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Original article

Influence of two substrates on the performances of Archachatina marginata's adults and juvenile in Benin

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ABSTRACT

Laying performance of 168 breeders and growth rates of 420 juveniles on both substrates (Tectona grandis sawdust and compost) for 12 weeks was determined in African giant snails Archachatina marginata. The experimental design was a simple randomized block with two treatments and three repetitions. The substrate has a negative effect on laying rate and parasitic state. In compost breeding, the infection rate was significantly ($p<0.01$) very high compared to those grown on sawdust. Snails with the highest infection rate were laying fewer eggs twice significantly ($p<0.05$) than those whose infection rate was lower. The average daily gain on snails' juvenile breed on sawdust was the same with those which are been breed on compost. But the shell growth rate of juveniles reared on sawdust was 0.1 mm/day when those which were reared on compost have zero-growth ($p<0.05$). According with these results, Tectona grandis sawdust could may be recommended for African

giant snails breeding in Benin.

1. Introduction

The giant African snails, genus Achatine (Achatina achatina, Achatina fulica, Achatina panther, sp) or genus Archachatine (Archachatina marginata, Archachatina ventricosa, Archachatina sp) are edible terrestrial gastropods which live weight easily reaches 250 g (Afolayan and Ejidike 2000). They are often found in plant debris across the tropics during the rainy seasons (Adeyemo and Borire 2002; Sodjinou et al. 2003). As emphasized by several authors (Hodasi 1984; Agbelusi and Ejidike 1992; Afolayan and Ejidike 2000; Ekoué and Kuévi – Akue 2002), the African giant snail meat is very popular in Africa and have protein level between 37 and 51 % (Hardouin and Stievenart 1991; Hardouin et al. 1995; Afolayan and Ejidike 2000). Its annual consumption is about 17000 t/year in Ivory Coast, 300 t/year in Benin (Sodjinou et al. 2003), 30 t/year in Togo (Ekoué and Kuévi – Akue 2002) and 25 t/year for Ecuador region in Central African Republic (Mbétid – Bessane 2006). In addition to this exceptional nutritional value, African giant snail meat is an important ingredient in the preparation of various traditional medicines and ritual ceremonies (Agbelusi and Ejidike 1992; Codjia and Noumonvi 2001). Snails are also used in feed formulation for monogastric livestock because their shell is a source of calcium and crushed flesh can replace fishmeal (Ekoué and Kuévi – Akue 2002). Stievenart and Hardouin (1993) point out that giant African snail meat is a food source that can provide lysine in the diet of people who suffer of protein malnutrition in poor countries. African giant snails consumed in Benin are harvested largely in the nature and are collected in the wild. With the increasing of the population, the demand is now very strong. This situation has led into a drastic decline African giant snail's wild's livestock (Sodjinou et al. 2003). Also, African giant snails are threatened by extinction due to the use of pesticides and other farming products (Hardouin et al. 1995). Other factors such as climate change and especially urbanization disrupt the habitat of giant African snails. Faced with this danger, which potentially threatens the survival of those snails, we need to promote snail breeding. In Europe, several studies have been devoted to Helicidae. However in Africa, information about Achatinidae are not available. Most studies on these gastropod, have focused on biological, and partly to feeding methods in close captivity (Ayodele and Asimalowo 1999).

Different types of substrates can be used in breeding giant African snails. Indeed, those snailstake from their substrate minerals to form their shells and nutrients for weight growth. In addition, the substrate serves as refuge and bury the eggs until they hatch, but it can be an ideal gateway for parasites, infectious agents and other potential predators (Codjia and Noumonvi 2001; Koudandé et al. 2006). Thus, the objective of this paper is to evaluate the effect of the substrate on laying rate and parasitism performances of adult and juvenile Archachatina marginata respectively reared in close captivity in southern Benin.

2. Materials and methods

The study was conducted at Ever Green NGO's farm located in the town of Abomey – Calavi (6 ° and 26.36 ° north latitude and 20.15 longitude 2) in southern Benin. The climate is Guinean, with two rainy seasons (March-July and September-November) and two dry seasons (December to February and August). The annual average rainfall is 1200 mm and the annual average temperature varies between 27 and 31 ° C.

2.1. Experimental design

Two trials were conducted according to an experimental device randomized complete block with two treatments, substrates and 3 repetitions for 12 weeks excluding a period of two weeks to adapt snails to farming conditions.

The livestock management was the same for both tests. Thus 6 enclosures each having a surface area of 1.2 m2 and 0.2 m deep were used. The two substrates used and allocated to enclosure randomly were as follows: Tectona grandis sawdust with a neutral pH (and low parasitic infestation); compost first generation with a neutral

pH which is a natural environment for snails. The compost was produced on the farm with layers litter and Zea maize tops while the Tectona grandis sawdust was obtained in a semi-industrial sawmill wood located in the town.

Table 1
Substrates' comparison.

Parameters	Sawdust	Compost
substrate pH	7±0.0	7±0.5
Substrate temperature (°C)	26±0.0	26±1.6
Relative humidity of the air in the enclosure (%)	85±0.0	85±0.0

The 6 enclosures were cleaned, disinfected and allowed to crawl for 2 weeks before the start of the trial to minimize the predatory action of different enemy. The substrates had 20 cm of thickness. Each experimental enclosure was watered with 1 liter of water twice daily. This allowed watering soil aeration, sanitation and the fight against ants as already noted by other authors who have conducted experiments on the snail breeding in Benin (Koudandé and Ehouinsou 1995; Mensah and Alassane – Kpembi 1999; Codjia and Noumonvi 2001; Koudandé et al. 2006). Once a week, the substrate was aerated by stirring manually.

In each enclosure two plates for feed and drinking water were prepared. Snails were fed with 30 g of maize's bran and 10 g of chickens' eggshell flour as calcium source, and were watered ad libitum. Before the start of data collection, snails were subjected to an adaptation phase which lasts two weeks in the goal to acclimate them to the substrates and breading conditions. Both tests were conducted as follows:

2.2. First test conducted with 168 Archachatina marginata's adults

The mortality rate estimated at 10% in snails breeding has been taken in consideration in determining samples' numbers were done by Koudandé and Ehouinsou (1995), Mensah and Alassane – Kpembi (1999); Codjia and Noumonvi (2001) and Koudandé et al. (2006) in Benin. So in the goal to replace any deaths during the adaptation phase, 12% were added to the initial number. Therefore, for this first test instead of 150 adults of Archachatina marginata as original number, it is rather 168 snails, that's mine 28 per enclosure which were used. In the weekly substrate manual moving of each enclosure, eggs laid by snails were collected and incubated in plastic pots. Those plastic pots were filled with Tectona grandis's sawdust. Their bottom were perforated with several holes to allow flow after daily watering.

2.3. Second test conducted with 420 Archachatina marginata's juveniles

The juveniles' from each experimental block were bred in different enclosures. In each enclosure 70 juveniles totaling 420 juveniles born of adults of the first test were used. Here, the drinking water was served at will, but in small troughs to avoid drowning.

2.4. Data Collection

2.4.1. Usual data

On fact sheets for each enclosure, data collected were been marked; daily food refusal weighs with an electronic scale Salter brand range 2 kg and 1 g precision; weekly snails weighs with the same scale; weekly snails' shell with calipers square; snails' death; weekly recording of the number of eggs laid in each enclosure. Before starting the tests, the pH and the level of infestation of substrates from a primary sample of 50 g were determined (Table 1). Thus, 50 g of substrate were taken from 10 repetitions at 5 g out of the diagonals of each enclosure. In lab, the determination of pH using universal indicator pH 1-10 RotaTM and determining the level of parasitic infestation of the substrate were made on a second sample of 10 g taken from the mixture of 50 g the primary sample. The dead snails were removed from the enclosure.

2.4.2. Production parameters

ADG (Average Daily Gain) and mortality rate were the two production parameters calculated using Lhoste et al. (1993) formulas as follows:

Average Daily Gain (ADG): AWG = (Wf-Wi) /T and ADG = AWG/d, where:

ADG = Average Daily Gain (g/d); AWG = Average Week Gain; Wf = final weight; Wi = initial weight; T = time between weighing (days); d = 7 days

Shell Average Growth: ASG = (L2-L1) / T, where:

SAG = Shell Average Growth; T = time between shells measure out (days); L1: Initial shell average length; L2: Final shell average length.

Kouassi et al (2007) equations were used to calculate the monthly Egg-Laying rate and hatchability rate as follows:

Egg-Laying rate: Lc = Pt/(Sn x 100) where: Lc = Laying rate; Pt: total number of egg laid in an enclosure; Sn: number of snails in an enclosure.

Hatchability rate : Hr = Eh/(Eg x 100) where: Hr = Hatching rate; EG: number of hatched eggs; Eg: total number of eggs laid.

2.4.3. Eggs per gramme of feces

On scatologic exams, 10 g of snails' feces were been taken fortnightly on morning in each enclosure throughout tests duration. Feces were placed in numbered plastic bags, sealed in taking care to remove the air. Droppings and packed were sent within an hour at the Laboratory of Ethnopharmacology and Animal Health to find the eggs of nematodes', cestodes' eggs and coccidia oocysts. In the laboratory, 2 g of the primary sample were taken to achieve the scatological analysis. The quantitative method of Mac Master as the "Diagnosis of worm infections by stool examination" (Thys and Vercruysse 1990) adapted to the shellfish was used to do this. EPG (eggs per gramme of feces) was determined by the following formula:

EPG = [(n2 + n1) / 2] x 100, with n1 = number of eggs in the cell numbered 1 and n2 = number of eggs numerated in cell 2.

2.4.4. Statistical analysis

The variables taken into account were submitted to GLM using the package MASS (Venables and Ripley 2002) implemented in the software R (R Core Team 2014). Averages were then calculated and compared using the Wilcoxon test with the software R (R Core Team 2014). Means' hierarchical organizations were made with the HSD test using the package Agricolae (de Mendiburu of 2013).

3. Results

3.1. Substrate's effect on growth

The substrate had a significant effect ($p < 0.05$) on the various parameters calculated (Table 2, 3 & 4). Archachatina marginata's juveniles which were bred on Tectona grandis's sawdust had their shells longer than those which are bred on compost of 0.02 mm ($p < 0.05$). On the other hand, substrates had no effect on juveniles' ADG. All the snails had an average daily gain weight of 0.15 g/d.

Table 2
Results of analysis of variance of juveniles' ADG depending on the substrate.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
S	1.00000	0.00045	0.00046	0.03600	0.85100
Re	20.00000	0.24955	0.01248		

Sc: 0'***' ;0,001'**' ;0,01'*' ;0,05',' ;0,1'' ;1

S : Substrate ; Re : Residuals ; Df : Degree of freedom ;

Table 3
Results of analysis of variance of juveniles' ASG depending on the substrate.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
S	1.00000	0.00077	0.00077	4.61700	0.04410	*
Re	20.00000	0.00333	0.00017			

Sc: 0'***' ;0,001'**' ;0,01'*' ;0,05',' ;0,1'' ;1

S : Substrate ; Re : Residuals ; Df : Degree of freedom ;

The breeding method being the same, the growth induced by the substrate sawdust is much complete than that induced by the compost. The juveniles bred on sawdust had the same ADG than others and had the best shellfish performance ($p<0.01$).

Table 4
Outline averages of ADG and ASG with the Tukey HSD test on the threshold of $\alpha = 0.05$.

Pa	Gr	Trt	r	M	std	MS	HSD
ADG (g/d)							
	a	Saw	11	0.15	0.08	0.01	0.10
	a	Comp	11	0.15	0.14		
ASG (mm/d)							
	a	Saw	11	0.02	0.02	0.0001	0.01
	b	Comp	11	0.00	0.01		
Cm (%)							
	a	Saw	11	1.00	0.10	0.009	0.002
	b	Comp	11	24.0	0.10		

Means with the same letters are not significantly different.
Pa : Parameters ; Gr : Group ; Trt : Treatment; r : Number ; M : Means ; std : Standart Error ; MS : MS error ; HSD : Honestly Significant Difference ; Saw : Sawdust ; Comp : Compost ; Cm : Cumulative mortality

3.2. Substrates' effect on infestation level and laying performances

The laying cycle of adult intestinal parasites in the body of gastropods was irregular and high archachatines on compost were almost 3 times more infected than those reared on sawdust (Figures 1, 2 and 3 and table 5).

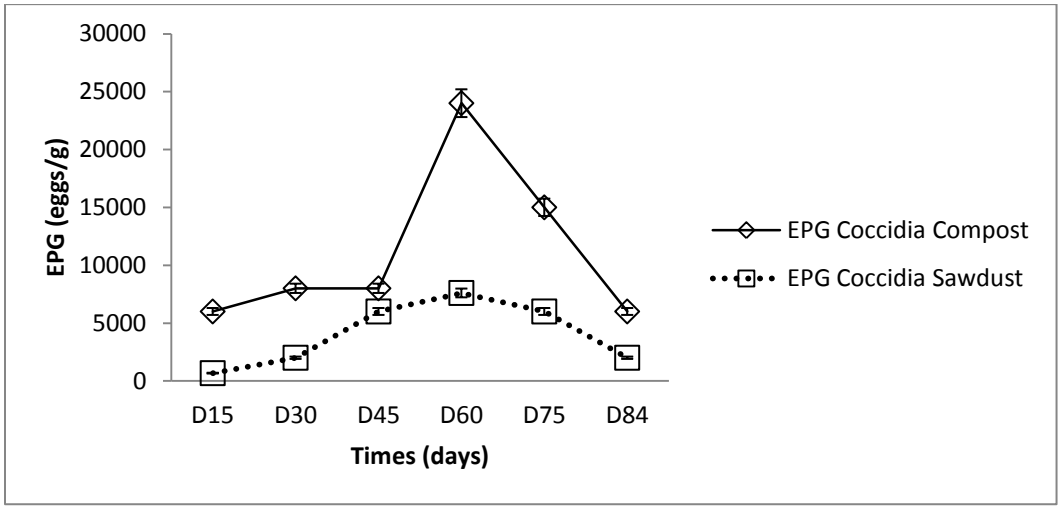

Fig. 1. Coccidia's EPG evolution of adults on both substrates.

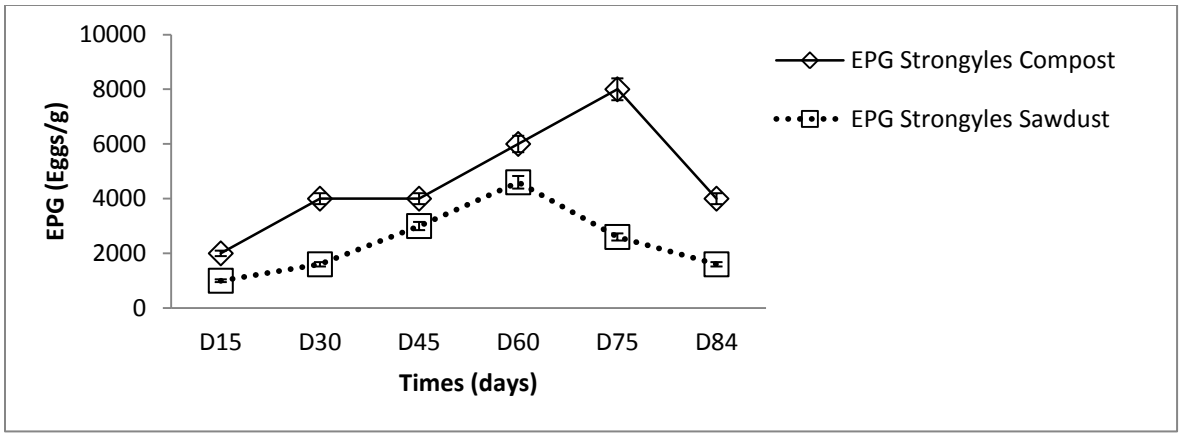

Fig. 2. Strongyles' EPG evolution of adults on both substrates.

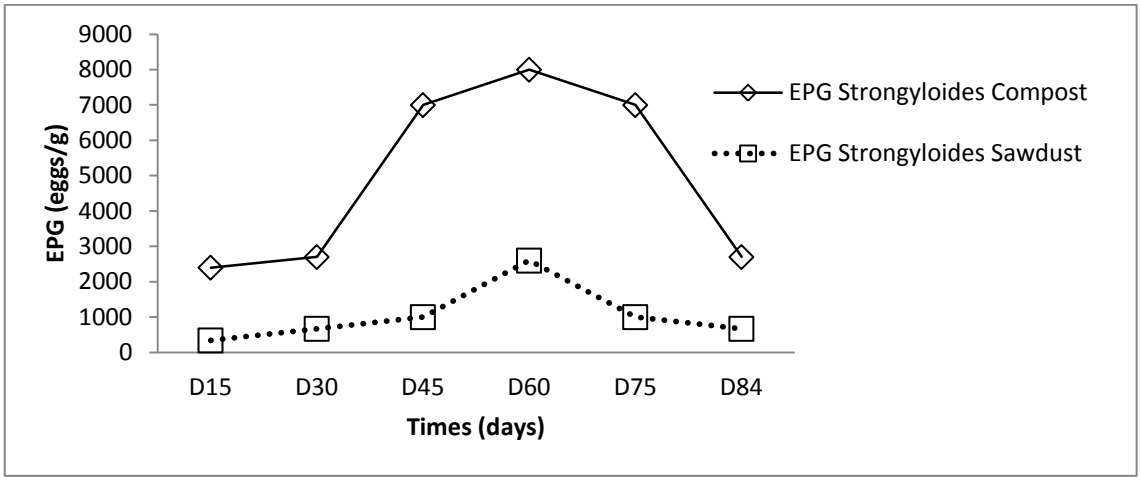

Fig. 3. Strongyloides' EPG evolution of adults on both substrates.

Of all nematodes' eggs encountered, coccidia' eggs were the most numerous with Eggs per Gramme of feces (EPG) values significantly higher of 39000 ± 5045 ($p < 0.05$) than those of strongyles in adults snails bred on sawdust and of 9870 ± 1527 than those of adults snails bred on compost. The EPG of strongyles and of strongyloides were virtually the same among snails bred on compost. By against snails bred on sawdust were significantly ($p < 0.05$) twice parasitized by strongyles than by strongyloides. Cumulative mortality rates were significantly ($p < 0.05$) higher on compost's snails than those bred on sawdust (Table 2).

The Archachatina marginata are oviparous hermaphroditic snails. Reproduction of progenitors is influenced by the type of substrate. Laying rate of broodstock installed on sawdust, less parasitized, was 38.77 ± 3.7 % higher ($p < 0.05$) than those installed on compost snails that were recognized as three more infected than previous ($p < 0.05$). This effect is confirmed in the hatching rates that was significantly lower of 37.5 ± 6.1% ($p < 0.05$) in compost's snails than sawdust's.

4. Discussion

The average daily gain obtained shows that Archachatina marginata's juveniles bred on the Tectona grandis's sawdust have better shell growth performances than those installed on compost. The shell growth can be explained by the fact that the juveniles entered in shell growth period. However, the fact remains that juvenile bred on sawdust have a better shell growth ($p < 0.05$). Indeed, the comfort of the substrate, its ventilation's level and its hygiene degree seem to be the reason why snails had better growth performance, knowing that the

breeding method is the same. The compost is a substrate rich in microorganisms and parasites that will surely have a negative effect on juveniles' complete growth. However, these results do not confirm those of Ayodele and Asimalowo (1999) who found that snail growth appears strongly related to the organic matter content and to a lesser extent in the calcium content of the food and the breeding's substrate. Ategbo and Zongo (2000) had shown the substrate's water level importance on the snails's growth. Otchoumou et al. (2004) are agreeing with Ategbo and Zongo (2000) and have shown the capabilities of shell repair of giant snails on different substrates. In Ivory Coast, for Kouassi et al. (2007), the ideal substrate for Archachatina ventricosa breeding in captivity is a mixing of a cassava plantation's soil and 10% of sawdust. African giant snails' growth induced by such a substrate is similar to that observed in snails bred on rainforests' soils. Indeed, Archachatina spp are subservient in rainforests' soil constantly renewed by the decomposition of various organic matters (Ejidike 2004). However, the environment for a better growth of A. marginata among the two substrate studied in the context of our study is the Tectona grandis's sawdust. This substrate although it is low in nutrients ensures in a one hand a good growth but more importantly in a second hand promotes a low mortality rate. This sawdust which is used is more efficient than the compost which is a natural substrate rich in organic matter usually used in snails' breeding. This organic matter may contain nutrients for growth, but also may contain microorganisms and parasitic pathogens for snails.

Table 5
Substrates' effect on infestation level and laying performances

Pa	Grps	Trt	r	M	Grps M	std	p.chisq	LSD
EPD Coccidia (eggs/g of feces)								
	a	Saw	6	4045.00	4.17	2829.73	0.022	3.44
	b	Comp	6	11166.67	8.83	7111.02		
EPD Strongyles (eggs/g of feces)								
	a	Saw	6	2400.00	4.5	1302.31	0.05	3.91
	b	Comp	6	4666.67	8.5	2065.59		
EPD Strongyloides (eggs/g of feces)								
	a	Saw	6	1046.67	3.67	800.04	0.006	2.72
	b	Comp	6	4966.67	9.33	2620.43		
Laying rate (%)								
	a	Saw	6	89.00	9.5	1.55	0.003	2.15
	b	Comp	6	51.33	3.5	4.93		
Hatching rate (%)								
	a	Saw	6	88.33	9.5	2.58	0.003	2.15
	b	Comp	6	50.67	3.5	8. 01		

Means with the same letters are not significantly different..
Pa : Parameters ; Grps : Groups ; Trt : Treatment; r : Number ; M : Means ; Grps M: Groups' means; std : Standart Error ; p.chisq: probability; HSD : Honestly Significant Difference ; Saw : Sawdust ; Comp : Compost

Certainly, sawdust is richer in lignin and the level of nutrient it contains is very small. More preferably, the lignin does not degrade quickly in the soil. Moreover, according to Ejidike (2004) weight gain of African giant snails is lower on soils rich in calcium oxide. Yet African giant snails draw calcium in the soil (Koudandé et al. 2006) and fond of lateritic soil and / or water-rich limestone (Chevalier 1992). Indeed, the calcium oxide may be less well absorbed than other forms of Ca. In general, Archachatina spp growth is under humidity's influence, the availability of food resources, and the availability of easily assimilated calcium source, and the ambient temperature and predators' density. According to Stiévenart (2000), Ireland (1991) and Ebenso and Okafor (2002), average daily gain of gastropods is inversely correlated with the ambient temperature. Other physical variables that are highly correlated (R2 = 98.0) with weight gain are relative humidity and rainfall. Regarding the shell growth, in contrast to our findings, it follows the same trend as weight gain. It depends on good farming conditions (Stiévenart 2000, Ireland 1991; Ebenso and Okafor 2002). Finally, Ejidike (2004) showed the importance of diet in addition to all these factors mentioned above. He is follows with Ademolu and Idowu (2005) who worked on the influence of several rations on Archachatina marginata's reproduction.

Juveniles' breed on sawdust growth confirms the results from the tests of infestations. Their best performances could be explained by the level of infestation which is three times lower than those which are breed

on compost. Indeed, compost's juveniles have a high rate of infestation of coccidia (Average EPG = 11166.7 ± 14.1 against 4045 ± 8.9) of strongyles (Average EPG = 4666.7 ± 7.6 against 2400 ± 6.0) of Strongyloides (Average EPG = 4966.7 ± 8.5 against 1046± 4.7) ($p < 0.05$). Substrates's infestation level also affects reproductive performance of broodstock. Snails on sawdust have a higher laying rate ($p < 0.05$) than those on compost. These EPG's values match those obtained by Hounzangbé-Adoté and Meyer (1996) and Azando et al. (2011) on small ruminants. Compost's high level of infestation would be the main cause of low juveniles' shell growth and adults' laying rate performances. It must be noted that compost's high level of infestation has decrease snails hatching rate. Indeed, whatever the speculation, the substrate always has an influence on the parasitic state thus the livestock performance. Krieg et al. (1988) showed through livestock trials the importance of the rough substrate in breeding sows. Their test revealed the effect of the substrate in the vitelline retention. Also, Vanessa and Michel (1999) have demonstrated that the substrate would contribute to the well-being of pets. They showed the importance of sawdust in the cages of laying hens. A good substrate is essential to the success of exploitation. This is even truer that a poor substrate induces a high animals' infestation rate. One of the immediate consequences of this state of affairs seems to be growth performance declining and a high mortality rate. It is clear from our work that the mortality rate of snails on sawdust is lower than that of snails on compost. Indeed, control of parasites of livestock is often essential otherwise penalize growth performance of these animals (Vandiest et al. 2007).

5. Conclusion

This work contributed to the specific knowledge on laying performances, weight and shell growth and finally the level of parasitism of snails bred with the same method but on two different substrates. It is clear from this experiment that sawdust is a substrate suitable for the breeding of the giant African snail. To better understand the strategic approaches, we suggest that the inventory of parasites' eggs in the feces of snails to be extended to other nematodes on the first hand and on the second that tests on juveniles should be considered in order to study their livestock response to various substrates and diets and finally that the parameters which are observed must be studied on three production cycles.

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