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The combined effect of stocking density and dietary calcium content on egg-laying performance of *Achatina fulica*

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Abstract

The aim of this study was to determine the appropriate amount of dietary calcium to reduce the impact of density on laying performance in *Achatina fulica*. To do this, 1380 breeding animals were divided into 8 batches reared at different densities. For each rearing density, the breeding stock were classified into 4 sub-lots and fed diets differing in their calcium content. The reproductive performance of these breeders was determined and compared as a function of rearing density and dietary calcium level. The results showed that for a diet with a low calcium source content (10%), the density above 25 breeders per square metre led to a significant drop in the laying rate (13 ± 3.7% to 9.36 ± 4.13%) and the number of eggs per clutch (111.23 eggs/clutch to 98 eggs/clutch). However, for a diet with a high calcium source content (40%), the rearing density had virtually no impact on the reproductive performance of *Achatina fulica*. This study shows that, in order to make his facilities profitable, the farmer can place 40 breeders per square metre of rearing enclosure, provided that their feed contains sufficient calcium (40% oyster shell).

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Introduction

Snail meat is a real source of animal protein, with an average content of 74.6% (Fabguaro *et al.*, 2006), as well as essential amino acids such as lysine, leucine and phenylalanine (Otchoumou *et al.*, 2010). It is also a valuable source of macronutrients and income for many households (Brescia and Chardonnet, 2002). Faced with increased pressure from the harvesting of these molluscs in West Africa and the destruction of their natural habitats, snail farming appears to be a necessary solution to compensate for seasonal deficits and ensure the survival of this animal species in the face of human activity (Otchoumou, 1997). Achatinidae farming therefore aims to progressively reduce this pressure by developing rational production techniques, including the development of effective diets and the determination of adequate stocking densities (Karamoko *et al.*, 2011, Aman *et al.*, 2023) to improve growth and optimal reproduction in these animals. Several studies have shown that overcrowding results in slower snail growth and poor reproductive performance.

This has been justified by the fact that overcrowding in snails leads to changes in food quality (Thomas *et al.*, 1975) resulting from an accumulation of faeces and mucus in the food (Dan and Bailey 1982). This has been justified by the fact that overcrowding in snails leads to changes in food quality (Thomas *et al.*, 1975) resulting from an accumulation of faeces and mucus in the food (Dan & Bailey 1982). High densities also lead to an increase in the amount of mucus secreted, making it difficult for snails to move around in search of food and to exploit the environment. The aim of the breeder, in order to make his facilities profitable, is to place as many breeding animals as possible per square metre of breeding enclosure, provided that this density does not cause any disturbance to the animals.

It was therefore with the aim of helping breeders to find solutions to the problem of stocking density that this study was initiated. The main objective of this work is to minimise the effect of overcrowding on laying performance through dietary calcium levels.

Material and methods

Material

Study site

This study was carried out at the "Choice" farm located in Oussou, a village in the Toumodi sub-prefecture. The department of Toumodi is located at the base of the “V-Baoulé”, in the Bélier region. The “Oussou” village is characterised by transitional vegetation between the forest in the south and the savannah in the north. The work lasted around 6 months.

Biological material

The biological material used in this study consisted of snails (*Achatina fulica*) of reproductive age (6 months), with a average live weight (65 ± 1,2 g) and a average shell length (8 ± 0,2 cm) (Fig. 1) relatively identical.

Feed

The animals were fed concentrated diets made from cereal meal (maize), agri-food by-products (wheat bran and soybean meal) and calcium sources (oyster shell powder).

Breeding enclosure

Cement brick enclosures measuring 4 per square metre were used to rear the snails. These enclosures are fitted with mosquito net-type covers to prevent the snails from escaping and to ensure that the enclosure is well ventilated. The bottoms of these enclosures are filled with compost and covered with dry cocoa leaves. A straw-roofed shed houses the breeding pens to protect the snails from the weather (Fig. 2).

Measuring instrument

A sartorius balance with a precision of 0.1g and a capacity of 500g was used to weigh the animals, eggs and diets' ingredients (Fig. 3). A werckmann electronic caliper, accurate to 1/10 of a millimetre, was used to measure snail and egg size (Fig. 4).

Methods

Constitution of experimental batches

One thousand three hundred and eighty (1380) *Achatina fulica* were selected, weighed and measured. These animals of approximately identical weight and shell length were randomly divided into seven batches according to the different rearing densities considered: 10 snails per square metre, 15 snails per square metre, 20 snails per square metre, 25 snails per square metre, 30 snails per square metre, 35 snails per square metre and 40 snails per square metre. Each batch was in turn divided into 4 sub-batches according to four diets differing in their calcium source content.

Preparing snail diets and feeding snails

Four feeds were made at the base in identical proportions by mixing maize meal (22%), soya cake (35%), wheat bran (40%) and premix (3%). Oyster shell powder was added to this feed at different rates (10%, 20%, 30% and 40%) to produce four diets with different. For diet R1, 10% shell powder was added to the feed, 20% for diet R2, 30% for diet R3 and 40% for diet R4. Each diet was associated with different rearing densities (10 snails per square metre, 15 snails per square metre, 20 snails per square metre, 25 snails per square metre, 30 snails per square metre, 35 snails per square metre and 40 snails per square metre. The animals were fed ad libitum every 2 days and watered daily morning and evening.

Estimating the egg-laying performance of snails

Every 12 days, the substrates of each batch of snails were searched to collect eggs per clutch. Eggs found grouped together in the same place were considered to constitute a clutch.

These eggs, collected according to the diet and rearing density of the breeders, were weighed and 10 (eggs) were chosen at random to be individually weighed and measured lengthways (large diameter) and widthways (small diameter). The eggs from each clutch were then incubated in plastic bowls lined with moist coconut pulp fibres. These bins are fitted with perforated lids (Fig. 5) to prevent the hatched from escaping. After 16 days of incubation, the bowls were opened and the hatchlings counted. The number of eggs laid per breeding density was also counted.

Expressing results

For each rearing density and depending on the calcium source content of the diet, the laying rate, the number of eggs per laying and the average egg hatching rate were determined according to the following formulae:

Laying rate = Number of eggs laid/number of spawners

Average number of eggs per clutch = Total number of eggs laid/number of clutches

Hatching rate = Total number of eggs hatched / Total number of eggs incubated

Statistical analysis

Statistical analysis of the data was carried out using Statistica version 7.1 software. The number of eggs per clutch, the laying rate and the hatching rate were compared as a function of the diet and rearing density of the breeding snails using the Schéffé test at $P<0.05$.

Results

Laying performance of snails fed a diet containing 10% oyster shell (R1) as a function of rearing density

Table 1 records the number of eggs per clutch, the laying rate and the hatching rate of the eggs of snails fed a diet containing 10% oyster shell powder.

Analysis of the results shows an identical number of eggs per laying, at rearing densities D1 (10 snails per square metre), D2 (15 snails per square metre), D3 (20 snails per square metre) and D4 (25 snails per square metre). The number of eggs counted per clutch was 111.17 ± 1.52 for D1, 111.87 ± 3.90 for D2, 111.63 ± 2.01 for D3 and 111.23 ± 2.54 for D4. Increasing the rearing density from D5 (30 snails per square metre) led to a significant reduction in the number of eggs per clutch. Consequently, the lowest oviposition rate (7.5 ± 3.43%) was observed in snails exposed to the highest stocking density, (D7, 40 snails per square metre). The highest egg-laying rate (15.24 ± 3.23%) was for snails reared at the lowest density (10 breeders per square metre) and the lowest (7.35 ± 3.43) for snails reared at the highest density (40 breeders per square metre). The statistical analysis indicates a significant drop in the egg laying rate of snails from density D3 (14 ± 3.12%) to D7 (7.35 ± 3.43). Also, the hatching rate experienced a significant decrease (from 60 ± 3.33% to 22 ± 6.52) with the increase in rearing density from D1 to D7.

Table 1. Reproductive performance of *A. fulica* subjected to a diet with 10% calcium source diet (R1) as a function of rearing density.

	R1-D1	R1-D2	R1-D3	R1-D4	R1-D5	R1-D6	R1-D7
Number of eggs	111,17ᵃ ±1,52	111,87ᵃ ±3,90	111,63ᵃ ±2,01	111,23ᵃ ±2,54	98ᵇ ±7,26	95,43ᵇ ±7,64	94,47ᵇ ±8,31
Laying rate (%)	15,24 ± 3,23ᵃ	15,18 ± 4,22ᵃ	14 ± 3,12ᵇ	13 ± 3,7ᵇ	9,36 ± 4,13ᶜ	8,45 ± 3,27ᶜ	7,35 ± 3,43ᵈ
Hatching rate (%)	60 ± 8,33ᵃ	59 ±6,84ᵃ	41 ± 7,13ᵇ	39 ± 13,17ᵇ	30 ± 7,48ᶜ	28 ± 7,56ᶜ	22 ± 6,52ᵈ

R1: 10% calcium source diet,
D1: Density of 10 snails per square metre, D2: Density of 15 snails per square metre,
D3: Density of 20 snails per square metre, D4: Density of 25 snails per square metre,
D5: Density of 30 snails per square metre, D6: Density of 35 snails per square metre,
D7: Density of 40 snails per square metre

NB: The mean values of the same line indexed by the same alphabetical letters are not statistically different using Scheffé test at P<0.05.

Table 2. Reproductive performance of *A. fulica* subjected to a diet with 20% calcium source diet (R1) as a function of rearing density.

	R2-D1	R2-D2	R2-D3	R2-D4	R2-D5	R2-D6	R2-D7
Number of eggs	127,4ᵃ ±9,1	128,73ᵃ ±8,78	127,33ᵃ ±11,12	128,27ᵃ ±9,96	119,63ᵃ ±16,83	110,43ᵇ ±15,75	98,77ᵇ ±11,69
Laying rate (%)	19,22 ± 6,21ᵃ	19,32 ± 5,41ᵃ	18,87 ± 6,56ᵃ	16,41 ±5,65ᵇ	13,85 ± 5,77ᶜ	13,57 ± 6,10ᶜ	12,66 ± 6,11ᶜ
Hatching rate (%)	70 ±12,4ᵃ	70 ± 9,9ᵃ	71 ± 11,62ᵃ	65 ± 11,2ᵇ	63 ± 12,3ᵇ	64 ± 9,3ᵇ	60 ± 11,4b

R2: 20% calcium source diet
D1: Density of 10 snails per square metre, D2: Density of 15 snails per square metre, D3: Density of 20 snails per square metre, D4: Density of 25 snails per square metre, D5: Density of 30 snails per square metre, D6: Density of 35 snails per square metre, D7: Density of 40 snails per square metre.

NB: The mean values of the same line indexed by the same alphabetical letters are not statistically different using Scheffé test at P<0.05.

Egg laying performance of A. fulica subjected to a diet with 20% calcium source (R1) depending on the rearing density

Egg production per clutch was statistically similar for snails receiving a diet enriched with 20% oyster shell at stocking densities D1 (10 breeders per square metre), D2 (15 breeders per square metre), D3 (20 breeders per m²), D4 (25 breeders per square metre) and D5 (30 breeders per m²) (Table 2). A significant reduction in the number of eggs per clutch was observed at densities D6 (110.43 ± 15.75 eggs) and D7 (98.77 ± 11.69 eggs). The egg-laying rates remained statistically unchanged and higher at densities D1 (19.22 ± 6.21 %), D2 (19.32 ± 5.41%) and D3 (18.87 ± 6.56 %) compared to those observed in snails subjected to densities beyond D3 (20 reproductives per m²). It is evident that the laying rates were lower at densities D4 (16.41 ± 5.65%), D5 (13.85 ± 5.77%), D6 (13.57 ± 6.10%) and D7. (12.66 ± 6.11%). The highest hatching rates were observed at densities D1, D2 and D3, with values of 70 ± 12.4%, 70 ± 9.9% and 71 ± 11.62%, respectively. The lowest hatching rates were observed at densities D4 (65 ± 11.2%), D5 (63 ± 12.3%), D6 (64 ± 9.3%) and D7 (60 ± 11.4%).

Table 3. Reproductive performance of *A. fulica* subjected to a diet with 30% calcium source diet (R1) as a function of rearing density.

	R3-D1	R3-D2	R3-D3	R3-D4	R3-D5	R3-D6	R3-D7
Number of eggs	142[a] ±7,38	143[a] ±6,84	142[a] ±6,63	144[a] ±6,35	143[a] ±6 ,58	143[a] ±6,58	142[a] ±8,04
Laying rate (%)	32,10 ± 7,61[a]	32,21 ± 7,15a	32,18 ± 6,17[a]	31,14 ± 6,41[a]	31,09 ± 6,41[b]	30,97 ± 6,14[b]	31,96 ± 5,17[b]
Hatching rate (%)	81 ± 11,2[a]	80 ± 12,35[a]	80 ± 14,02[a]	80 ± 11,2[a]	81 ± 9,89[a]	80 ± 11,33[a]	79 ± 7,98[a]

R3: 30% calcium source diet

D1: Density of 10 snails per square metre, D2: Density of 15 snails per square metre, D3: Density of 20 snails per square metre, D4: Density of 25 snails per square metre, D5: Density of 30 snails per square metre, D6: Density of 35 snails per square metre, D7: Density of 40 snails per square metre.

NB: The mean values of the same line indexed by the same alphabetical letters are not statistically different using Scheffé Test at P<0.05.

Table 4. Reproductive performance of *A. fulica* subjected to a diet with 40% calcium source diet (R4) as a function of rearing density.

	R4-D1	R4-D2	R4-D3	R4-D4	R4-D5	R4-D6	R4-D7
Number of eggs	164,93[a] ±6,69	162,5[a] ±8,44	163,47[a] ±10,97	164,97[a] ±6,18	165,8[a] ±13,52	167,73[a] ±10,62	164,87[a] ±13,12
Laying rate (%)	40,1 ± 9,20[a]	40,15 ± 11,02[a]	40,11 ± 10,85[a]	40,17 ± 9,77[a]	41,94 ± 9,34[a]	40,24 ± 8,52[b]	40,17 ± 9,43[b]
Hatching rate (%)	83 ± 14,33[a]	82 ± 12,45[a]	84 ± 13,22[a]	83 ± 14,26[a]	82 ± 12,50[a]	81 ± 13,45[a]	80 ± 18,14[a]

R4: 40% calcium source diet

D1: Density of 10 snails per square metre, D2: Density of 15 snails per square metre, D3: Density of 20 snails per square metre, D4: Density of 25 snails per square metre, D5: Density of 30 snails per square metre, D6: Density of 35 snails per square metre, D7: Density of 40 snails per square metre.

NB: The mean values of the same line indexed by the same alphabetical letters are not statistically different using Scheffé Test at P<0.05.

Egg-laying performance of A. fulica subjected to a diet with 30% calcium source diet (R1) as a function of rearing density

Table 3 summarises the egg-laying performance of snails fed a concentrated diet with 30% oyster shell powder as a function of rearing density.

Analysis of the results shows no significant differences between the different rearing densities with regard to the number of eggs per spawner, ranging from 142 ± 8.04 (D7) to 144 ± 6.35 (D4). There was also no significant difference between densities in terms of egg hatching rates. In fact, the egg hatching rate varied between 79 ± 7.98% (D7) and 81 ± 11.2% (D1). On the other hand, lower egg-laying rates were recorded in snails subjected to densities D5 (31.09 ± 6.41%), D6 (30.97 ± 6.14%), and D7 (31.96 ± 5.17%).

Egg-laying performance of A. fulica subjected to a diet with 40% calcium source diet (R1) as a function of rearing density

No difference was observed between the different rearing densities when considering the number of eggs per clutch, which ranged from 162.5 to 167.7 (Table 4). Statistically identical egg-laying and hatching rates were recorded irrespective of the breeding density.

The snail laying rate for all densities varied between 40.1 ± 9.20 and 41.94 ± 9.20% and the hatching rate between 80 ± 18.14 and 84 ± 13.22%. However, egg-laying rates were lower at densities D5 (40.17 ± 9.43%) and D6 (40.24 ± 8.52%). On the other hand, it was higher at densities D1 (40.1 ± 9.20%), D2 (40.15 ± 11.02%), D3 (40.11 ± 10.85%), D4 (40.17 ± 9.77%) and D5 (41.94 ± 9.34%).

Fig. 1. Snail *Achatina fulica*, Breeder.

Discussion

The results of this study showed that dietary calcium density and rate have a major impact on the reproductive performance of *A. fulica*. Indeed, when snails were fed diets with identical calcium content, those reared at high densities showed lower egg-laying rates, number of eggs per laying and hatching rates than snails reared at moderate densities.

Fig. 2. Snail farm under hangar.

This situation could be explained by an alteration in the quality of the food, caused by the accumulation of droppings and mucus in the food when snails are reared in overcrowded conditions (Thomas *et al.*, 1975, Dan and Bailey 1982). Excessive mucus in the snails' habitat makes it difficult for them to move around to feed.

Fig. 3. Electronic scale.

It is also possible that high densities have a negative impact on the level of oxygen present in the environment, as noted by Cameron and Carter (1979) in *Helix aspersa*. Analysis of the results also showed that, at identical rearing densities, snails fed low-calcium diets had the lowest egg-laying performance.

Fig. 4. Werckmann electronic caliper.

These results corroborate those of Thiengo *et al.* (2007) who showed that a diet rich in calcium contributes to better eggshell formation in snails, which in turn improves embryo survival and increases the hatching rate. Furthermore, our results are in agreement with those of Oosterhoff (1977) who revealed that density has an impact on the reproduction of *Cepaea nemoralis* and those of Karamoko *et al.* (2011) who showed that in *Limicolaria flammea* a decrease in density leads to an increase in the oviposition rate and the number of eggs per oviposition. According to these authors, when the density is low, the snail feeds better, which enables it to improve its reproductive performance.

Fig. 5. Incubation bowl.

Over-density and low dietary calcium resulted in a low egg hatching rate (22 ± 6.52%) in *A. fulica*. These results corroborate those of Karamoko *et al.* (2011) who showed that high density resulted in a low hatching rate in *Limicolaria flammea*.

This suggests that the quality of eggs laid by snails is influenced not only by dietary calcium content but also by stocking density. Karamoko *et al* (2011) also showed that high density resulted in a low hatching rate in *Limicolaria flammea*. Analysis of the results of this study also revealed that increasing the dietary calcium level reduced or even cancelled out the effect of over-density on the egg-laying performance of *A. fulica* in intensive rearing. This suggests that increasing dietary calcium content plays an anti-stress role in *Achatina fulica*. Indeed, according to Noumonvi *et al.* (2012), overdensity can create stress and physiological disturbances in snails.

Conclusion

At the end of this study, it should be emphasized that rearing density and dietary calcium content have a significant impact on the spawning performance of *A. fulica* breeders. Excessive densities lead to poor egg-laying performance in this mollusk, when the feed is low in calcium. Increasing the level of calcium in the diet of these molluscs helps to mitigate the negative impact of high rearing densities on their egg-laying performance. The results of this study show that a density of 10 to 15 snails per square metre requires a minimum proportion of edible oyster shell of 10%. For a density of 20 to 25 snails per square metre, the appropriate proportion of edible oyster shell powder is 20%. On the other hand, for a rearing density of between 30 and 35 snails per square metre, the dietary calcium source content needs to be 30% and for a density of 40 snails per m^2, the minimum content is 40%.

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Conflict of interest

The authors of this article declare that they have no conflict of interest.

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