake was significantly higher (P < 0.05) in animals receiving 0.75% *T. vulgaris*, compared to other treatments. Likewise, the values of cardiac and pedal mass were higher in animals receiving 0.25% inclusion of *T. vulgaris* in their diet. Low relative weight variations in the internal organs across treatments were observed but significant differences occurred regarding only the shell weight and ovotestis. The results obtained from the proximate analysis of snail flesh show that dry matter was significantly (P < 0.05) higher in animals receiving 0.25% *T. vulgaris* in their diet. Minerals analyzed were also significantly affected (P < 0.05) by the inclusion of *T. vulgaris* in the diet. In conclusion, incorporation of *T. vulgaris* leaf powder at 0.25% can be recommended in the diet of snails.

Keywords: Archachatina marginata, growth, nutrition value, Thymus vulgari

### Melhoria da produção do Caracol Gigante da África Ocidental (Archachatina marginata) com Thymus vulgaris (Lamiaceae) na região sudoeste de Camarões.

**Resumo.** O estudo foi conduzido para avaliar o efeito da farinha de folhas de *Thymus vulgaris* (tomilho) na produção de *Archachatina marginata* (caracol gigante da África Ocidental). Duzentos e vinte e quatro caramujos adultos foram divididos em quatro tratamentos de 14 indivíduos e quatro repetições. O tratamento  $T_0$  recebeu apenas a dieta experimental, enquanto os outros três receberam a dieta com diferentes níveis de inclusão de farinha de folhas de *Thymus vulgaris* a 0,25%, 0,50% e 0,75%. A ração e sobras foram quantificadas diariamente usando uma balança eletrônica. Os resultados obtidos mostraram que o consumo de ração foi significativamente maior (P < 0,05) nos animais que receberam 0,75% de *Thymus vulgaris* em comparação com os demais tratamentos. Da mesma forma, os valores do peso do coração e da massa do pé foram maiores nos animais que receberam inclusões de 0,25% de *Thymus vulgaris* na dieta. Foram observadas baixas variações no peso relativo dos órgãos internos entre os tratamentos, mas ocorreram diferenças significativas apenas em relação ao peso da concha e o oviduto. A análise proximal da carne de caracol mostrou que a matéria seca foi significativamente (P < 0,05) mais alta nos animais que receberam 0,25% de *Thymus vulgaris* na dieta. Os minerais analisados também

foram significativamente (P < 0,05) afetados pela inclusão de *Thymus vulgaris* na dieta. Em conclusão, a incorporação de farinha de folhas de *Thymus vulgaris* a 0,25% pode ser recomendada na alimentação de caramujos.

Palavras-chave: Archachatina marginata, crescimento, valor nutricional, Thymus vulgaris

#### Introduction

The increasing demand for animal protein in the developing country has not only been on the rise, but has far surpassed supplies (Gandhi & Zhou, 2014). The low level of animal protein production and the rapid increase in the cost of animal protein source have put protein of animal origin out of the reach of an average African in general and Cameroonian in particular (Ani & Ugwuowo, 2011). Thus, the low intake of animal protein in the diet of many people demands that efforts should be directed to the rearing of animals that are highly prolific and highly desirable, especially with a rapid turnover rate at a very low cost. (Christiana et al., 2015) observed that there is also a need to diversify livestock production with an emphasis on micro livestock to find the solution to the problem of acute shortage of animal protein in the diets of man. Unfortunately, a rich alternative source of animal protein that has been silent for years in Africa, particularly in Cameroon is non-conventional species among other snails (Adia et al., 2018). Snail flesh is tasty, tender, and nutritious and contains almost all the amino acids required by humans (Nwogor, 2015). Snail flesh compares favorably with other conventional sources of animal protein like beef, pork, and poultry meat (Tchowan et al., 2022). Developing the snail farm is the modern means of bridging the protein deficiency gap presently prevailing in many countries (Christiana et al., 2015). In order to make snail supply sufficient, its domestication is very vital to supplement the conventional method of picking snails from the wild. (Abiona et al., 2013) state that growth rate and reproduction are two major challenges related to snail domestication especially when commercial production is the purpose. The same author reports that the feeding type given to the snails greatly influences the growth rate and reproductive apparatus of snails (Abiona et al., 2013). To meet the production target, therefore, Abiona et al. (2013) observed that it is paramount to source feed rich in substances that could promote growth and reproductive activity. Farmers often use antibiotics to improve the production performance of the species and hormones to stimulate growth (Castanon, 2017). Unfortunately, the international feed industry is facing the challenge of awareness among consumers of meat on the risk of bringing about antibiotic resistance in pathogenic microbiota through antibiotics used in animals (Madhupriya et al., 2018). There is a need for sourcing available, accessible, cost-effective, and efficacious alternatives. One of the possible alternative sources of this is Thymus vulgaris. Thymus vulgaris contains many active principles known to have disease-preventing and health-promoting properties (Kuete, 2017; Reddy et al., 1998).

These include thymol, and carvacrol, which have been reported to have antibacterial (Yakhlef et al., 2011), antifungal (Katooli et al., 2012), anti-coccidian (Abou-Elkhair et al., 2014), antiviral and antioxidants properties (Cerda et al., 2013; El Bouzidi et al., 2013; Ozen et al., 2011; Sarikurkcu et al., 2010). It has also been shown to have several health benefits, including its ability to boost the immune system and improve digestion (Toukala et al., 2020). In animal nutrition, thyme extracts have been shown to improve growth performance, feed efficiency, and immune response in poultry and fish (Ndzi et al., 2016). Its phenolic compounds act as appetite stimulants and increased feed intake as well as the secretion of endogenous digestive enzymes and strengthen the immune system when added to poultry feed (Gumus & Gelen, 2023). Its aqueous extracts improve some reproductive parameters such as ejaculate volume, sperm concentration, and testes weights in broilers (Shanoon & Jassim, 2012). However, information on the use of *T. vulgaris* as a natural phytogenic feed additive in snail production is scarce, having highlighted the acclaimed nutritional importance of T. vulgaris, it is imperative that this be investigated. This research project aimed at promoting a better knowledge of T. vulgaris leaf powder as a feed additive on the production performance of African Land Snails (Archachatina marginata), specifically to assess the effect of T. vulgaris (Lamiaceae) leaf meal as a natural phytogenic feed additive on the growth performance, and proximate composition of the flesh of Giant African Land Snail.

#### Materials and methods

#### Period and study area

This study was carried out between June 2022 and October 2022 at the sub-station of the Faculty of Agriculture Research and Teaching farm, located in Molyko, Buea, South-West Region. Buea lies within Longitude 9°14'27.60" to 9°19'27.60" E and Latitude 4°09' 9.72" to 4°12' 9.72 North and on an altitude of 970-4095 m above sea level. Buea is characterized by a typical climate with two main seasons (the dry season ranging from November to mid-March and the rainy season prevailing from mid-March to October). The annual rainfall is 3000-5000 mm, temperature ranges from 20-30 °C, and relative humidity is 85-95%. Wind speed ranges from 7 to 11 km/hr, with rainforest vegetation and volcanic soils.

#### Experimental diet

The ingredients and nutritional values of the snail diets are summarized in <u>table 1</u>. The feed was formulated weekly to avoid rancidity. The feed was stored in labelled plastic containers and classified according to the treatments at room temperature.

Table 1. Ingredients and nutritional values of snail diet

Food ingredients	%
Corn	22.40
Wheat offal	16.00
Soybean	10.60
Cotton meal	13.40
Shell	22.53
Fishmeal	9.70
Palm oil	4.50
Vitamin premix	0.87
Total	100.00
Nutritional value, g/DM	
Crude protein	22.00
Metabolizable energy	2601.77
Fat	8.42
Calcium	9.62
Phosphorus	0.87
Lysine	1.18
Methionine	0.49

#### Preparation of plant

The leaves of *T. vulgaris* (Figure 1a) were purchased from the local market. The plants were sundry (Figure 1b) at ambient temperature for seven days. Dried samples were ground using a hammer mill, and the powder obtained was put into labeled plastic bags (Figure 1c) and placed in a sealed plastic bucket.



Figure 1. Leaves of thyme (T. vulgaris) under different conditions. Fresh (a), dried (b), and powder leaves (c).

#### Animal Equipment and Housing

A total of 224 snails (Figure 2) aged at least six months, weighing between 70-8 g, free of injury or breakage were purchased in Muea Market of the Buea Municipality. The snails were placed in perforated plastic buckets (45 cm in diameter and 30 cm deep each) equipped with a plastic feeder and a drinker of 5 cm in diameter. The bottom of each bucket consisted of a 10 cm thick loose soil substrate previously

disinfected with virunet, two weeks before the animals were introduced. Virunet is a broad-spectrum disinfectant that kills all types of bacteria, viruses, and fungi. 0.1 g of virunet was mixed with 0.5 L of water and spread on the substrates using a sprayer. The buckets were covered with mosquito-type netting (1 mm mesh) constituting an antileak device and then placed in a block building 7 m long by 6 m wide with the floor made of cement) covered with a metal sheet at room temperature and natural lighting.



Figure 2. Adult Giant African Land Snails (Archachatina marginata)

#### Conduct of the experiment and data collection

Two hundred and twenty-four adult snails (*Archachatina marginata*) were randomly partitioned into four treatments and four repetitions (Figure 4) of 14 comparable snails (weight and size). Treatment  $T_0$  (control) received only the experimental diet (Table 1) while three other treatments received the same diet with different inclusion of *T. vulgaris* leaf powder at 0.25%, 0.50%, and 0.75%. Diet and fresh pawpaw leave (staple diet) were measured and served daily, and the leftover were quantified using an electronic weighing 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) and the animals were monitored for a period of 12 weeks. At the end of the experiment, the animals of each replicate were sacrificed, the shell, soft tissue, pedal mass, total meat, and ovotestis were collected for evaluation of carcass characteristics and relative weight (Apata et al., 2015). The samples of the pedal mass were transported to the Laboratories of Animal Nutrition and Feeding and Soil Sciences of the Faculty of Agronomy and Agricultural Sciences of the University of Dschang-Cameroon, to determine, respectively the proximate composition and the mineral content of the carcass.

#### Growth characteristics

The characteristics of growth considered are:

- Feed intake = Quantity of feed served-leftover
- Leave intake = Quantity of leave served-leftover
- Carcass characteristics
- Relative weight of organs (RWO)
- RWO = weight of organ considered
- Live weight

#### Analysis of proximate composition

The quantities of dry and organic matter, protein, lipid, and ash were determined according to the method proposed by <u>AOAC</u> (2005). Snails flesh was analyzed and the analysis was repeated three times for each sample. The proximate composition analysis of snail flesh (*A. marginata*) was about the estimation of the whole nitrogen by way of the Kjeldhal method and the crude fibre and fat by the methods of <u>AOAC</u> (2005). The quantity of dry matter was estimated on a fraction of the sample which had been dried in the hot room. The ashes were determined after incineration of the dry material under  $550^{\circ}$  C for 24 hours.

The level of minerals in the ash was determined according to the method proposed by Pauwels (Pauwels & Verloo, 1992). The minerals were analyzed from solutions obtained by dry-ashing the samples at 550° C and dissolving the ash in standard flasks with distilled, de-ionized water containing a few drops of concentrated hydrochloric acid. Phosphorus was determined calorimetrically with KH2PO4 as a standard. Sodium and potassium were determined using a flame photometer, NaCl and KCl were used to prepare the standards. All other minerals were determined by means of an atomic absorption spectrophotometer at the Laboratories of Animal Nutrition and Feeding and Soil Sciences of the Faculty of Agronomy and Agricultural Sciences of the University of Dschang-Cameroon.

#### Statistical analyses

Data from the growth, carcass, and relative weight of organs, proximate composition were analyzed using One-way analysis of variance (ANOVA) to compare the means and when the differences were significant, Duncan's test was used to separate them at the 5% level with Statistical Package for Social Sciences (SPSS) version 20.0.

#### Results

# *Effects of different concentration of Thymus vulgaris as feed additive on the leave intake of Giant African Land Snails (Archachatina marginata)*

The effects of different concentration of T. *vulgaris* on leave intake is illustrated in <u>figure 3</u>. It is revealed that, irrespective of the period of the experiment, the trend, profile, and shape of the curve are comparable among treatments.

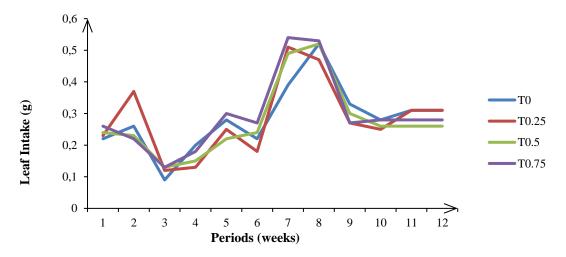


Figure 3. Effects of different concentration of Thymus vulgaris on the evolution of leaf intake of Archachatina marginata

When we consider the period of the experiment, at the fifth period of the experiment, animals that received 0.75% *T. vulgaris* recorded the highest value of leave intake compared to other treatments. However, no significant difference (P > 0.05) was recorded among treatments.

At the end of the experiment, animals in the control group ( $T_0$ ) and animals that received 0.25% *T*. *vulgaris* recorded the highest value of leave intake followed by treatment that received the highest *T*. *vulgaris* ( $T_{0.75}$ ) in the diets.

## Effects of different concentration of Thymus vulgaris on the evolution of feed intake of Archachatina marginata

The evolution of feed intake in relation to the proportion of *T. vulgaris* in the diet is presented in <u>figure 4</u>. We can observe that, irrespective of the period of the experiment, the evolution of feed intake decreases with the period of trial.

When we consider the period of the experiment, at the second period of the trail, animals that received 0.75% *T. vulgaris* recorded the highest value of feed followed by the treatment that received 0.50% *T. vulgaris*.

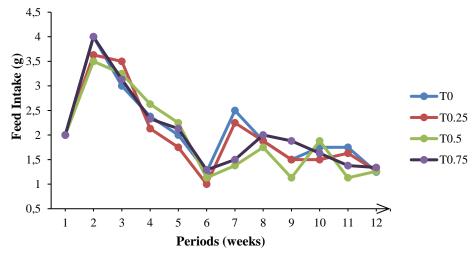


Figure 4. Effects of different concentration of Thymus vulgaris on the evolution of feed intake of Archachatina marginata.

At the 7<sup>th</sup> period of the experiment, the significantly highest value (P < 0.05) of the feed intake was registered in the control group. The lowest value was obtained in the treatment that received 0.50% *T. vulgaris*.

At the end of the experiment, it appears that animals that received the highest proportion of *T*. *vulgaris* ( $T_{0.75}$ ) obtained the highest value of feed intake followed by the animals that received 0.25% of *T*. *vulgaris* in the diet.

#### Effects of different concentration of Thymus vulgaris on the Characteristics of the carcass of Snail

The effect of different concentration of *T. vulgaris* on the characteristics of the carcass is presented in <u>table 2</u>. We can realize that the characteristic of the carcass was affected by *T. vulgaris* in the diet. The significantly (P < 0.05) highest value of the live weight was recorded in the control group ( $T_0$ ) compared to other treatments. The significantly (P < 0.05) lowest value was recorded in the treatment that received the highest proportion of *Thymus vulgaris* ( $T_{0.75}$ ). The highest values for the head, stomach, and soft tissues, were registered in the control group ( $T_0$ ) compared to other treatments. However, no significant differences were registered among the treatments.

<b>Relative Weight</b>	Treatments, %Thymus vulgaris				
	<b>T0.00</b>	Т0.25	Т0.50	T0.75	
Live Weight	88.96±5.50 <sup>a</sup>	87.74±4.55 <sup>ab</sup>	82.98±6.24 <sup>ab</sup>	82.38±6.99 <sup>b</sup>	
Shell weight	19.94±3.20 <sup>a</sup>	18.63±3.78 <sup>a</sup>	20.22±1.91ª	19.05±4.12 <sup>a</sup>	
Intestines	11.77±0.96 <sup>a</sup>	11.81±2.19 <sup>a</sup>	12.31±1.05 <sup>a</sup>	$10.83 \pm 3.44^{a}$	
Head	2.21±0.35 <sup>a</sup>	2.01±0.65 <sup>a</sup>	$1.80\pm0.47^{a}$	$1.92 \pm 0.20^{a}$	
Heart	$0.18{\pm}0.15^{a}$	1.11±0.35 <sup>a</sup>	0.81±0.33 <sup>a</sup>	$0.79 \pm 0.32^{a}$	
Stomach	$1.15 \pm 0.59^{a}$	1.11±0.35 <sup>a</sup>	0.81±0.33 <sup>a</sup>	$0.79 \pm 0.32^{a}$	
pedal mass	16.11±3.50 <sup>a</sup>	17.38±2.92 <sup>a</sup>	16.84±2.50 <sup>a</sup>	16.50±3.37 <sup>a</sup>	
Ovotestis	1.58±0.34 <sup>ac</sup>	$1.10\pm0.49^{b}$	1.17±0.400 <sup>ab</sup>	1.88±0.48°	
Soft tissue	$1.34{\pm}0.55^{a}$	$1.28\pm0.42^{a}$	1.06±0.35 <sup>a</sup>	$1.02 \pm 0.0.33^{a}$	
Gonadosomatic index	$0.01 \pm 0.00^{ab}$	$0.01 \pm 0.00^{b}$	$0.01 \pm 0.05^{b}$	$0.02 \pm 0.00^{a}$	

**Table 2.** Effect of different concentration of *Thymus vulgaris* on characteristics of the carcass of *Archachatina marginata*

**a**, **b**, means along the same row having different superscripts are significantly different (P < 0.05).

The highest value of the heart was registered in animals that received 0.25% of *T. vulgaris* in the diet compared to three other treatments. However, no significant differences were observed among the treatments. The highest weight of the pedal mass was obtained in the treatment that received 0.25% *T. vulgaris* in their diet followed by the treatment that registered 0.50% *T. vulgaris*. The lowest value for the pedal mass was recorded in the control group ( $T_0$ ). The value of the ovotestis increases significantly

(p<0.05) in the treatment that received the highest *Thymus vulgaris* and the lowest value in the treatment that received 0.25% *T. vulgaris* in the diet.

# Effects of different concentration of Thymus vulgaris on relative weight of the organs of Archachatina marginata

The effect of different concentration of *T. vulgaris* on the relative weight of the organs is presented in <u>table 3</u>. It appears that the relative weight of organs was affected by *T. vulgaris* in the diet. The highest value of the live weight was noted (p<0.05) in the control group ( $T_0$ ) compared to other treatments.

The highest values of shell weight and intestines were obtained in the treatment receiving 0.50% *Thymus vulgaris* compared to other treatments. The lowest values were registered in the treatment that received 0.25% of *T. vulgaris* and the control group (T<sub>0</sub>).

The highest values of the heart, pedal mass, and gonad were recorded in the treatment that received the highest percentage of *T. vulgaris* ( $T_{0.75}$ ) compared to other treatments. The lowest values of the heart were obtained in the control group ( $T_0$ ), while the lowest of the pedal mass and gonad were noted in the treatment that received 0.50% *T. vulgaris* and the treatment that registered 0.25% *T. vulgaris* in the diet. The highest value of the stomach was registered in animals that received 0.50% and 0.75% *T. vulgaris* compared to other treatments. The highest value of the soft tissue was recorded in the control group ( $T_0$ ) and the treatment that received 0.25% *T. vulgaris* compared to other treatments otherwise comparable.

Table 3. Effect of different concentration of Thymus vulgaris on relative weight of the organs of Archachatina marginata

Relative Weight	Treatments, % Thymus vulgaris				
	Т0.0	T0.25	Т0.50	Т0.75	
Live weight	$88.96 \pm 5.50^{a}$	87.74±4.55 <sup>ab</sup>	82.98±6.24 <sup>ab</sup>	82.38±6.99 <sup>b</sup>	
Shell weight	$0.22\pm0.29^{a}$	0.21±0.05ª	0.24±0.02 <sup>a</sup>	0.23±0.04ª	
Intestines	0.13±0.01ª	$0.13\pm0.02^{a}$	0.14±0.01 <sup>a</sup>	0.13±0.04 <sup>a</sup>	
Head	$0.03\pm0.01^{a}$	0.02±0.01ª	0.02±0.01ª	$0.02 \pm 0.00^{a}$	
Heart	0.002±0.001 <sup>a</sup>	0.002±0.001ª	0.003±0.019 <sup>a</sup>	0.203±0.054ª	
Stomach	$0.013 \pm 0.007^{a}$	0.013±0.004 <sup>a</sup>	$0.009 \pm 0.004^{a}$	0.009±0.005ª	
Pedal mass	$0.181 \pm 0.036^{a}$	0.198±0.035ª	0.022±0.018 <sup>a</sup>	0.203±0.055ª	
Gonad	$0.017 \pm 0.004^{ab}$	$0.012 \pm 0.006^{a}$	0.014±0.005 <sup>a</sup>	$0.023 \pm 0.005^{b}$	
Soft tissue	$0.015 \pm 0.006^{a}$	0.015±0.005 <sup>a</sup>	0.012±0.004 <sup>a</sup>	0.012±0.005ª	

**a**, **b** on the same line, the values assigned to the same letter do not differ significantly (P > 0.05). **n** = number of snails.

Effects of different concentration of Thymus vulgaris in the diet of snails on proximate composition of snail flesh

The effect of different concentration of *T. vulgaris* on the proximate composition of snail flesh is summarized in figures 5 to 9.

#### Moisture content

The effects of different concentration of *T. vulgaris* on moisture content are shown in <u>figure 5</u>. The moisture content is influenced by the *T. vulgaris* in the diet.

The significantly (P < 0.05) highest values are obtained in the animal receiving 0.5% and 0.75% *T. vulgaris* in the diet compared to animals in the control group (T<sub>0</sub>) and animals that received 0.25% *T. vulgaris*.

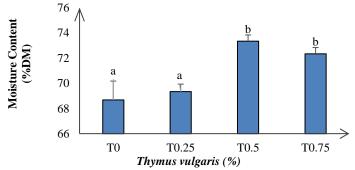


Figure 5. Effect of different concentration of *Thymus vulgaris* on the moisture content of snail flesh.

#### Fat, %/DM

The effect of different concentration of *T. vulgaris* on fats in the diet of snails is illustrated in <u>figure</u> <u>6</u>. We can notice that the fat content is comparable among treatment.

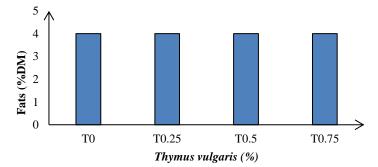


Figure 6. Effect of different concentration of Thymus vulgaris on fat content of snail flesh

#### Crude protein, %/DM

The effects of different concentration of *T. vulgaris* on crude protein is presented on <u>figure 7</u>. The significantly (P < 0.05) highest value was obtained in the animal receiving 0.5% *T. vulgaris* followed by animals receiving highest proportion of *T. vulgaris* (T<sub>0.75</sub>) in the diet compared to animals in the control group (T<sub>0</sub>). The significantly (P < 0.05) lowest value was registered in the treatment that received 0.25% *T. vulgaris* in the diet.

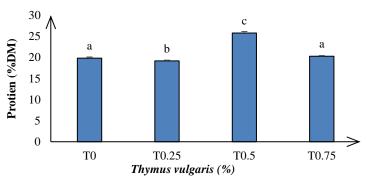


Figure 7. Effect of different concentration of Thymus vulgaris on protein content of snail Archachatina marginata flesh.

#### Ash content

The effects of different concentration of *T. vulgaris* on ash content is shown in Figure 8.

The significantly (P < 0.05) highest value of the ash content was recorded in the animal receiving 0.50% *T. vulgaris* in the diet followed by the animals in the control group (T<sub>0</sub>) and treatment that received the highest level of *T. vulgaris* (T<sub>0.75</sub>) in the diet of snails compare to others. The significantly (P < 0.05) lowest value of the ash content was registered in the animal receiving 0.25% *T. vulgaris* in the diet.

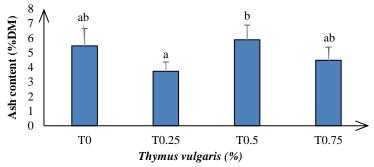


Figure 8. Effect of different concentration of *Thymus vulgaris* on ash content of snail flesh.

#### Dry matter

The effect of different concentration of *T. vulgaris* on dry matter content is presented Figure 9. We can also observe that; the values obtained for dry matter decreased with increasing levels of *T. vulgaris* in the diet of snails. The significantly (P < 0.05) highest value of the dry matter was recorded in the animal receiving 0.25% *T. vulgaris* in the diet followed by the animals in the control group ( $T_0$ ) and the treatment receiving 0.5% *T. vulgaris* otherwise comparable.

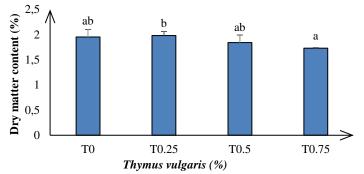


Figure 9. Effect of different concentration of Thymus vulgaris on dry matter content of snail flesh.

The significantly (P < 0.05) lowest value of the dry matter was obtained in the animal receiving the highest proportion ( $T_{0.75}$ ) of *Thymus vulgaris* in the diet.

#### Effects of different concentration of Thymus vulgaris on the mineral of the snail flesh

The effect of different concentration of *T. vulgaris* on the mineral content of snail flesh is summarized in <u>table 4</u>. We can observe that: the mineral content of snail flesh is affected by *T. vulgaris*.

The animals in the control group ( $T_0$ ) recorded the significant (P < 0.05) highest value for ash, K, P, and Zn. The highest values for Mg and Na were obtained in the treatment receiving 0.5% *T. vulgaris* compared to other treatments.

The levels of Zn and Fe were significantly (P < 0.05) higher in the animals receiving 0.25% *T. vulgaris*. The significant (P < 0.05) highest value for Ca was recorded in the treatment with the highest proportion (T<sub>0.75</sub>) of *T. vulgaris* in the diet. The lowest value was registered in those receiving 0.25% *T. vulgaris*.

Minerals -	Treatments, % Thymus vulgaris				
	Т0.0	T0.25	Т0.5	T0.75	
Ash (%)	6.12±0.11 <sup>a</sup>	4.12±0.11 <sup>b</sup>	5.04±0.05°	4.14±0.16 <sup>b</sup>	
K(mg/kg)	9104.75±200.36 <sup>a</sup>	8252.72±29.15 <sup>b</sup>	7093.95±0.03°	$936.04 \pm 27.800^{d}$	
Na (mg/kg)	41.03±2.39 <sup>a</sup>	$70.43 \pm 2.48^{b}$	105.12±1.53°	40.24±1.72 <sup>a</sup>	
P (mg/kg)	11692.86±45.53ª	9625.23±50.88 <sup>b</sup>	5838.11±46.19°	9455.91±46.32 <sup>d</sup>	
Zn (mg/kg)	2119.32±45.53ª	2178.39±51.04ª	881.03±46.19 <sup>b</sup>	1843.83±46.32°	
Ca (mg/kg)	13828.95±91.06 <sup>a</sup>	12202.15±102.05 <sup>b</sup>	13827.74±92.39 <sup>a</sup>	27427.23±92.64°	
Mg (mg/kg)	5242.77±91.06 <sup>a</sup>	1381.24±101.89 <sup>b</sup>	7673.99±92.39°	4002.16±92.64 <sup>d</sup>	
Fe (mg/kg)	19.91±2.39ª	302.03±0.03 <sup>b</sup>	72.72±1.55°	92.94±1.71 <sup>d</sup>	

Table 4. Effect of different concentration of Thymus vulgaris on the minerals of snail flesh

**a**, **b**, **c** and **d** on the same line, the values assigned to the same letter do not differ significantly (P > 0.05). **n** = number of snails.

#### Discussion

The effects of different concentration of *Thymus vulgaris* on leave and feed intake in relation to different proportions of *T. vulgaris* were highest in animals that received 0.75%. The result was in line with Darmawan et al. (2022) who concluded that the inclusion of phytogenic extracts of *T. vulgaris* in the diet of laying hens had a positive effect on productive performance, feed efficiency, egg mass, immunity, and antioxidant activity without interference with egg quality. The results are also in agreement with that of Toukala et al. (2020) who investigated the effects of feed supplementation of *T. vulgaris* powder on growth performance, carcass characteristics, and blood profiles of maturing Cameroonian local Kabir chicken. The results were also consistent with (Reddy et al., 1998) who

reported that *T. vulgaris* supplementation in broiler diets can increase feed intake as well as the secretion of endogenous digestive enzymes and strengthen the immune system when added in poultry feed. The results can be justified by the fact that *T. vulgaris* contains thymol and carvacrol which improve digestive function and increase feed intake in livestock.

Furthermore, *T. vulgaris* contains compounds that have immunostimulatory properties, which may improve the health and well-being of snails (<u>Hazzit et al., 2009</u>; <u>Kuete, 2017</u>). This, in turn, could have led to an increase in appetite and feed intake (<u>Abdel-Ghaney et al., 2017</u>; <u>Cross et al., 2007</u>). Thymol and carvacrol have been shown to stimulate the digestive system and enhance the absorption of nutrients, which could promote an increase in feed intake and a palatable-enhancing effect, making the leaves more appealing to the animals (<u>Abdel-Ghaney et al., 2017</u>; <u>Monteschio et al., 2017</u>; <u>Souza et al., 2019</u>).

Higher values of live weight, head, stomach, and soft tissues (except heart, pedal mass and gonad) were registered in the control group ( $T_0$ ) compared to other treatments. These results corroborate the other findings of (<u>Ali et al., 2007</u>) who reported that hens fed *T. vulgaris* had no significant effect on carcass parameters and internal organs.

Similar studies conducted by Ayoola et al. (2014), the addition of T. vulgaris to the diet of broiler chicken led to decreased feed intake and body weight due to the antioxidant and antimicrobial properties of these compounds. Also, the data by Ocak et al. (2008) observed no significant statistics in carcass weight, dressing yield, the relative weights of the edible parts and small intestine, and length of small intestine tract of broilers chickens fed diets supplemented with dry thyme powder. Dahal & Farran (2011) noticed no effect of feeding a thyme-containing diet on carcass traits of broiler chicks. The present studies are also in agreement with those obtained by Amouzmehr et al. (2012) who demonstrated that carcass characteristics of broiler chickens were not affected by feeding diets containing thyme powder. The results of the study suggest higher values (except heart, pedal mass, and gonad values) in the negative control compared to the other treatments suggesting growth suppressing effects of T. vulgaris additives on the snail's internal organs. The possible reason for the observed results could be that the T. vulgaris may have certain chemical compounds that are not conducive to the growth and development of snails. Thymol and carvacrol have been reported to have anti-inflammatory and antioxidant effects, which may contribute to their growth-suppressing effects. The results of the study also suggest that the addition of T. vulgaris to the diet of the snails had a negative effect on their growth and development. The possible reasons for this could be that T. vulgaris contains compounds such as thymol, and carvacrol, which may have antimicrobial and antioxidant properties. However, these same compounds may also have negative effects on the snails' digestive system and nutrition absorption, leading to decreased growth and development.

The relative weight of internal organs did not significantly vary with treatment, except for the live weight and the gonad, corroborating the findings of Adam et al. (2020) who report that different levels of thyme powder or essential oils of thyme had no marked effect on the relative weight of the dressing, proventriculus, gizzard, liver, heart, pancreas and intestinal tract. This result is also in agreement Ghalamkari et al. (2012) who studied the effects of thyme powder extract or essential oils on broiler chickens' weight of organs and observed no significant effects. These results signify the suppressing effects of *T. vulgaris* in the snail's internal organs. The numerical increase of heart, pedal mass, and ovotestis values provides an indication of the immune-stimulating potentials of the plant on these organs.

The moisture increased with the *T. vulgaris* in the diet, the lowest of which was recorded in the control group ( $T_0$ ). Our results are similar to those of <u>Shirmohammadi et al.</u> (2016) who reported that supplementation of thyme increased the moisture of the thigh muscle significantly, compared with the control. The results obtained may be a result of *T. vulgaris* containing antimicrobial properties which have been shown to improve growth performance, feed efficiency, and immune response in poultry and fish. The significantly highest value for moisture content increase with increasing *T. vulgaris* in snail diet may indicate that the inclusion of *T. vulgaris* leads to higher water retention or reduced nutrient absorption, resulting in higher moisture content in snail tissues (<u>Brewer, 2011</u>; <u>Lee et al., 2007</u>; <u>Niki, 1999</u>; <u>Viuda-Martos et al., 2011</u>; <u>Wojdyło et al., 2007</u>).

The fat content was not affected by *T. vulgaris* in the diet. <u>Radwan et al.</u> (2008) reported decreased fat content at 1% *T. vulgaris*. This may be due to several reasons: *T. vulgaris* may not directly affect

lipid metabolism (<u>Radwan et al., 2008</u>) or deposition in snails, as well as snails, may have a natural ability to regulate their fat stores and maintain a constant fat content despite dietary changes.

The values of crude protein and ash content in the flesh of the snail were highest in the animal receiving 0.5% *T. vulgaris*. Our results contradict that of <u>Shirmohammadi et al.</u> (2016) who studied the effects of *T. vulgaris* and *Mentha pulegium* on color, nutrients, and peroxidation of meat in heat-stressed broilers and reported that no significant differences between treatments were observed for the ash, ether extract, and crude protein content of the thigh muscle. These results could be explained by the fact that the inclusion of *T. vulgaris* in the snail's diet may have multiple effects on protein metabolism, resulting in increased protein and ash levels.

The effects of *T. vulgaris* on dry matter reveals that; the values obtained for dry matter decreased with increasing level of *T. vulgaris* in the diet of snails. A similar observation was also made in the broiler and revealed that the dry matter content of excreta was not significantly influenced by dietary treatments compared to the control group (Sarica et al., 2005). Also, Ebrahimi et al. (2018) found that the addition of thymol to the diet of dairy cows led to decreased dry matter intake and milk production. The results can be justified by the fact that *T. vulgaris* is an herb that has been used for its medicinal and flavoring properties. When snails consume a diet that contains increasing levels of *T. vulgaris*, their dry matter content decreases. This may be because the herb contains compounds that reduce the ability of snails to retain water, leading to a decrease in dry matter content.

This study found that the mineral content of snail flesh was affected by the concentration of *T*. *vulgaris* in the snail's diet. The highest values for ash, K, and P were recorded in animals in the control group (T<sub>0</sub>). This result could be because, at higher levels of inclusion, the herbs may have adverse on the snail's health and reduce their ash, K and P content. On the other hand, the higher values for Mg and Na were obtained in the treatment receiving 0.5% *T. vulgaris*. The levels of Zn and Fe registered the highest values in the animals receiving 0.25% thymus vulgaris. The highest value for Ca was recorded in the treatment with the highest proportion (T<sub>0.75</sub>) of *T. vulgaris* in the diet. Kerstetter et al. (2005) reported that increasing the energy level in the diet decreases the intestinal absorption of calcium and iron, with an increase in phosphorus. These results are contrary to Tchowan et al. (2022) who report that the level of calcium in the ash decreased while the level of phosphorus increased with the increasing energy level in the diet. Radwan et al. (2008) reported that the improvement in the antibody titter values may be due to the high level of iron in *T. vulgaris* leaves which may affect the transport of oxygen needed for hemoglobin synthesis in blood.

#### Conclusion

At the end of the study on the effect of different concentration of *Thymus vulgaris* leaf powder as feed additive on performance of Giant African Land Snail (*Archachatina marginata*), the main conclusions are as follows: the inclusion of graded levels of *T. vulgaris* in the diet of snails affected the growth performance, carcass and bromatological characteristics and minerals of the snail flesh. *T. vulgaris* leaf meal can be added to the diet of mature snails up to 0.25% inclusion levels.

#### **Data Availability**

The data used to support the findings of this study are available from the corresponding author upon request.

#### **Conflicts of Interest**

The authors declare that they have no conflicts of interest.

#### **Credit Author statement**

Tchowan Guy Merlin designed the study and carried out the experimental protocol. Analyzed and interpreted the results. Jam Jude Jua participated in data collection and data analysis. He wrote the first draft. Ferdinand Ngoula analyzed and interpreted the results. Tchoumboué Joseph conceptualized the study and supervised the field. All authors read and approved the final manuscript.

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