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Effect of Eggshells As Calcium Sources on The Growth Performance of Offspring Land Snails (*Cornu Aspersum* Müller, 1774)

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ABSTRACT: In this study, the effect of eggshell added to the diet as a calcium source on the growth performance of juvenile land snails (*Cornu aspersum*) was evaluated. In the study, 3 experimental diets were prepared using eggshells added to the diets in two different ways: with (ES) and without (MES) membrane with limestone (LMS). Experimental diets were prepared as isonitrogenous (~15%), isolipidic (~3%) and isocalcium (~13%). The experiment was conducted for 45 days and the growth rates of the snails were monitored by interim weighing at 15-day intervals. At the end of the experiment, the best growth, weight gain and SGR were determined in the group fed with membrane eggshell supplemented diet (P<0.05). FCR was better in the eggshell groups (ES and MES) than in the limestone supplemented group. No mortality occurred in any group during the study period. According to the results of the study, it is thought that eggshell can be added to the diet of juvenile land snails as a calcium source and recycling of waste eggshells will benefit the environment and economy.

Keywords- Offspring land snail, *Cornu aspersum*, eggshell, calcium sources.

Kalsiyum Kaynağı Olarak Yumurta Kabuklarının Yavru Kara Salyangozunun (*Cornu Aspersum* Müller, 1774) Büyüme Performansı Üzerine Etkileri

ÖZET: Bu çalışmada, kalsiyum kaynağı olarak yeme ilave edilen yumurta kabuğunun yavru kara salyangozların (*Cornu aspersum*) büyüme performansları üzerindeki etkisi değerlendirilmiştir. Araştırmada limestone (LMS) ile membranlı (ES) ve membransız (MES) olarak iki farklı şekilde yemlere ilave edilen yumurta kabukları kullanılarak 3 deneme yemi hazırlanmıştır. Deneme yemleri protein, lipid ve kalsiyum oranları eşit olacak şekilde hazırlanmıştır. Deneme 45 gün süre ile yürütülmüş ve 15 günlük periyodlar halinde ara tartımlar yapılarak salyangozların büyüme oranları takip edilmiştir. Deneme sonunda en iyi büyüme, ağırlık artışı ve SGR membranlı yumurta kabuğu ilave edilmiş yemle beslenen grupta belirlenmiştir (P<0.05). FCR'ın yumurta kabuğu gruplarında (ES ve MES) limestone ilaveli gruptan daha iyi olduğu tespit edilmiştir. Araştırma süresince bütün gruplarda herhangi bir ölüm olmamıştır. Araştırma sonuçlarından, yavru kara salyangozu diyetlerine kalsiyum kaynağı olarak yumurta kabuğu ilavesinin yapılabileceği gibi atık yumurta kabuklarının geri dönüştürülerek çevreye ve ekonomiye fayda sağlayacağı düşünülmektedir.

Anahtar Kelimeler- Yavru kara salyangozu, *Cornu aspersum*, yumurta kabuğu, kalsiyum kaynakları.

1. Introduction

Due to the rapidly increasing human population, the need for animal protein is expected to increase by 25-70% by 2050 (Forte et al., 2016; Hua et al., 2019). Aquaculture products

contain high quality protein and have an important role in meeting the global protein demand (Yu et al., 2024; Melenchón et al., 2020). In addition, proteins from aquatic sources have different quality, flavour and nutritional value with high bioavailability compared to proteins from terrestrial animals and plants (Han et al., 2022). Furthermore, snail meat is an important source of protein for people in rural communities, where the majority cannot afford the cost of animal meat (Ebenso, 2003; Fagbuaro et al., 2006).

Snails have been consumed by humans as food and medicine for thousands of years (Ghosh et al., 2016; Cilia and Fratini, 2018). Land snail farming, also known as heliciculture, involves the collection, breeding and cultivation of edible land snail species for various purposes, including food and cosmetic uses (Danilova, 2022). In general, heliciculture represents an investment activity with limited fertiliser and energy inputs, low mechanisation level, low technology and low capital (Forte et al., 2016). Therefore, land snail farming is increasing in Turkey and Europe (Çelik et al., 2018, 2022). In the world, countries with high consumption are France, Italy, Germany, England, Switzerland, Belgium, Hungary, Austria, Spain, North African countries and Japan. Land snail hunting is carried out in Adana, Afyon, Aydın, Amasya, Balıkesir, Bilecik, Bursa, Edirne, Hatay, Isparta, Kocaeli, Konya, Niğde, Osmaniye, Samsun and Tokat in Turkey. Land snails, which are consumed in minimal amounts in our country, are collected and processed by export-approved processing and evaluation facilities and made ready for export. They are exported in various forms, such as live, boiled, frozen, canned or just empty shells, to countries such as France, Italy, Germany, England, EU countries, the USA, Switzerland, South American countries, Lebanon and China, thus generating significant amounts of foreign exchange for our country (Köseoğlu, 2020). 4-5 tons of product can be obtained from a 1000 square meter greenhouse and a good business can make a profit of 1-1.5 euros per kilogram (Oktay, 2022).

The shell of the land snail is a one-piece helical exoskeleton composed of several layers of calcium carbonate (Liew and Schilthuizen, 2014). The shell has the task of protecting the organism against environmental conditions and predators. So much so that it has been reported to help the snail survive in the digestive system of the predator even when swallowed (Ito et al., 2023). Calcium, which snails need for shell development and other metabolic processes, is the most important macro-element of their diet (Juříčková et al., 2008). Limestone is generally used as a source of calcium in snail farms (Forte et al., 2016; Çelik et al., 2024). However, eggshells, wood ash, mussel shells and bones have also been reported to be used as calcium sources (Rygało-Galewska et al., 2022).

Egg shell consists of approximately 85-95% calcium carbonate, 1.4% magnesium carbonate, 0.76% phosphate and 4% organic matter. In addition, eggshells contain trace amounts of elements such as sodium, potassium, zinc, manganese, iron and copper (Chojnacka, 2005). Eggshells are used in many fields such as agriculture and horticulture, animal nutrition, construction and material science, environmental science, food industry, biomedical applications, but the amount that is not used and goes to waste is also quite high. For example, Okur et al. (2019) used chicken egg shells for Cr (III) removal from water, since it is an alternative material that can be used in the adsorption of heavy metals thanks to its porous structure. According to the data of the Turkish Statistical Institute, 1.7 billion eggs are produced in Turkey in February 2024 and approximately 100 thousand tonnes of these eggshells go to waste. Therefore, the utilisation of eggshells is very important.

In the present study, the shells, which are the waste material of eggs, were added to snail feeds as a calcium source and their effects on the growth performance of juvenile black snails (*Cornu aspersum*) were investigated.

2. Material and Methods

2.1. Experimental design

The experiment was carried out between December 2021 and February 2022 in the laboratory of Sinop University Faculty of Fisheries. Approximately 1-month-old baby snails with an average weight of 0.16 g, produced in the laboratory, were used in the experiment. A total of 162 baby snails, 18 in each replication, were used for the experiment, which was designed as 3 groups and 3 replications (Figure 1).



Figure 1. Experimental design (Original)

Şekil 1. Deneme dizaynı

The experiment was started under semi-controlled laboratory conditions, under natural photoperiod, avoiding direct sunlight, and the temperature of the laboratory was kept constant with air conditioning (21°C) and 20*15*10 cm translucent plastic experimental boxes were used for the experiment; 3 cm wet natural pre-sterilized soil was placed at the bottom, a petri dish filled with water, and a petri dish for the feeding area. The experimental boxes were cleaned daily to avoid the negative effects of feces, mucus, and food residues. The relative humidity of the plastic boxes was kept between 80%-85% by spraying water twice a day during the active and resting periods of the snails. Relative humidity and temperature in the experimental boxes were measured with a Diwu HTC-1 laboratory-type temperature and humidity meter.

2.2. Diet preparation and feeding

The formulation and nutritional composition of the experimental feeds are given in Table 1. Experimental diets were prepared as isonitrogenous (~15%), isolipidic (~3%) and isocalcium (~13%). Before preparing the experimental feeds, all raw materials were ground, sieved and weighed, and all materials were mixed homogeneously to prepare the feeds. Eggshell waste, which constitutes the main material of the study, was first washed one by one, boiled, and dried in the oven. Then, it was ground and sieved to make it usable. Membraneless egg shells were subjected to the same process by removing their membranes.

The prepared feeds were stored in glass containers at +4°C until used in the study (Figure 2).

Table 1. Formulation (g kg^{-1}) and proximate composition (%) of the experimental diets
Çizelge 1. Deneyisel diyetlerin formülasyonu (g kg^{-1}) ve besin bileşimi (%)

	Experimental Diets		
	LMS	ES	MES
Nutrients (g kg^{-1})			
Extracted soybean meal	181	181	181
Corn meal	270	270	270
Wheat flour	277	277	277
Limestone (LMS)	200	-	-
Eggshell (ES)	-	200	-
Membraneless eggshell (MES)	-	-	200
Sunflower oil	20	20	20
Dicalcium phosphate	50	50	50
Vitamin premix(*)	1	1	1
Mineral premix(*)	1	1	1
Proximate composition (%)			
Moisture	7.84	6.91	5.23
Protein	15.42	15.91	15.85
Lipid	3.24	3.10	3.34
Ash	26.88	27.54	27.31
Calcium	13.04	12.55	12.69
NFE ¹	46.62	46.54	48.27
Gross energy (kJ g^{-1}) ²	12.85	12.83	13.13

* Vitamin-mineral premix (mg/kg premix): vitamin A, 210000 IU; Vitamin D₃, 35000 IU; vitamin E, 7000 mg; vitamin K₃, 322 mg; vitamin B₁, 588 mg; vitamin B₂, 252 mg; vitamin B₆, 294 mg; vitamin B₁₂, 826 mcg; niacin, 1400 mg; biotin, 7583 mcg; 182 mg folic acid, pantothenic acid, 1722 mg; inositol, 17220 mg; vitamin C, 933.31 mg; Ca, 1414mg.

¹NFE=100-(%protein+ %lipid+ %ash+ %moisture)

²Gross energy is calculated according to 23.6 kJ g^{-1} protein, 39.5 kJ g^{-1} lipid and 17 kJ g^{-1} NFE



Figure 2. Preparation of experimental diets

Şekil 2. Deneme yemlerinin hazırlanması

Snails were fed *ad libitum* with artificial feed in the form of flour prepared according to organic feeding rules (Figure 3). The prepared feeds were weighed daily in certain quantities. Slime wastes contaminating the feed were ignored in FCR calculations. Lettuce, green apple, carrot or cucumber were given alternately with the feed once a week.



Figure 3. Feeding offspring land snails and removing spent feed

Şekil 3. Yavru kara salyangozlarının yemlenmesi ve biten yemlerin alınması

2.3. Measurements and Analyses

All juvenile snails were individually weighed using a digital balance (± 0.0001 precision) at the beginning of the experiment and at 15-day intervals throughout the experiment. ImageJ software (Version 1.54) was used for image analysis to obtain juvenile sizes (Figure 4). At the end of the experiment, weight gain (WG, g), specific growth rate (SGR, %) and feed conversion ratio (FCR) were determined according to the following formulae;

Weight gain (WG, g) = Final body weight - initial body weight

Specific growth rate (SGR, %) = $[(\ln \text{ final wet weight} - \ln \text{ initial wet weight}) / \text{days}] \times 100$

Feed conversion rate (FCR) = Total feed intake / weight gain

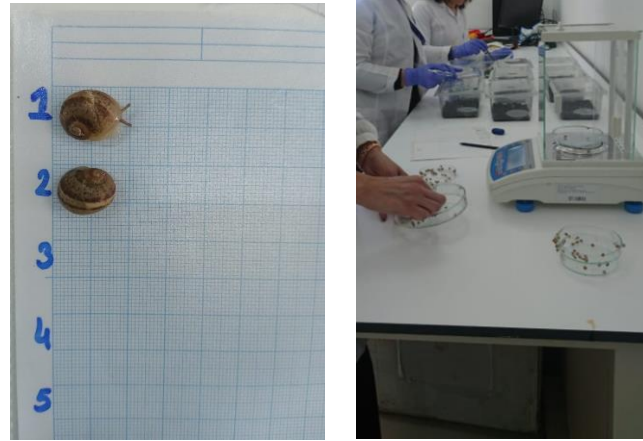


Figure 4. Measurement of length and weight of offspring land snails used in the experiment
 Şekil 4. Denemede kullanılan yavru kara salyangozlarının boy ve ağırlıklarının ölçülmesi

2.4. Statistical Analyses

Anderson-Darling and Levene's tests were used for homogeneity of variances and equality of variance of groups, respectively. Square root transformations of percentage data, and the significance of differences in growth and feed utilization variables among the treatments were tested using a one-way analysis of variance (ANOVA), followed by Tukey's method for multiple comparisons. Differences were considered significant when $P < 0.05$. Statistical analyses were performed using Minitab 17 software for Windows.

3. Results

At the end of the experiment, the best growth performance of juvenile land snails was determined in the group fed with membrane eggshell (ES) supplemented diets ($P < 0.05$), followed by the groups fed with membrane-free eggshell (MES) and limestone (LMS) supplemented diets. There was no statistical difference between LMS and MES groups ($P > 0.05$). At the end of the experiment, shell length was determined in ES, MES and LMS groups, respectively. Weight gain was better in ES group than MES and LMS groups ($P < 0.05$). SGR ratio was highest in ES group, while it was equal in MES and LMS groups. FCR was equal in ES and MES groups and higher in LMS group ($P < 0.05$). There was no mortality in all groups during the study. Survival rate was determined as 100%.

Table 2. Growth parameters of offspring land snails fed the three different experimental diets

Çizelge 2. Üç farklı diyetle beslenen yavru kara salyangozlarının büyüme parametreleri

	Experimental Diets		
	LMS	ES	MES
Initial weight (g)	0.16±0.00	0.16±0.00	0.15±0.00
Final weight (g)	1.30±0.11 ^a	1.43±0.25 ^b	1.31±0.09 ^a
Final shell length (cm)	17.00±0.63 ^a	17.40±1.05 ^b	17.24±0.66 ^{ab}
Weight gain (WG, g)	1.15±0.11 ^a	1.28±0.25 ^b	1.16±0.08 ^a
SGR (%)	4.93±0.15 ^a	5.02±0.34 ^b	4.92±0.11 ^a
FCR	1.12±0.10 ^b	1.10±0.13 ^a	1.10±0.04 ^a
Survival (%)	100	100	100

Data are reported as mean ± standard errors of three replicates (3). Means with different superscript letter in a row are significantly different ($p > 0.05$). WG: Weight gain, SGR: Specific growth rate, FCR: Feed conversion rate.

Offspring land snails with an initial weight of 0.16 g were measured at 15-day intervals throughout the experiment (Figure 1). On the 45th day, the study was terminated when regression was observed in the snails. Offspring land snails reached the best growth level on the 30th day.

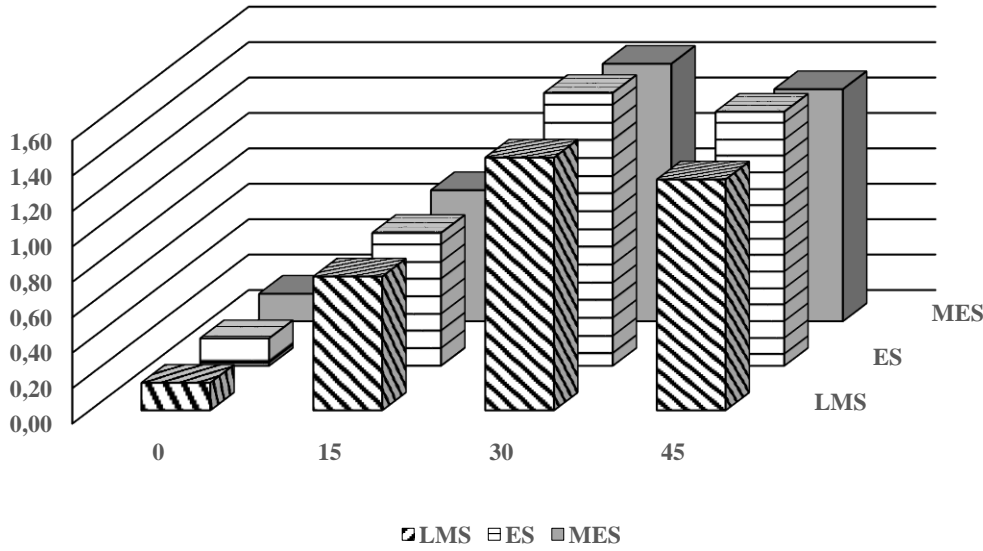


Figure 1. Changes in average weight (g) of offspring land snails fed three different diets for the 6-weeks

Şekil 1. Üç farklı diyetle 6 hafta boyunca beslenen yavru kara salyangozlarının ortalama ağırlıklarındaki (g) değişimler

4. Discussion

The diet of juvenile land snails is a determining factor in their growth performance. Calcium in eggshell is known to play a critical role in shell development and health of snails (Zhang et al., 2020). Eggshell provides a calcium-rich alternative (Amaka et al., 2020), while the addition of limestone provides pH regulation and mineral support (Boyles and Willis, 2010; Jones, 2013). Assessing the effects of these factors on the growth performance, shell length and general health status of juvenile snails can make important contributions to the development of both feeding and rearing strategies.

In the present study, snails fed with ES diet generally exhibited the highest final weights and shell lengths, followed by MES diet. This suggests that ES diet may support growth better in snails compared to LMS and MES diets. In land snail culture studies conducted to date, different calcium sources have been used as feed raw materials and their differences in terms of bioavailability have been demonstrated (Ebenso, 2003; Didier et al., 2015; Egonmwan, 2008; Rygało-Galewska et al., 2023). Snails on the ES diet also showed the highest weight gain and specific growth rate (SGR), indicating that this diet potentially provides better nutrition or digestibility for the snails. In a study comparing eggshell, seashell and limestone, it was reported that snails fed with eggshell containing feed showed better growth than other groups, which may be due to the richer nutritional content of eggshell compared to seashell and limestone (Çelik et al., 2024). Yüceer (2021) reported that the nutritional content of membraned eggshells is better than membraneless eggshells. Markoglou et al. (2011) reported that there was no difference in the growth performance of *Helix aspersa* fed diets with different calcium levels (9%, 11%, 13%) and limestone supplementation. Niba et al. (2022) reported that the giant African land snails

(*Archachatina marginata*) fed with feeds containing different calcium sources (snail shells, eggshells and limestone) showed better growth than those fed with natural plant materials (natural plant materials of pawpaw leaves and watermelons), that the best growth was in snails fed with feed containing snail shells, and that the least growth was in the group fed with limestone among the calcium sources used. Similar to this study, the lowest growth performance in the current study was determined in the group fed with limestone-added feed.

Calcium is undoubtedly one of the most important elements for snails. It is known that calcium deficiency or excess causes negative effects on the development of snails (Egonmwan, 2008). Calcium sources used in snail farming have a very wide distribution. Studies have shown that organic source calcium such as eggshell and snail shell added to feeds are generally better in terms of bioavailability in the development of snails (Niba et al., 2022; Çelik et al., 2024). In our study, the better growth of snails fed with feed containing membraned eggshells supports many previous studies. These results indicate that the ES diet may be optimal among the three tested diets for promoting growth and effective feed conversion in juvenile land snails. From the findings obtained from the study, it can be said that membraned eggshells can be a good source of calcium for snail farming. In the present study, ES and MES diets had lower FCR values compared to the LMS diet. This suggests that diets prepared with the addition of eggshells with or without membranes may be converted into snail biomass more efficiently. Niba et al. (2022) reported that the FCR value of the Giant African Land Snails fed with diets containing natural plant materials of pawpaw leaves and watermelons was better than that of those fed with diets containing snail shells, egg shells and limestone. Rygała-Galewska et al. (2023) reported that garden snails (*Cornu aspersum*) fed diets with different amounts of Ca carbonate (8.9%, 13.2%, 17.3%, 20%) had the highest FCR in the 20% supplemented group with 1.28 kg of feed per kg of snail body weight, while the FCR in the 13.2% Ca carbonate supplemented group was 1.10. Considering the Ca ratio of the current study, it was determined that it was similar. The findings obtained from the current study can be translated into practical applications in terms of snail farming and serve as an example for future studies. However, it is thought that more detailed research on this subject will help determine the most appropriate rate of eggshell use for offspring land snails. The findings obtained from the current study can be translated into practical applications in terms of snail farming and serve as an example for future studies. It is also thought that this study will contribute to both the aquaculture sector and the national economy by encouraging more use of eggshells, which are waste, in snail farming.

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