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## **RESPONSE OF GIANT AFRICAN LAND SNAILS (***ACHACHATINAMARGINATA***) HATCHLINGS FED MUNICIPAL ORGANIC WASTE, WITH DIFFERENT FOLIAGE AND FORAGE.**

# SHORT TITLE: MUNICIPAL ORGANIC WASTE, DIFFERENT FOLIAGE AND FORAGE FED AFRICAN GIANT LAND SNAILS.

<sup>1</sup>Etim, E. A.,<sup>1</sup>Agida, C. A.,and<sup>1&2</sup>Anigbogu, N. M

<sup>1</sup>Department of Animal Nutrition and Forage Science, MichaelOkpara University of Agriculture, Umudike, P.M.B. 7267 Umuahia, Abia State, Nigeria.

<sup>2</sup>Coordinator, Life-Enzyme and Fine Chemical Research, (Waste Management, Recycling & Utilization) **Corresponding Author's:** E- mail: agidachrisagboje@gmail.com; Phone: (+234)8067929337

### ABSTRACT

Response of Giant African Land snails (Achachatinamarginata) hatchlings fed municipal organic waste, with different foliage and forage, using Completely Randomized Design was studied. The research had fourtreatments five replicates each with twenty five hatchlings per replicate the experiment lasted for 120 days. There were significant differences (p<0.05, p<0.01) among treatment levels. Final weight gained, weight gained and average daily weight gained of snail fed experimental diets were found to be significantly (p<0.05, p<0.01) higher for snails on diet 1 and 4. Feed Conversion Ratio were best observed among snails fed diets 1 and 4 respectively and were statistically and numerically the same. Carcass analysis of were highly significant (p<0.01) among snails fed the diets, snails fed diet 1 and 4 had better dressing percentage compare to other treatments. Cost of feed/kg, cost of feed consumed, and cost of weight gained showed highest (p<0.05, p<0.01) values for diet 2, 3, 1 and 4 respectively. Gross profit, profit and return on investment were significant (p<0.05, p<0.01) among treatments with the analytical values being recorded for snails fed diet 1, 4, 2 and lastly 3. The cost efficiency (p<0.01) was also found to have the best values with snails on 1, 2, 3 and 4 respectively, cost efficiency is an investment analysis which indicates in monetary terms the amount derived to everyone naira invested. Foliage materials especially from pawpaw, banana, cocoyam, plantain, spinach together with municipal organic waste can be fed to achieve better performance without any adverse effects.

Keywords: Municipal organic waste, foliage, forage, giant African land snails, hatchlings

#### INTRODUCTION

Snails belong to a group of invertebrate animal known as mollusc. It is among the mollusks that carries shell. Others in this group include slugs, squid and cuttlefish. They belong to the family Achatinidae have species such as: Giant which snail African land (Achatinaachatina), Giant snail (Archachatinamarginata) and Garden snail (Achatinafulica) (Okonet al., 2012).

Animal growth depends greatly on the level of protein intake and utilization of other nutrient (Ejidike, 2004)).

Municipal organic wastes (MOW) consist of market garbage, kitchen garbage, papers, saw dust etc. They constitutes about 80% of 96,360,000 tones estimated municipal solid waste MSW) produced in Nigeria annually (Anigbogu and Chukwurah, 2014). They contain nutrients. If they are used as alternative feed, they will help in limiting environmental pollution and minimize the excess expenditure by government (Anigbogu *et al.*, 2019).Anigbogu*et al.*, (2019) further reported that after manual sorting and magnetic separation of the waste, 80% as sorted was municipal organic waste (MOW), while 7.708 (PW), which is about 8% of total MOW found in Nigeria in the year 2011, these could be recycled into reusable paper. It is good to note that, delivering these 7.708 million tons of PW annually from Nigeria alone to paper factories for recycling will save 122.36 million trees from being cut down each year. This could help to improve the environmental condition, for better health, thereby reducing the high level of the green-house gases and other nitrogenous compounds accumulation in the environment. Similarly, some cost associated with harvesting, storing, and processing crop residues for feeding farm animals may be eliminated if MOW feeds emerge as an acceptable feed alternative (Anigbogu*et al.*, 2019).

million tons of the MOW was noted as paper waste

#### MATERIALS AND METHODS Experimental site

The study was carried out at the Teaching and Research Farm, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria; located on Latitude  $05^0$  21'N and Longitude  $07^0$  33'E. It is approximately 122m above sea level with maximum

and minimum temperature of  $27 - 37^{\circ}$ C and  $20 - 26^{\circ}$ C, respectively. The relative humidityrangedfrom 50 - 90% with an annual rainfall of about 2177mm (NRCRI, 2014).

#### Experimental animals, management and diets

Fifty matured snails (Archachatinamarginata) sourced from a farm in IkotEkpene in AkwaIbom state of Nigeriawere used as parent stock in multiplication of five hundred hatchlings used in this study. The snails were confined in a wooden hutch (1 x 1 x 1) m where they laid a total of 2750eggs. They eggs were incubated in arectangular plastic bowl (60 x 45 x 45 cm) that was perforated to enhanced drainage. The eggs hatched between 30-32 days and the hatchlings werecollected and housed using 20 rectangular plasticbowl (60 x 45 x 45 cm) that were perforated to enhance easy drainage and flow of air, 136 hatchlings were collected from each rectangular plastic buckets. Each plastic bowlwas half filled with the prepared media (loamy soil 60%, saw dust 20% and poultry droppings 20%) it was allowed to ferment for 10 days before used Anigbogu (2016), and the hatchlings in the plastic bowlwatered 2 times daily to keep the soil moistened to prevent the snail from aestivating. The plastic bowl were placed on a raised wooden platform and surrounded by a shallow gutter filled with water mixed with used engine oil to prevent predators like ants from having access to the snails.

#### **Experimental diets**

The treatments consisted of Diet 1 = 100%Municipal organic wastes; Diet 2 = 100% Grasses ( 20% Signal grass, 20% Guinea grass, 20% Stubborn grass, 20% Elephant grass, 20% Carpet grass); Diet 3 = 75% grasses ( 15% Signal grass, 15% Guinea grass, 15% Stubborn grass, 15% Elephant grass, 15% Carpet grass) and 25% Centro; Diet 4 = 100%Miscellaneous plants( 20% Gourd leaves, 20% Pawpaw leaves, 20% Cocoyam leaves, 20% Bush green leaves, 20% Plantain laves).

#### Collection of Municipal Organic Waste (MOW)

Municipal Organic Waste was collected at the end of every market day using basket. They were made up of biodegradable waste (BDW) and recyclable waste (RW). Since it was a food market, about 98% of the waste were biodegradable waste (contains waste food items, vegetables and green waste etc) and about 2% were recyclable waste (plastic, paper, cloths, aluminium cans etc). These 2% recyclable waste were hand-sorted every day as they were collected from the bulk of the waste to have about 98% of BDW of the MOW used. After sorting, they were chopped into piece using knife before fed to hatchlings.Feed and water were given to the snails *ad libitum*by 6pm each day.

Table 1: Composition of	f experimental (	diets fed Gian	t African land	snail (Archachatinamarginat	<b>a</b> )
				Treatments	

	Treatments					
Feed	1	2	3	4		
Municipal organic waste (MOW)	100.00	_	_	_		
Grasses:						
Signal grass (Brachiariadecumben)	_	20.00	15.00	_		
Guinea grass (Pannicum maximum)	_	20.00	15.00	_		
Stubborn grass (Eleucineindica)	_	20.00	15.00	_		
Elephant grass (Pennisetumpurpureum)	_	20.00	15.00	_		
Carpet grass (Axonopuscompresus)	_	20.00	15.00	_		
Legume:						
Centro (Centrosema pubescence)	_	_	25.00	_		
Miscellaneous plants:						
Gourd leaf (Adenoupusbreviflorus)	_	_	_	20.00		
Pawpaw leaf (Carica papaya)	_	_	_	20.00		
Bush green leaf (Amaranthusspinosus)	_	_	_	20.00		
Plantain leaf (Musa spp)	_	_	_	20.00		
Cocoyam leaf (Colocosiaesculenta)	_	-	_	20.00		
TOTAL	100.00	100.00	100.00	100.00		
Chemical analysis						
Dry matter (%)	91.88	87.37	84.74	83.08		
Crude protein (%)	12.25	8.20	12.47	20.20		
Ether extract (%)	17.90	2.50	2.68	4.24		
Crude fibre (%)	16.90	33.40	33.00	15.32		
Nitrogen free extract (%)	7.80	47.50	43.60	47.80		
Ash (%)	9.29	8.40	8.25	12.32		
Metabolizable energy (Kcal/gDM)	2473.84	3018.14	3069.47	3052.44		
Ca (%)	1.89	0.50	0.63	1.45		
P (%)	0.29	0.22	0.24	0.45		

#### Body weight and feed intake

The initial body weight was obtained and subsequently weighed at weekly intervals till the end of the experiment. A given quantity of each experimental feed was weighed daily for each replicate, then fed, and left-over collected and weighed were subtracted from the initial quantity to obtain the total feed intake. A sensitive weighing balance was used in the measurement.

#### Feed conversion ratio and Mortality (%)

The feed conversion ratio (FCR) was computed from feed intake over weight gained. There was no

mortality during the course of the experiment.

#### **Carcass evaluation**

At the end of the study, 40 snails were randomly selected from each treatment at 10 snail each per replicate for carcass evaluation. The visceral, shell and edible portion were separated and weighed, from where the dressing percentage was obtained. Parameters measured were as described by Okeke, (2007) as follows: Shell % equal to weight of shell divided by live weight of snail multiply by 100, Visceral % equal to weight of visceral divided by live weight of snail multiply by 100, Visceral % equal to weight of snail multiply by 100, Visceral % equal to weight of snail multiply by 100, Dressing % equal to weight of snail multiply by 100, Shell thickness was measured by placing the upper portion of the shell between the jaws of a Micrometer screw gauge and reading obtained in centimeter (cm).

#### Cost benefit analysis

The cost benefit analysis was computed based on the method as described by Anigbogu, (2003). Cost of feed/kg equal to cost of 100kg of feed divided by100, cost of total feed consumed equal to cost of feed multiply by feed intake, cost of animal equal to cost of individual animal divided by Initial weight of animal, cost/weight gain equal to Cost of feed multiply by FCR, cost of production per animal equal to cost of individual animal plus total cost of feed consumed, gross profit equalto cost of animal multiply by final weight, profit equal to gross profit subtracted by cost of production, % return on investment equalsto grossprofit divided by cost of production multiply by 100, cost efficiency equal gross profit divided feed cost.

#### Experimental design and statistical analysis

Five hundred (500) hatchlings were selected and randomly divided into four (4) groups of 125 hatchlings each and were replicated five (5) times with 25 hatchlings per replicates, using Completely Randomized Design. The data collected were subjected to analysis of variance (ANOVA) as described by Steel and Torrie (1980). Means were separated using Duncan Multiple New Range Test (DMRT) as described by Duncan (1955).

#### **RESULTS AND DISCUSSION**

The result for weight gain, feed intake and feed conversion ratio of snails fed experimental diets are presented in Table 2.

Weight gain were significant (p<0.05) among treatment levels. Snails on diet 1 gave a better numerical value of weight gain as fed municipal organic waste (MOW). Source of municipal organic waste are rich edible products like vegetables and kitchen waste, these products are important source of biodegradable municipal solid waste readily available and are rich in nutrients easily digestible and utilizable by the animals (Anigbogu et al., 2019). The source of nutrient found in diet 4 may also contain rich essential nutrients as its required forms digestible crude fibre capable contain of demonstrating better performance as compared to snails fed diet 2 and 3 respectively. The growth performance differs from that reported by Ejidike, (2004)that the growth performance of Achachatinamarginatahatchlings fed diet with 15% crude protein performed lower than those on 20 and 25% crude protein.

Average daily feed intake were significant (P<0.05) among treatments. Snails on diet 3 had the highest daily feed intake (3.08g). The high feed intake of the snails noted on diet 3 indicates a better acceptability of the feed and good forage combination. Feed intake is subject to palatability, and animals are known to eat more when feed is palatable and tasty (Alikweet *al.*, 2013). Reduction in feed intake in diet 2 may be due to the fibrous nature of the diet (Akpanet *al.*, 2012). It has been observed that feed intake tend to reduce when the fiber content in the diet increase animal growth performance depends greatly on the level of protein-energy intake and a balance nutrient utilization (Ejidike, 2004).

Average daily weight gain were significant (P<0.05) among treatments. Snails on diet 4 had significantly (P<0.05) higher weight gain than those in other treatments, though comparable (P>0.05) to diet 1. Snails on diet 2 had the lowest weight gain, but with comparable weight gain (P>0.05) with those on diet 3. The highest gain in weight found in diets 4 and 1 could be attributed to high crude protein and energy content of the diets (Odo and Orji, 2010), and what was consumed was well utilized by the snails (Alikweet al., 2013). The low weight gain of snails in diet 2 and diet 3 may be due to the high level of fiber and anti-nutrients of the diets (Aniet al., 2014). High dietary fiber is known to limit the availability of nutrients, especially energy and protein. Such depression in apparent nutrient digestibility and retention was attributed to high rate of passage of digester and excessive nutrient excretion in animals fed high fiber diets (Macdonald et al., 2002).

**Feed Conversion Ratio** differed significantly (P< 0.05) among treatments. Snails on diet 3 had higher value than diet 2 but were statistically similar. Diet 1 and diet 4 had the best feed conversion ratio but were statistically and numerically the same. This implies that, they had similar influence on the growth performance of the snails, and the snails were efficient in utilizing the feeds than others (Ejidike,

2004). The poorest feed conversion ratio was recorded on diets 3 and 2. This is an indication that snails on diets 3 and 2 were not efficient in converting their diets to meat as those in other diets. This suggestion agrees with reports made by (Akpan*et al.*, 2012) andOkpez*eet al.* (2007) that poor forage utilization resulted in depression in growth performance.

#### CARCASS CHARACTERISTICS

The results of dress weight, shell percentage, visceral percentage, dressing percentage and shell thickness of snails fed experimental diets are presented in Table 3.

Average live weight were significant differences (P<0.05) in live weight among treatments. Diet 4 was highest followed by diets 1 and 3 respectively. Diet 2 had the least live weight. The high live weight recorded in diets 1 and 4 could be as a result of increased growth performance which is attributed to high nutrient absorption and utilization on account of low fiber content of the diets (Ani*et al.*, 2014).

**Dressedweight** were significant (P<0.05) among treatments. Diet 4 was highest followed by diets 1 and 2, respectively; while diet 3 had the least dress weight. The high dress weight recorded in diets 4 and 1 could be traced to better growth performance which is attributed to high protein and energy content of the diets as recorded in this study. This suggestion is corroborates the report of Anigbogu (2003) that high crude protein enhances animal growth performance and high meat yield.

Average shell percentage values differed significantly (P<0.05) among treatments. The snails on diet 2 had the poorest shell percentage while those on diet 4 had the highest, snails on diet 1 and 3 were statistically (p>0.05) similar. The highest shell percentage recorded in diet 4 could be attributed to high calcium/phosphorus content of the diet (Mogboet al., 2014). This is an indication that, what was consumed was well utilized by the snails to build up the shell and make room for growth of internal mass (Alikweet al., 2013). The least shell percentage observed in diet 2 could be as a result of low level of calcium/phosphorus content (Ubuaet al., 2013).

Average visceral percentage were significant (P<0.05) among treatments. Snails on diet 1 had the least value and were followed by snails on diet 3 and 4 were statistically (p>0.05) the same. The highest visceral percentage value was recorded in diet 2.

Average dressing percentage showed significant (P<0.05) differences among treatments as in Table 3. Snails on diet 4 had significantly (P<0.05) higher dressing percentage than others. The least dressing percentage value was observed in diet 2 followed by diet 3. The higher dressing percentage observed in diet 4 and 1 are attributed to the fact that snails fed diets 4 and 1 had superior live weights. As a general rule, heavier animals are expected to have higher dressing percentage than lighter ones. As stated

earlier, diets 4 and 1 contained high quality protein and energy levels and this could have met the snails' protein and energy requirement for optimal growth.

**Shell thickness** showed significantly higher difference at (P<0.05) for diet 4 and was followed by diet 1, while diets 2 and 3 respectively were statistically (P>0.05) comparable. The superiority of the shell thickness of snails in diet 4 could be attributed to calcium/phosphorus availability which enhanced growth performance on this diet. The least value obtained in diet 2 may be a reflection of the low calcium/phosphorus composition of the diet (Babalola*et al.*, 2015).

### **COST BENEFIT ANALYSIS**

The results for cost of feed, cost of feed consumed, cost/gram of snail, cost/gram weight gain, cost of production/snail, gross profit, profit return on investment, and cost efficiency of snails fed experimental diets are presented in Table 4.

**Cost of feed per kilogram** of diets 1, 2, 3 and 4 were significant (p<0.05). The lowest cost of feed recorded in diet 4 was as a result of the low cost of acquiring the feed components when compared to other diets. This agrees with the report made by Ojebiji*et al.*, (2011) who opined that, low cost of feed will led to the reduction in the production cost. This has the potential to increase farmers' profit margin and stimulate increased snail production, because snail feeds are easily accessible as compare with other livestock in the tropics especially the sub-Sahara Africa (Anigbogu*et al.*, 2009).

**Cost of feed consumed**were significant (P<0.05) among treatments. Snails fed diets 2 and 3, respectively had higher cost of feed consumed than snails on other diets. However, they were statistically (P>0.05) the same. The high cost of feed in diets 2 and 3 amount to high cost of production which brings about poor return on investment as witnessed in this study, this was also noted by Anigbogu and Ezekwem (2013) in a similar study with goats.

The values for **cost per gram weight gain** showed significant (P<0.05) differences among treatments. Cost per gram weight gain of snail fed diet 2was significantly higher, though similar to snails fed diet 3 but differed (P<0.05) to snails fed diet 1 and 4, respectively. The best cost per gram weight gain was recorded in diet 4.This may be due to the improved feed conversion ratio (FCR) as reported by Ani*et al.* (2014).They attributed reduction in feed cost per kg weight gain of birds to enhanced feed utilization and weight gain. The highest feed cost per gram weight gain was observed in diet 2. This could be as a result of high cost of feed per kg as revealed in this work, and also noted by Anigbogu and Ezekwem (2013) in a similar study with goat.

**Cost of production**showed significant differences (P<0.05) among treatments. The highest cost of production observed in diet 2 though similar (P>0.05) to snails fed diet 1 and diet 4 respectively, but differed (P<0.05) with the snails fed diet 3. The

high cost of production in diet 2 is traced to high cost of feed/kg as revealed in this work and also indicated by Anigbogu and Ezekwem (2013).

**Gross profit** realized differed significantly (P<0.05). The best gross profit was observed in snails fed diet 1 and was statistically (P>0.05) similar to diet 4, while the poorest was obtained from diet 3, but was not significantly different from diet 2. High gross profit recorded in diets 1 and 4, respectively could be as a result of low cost of feed per kg and low cost of feed consumed per animalsas revealed in this study. Anigbogu*et al.* (2009) opined that, the low cost of feed consumed by animal has the potential to increase farmers' profit margin, when goats were fed sawdust as based feed.

**Profit**realized differed significantly (P<0.05) among the treatments. Snails fed diet 1had the highest profit followed by diet 4. Snails on diet 3 had the lowest and were comparable to snails fed diet 2. The highest profit on diet 1 could be traced to low cost of feed consumed and better performances as witness in this work. This is in line with the findings of Aniet al. (2014), who observed low cost of feed consumed per animal with an increase inclusion of nonconventional feed material in ruminant feed. They concluded that, low cost of feed consumed per animal could increase farmers profit and stimulate increase animal production. The poor profit obtained in diet 3 is attributed to high cost of feed per kg and high cost of feed consumed per snail as revealed in this work.

**Return on investment** differed significantly (P<0.05) among treatments. The return on investment was highest with diet 1, followed by diet 4, diet 3 and diet 2, respectively. The lowest value obtained from diet 2 implies a poor saving, this could be as a result of high cost of acquiring diet 2 compared to the low cost of acquiring diet 4.

Cost Efficiency Ratiowas also significantly different (P< 0.05) among treatments. Economic efficiency was high in diet 4, followed by diet 1, diet 3 and diet 2, respectively. The highest value obtained from diet 4could be an indication of the low cost of production and high profit earlier reported in this study. Cost benefit analysis on snails fed experimental diets indicated that municipal organic wastes (MOW), which are readily available and can be obtained from the market and kitchen wastes are rich in biodegradable organic composite waste materials. It can reduce relatively the inherent high cost of purchasing forage fodder for snail production. This can also help in recycling municipal organic waste as feed stuff for snails and encourage urban dwellers to venture into small scale snail farm production in other to meet their immediate household protein needs especially for low income urban dwellers.

#### CONCLUSION AND RECOMMENDATION

The information presented in this study herein; has strongly shown that giant African land snail (*Archachatinamarginata*) hatchlingsrespond better on diet 4 (Miscellaneous plants) and diet 1 (Municipal organic wastes) considering the parameters measured. It is therefore recommended that they be used in snail production since they have no depressive effect and are available having no competition between man and animal.

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 Table 2: Performance of snails fed the experimental diets

	Experimental Diets					
Parameters	1	2	3	4	SEM	SIG.
Initial weight/snail (g)	12.13	12.38	11.50	12.13	0.07	NS
Weight gain/snail (g)	19.87ª	11.12 <sup>c</sup>	13.70 <sup>b</sup>	18.57ª	0.55	**
Final weight/snail (g)	32.00 <sup>a</sup>	23.50 <sup>d</sup>	25.20 <sup>c</sup>	30.70 <sup>b</sup>	0.83	**
Average Daily weight gain/snail (g)	0.16 <sup>ab</sup>	0.12 <sup>c</sup>	0.13 <sup>bc</sup>	0.17 <sup>a</sup>	0.007	**
Average Daily feed intake /snail (g)	2.79 <sup>b</sup>	2.89 <sup>b</sup>	3.08 <sup>a</sup>	2.96 <sup>b</sup>	0.03	*
Feed conversion ratio	0.14 <sup>a</sup>	0.18 <sup>b</sup>	0.19 <sup>b</sup>	0.14 <sup>a</sup>	0.006	*

a, b, c, d means within the same row, with different superscripts were significantly different  $(p<0.05)^*$ ,  $(p<0.01)^{**}$ , SEM = standard error mean, NS = not significant

Table 3:	Carcass	analysis (	of snails	fed the	experimental	diets
		•/				

Table 5. Carcass analysis of shans led the experimental dets								
	Experimental Diets							
Parameters	1	2	3	4	SEM	SIG		
Av. live weight (g)	30.25 <sup>b</sup>	27.50 <sup>d</sup>	28.50 <sup>c</sup>	32.75 <sup>a</sup>	0.46	**		
Dressed weight (g)	13.10 <sup>b</sup>	12.20 <sup>c</sup>	12.10 <sup>c</sup>	15.10 <sup>a</sup>	0.13	**		
Av. shell percentage/snail (%)	25.00 <sup>b</sup>	21.81 <sup>d</sup>	25.96 <sup>b</sup>	27.78 <sup>a</sup>	0.50	**		
Av. visceral percentage/snail (%)	25.09 <sup>a</sup>	20.00 <sup>c</sup>	21.75 <sup>b</sup>	21.98 <sup>b</sup>	0.42	**		
Av.dressing percentage/snail (%)	43.30 <sup>b</sup>	40.36 <sup>c</sup>	42.45 <sup>b</sup>	46.10 <sup>a</sup>	0.51	**		
Shell thickness (cm)	0.09 <sup>b</sup>	0.07°	$0.08^{bc}$	0.12 <sup>a</sup>	0.01	**		

<sup>a,b,c,d</sup> means within the same row, with different superscripts were significantly different (p<0.05)\*, (p<0.01)\*\*, SEM = standard error mean.

## Table 4: Cost benefit analysis of snails fed the experimental diets

	Experimental Diets						
Parameters	1	2	3	4	SEM	SIG.	
Cost of feed/kg( <del>N)</del>	0.67°	0.83ª	0.75 <sup>b</sup>	0.58 <sup>d</sup>	0.02	**	
Cost of feed consumed/snail (N)	1.90 <sup>b</sup>	2.40 <sup>a</sup>	2.31 <sup>a</sup>	1.72 <sup>b</sup>	0.008	*	
Cost/g of snail ( <del>N)</del>	1.42	1.42	1.42	1.42	0.005	NS	
Cost/g weight gain ( <del>N)</del>	$0.09^{b}$	0.15 <sup>a</sup>	0.14 <sup>a</sup>	$0.08^{b}$	0.007	*	
Cost of production/snail (N)	17.90 <sup>a</sup>	18.41 <sup>a</sup>	17.08 <sup>b</sup>	17.90 <sup>a</sup>	0.10	*	
Gross profit ( <del>N)</del>	551.19 <sup>a</sup>	413.12 <sup>b</sup>	411.52 <sup>b</sup>	528.80 <sup>a</sup>	14.99	*	
Profit ( <del>N)</del>	533.29 <sup>a</sup>	394.71°	394.44°	510.80 <sup>b</sup>	15.00	**	
Return on investment (%)	3079.27 <sup>a</sup>	2244.00 <sup>d</sup>	2409.37°	2954.19 <sup>b</sup>	87.63	**	
Economic efficiency	30.79 <sup>b</sup>	22.44 <sup>d</sup>	24.09 <sup>c</sup>	29.54 <sup>a</sup>	7.969	**	

<sup>a,b,c,d</sup> means within the same row, with different superscripts were significantly different  $(p<0.05)^*$ ,  $(p<0.01)^{**}$ , SEM = standard error mean.