Wiadomości Zootechniczne, LV (2017), 4: 3–9

Calcium, crude ash and crude protein content in the visceral sac and shell of the edible garden snail (*Cornu aspersum* synonym *Helix aspersa*) farmed in confined conditions

Maciej Ligaszewski, Przemysław Pol

National Research Institute of Animal Production, Department of Production Systems and Environment, 32-083 Balice n. Kraków, Poland

Calcium regulation plays a particularly important role in the development of shell snails and in the life cycle of their adult individuals due to the need to build and repair shells, which consist of calcite and aragonite crystalline forms of calcium carbonate. The calcium content in the shells of the edible garden snail (*Cornu aspersum*) is 30.0–40.0% on average (Ligaszewski et al., 2009). The number and the ability to increase the size of calcic cells of the hepatopancreas, which are responsible for storing and releasing calcium into the body, determine the efficiency of the osmoregulation process and the resistance of snails to thermal stress (Scheil et al., 2010). Calcium accumulates in the hepatopancreas as intercellular granules containing an inorganic, amorphous deposit of this element and phosphorus and magnesium (Almedras & Porcel, 1992). For these reasons, the standard content of calcium in a pure ingredient (depending on the developmental stage of the snails) ranges from 10.0% to 11.0% (Ligaszewski & Pol, 2017), and discretionarily even more by 2–3%, in feed mixtures for edible land snails in production farms, in particular two subspecies of the garden snail (*Cornu aspersum*), i.e. the North-African subspecies of large garden snail *Cornu aspersum maxima* and small garden snail *Cornu aspersum aspersum*. However, too much calcium deposited in the snail carcasses results in a deterioration in the consumption quality of their meat. This is due to the fact that they accumulate, on average, 10 times more calcium in their tissues than other farm animals, which is also accompanied by an increased accumulation of other metallic elements, including heavy metals (Toader-Williams & Golubkina, 2009). Poland is a major producer and exporter of both subspecies of *Cornu aspersum* in Europe. The hepatopancreas, which is the main deposit of calcium and other metals in the body of the snail, accounts for several dozen percents of the mass of the visceral sac. Therefore, the main objective of the study was to assess the difference in calcium content in the visceral sacs of *Cornu aspersum aspersum* and *Cornu aspersum maxima* as well as the content of crude ash, which is a deposit of calcium and other metals, including heavy metals and phosphorus. In addition, the content of calcium in shells and total protein in the visceral sacs of the experimental snails was also investigated.

Material and methods

The 2-week hatch of *Cornu aspersum aspersum* and *Cornu aspersum maxima* was placed in plastic two-piece trays specially designed for snail breeding. Five repeats (trays) were used for each of the five nutritional treatments (Tab. 1), which corresponded to the number of 25 experimental trays. The hatching density was 200 individuals per 1 m^2 of the inner area of the tray, i.e. 100 individuals per 1 tray. The initial number of experimental animals was 2,500. The basic experimental feed (no. I) was a dry, plant-based feed composed of fine cereal, butterfly and soybean meals, and the main source of calcium was ground chalk. This feed was modified to create four additional feed treatments (no. II–IV) through the use of appropriate additives. It contained various proportions of agricultural chalk, 1-calcium phosphate and post-

extraction soybean meal. All feeds were similar to isoproteic feed but differed in the percentage content of calcium and phosphorus. The study was conducted over a period of 9 weeks until somatic maturity was reached in most of the snails that survived until the end of the experiment. The mortality of the experimental snails in individual trays was within the range of 23.0–38.0%, but no statistically significant differences ($P<0.05$) between the different feed treatments were found in this regard. There were also 2–3% of not fully grown, underdeveloped individuals. The mature individuals from each tray were first hibernated for 3 days and then killed in boiling water. Visceral sacs were separated for further examination from the carcasses of the killed snails, which had been completely removed from the shells with tweezers. Appropriate bulk samples were created from the visceral sacs and the shells of the snails collected from the individual trays. The samples were then subjected to chemical analyses. The calcium content was determined by the titration method according to Polish standard PN-93 R-64750. Crude ash in shells and visceral sacs was determined by weight method by drying and roasting the samples at the temperature of approx. 580^{o}C into a solid mass and then grinding in a mortar into powder and incinerated in a muffle furnace until a uniform white or greyish-white ash without dark spots was obtained. The crude protein content was examined by nitrogen determination using the Kjeldahl method and converting it to protein using the factor of 6.25 (PN-75 A-04018). The statistical significance of the differences in the chemical composition of visceral sacs and shells between the two subspecies fed with the same experimental feed treatments and between the results of feeding with all the experimental feed treatments was examined using the Tukey's honest significance test.

Table 1. Selected components of the five feed treatments for snails

Feed no.	Crude protein (%)	Calcium (%)	Phosphorus (%)	Ca : P	Ca: protein
I	17.4	10.0	0.6	16.6	0.6
II	17.3	11.6	0.6	19.3	0.7
III	17.1	13.5	0.6	22.5	0.8
IV	17.2	11.4	1.0	11.4	0.7
V	17.1	13.4	0.8	16.8	0.8

Results

I. Calcium content in shells and visceral sacs of Cornu aspersum

Cornu aspersum aspersum shells were found to be higher in calcium than *Cornu aspersum maxima* shells for all the feed treatments. The differences ranged between 0.07 and 27.1%, with the difference between the snails fed with feed no. I was statistically significant ($P<0.05$) (Tab. 2). An opposite difference direction was found between calcium content in the visceral sacs of both subspecies, which was 30.0–83.3% higher in *Cornu aspersum maxima* than in *Cornu aspersum aspersum.* This was a highly statistically significant difference ($P<0.01$) for feeding with feed no. II and statistically significant difference ($P<0.05$) in the snails fed with feeds no. III and V.

In the case of *Cornu aspersum aspersum*, the highest calcium content was observed in the visceral sac of snails fed with feed no. IV, which was characterized by low calcium concentration, the highest phosphorus content and the lowest calcium-to-phosphorus ratio from all the experimental feeds, in comparison to the other experimental groups. In comparison to the snails fed with feed no. I, it was a statistically significant difference ($P<0.05$). In contrast, the occurrence of such statistically significant differences was not found in the shells of both *Cornu aspersum* subspecies and in the visceral sac of *Cornu aspersum maxima.*

Table 2. The calcium content of *Cornu aspersum* shell and visceral sac

Feed mixture		Shell Ca (%)		Visceral sac Ca (%)	
No.	Ca content (ascending) (%)	*Cornu aspersum aspersum*	*Cornu aspersum maxima*	*Cornu aspersum aspersum*	*Cornu aspersum maxima*
I	10.0	35.2^{a}	27.7^{b}	0.6^{1}	0.9
IV	11.4	27.0	26.8	1.0^{2}	1.3
II	11.6	25.6	25.1	0.7^{A}	1.0^{B}
V	13.4	29.1	25.2	0.7^{a}	1.1^{b}
III	13.5	29.7	24.2	0.6^{a}	1.1^{b}

A, B – highly significant differences between subspecies (P<0.01).
a, b – significant differences between subspecies (P<0.05).
I, II, III – highly significant differences between feed treatments (P<0.01).
1, 2 – significant differences between feed treatments (P<0.05).

II. Content of crude ash in the shells and visceral sacs of Cornu aspersum

The examination of the crude ash content in the shells did not find statistically significant differences between the *Cornu aspersum subspecies* (Tab. 3) or between feed treatments for each of these subspecies.

Like in the case of calcium content, the crude ash content in the visceral sacs of *Cornu aspersum maxima* was higher in all feed treatment than in *Cornu aspersum aspersum*. These differences ranged from 25.0 to 64.3%, with the difference being statistically significant (P<0.05) for snails fed with feed no. I and highly significant (P<0.01) in relation to feeds no. II and V.

The visceral sacs of the *Cornu aspersum aspersum* snails fed with feed no. IV, which was characterized by a relatively low calcium concentration but the highest phosphorus content of all experimental feeds, were found to have the highest crude ash content, and the difference it was statistically significant (P<0.05) for feeds no. I and III and highly significant (P<0.01) for feeds no. II and V. The visceral sacs of *Cornu aspersum maxima* did not show statistically significant differences in ash content between the various dietary treatments.

Table 3. The crude ash content of *Cornu aspersum* shell and visceral sac depending on some nutrients of the feed mixture for snails

Feed mixture		Shell – Crude fat (%)		Visceral sac – Crude ash (%)	
No.	Ca content (ascending) (%)	*Cornu aspersum aspersum*	*Cornu aspersum maxima*	*Cornu aspersum aspersum*	*Cornu aspersum maxima*
I	10.0	63.1	59.8	$2.5^{a.\ II}$	4.1^{b}
IV	11.4	51.3	61.2	$3.6^{I.\ 1}$	4.5
II	11.6	56.3	60.9	$2.9^{A.\ 2}$	3.8^{B}
V	13.4	63.2	60.8	2.8^{A}	4.6^{B}
III	13.5	59.4	66.3	2.6^{II}	3.6

For notes see Table 2.

III. Crude protein content in visceral sacs of Cornu aspersum

The crude protein content of the visceral sacs of *Cornu aspersum aspersum* fed with feeds no. I and V was higher in a statistically very significant way (P<0.01) than in the visceral sacs of *Cornu aspersum maxima* (15.7% and 12.6% respectively) and was higher by 9.7% but in a statistically insignificant way in the snails fed with feed no. II (Tab. 4). The content of this element in the visceral sacs of both snail subspecies fed with feeds no. III and IV was at the same level.

The visceral sacs of *Cornu aspersum aspersum* fed with feed no. III showed a lower protein content in a statistically significant way (P<0.05) than in those parts of the carcasses of the snails fed with feeds no. IV and V. This could be attributed to two factors related to the quality of this feed compared to the other experimental feeds: the high calcium-to-phosphorus and calcium-to-protein content ratios. Similarly, in the case of *Cornu aspersum maxima,* the protein content of the visceral sacs of the snails fed with feed no. III was statistically significantly (P<0.05) lower than that of the snails fed with feed no. IV.

Table 4. The crude protein content of *Cornu aspersum* visceral sac depending on some nutrients of the feed mixture for snails

Feed mixture		Visceral sac	
		Cornu aspersum aspersum	*Cornu aspersum maxima*
No.	Ca content (ascending) (%)		
I	10.0	25.1^{A}	21.7^{B}
IV	11.4	25.4^{1}	25.6^{1}
II	11.6	23.8	21.7
V	13.4	25.9$^{A.1}$	23.0^{B}
III	13.5	20.6^{2}	20.7^{2}

For notes see Table 2.

Discussion

Evolutionary genetics does not take into account the division of *Cornu aspersum* into subspecies but deals with the phylogeny of its African (*Cornu aspersa maxima*) and European (*Cornu aspersum aspersum*) natural populations. Therefore, it was found that most of the natural populations of *Cornu aspersum* that are the oldest in Europe from the viewpoint of phylogenetics (Italian and Spanish) are closely related to the populations of the Northwest Africa (Algeria) and come from these populations (Guiller & Madec, 2010). The gradual expansion of this species from Africa to the Mediterranean Africa to the Mediterranean and then Atlantic zone of Europe, which occurred in the period of Pleistocene to Holocene, undoubtedly required physiological and anatomical adaptation of the phylogenetically younger populations of *Cornu aspersum* to the more severe micro-climate conditions than those in Africa. As mentioned in the introduction, the calcium regulation in land snails has a huge impact on the thermal shock prevention. The calcic cells in hepatopancreas play a special role here (Scheil et al., 2010). It has been mentioned that there were numerous intercellular granular structures composed of inorganic forms of calcium and smaller amounts of other metals and phosphorus (Almedras & Porcel, 1992). As found in the research described here, the calcium and crude ash contents of the visceral sacs of the *Cornu aspersum maxima* snails were higher than in the snails of the European subspecies *Cornu aspersum aspersum* that were fed with the same feed treatments and kept under the same micro-climate conditions. It is clear that the experimental population of *Cornu aspersum maxima*, which comes from the warm climate zone but has been kept for at least 25 generations in temperate climate conditions, kept its genetically determined adaptive ability to live in hot climate conditions. The higher calcium content in the body compared to the European subspecies of *Cornu aspersum aspersum* is an

intermediate proof of this claim (in the context of the research discussed here). Perhaps, due to a lower demand for calcium in the body of *Cornu aspersum aspersum*, it accumulated in a higher percentage amount in the shell of this subspecies than in the shells of *Cornu aspersum maxima*, because it was stored in the extracellular structures to a lesser degree. However, the above differences in calcium content in the shells of both subspecies were rarely statistically significant. On the other hand, it was found in other research that shells of *Cornu aspersum aspersum* (synonym *Helix aspersa aspersa*) exhibited less mechanical strength than shells of *Cornu aspersum maxima* despite a higher percentage content of calcium (Ligaszewski et al., 2009). *Cornu aspersum aspersum* may have demonstrated a decreased ability or physiological need to store more calcium in the body in the presence of the increased amount of phosphorus in the feed (feed no. IV) during the study. It should be noted that the meat of *Cornu aspersum aspersum* is considered by Western European consumers to be more delicate and not as hard as *Cornu aspersum maxima*. As a consequence, the carcases of the European subspecies of *Cornu aspersum* are eaten in entirety together with the visceral sac, whereas a part of the visceral sac of the African subspecies, including the hepatopancreas, which is not considered an edible part, is generally removed in the initial cooking treatment. Perhaps this is indirectly related to the presence of more calcium and other mineral components that can be stored in the form of more numerous or larger hard granular intercellular structures in the latter subspecies that comes from the warmer climate zone. The percentage content of crude protein in the visceral sacs of *Cornu aspersum aspersum* was higher in most comparisons and never lower than in the visceral sacs of *Cornu aspersum maxima*, which is a confirmation of previous studies which had been conducted in pen and greenhouse farming conditions on snails fed with feeds with different protein content (Ligaszewski & Pol, 2016). In addition, by comparing the obtained results with the above-mentioned literature data, it can be concluded that the protein content in snail visceral sacs increased – starting from the individuals bred in pens (13.1–13.9%), to greenhouse snails (14.8–16.8%), to the highest value, which was reached by the molluscs bred in the trays discussed here (20.6–25.1%). This was probably due to the fact that the above-mentioned farming systems provided snails with a decreasing access to the nutrients contained in the soil and the relatively low-protein pasture vegetation used in greenhouses and pens which was rich in fibre in the older stage of development. On the other hand, the share of the high-protein feed mixture increased until it reached 100% in the total feed dose of the snails from tray farming. While comparing the effect of the composition of the feed on the protein content of the visceral sacs of both subspecies fed with feed no. III, a decreased protein level was found – sometimes in a statistically significant way ($P<0.05$) – compared to the results of feeding with other feeds. The major characteristic of this feed in terms of its nutritional value was the significantly higher calcium-to-phosphorus ratio and the highest (in addition to feed no. V) calcium-to-protein ratio, compared to the other feeds. However, determination of any causal relationship requires further research.

Recapitulation

The African snail *Cornu aspersum maxima* and the European snail *Cornu aspersum aspersum* were studied for calcium and crude ash content of the shells and visceral sacs as well as for crude protein content of the yolk sacs. The snails of both *Cornu aspersum* subspecies received five different, almost isoproteic (from 17.1 to 17.4%) feeds differing in calcium (from 10.0 to 13.5%) and phosphorus content (from 0.6 to 1.0%). The experimental snails were obtained from farm populations maintained since 1996 on an experimental farm owned by the National Research Institute of Animal Production in Balice near Kraków. The testing was carried out on three-week-old snails maintained as part of a tray experiment over the next 8 weeks until most of them had reached somatic maturity. Regardless of the dietary treatment, the shells of *Cornu aspersum maxima* snails had lower calcium percentage compared to the shells of *Cornu aspersum aspersum*. Conversely, the visceral sacs of the European subspecies *Cornu aspersum aspersum* always contained less calcium and crude ash compared to the second African subspecies. As described in the discussion of the results, these differences were caused by the superior, genetically perpetuated

adaptation of the African subspecies to thermal stress, compared to the adaptation of the European subspecies, However, from the point of view of food industry, because of the greater presence of mineral granules containing calcium, phosphorus and often also heavy metals, the visceral sacs of *Cornu aspersum maxima* are less useful for consumption and less appreciated organoleptically by consumers than the visceral sac of the European *Cornu aspersum aspersum*, whose carcasses are consumed in entirety, including the visceral sac. In almost every feeding treatment, the visceral sacs of *Cornu aspersum aspersum* contained more protein than the visceral sacs of the second African subspecies. It was also found that the high calcium-to-phosphorus and calcium-to-protein ratios in the feed could be correlated with lowered total protein levels in the visceral sacs of both *Cornu aspersum* subspecies. In the presented study, the high phosphorus content in the feed, even when it had a relatively low content of calcium, could increase the mineral deposition of calcium in the extracellular spaces of the hepatopancreas in both subspecies of *Cornu aspersum*. These issues require further research.

References

Almedras A., Porcel D. (1992). A structural and microanalytical (EDF) study of calcium granules in the hepatopancreas of *Helix aspersa*. Comparative Biochemistry and Physiology, 103 A (4): 757–762.

Guiller A., Madec L. (2010). Historical biogeography of the land snail *Cornu aspersum*: a new scenario inferred from haplotype distribution in the Western Mediterranean basin. BMC Evolutionary Biology, 10: 18. Published online.

Ligaszewski M., Pol P. (2016). Ocena wpływu różnych systemów chowu ślimaka szarego (*Helix aspersa*) na wartość odżywczą i wydajność jego mięsa. Wiad. Zoot., LIV, 3: 18–34.

Ligaszewski M., Pol P. (2017). Produkcja towarowa ślimaka szarego (*Helix aspersa*) z odniesieniem do możliwości produkcji ślimaka winniczka (*Helix pomatia*). W: Mięczaki – potencjalne zagrożenie dla zdrowia konsumenta (monograph). K.S. Szkucik (ed.), pp. 23–38. Publ. Lubelskie Towarzystwo Naukowe, Lublin.

Ligaszewski M., Surówka K., Stekla J. (2009). The shell features of *Cornu aspersum* (synonym *Helix aspersa*) and *Helix pomatia*: Characteristics and comparison. American Malacological Bulletin, 27 (1/2): 173–181.

Scheil A.E., Köhler H.-R., Triebskorn R. (2010). Heat tolerance and recovery in Mediterranean land snails after pre-exposure in the field. Journal of Molluscan Studies, 77 (2): 165–174.

Toader-Williams A., Golubkina N. (2009). Investigation upon the edible snail's potential as source of selenium for human health and nutrition observing its food chemical contaminant risk factor with heavy metals. Bulletin UASVM Agriculture, 66 (2): 495–499.

CALCIUM, CRUDE ASH AND CRUDE PROTEIN CONTENT IN THE VISCERAL SAC AND SHELL OF THE EDIBLE GARDEN SNAIL (*Cornu aspersum* synonym *Helix aspersa*) FARMED IN CONFINED CONDITIONS

Summary

The African snail *Cornu aspersum maxima* and the European snail *Cornu aspersum aspersum* were studied for calcium and crude ash content of the shells and visceral sacs as well as for crude protein content of the yolk sacs. The snails received five different, almost isoproteic (from 17.1 to 17.4%), feeds differing in calcium (from 10.0 to 13.5%) and phosphorus content (from 0.6 to 1.0%). Regardless of the dietary treatment, the shells of *Cornu aspersum maxima* snails had lower calcium percentage compared to the shells of *Cornu aspersum aspersum*. Conversely, the visceral sacs of the European subspecies *Cornu aspersum aspersum* always contained less calcium and crude ash compared to the second African subspecies. The latter trend for differences must have been caused by the genetically perpetuated adaptation to thermal stress in the African subspecies, which is associated with the extent of calcium deposition in the body. In almost every feeding treatment, the visceral sacs of *Cornu aspersum aspersum* contained more protein than the visceral sacs of the second African

subspecies. In the present study, the high phosphorus content in the feed, even when it had a relatively low content of calcium, could increase the mineral deposition of calcium in the extracellular spaces of the hepatopancreas in both subspecies of *Cornu aspersum*. The present results may be important for the consumers of *Cornu aspersum* because they relate to snail meat quality.

Key words: *Cornu aspersum*, heliculture, snail feeding, feed composition, carcass quality