Polish Journal of Veterinary Sciences Vol. 22, No. 2 (2019), 259–262

DOI 10.24425/pjvs.2019.127094

Original article

The concentration of urea in hemolymph as a marker of health in *Lissachatina fulica* and *Cornu aspersum* edible snails – a preliminary study

J. Ziętek[1], L. Guz[2], A. Wójcik[1], S. Winiarczyk[1], Ł. Adaszek[1]

[1] Department of Epizootiology and Clinic of Infectious Diseases, Faculty of Veterinary Medicine, University of Life Sciences in Lublin, Głęboka 30, 20-612 Lublin, Poland
[2] Institute of Biological Bases of Animal Diseases, Subdepartment of Fish Diseases and Biology, Faculty of Veterinary Medicine, University of Life Sciences in Lublin, Akademicka 12, 20-950 Lublin, Poland

Abstract

Edible snails are kept in farms in many countries worldwide. As farm animals, they are an object of interest of veterinary studies and applied biology. There is a large demand for tests which would help identify their health and well-being. The objective of this study was to assess the usefulness of determining the concentration of urea in hemolymph as a marker of health of the *Lissachatina fulica* and *Cornu aspersum* edible snails. The observation covered snails from four farms marked from A to D, in which numerous deaths (farm A) and decreased body weight gain (farms B and C) were observed. In experimental farm D we observed a group of snails subjected to stress and a control group maintained in correct conditions. High concentrations of urea were found in the hemolymph of all farm animals from farms A, B and C, as well as in those subjected to food deprivation in farm D (on average from 96 mg/dl in farm D to 320 mg/dl in farm A). On the other hand, in controls from group D, the concentration of the parameter in question was much lower (< 2.0 mg/dl). The results obtained indicate that the urea concentration is a non-specific marker of pathological conditions in snails, and that the continuous monitoring of this parameter makes it possible to demonstrate irregularities in farming and introduce appropriate and early measures to eliminate such disturbances.

Key words: Lissachatina fulica, Cornu aspersum, hemolymph, urea, markers

Correspondence to: Ł. Adaszek, e-mail: ukaszek0@wp.pl

Introduction

Lissachatina fulica and *Cornu aspersum* snails are molluscs of economic value. They are used in the food industry as well as in the cosmetics and pharmaceutical sectors as a source of mucus and the medicinal substances contained therein. At present, in Central and Eastern Europe only a few snail farms and breeders are covered by constant veterinary care, and diagnosing diseases in these molluscs requires further research.

In our earlier studies, we have determined the standards of basic biochemical parameters of hemolymph in the selected species of snails kept on farms, allowing the health monitoring of the molluscs (Ziętek et al. 2017, Ziętek et al. 2018). Our observations indicate that urea can be a disease marker in snails. In *Lissachatina fulica* and *Cornu aspersum* snails, protein metabolism leads to the production of uric acid and urea. (Tunholi et al. 2011). When molluscs' metabolism is normal and when their diet is right, the concentration of urea in their hemolymph is very low, with the concentration of uric acid being higher (Becker 1980, Pinheiro et al. 2001).

When catabolic reaction predominates in a snail body and given the increase in protein metabolism connected with the breakdown of structural proteins, one can observe an increase in the concentrations of both uric acid and urea in hemolymph (Bandstra et al. 2006, Bislimi et al. 2012).

In parasitemic, diseased or unhealthy snails it was possible to observe greater quantities of protein breakdown products, manifesting in increased concentrations of uric acid and urea (Tunholi- Alves et al. 2015). Our observations indicate that the concentration of urea in hemolymph in cachectic animals and in those with helminthiasis or infectious diseases, increased by 300 to 1000% when compared with healthy specimens. Due the relative ease of hemolymph collection and the possibility of measuring urea using mammalian serum biochemistry analysers, the concentration of this parameter can prove useful when evaluating the health of farmed and laboratory snails.

The objective of this study was to assess the usefulness of determining the concentration of urea in hemolymph as a marker of health of the *Lissachatina fulica* and *Cornu aspersum* edible snails.

Materials and Methods

The tests were conducted on the *Cornu aspersum maximum* snails from an industrial farm – farms A and B (60-specimen batches), and an amateur *Lissachatina fulica* farm marked as farm C (48 specimens), and an experimental *Lissachatina fulica* farm (farm D – 20 specimens). Snails from farm A were sent to the Department of Epizootiology and Clinic of Infectious Diseases to determine the cause of frequent deaths (up to 80% per batch) after hibernation. On the other hand, in the case of farm B, the problem presented was lower body weight gain, decreased activity and deaths (up to 30% of the rout). The *Lissachatina fulica* snails from farm C displayed inhibited growth and nematodes present in faeces. Individual deaths were also recorded in this group.

Faeces of animals from the three farms were collected for microbiological tests and parasitological examination (the flotation method), while hemolymph was collected for biochemistry tests – determining the urea concentration.

The hemolymph was sampled from body cavities. A 0.7 mm needle was inserted into the side of the animal not covered with the shell, at the interface of the foot and mantle, opposite the lung, to a depth of approximately 1 cm, directing the needle towards the back of the shell. If no hemolymph flow occurred, the snail food was pressed gently. The hemolymph was collected into clean test tubes. In the case of less active animals, which did not leave their shells, the hemolymph was collected utilising a method developed by the researchers for the purposes of previous studies (Ziętek et al. 2017). The biochemical testing was conducted using the BS-130 biochemistry analyser.

From among the 20 snails from experimental farm D, ten were forced to remain constantly active through constant supply of water (spraying) and keeping them in an environment with a temperature of 25ºC for 2 weeks. Over that time, they received no food, and their body weight dropped by about 5%. After this period of fasting, hemolymph was collected from the animals and the urea concentrations were determined. Next, a diet consisting in 40% carbohydrates and 18% protein was administered. After 7 days of feeding, hemolymph was collected from all specimens and the urea concentrations were measured. The remaining 10 animals constituted the control group, which were kept in the correct conditions. Hemolymph was collected from this group of snails twice, at the same time as from those deprived of food, and the urea concentrations were determined.

Results

The information obtained from interviews and clinical examination indicated that poor animal welfare, excessively high temperatures in the cold store (12ºC) and high humidity contributed to poor body weight

Table 1. The concentration of urea in the *Lissachatina fulica* and *Cornu aspersum* snails from farms A, B and C.

Species	Number of studied specimens	Urea – arithmetic mean	standard deviation	notes
Cornu aspersum (farm A)	40	330 mg/dl	63	Poorly hibernating animals
Cornu aspersum (farm B)	20	160 mg/dl	93	Animals infected with *Pseudomonas ssp.*
Lissachatina fulica (farm C)	60	320 mg/dl	42	Animals with severe helminth infections

Table 2. The concentration of urea in the *Lissachatina fulica* snails from farm D.

Experiment stage	Number of specimens	Urea – arithmetic mean	Standard deviation
Animals after 2 weeks of food deprivation	10	96 mg/dl	42
Animals after 7 days of an intensive feeding programme	10	1.8 mg/dl	4

gains in farm A, the lack of an epiphragm, and increased activity of the molluscs during hibernation. A fecal bacteria test of these specimens revealed the presence of saprophytic microflora only. The average concentration of urea in the hemolymph of these specimens was 330 mg/dl. *Pseudomonas spp,* was cultured from the faeces of the specimens from farm B. Infection with these microbes was regarded as the cause of decreased body weight gains and deaths, as they act as bacterial pathogens that affect molluscs (Pitt et al. 2015). The average concentration of urea in the hemolymph of these specimens was 160 mg/dl. The parasitological examination of the faeces of poorly hibernating snails and of those with bacterial infections revealed the presence of small numbers of saprophytic nematodes.

In the case of animals from farm C, their bacterial test revealed the presence of only saprophytic microflora in their feces, while the parasitological examination indicated substantial nematode infestations, which were regarded as the cause of decreased body weight gain. The average concentration of urea in the hemolymph of these specimens was 320 mg/dl.

In the case of experimental animals (farm D), in the hemolymph of animals deprived of food and subjected to stress the concentration of urea increased to an average of 96 mg/dl after 10 days of fasting. Following the administration of a correct diet and welfare, it dropped to 1.8 mg/dL after seven days. Among the controls kept in correct conditions and appropriately fed, the average concentration of urea was below 2 mg/dL throughout the entire observation period.

The results obtained, urea concentrations in the hemolymph of snails with various pathologies, and subjected to experimentation are presented in Tables 1 and 2.

Discussion

The results of the study indicate that urea can be a marker of pathologies in snails. Our observations indicate that increased concentrations of this parameter are noted in the hemolymph of poorly hibernating molluscs, with bacterial infections and parasitic infestations, as well as in those deprived of food or subjected to stress.

Our earlier studies demonstrated that the normal concentration range of urea in hemolymph of *Cornu aspersum* is 3-6 mg/dl (Ziętek et al. 2017, Ziętek et al. 2018). In our studies, these values were exceeded by as much as 1000% in poorly hibernating specimens.

Urea concentration can also be used as a marker of helminthiasis. It was demonstrated that the concentration of urea in hemolymph of *Lissachatina fulica* infected with *Angiostrongylus cantonensis* was elevated (Tunholi-Alves et al. 2015). Therefore, this parameter can be used as a screening test in preliminary identification of parasitic invasions. It is worth highlighting that *Lissachatina fulica* frequently act as hosts of the invasive forms of *Angiostrongylus cantonensis* (Toma et al. 2002). Research conducted by other authors indicate that when the body is damaged by parasites, protein catabolism increases, which is manifested by, among other things, elevated transaminase and uric acid levels (Bandstra et al. 2006, Tunholi-Alves et al. 2015).

The objective of this study was to develop a method of assessing the homeostasis of snails that would be as simple as possible and require a small amount of the biological material (hemolymph). For this reason, urea was regarded as a useful marker. Mammalian serum biochemistry analysers constitute standard equipment in many veterinary clinics and laboratories, the amount of hemolymph required for testing ranges from 10 to 100 microlitres (depending on the type of equipment), and the test cost is 1 to 2 Euro.

To sum up, it should be stated that marking urea concentration in hemolymph can be used to periodically monitor the health of molluscs, while any deviation from the reference values of this parameter should spur farmers and veterinarians to search for its cause and early action.

References

Bandstra SR, Fried B, Sherma J (**2006**) High-performance thin-layer chromatographic analysis of neutral lipids and phospholipids in *Biomphalaria glabrata* patently infected with *Echinostoma caproni.* Parasitol Res 99: 414-418.

Becker W (**1980**) Metabolic interrelationship of parasitic trematodes and molluscs, especially *Schistosoma masoni* in *Biomphalaria glabrata.* Z Parasitenkd 63: 101-111.

Bislimi KR, Bojniku H, Gashi A, Grapci L, Halili J, Mazreku I, Halili F (**2012**) Seasonal changes in the hemolymph biochemistry of the snail *Helix aspersa*. FASEB J 26: 735.

Pinheiro J, Gomes EM, Chagas GM. (**2001**) Aminotranferases activity in the hemolymph of *Bradybaena similaris* (Gastropoda, Xanthonychidae) under starvation. Mem Inst Oswaldo Cruz 96: 1161-1164.

Pitt SJ, Graham MA, Dedi CG, Taylor- Harris PM, Gunn A (**2015**) Antimicrobial properties of mucus from the brown garden snail *Helix aspersa.* Br J Biomed Sci 72: 174-181.

Toma H, Matsumura S, Oshiro C, Hidaka T, Sato Y (**2002**) Ocular angiostrongyliasis without meningitis symptoms in Okinawa, Japan. J Parositol 88: 211-213.

Tunholi VM, Lustrino D, Tunholi-Alves VM, Mello-Silva CC, Maldonado A Jr, Pinheiro J, Rodrigues M (**2011**) Biochemical profile of *Biomphalaria glabrata* (Mollusca: Gastropoda) after infection by *Echinostoma paraensei* (Trematoda: *Echinostomatidae*). Parasitol Res 109: 885-891.

Tunholi-Alves VM, Tunholi VM, Amaral LS, Mota EM, Maldonado A Jr, Pinheiro J, Garcia J (**2015**) Biochemical profile of *Achatina fulica* (Mollusca: Gastropoda) after infection by different parasitic loads of *Angiostrongylus cantonensis* (Nematoda, *Metastrongylidae*). J Invertebr Pathol 124: 1-5.

Ziętek J, Guz L, Panasiuk K, Winiarczyk S, Adaszek Ł (**2017**) New intravital method for hemolymph collection from *Cornu aspersum* snails and the establishment of standards for selected biochemical parameters of their hemolymph. Med Weter 73: 366-369.

Ziętek J, Guz L, Winiarczyk S, Szkucik K, Ziomek M, Wysokowski M, Madany J, Adaszek Ł (**2018**) Study on establishing normal ranges of chosen biochemical parameters of haemolymph of *Cornu aspersum maxima* and *Cepaea nemoralis* gastropods. Pol J Vet Sci 21: 445-449.