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Original Research Article

EVALUATION OF DIFFERENT CHEMICAL COMPOSITIONS IN EGGS OF THE HERMANN'S TORTOISE (*Testudo hermanni*)

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Abstract: In this study, Hermann's tortoise (*Testudo hermanni* boettgeri) eggs were studied. The aim was to evaluate the basic composition, amino and fatty acid profiles, as well as the presence of certain trace elements in the eggs. The average size of THB eggs was 29.9 × 39.5 mm and weight 20.7 g. The shell accounted for 12.5 %, albumen 46.9 % and yolk 40.6 % of the entire THB egg. The refractive index was 1.3341 in the albumen and 1.5120 in the yolk. The albumen contained 98.2 % water, 0.9 % proteins, 0.7 % ash and traces of fat. The yolk contained 60.6 % water, 21.0 % protein, 14.2 % fat, and 4.0 % ash. The pH of albumen was 8.8.
The fatty acid composition was measured in egg yolk; oleic acid, palmitic acid, palmitoleic acid and, vaccenic acid were the most abundant. The amino acid composition was measured in egg albumen, and 18 amino acids were detected. The major amino acids present in albumen were glutamic acid, aspartic acid, leucine, phenylalanine, lysine, glycine, serine, threonine, and alanine. Concentrations of the trace elements, in descending order were in the shell: iron (Fe), nickel (Ni), copper (Cu), manganese (Mn), cobalt (Co), zinc (Zn), chromium (Cr), arsenic (As) and selenium (Se); in the albumen: Fe, Cu, Cr, Se; and in the yolk: Fe, Zn, Cu, Mn, Se, Cr, Ni, Pb, Co.
The purpose was to determine the chemical composition the of eggs, which can be used as a basic study for further comparison with free-living tortoises, and also as a comparison for the possible impact of environmental pollution.

Key words: *Testudo hermanni*; eggs; chemical composition; fatty acids; amino acids; trace elements

Introduction

Hermann's Tortoise (*Testudo hermanni*) (TH) is a medium-sized terrestrial species that can be found in nature in the European Mediterranean area. Currently, two subspecies are distinguished: *Testudo hermanni* hermanni (THH) in Western Europe and *Testudo hermanni* boettgeri (THB) in Eastern Europe (1). Scientists disagree about the existence of a third subspecies *Testudo hermanni* hercegovinensis (2).

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Egg reproduction is common for all tortoises. The size and shape of turtle eggs can vary within species and even for individual clutches. The clutch size ranges from only one or two eggs in the Asian black marsh turtle (*Siebenrockiella crassicollis*) or the pancake tortoise (*Malacochersus tornieri*) to over hundred eggs in sea turtles (3). THH lay a maximum of seven eggs per clutch and THB nine eggs; a mean clutch size is 3.3 and 4.3 eggs, respectively. Most frequently, TH lay one to two clutches per year, rarely three (1). Among turtles, the weights of their eggs range from 2.2 g in the Common musk turtle (*Sternotherus odoratus*) to

110 g in the Galapagos giant tortoise (*Geochelone elephantopus*) (3). In comparison to turtle eggs, the average weight of chicken eggs is 58 g (4). Typical reptile eggs are in most cases symmetrical, i.e. spherical or elliptical and are not tapered (5). TH eggs are white, hard-shelled, and almost elliptical in shape (1). Typical avian eggs are asymmetrical and tapered at one end (5). Reptiles exhibit a greater range in eggshell type then avian eggs (5). Irregularly shaped eggs occur commonly in turtles from the Flat-headed turtle (*Platemys*), the Neotropical wood turtles (*Rhinoclemmys*), the Freshwater terrapins from genus *Heosemys* and the genus *Melanochelys*. Pillow-shaped (i.e. elliptical in cross-section) eggs are the rule in the Hinged tortoises (*Kinixys* sp.). Most Testudinidae produce elliptical eggs, but some larger species, e.g. the African spurred tortoise (*Geochelone sulcata*), produce spherical eggs. Regardless of the actual size or shape of the egg, or the type of eggshell, the proportions of egg components are relatively constant among the species (5).

All reptile eggs have an outer shell that is either flexible (soft-shelled) or rigid (hard-shelled). The eggshell consists of a fibrous inner layer (egg membrane) that contains proteins and an outer layer that is primarily composed of calcium carbonate. The main functions of the eggshell are to protect the contents from mechanical and microbial influences and to serve as a mineral reservoir for the growing embryo. The eggshell contains pores through which, especially in hard-shelled eggs, moisture and gases are exchanged (3). The structure of the egg shell ranges from pliable to brittle in the former and entirely brittle in the latter (6). The reptile eggs have a thick fibrous membrane between the inner surface of the shell and albumen. Shortly after laying, contraction of the yolk and other contents causes an air cell to appear, usually at one end. This effect can be seen quite clearly if the egg is examined against a bright light source (7). The same effect is seen in the chicken egg (8).

A tortoise egg's albumen accounts for about 58 % of the entire egg weight and has a protein content of 10-12 %, comprising mainly ovalbumin, ovotransferrin, ovomucoid, globulins and lysozyme (9). The albumen fraction is an important reservoir of water (6). Chicken albumen consists primarily of about 90 % water into which 10% proteins are dissolved (including albumins, mucoproteins, and globulins). Unlike the yolk, which is high in fats, albumen contains almost no fat, and the carbohydrate content is less than 1 % (10). Chalazae are absent in reptile eggs and present in bird eggs (11; 12).

Reptile eggs contain large amounts of yolk, ranging 32-55 % for turtles and 72-99 % for lizards and snakes. The eggs contain a considerable 50-70 % more yolk than what the embryo requires for its development. The surplus yolk serves as an energy reserve and an important reservoir for vitamins, minerals, and trace elements for the newly hatched animal during the first few weeks of life (3). The yolk membrane, also called the vitelline membrane, surrounds the white and yellow yolk material on which the embryonic disc is situated. Morphologically, several structures are seen in the yolks of reptiles, birds, and five mammal species of monotremes (amniotic eggs) (5). The latebra, the neck of the latebra, the nucleus of the pander, and the embryonic disc originate from the white yolk, which represents only 2 % of the whole yolk (11; 12).

The chemical compositions of turtle eggs have not yet been investigated in detail, especially because the eggs are not commercially used for human consumption, as chicken eggs are. Only a few articles are found on this topic (13; 14). The aim of our study was to evaluate the chemical composition, amino and fatty acid profiles, and certain trace elements of the Hermann's tortoise eggs. The purpose was to determine the chemical composition of eggs, which can be used as a basic study for further comparison with free-living tortoises, and also as a comparison for the possible impact of environmental pollution.

Materials and methods

All tortoise species of the family Testudinidae have been included in Appendices of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) since 1977. The species Hermann's tortoise (*Testudo hermanni*) is covered by Regulation (EC) No. 338/97 and listed in Annexes A. It is also strictly protected by Council Directive 92/43/EEC, the "Habitats Directive". According to the national legislation, the breeder has obtained a permit for the breeding of Hermann's tortoises issued by the Ministry for the Environment and Spatial Planning.

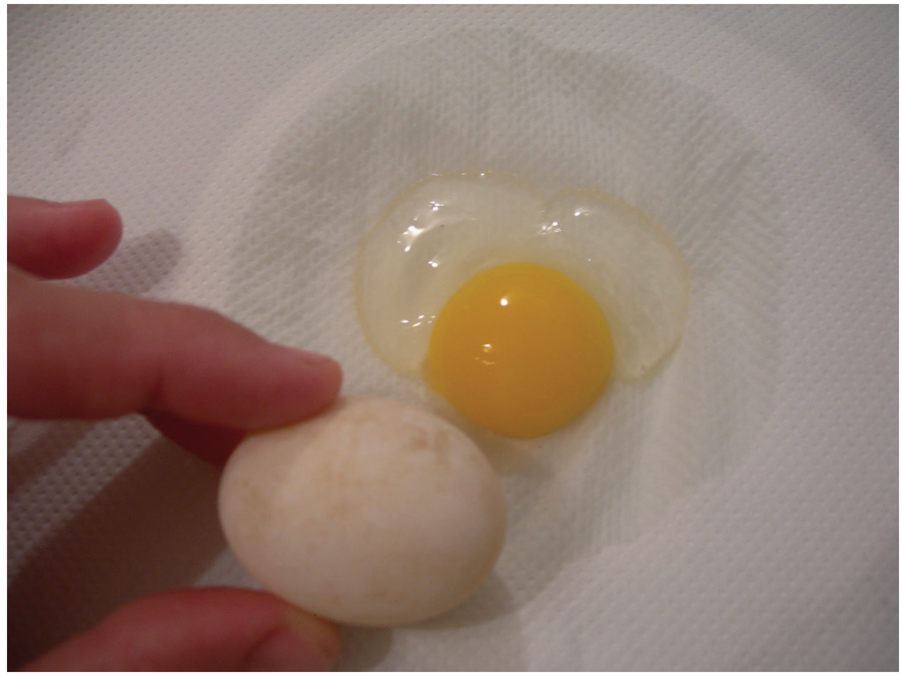

Figure1: Hermann's eggs

All the Hermann's tortoises in this study are captive bred in Slovenia, and they are born and bred in captivity in accordance with the conditions laid down in Article 54 of the Commission Regulation (EC) No 865/2006 and in CITES Res. Conf. 10.16 (Rev.). To use the eggs of protected species for scientific purposes, according to the national legislation (nature conservation or veterinary), no special permit is required. The farm of Hermann's tortoises was designated for the trade of tortoises as pet animals. The tortoises live throughout the year in outdoor enclosures. The average age of females was 30 years, and their average weight was over 2000 g. The diet of our breeding group was based on grass with additional seasonal vegetables and fruits.

In June 2014, eggs from three clutches of THB (N=15) were collected (Fig. 1). Eggs were laid within 24 hours. The next day, each egg was weighed at a precision of 0.01 g and measured with a vernier calliper with the precision of 0.05 mm. The yolk was separated from the albumen, and yolk, albumen, and shell from each egg were weighed individually. After that, the pooled samples of the shell, albumen and yolk were prepared for further analyses by homogenizing fractions from fifteen eggs with a laboratory blender. The pooled samples were prepared because the contents of one egg would not be enough for all the intended analyses. The refractive index value of albumen and yolk was measured with the Abbe refractometer, and the pH of albumen was determined using PHM210 standard pH meter (Meter Lab) immediately after the preparation of pooled samples. For further analyses samples were stored in a freezer (-20 °C).

For the determination of chemical and fatty acid composition, the contents of amino acids and trace element validated methods were used. Analyses were performed in duplicates. Moisture was determined by drying to a constant weight at 102 °C. Total ash was determined by incineration in a muffle furnace at 550 °C. Proteins were estimated by measuring the total nitrogen via the Kjeldahl method and multiplying by 6.25. Total fat was determined via the Weibull-Stoldt method. For the determination of fatty acids in the yolk, gas chromatography with a flame ionisation detector (GC-FID) was used. Tryptophan in albumen was determined via high-performance liquid chromatography (HPLC) and the concentration of other amino acids in albumen via GC-FID. The concentrations of trace elements in the shell, albumen and yolk, were determined via inductively coupled plasma mass spectrometry (ICP-MS) after microwave digestion of the samples.

Data were analysed using Microsoft Excel software.

Results

The results of the arithmetic mean (am), standard deviation (SD), the minimum value (min), and the maximum value (max) for eggs length, width, shell thickness and weight of shell, albumen and yolk are presented in Table 1. The moisture, ash, protein and fat contents in albumen and yolk are presented in Table 2. In the albumen, the pH value was 8.8 and the refractive index value was 1.3341. In the yolk, the refractive index value was 1.5120.

The quantity of each fatty acid was expressed as the percentage of the total fatty acid content (Table 3). Oleic acid was the major acyl component of the yolk, forming almost half of the total fatty acid mass (53.26%). The yolk profile was dominated by monounsaturated fatty acids (76.96%); saturated fatty acids contributed 18.60%. The fatty acid profile of the yolk lipid differed slightly from the results of the THB egg yolk obtained from Speake et al. (13), as seen in Table 3.

The results of amino acid composition in albumen are presented in Table 4. Of the non-essential amino acids, glutamic acid, and aspartic acid dominated. The largest part of the essential amino acids was represented by leucine, phenylalanine, and lysine.

The concentrations of trace elements Pb, Cd, As, Se, Zn, Ni, Cu, Cr, Mn, Fe, and Co in the shell, albumen and yolk are shown in Table 5. The concentrations of trace element in the yolk were far higher than those from the albumen, except for Cr for which the concentration did not differ from those in the yolk. The results showed a considerable difference between shell trace element concentrations of As, Ni, Cr, Mn, Fe and Co versus yolk or albumen concentrations. Interestingly, the contents of Se and Zn were the highest in the yolk. Regarding toxic elements, Pb was found only in the yolk, while As was found only in the shell. Cd was not found in any fraction of the egg.

Table 1: Hermann's tortoise eggs characteristics and compositions (N=15)

Egg's characteristics	am (mm)	SD (mm)	min – max (mm)
Egg length	39.5	±1.3	37.3 – 42.7
Egg width	29.9	±1.0	26.8 – 30.9
Shell thickness	0.5	±0.07	0.4 – 0.6
Egg's compositions	am (g)	SD (g)	min – max (g)
Shell	2.6	±0.15	2.3 – 2.9
Albumen	9.7	±1.4	7.1 – 11.0
Yolk	8.4	±0.6	7.7 – 9.1
Whole egg	20.7	±1.4	17.2 – 22.4

am – arithmetic mean, SD – standard deviation, min – minimum value, max – maximum value

Table 2: Composition of the albumen and yolk of Hermann's tortoise eggs

Constituent	Albumen (%)	Yolk (%)	Albumen and Yolk together (%)*
Moisture	98.2	60.6	80.7
Ash	0.7	4.0	2.3
Protein	0.9	21.0	10.2
Fat	<0.5	14.2	<6.8

* the calculated value based on the data: the shell 12.5 %, the albumen 46.9 % and the yolk 40.6 % of the total content of eggs.

Table 3: Fatty acid composition of Hermann's tortoise egg yolk

Fatty acids	Percentage our results (MU)	Percentage ± SD Speake et al. (13)
Caprinic acid (C 10:0)	< 0.05	/
Lauric acid (C 12:0)	0.09 (±0.04)	/
Myristic acid (C 14:0)	0.92 (±0.17)	0.8 ± 0.1
Myristoleic acid (C 14:1)	0.13 (±0.04)	/
Pentadecylic acid (C15:0)	0.06 (±0.03)	/
Palmitic acid (C 16:0)	13.84 (±2.04)	14.1 ± 0.3
Palmitoleic acid (C 16:1)	12.58 (±1.97)	8.9 ± 1.2
Heptadecanoic acid (C 17:0)	< 0.05	/
Heptadecenoic acid (C 17:1)	0.20 (±0.05)	/
Stearic acid (C 18:0)	3.63 (±0.67)	5.3 ± 0.9
Oleic acid (C 18:1 n-9)	53.26 (±5.14)	45.6 ± 1.5
Vaccenic acid (C 18:1 n-7)	9.33 (±1.72)	9.4 ± 0.3
Linoleic acid (C 18:2 n-6)	1.78 (±0.33)	6.6 ± 1.5
α-linolenic acid (C 18:3 n-3)	0.67 (±0.13)	3.8 ± 0.6
γ-linolenic acid (C 18:3 n-6)	< 0.05	/
Octadecatetraenoic acid (C 18:4 n-3)	< 0.05	/
Arachidic acid (C 20:0)	0.06 (±0.04)	/
Eicosenoic acid (C 20:1 n-9)	1.40 (±0.26)	1.3 ± 0.1
Eicosadienoic acid (C 20:2 n-6)	0.06 (±0.04)	/
Eicosatrienoic acid (C 20:3 n-6)	0.13 (±0.04)	/
Arachidonic acid (C 20:4 n-6)	0.84 (±0.16)	1.5 ± 0.3
Eicosatetraenoic acid (C 20:4 n-3)	< 0.05	/
Eicosapentaenoic acid (C 20:5 n3)	0.18 (±0.05)	0.4 ± 0.0
Erucic acid (C 22:1 n-9)	0.06 (±0.04)	/
Docosapentaenoic acid (C 22:5 n3)	0.29 (±0.06)	0.6 ± 0.1
Docosahexaenoic acid (C 22:6 n3)	< 0.01	0.0
Lignoceric acid (C 24:0)	< 0.01	0.0
Tetracosenoic acid (C 24:1)	< 0.05	/
Conjugated linoleic acid	0.33 (±0.07)	/
Saturated fatty acids	18.60 (±2.16)	20.2
Mono-unsaturated fatty acids	76.96 (±5.76)	65.2
Poly-unsaturated fatty acids	4.28 (±0.41)	/
Total omega 3	1.14 (±0.15)	/
Total omega 6	2.81 (±0.37)	/
Total omega 3/ Total omega 6	0.41 (±0.08)	/

MU – measurement uncertainty, SD – standard deviation, / – not done

Table 4: Amino acid composition of Hermann's tortoise egg albumen

Amino acid	g/100 g (MU)
Tryptophan	< 0.01
Aspartic acid	0.101 (±0.014)
Threonine	0.052 (±0.009)
Serine	0.064 (±0.011)
Glutamic acid	0.115 (±0.015)
Proline	< 0.01
Glycine	0.072 (±0.011)
Alanine	0.042 (±0.008)
Valine	< 0.01
Isoleucine	< 0.01
Leucine	0.095 (±0.013)
Tyrosine	< 0.01
Phenylalanine	0.083 (±0.012)
Lysine	0.081 (±0.012)
Histidine	< 0.01
Arginine	< 0.01
Cysteine	0.0607 (±0.0074)
Methionine	< 0.001

MU – measurement uncertainty

Table 5: Eleven trace element concentration (mg/kg wet weight) in the shell, albumen, and yolk of Hermann's tortoise eggs

Element	Shell (mg/kg)	Albumen (mg/kg)	Yolk (mg/kg)
Pb	< 0.01	< 0.01	0.03
Cd	<0.005	< 0.005	< 0.005
As	0.068	< 0.01	< 0.01
Se	0.017	0.005	0.188
Zn	0.5	< 0.4	26
Ni	9.7	< 0.005	0.031
Cu	1.0	0.23	1.0
Cr	0.251	0.076	0.077
Mn	0.922	< 0.020	0.369
Fe	> 40	4.1	31
Co	0.873	< 0.002	0.005

Discussion

Relationships between egg size, egg components, and neonate size have been investigated across a wide range of oviparous taxa (15). The average size of THB eggs examined was 29.9 × 39.5 mm and weight 20.7 g (Table 1). Bertolero et al. (1) obtained a slightly lower average value: THB eggs measured 27.9 × 37.4 mm and weighed 17.1 g. Values for THB eggs, which were measured by Highfield, were bigger but lighter: 40 × 29 mm and weighing 12 to 14 g (7). In Greece, the measured average weight for THB was 17.8 g, ranging from 10.5 to 23.5 g (16). According to available literature, no data was found for shell thickness, but we think it is an important indicator of the relationship between the size of eggs and their weight. The average shell thickness for THB eggs in our study was 0.5 mm (Table 1).

In terms of overall turtle egg weight, the shell contributes about 11 to 12 % (range: 4.3-24.7 %), the albumen contributes about 46 to 47 % (range: 34.4-61.9 %), and the yolk contributes about 41 to 42 % (range: 32.1-55.0 %) (5). Wallace et al. (15) examined relationships between the egg weight, the egg composition and the hatchling size in the Leatherback sea turtles (*Dermochelys coriacea*). Albumen comprised 63 % of egg weight and explained most of the variation in egg mass, whereas yolk comprised only 33 %. However, Hewavisenthi and Parmenter (17) reported that the Flatback sea turtle (*Natator depressus*) eggs contained roughly equal proportions of yolk and albumen. The shell contributed approximately 5 %, the albumin 45 %, and the yolk 50 %. In our study, we found out that shell represented 12.5 %, albumen 46.9 % and yolk 40.6 %. The weight of albumen varied the most. Speake et al. (13) found 42.5 % yolk in the same species. Proportions of the Spur-thighed tortoise (*Testudo graeca*) eggs are the most similar to our results. Specifically, the eggs of the Spur-thighed tortoise consist of 16 % shell, 44 % albumen and 40 % yolk (7). Unlike reptilian eggs, chicken eggs consist of about 9.5 % shell, 63 % albumen, and 27.5 % yolk (18). From the results seen in tortoises, a greatly lower yolk content is seen in chicken eggs.

Turtle females supply eggs with more or less equal proportions of solid nutrients, but there may be considerable variations in the amount of water in the eggs. Among clutches of the Diamondback terrapin (*Malaclemys terrapin*) and the Common snapping turtle (*Chelydra serpentina*), small eggs tend to have proportionally less water than large eggs do (3). From the results of our study we calculated the composition of THB egg contains (the albumen and the yolk, without shell), moisture 80.7 %, ash 2.3 %, protein 10.2 %, and fat 6.8 %. According to available literature data, the composition of THB eggs has not yet been published. In comparison to the snail-eating turtle (also known as 'Rice Field Terrapin') (*Malayemys macrocephala*) eggs, less moisture (72.9 %) and ash (1.5 %) but more protein (12.4 %) and fat (8.6 %) were found (14). The composition of the Olive Ridley sea turtle (*Lepidochelys olivacea*) eggs was: moisture 43.4 %, ash 3.5 %, protein 49.2 %, and fat 3.9 % (19). The results of our study show a great difference especially in moisture and protein content in comparison to the sea turtle eggs. The main components of chicken eggs are moisture 74 %, proteins 12 % and fats 11 % (4). THB eggs contain less protein and fat, and they are more watery than chicken eggs.

Fatty and amino acid compositions of different species of turtle eggs were previously examined. Comparing our results detected in THB (Table 3) with results for the snail-eating turtle (14), the contents of fatty acids in THB eggs were lower for all fatty acids except for oleic acid, vaccenic acid, and eicosenoic acid. The ratio of omega-3/omega-6 fatty acid found in our study (0.41 %) was almost two times higher than in the snail-eating turtle (0.23 %). Thompson and Speake (20) interpreted that the fatty acid composition of yolk lipids is partly determined by maternal diet and partly by the expression of maternal biochemical pathways for the interconversion of fatty acids that have evolved to adjust the dietary supply to the embryonic requirements. Our results regarding fatty acid composition in the yolk in comparison to those of Speake et al. (13) differ slightly in palmitoleic acid, stearic acid, oleic acid, and arachidonic acid. The obvious difference is seen in linoleic acid and α-linolenic acid. The reason was probably due to differences in diet and environmental conditions. The diet of our breeding group is based on grass complemented with additional seasonal vegetables and fruits. It should be noted that *Testudo hermanni* has a postnuptial reproduction cycle which means after the completion of the reproductive phase an intense foraging period to permit nutritional accumulations for full gonadal growth in the late fall prior to the next hibernation occurs (21). For a more detailed examination among feeding of parents, environmental conditions and contents of female eggs would be required to make more detailed investigations. Linolenic acid formed 1.78 %, and α-linolenic acid formed 0.67 % of the total fatty acid composition of lipid in the yolk. Speake et al. (13) fed tortoises only with green plants, which contribute α-linolenic acid as the main polyunsaturated acid. The linolenic acid formed 6.6 %, and α-linolenic acid formed 3.8 %. The same as Speake et al. (13), we did not detect docosahexaenoic acid (DHA) in yolk which is essential for embryonic development.

There is very little detailed information available on the amino acid composition of the turtle eggs. Results from the present study are shown in Table 4. The essential amino acids profiles of egg albumen found in chicken and quail values were higher than in Hermann's tortoise egg. By comparing our results with the snail-eating turtle (14), lower values for arginine, prolyne, tyrosine, isoleucine, methionine, tryptophan and valine were found. Other amino acids were present in higher values: cysteine, aspartic acid, threonine, serine, glutamic acid, glycine, alanine, leucine, phenylalanine, lysine, and histidine. Further investigations considering the importance of amino acids for single turtle's species should be examined.

Very few published studies exist regarding tortoise eggs; it is thus difficult to compare the composition of relative trace elements. In our study, concentrations of the trace elements were shown in descending order in the shell: iron (Fe), nickel (Ni), copper (Cu), manganese (Mn), cobalt (Co), zinc (Zn), chromium (Cr), arsenic (As), selenium (Se); in the albumen: Fe, Cu, Cr, Se; and in yolk: Fe, Zn, Cu, Mn, Se, Cr, Ni, lead (Pb), Co (Table 5). Pb and cadmium (Cd) were not detected in the shell; Pb, Cd, As, Zn, Ni, Mn, Co were not detected in the albumen; Cd and As were not detected in the yolk. Lam et al. (22) also measured the presence of trace elements for the Green sea turtle (*Chelonia mydas*) in the shell and both composites of eggs. Their results were all higher except for Mn in the shell, Cu in albumen and Cu and Mn in the yolk, which were lower than our concentrations. The main reason to study elements in turtle eggs is pollution and its potential threat to sea turtles. The trace element concentrations found worldwide could also be a consequence of both diet and environmental pollution, as described Lam et al. (22) for the Green sea turtle (*Chelonia mydas*).

Concentrations of different amino acids, fatty acids and elements are found in different proportions in different turtle species. Farms that breed turtles are a unique living system, and further investigation needs to be done in this field, especially concerning the nutrition of breeding parents and its impact on the composition of eggs.

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References

1. Bertolero A, Cheylan M, Hailey A, Livoreil B, Willemsen RE. (2011). *Testudo hermanni* (Gmelin 1789) – Hermann's tortoise. In: Rhodin AGJ, Pritchard PCH, van Dijk PP, eds. Conservation biology of freshwater turtles and tortoises: a compilation project of the IUCN/SSC tortoise and freshwater turtle specialist group. [Online] Arlington : Chelonian Research Foundation, 2011: e059.1–059.20 (Chelonian research monographs, No. 5) http://www.iucn-tftsg.org/wp-content/uploads/file/Accounts/crm_5_059_hermanni_v1_2011.pdf

2. Fritz U, Auer M, Bertolero A, et al. A range-wide phylogeography of Hermann's tortoise, *Testudo hermanni* (Reptilia: Testudines: Testudinidae): implications for taxonomy. *Zool Scripta* 2006; 35: 531–43.

3. Köhler G. Incubation of reptile eggs: basics, guidelines, experiences. Malabar : Krieger Publishing, 2006: 9–13.

4. Belitz HD, Grosch W, Schieberle P. Food chemistry. 2nd ed. Berlin : Springer, 2009: 546–62.

5. Miller JD, Dinkelacker SA. Reproductive structures and strategies of turtles. In: Wyneken J, Godfrey MH, Bels V, eds. Biology of turtles:

from structures to strategies of life. Boca Raton : CRC Press, 2008: 225–78.

6. Booth DT, Thompson MB. A comparison of reptilian eggs with those of megapode birds. In: Deening D, Ferguson MWJ, eds. Egg incubation: its effects on embryonic development in birds and reptiles. 5th ed. Cambridge : University Press, 1991: 325–44.

7. Highfield AC. Practical encyclopedia of keeping and breeding tortoises and freshwater turtles. London : Carapace Press, 1996: 141–257.

8. Zeidler G. Shell eggs and their nutritional value. In: Bell DD, Weaver WD, eds. Commercial chicken meat and egg production. 5th ed. Norwell, Massachusetts : Kluwer Academic Publishers, 2002: 1109–28.

9. Prajanban BO, Shawsuan L, Daduang S, et al. Identification of five reptile egg whites protein using MALDI-TOF mass spectrometry and LC/MS-MS analysis. J Proteomics 2012; 75: 1940–59.

10. Hosen SMZ, Swati P, Dibyajyoti S. Artificial and fake eggs: dance of death. Adv Pharmacol Pharm 2013; 1: 13–7.

11. Okubo T, Akachi S, Hatta H. (1997). Structure of hen eggs and physiology of egg laying. In: Takehiko Y, Lekh RJ, Hajime H, Mujo K, eds. Hen eggs: basic and applied science. Boca Raton : CRC Press, 1997: 1–12.

12. Bellairs R, Osmond M. The atlas of chick development. 3rd ed. Oxford : Academic Press, 2014: 1–6.

13. Speake BK, Surai PF, Gore M. Lipid composition, fatty acid profiles, and lipid-soluble antioxidants of eggs of the Hermann's tortoise (*Testudo hermanni* boettgeri). Zoo Biol 2001; 20: 75–87.

14. Tunsaringkarn T, Siriwong W, Tungjaroenchai W. Chemical compositions of eggs from chicken, quail and snail-eating turtle. Thai J Agric Sci 2011; 44: 478–86.

15. Wallace BP, Sotherland PR, Tomillo PS, et al. Egg components, egg size, and hatchling size in leatherback turtles. Comp Biochem Physiol A Mol Integr Physiol 2006; 145: 524–32.

16. Hailey A, Loumbourdis NS. Egg size and shape, clutch dynamics, and reproductive effort in European tortoises. Can J Zool 1988; 66:1527–36.

17. Hewavisenthi S, Parmente CJ. Egg components and utilization of yolk lipids during development of the flatback turtle *Natator depressus*. J Herpetol 2002; 36: 43–50.

18. Li-Chan ECY, Powrie W, Nakai S. The chemistry of eggs and eggs products. In: Stadelman WC, ed. Egg science and technology. 4th ed. Binghamton, NY : Food Product Press, The Haworth Press, 1995: 105–75.

19. Castro-Gonzalez MI, Perez-Gil Romo F. Chemical composition of eggs of the Olive Ridley *Lepidochelys olivacea* (Testudines: Cheloniidae) and it's potential as a food source. Rev Biol Trop 2011; 59: 1729–42.

20. Thompson MB, Speake BK. Energy and nutrient utilisation by embryonic reptiles. Comp Biochem Physiol A Mol Integr Physiol 2002; 133: 529–38.

21. Blanvillain G, Owens DW, Kuchling G. Hormones and reproductive cycles in turtles. In: Norris DO, Lopez KH, eds. Hormones and reproduction of vertebrates. Volume 3: Reptiles. San Diego : Elsevier, 2011: 277–303.

22. Lam JC, Tanabe S, Chan SK, Lam MH, Martin M, Lam PK. Levels of trace elements in green turtle eggs collected from Hong Kong: evidence of risks due to selenium and nickel. Environ Pollut 2006; 144: 790–801.

DOLOČANJE RAZLIČNIH KEMIČNIH SESTAVIN V JAJCIH GRŠKE KORNJAČE (*Testudo hermanni*)

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Povzetek: Namen raziskave je bil proučiti sestavo jajc grške kornjače (Testudo hermanni boettgeri), vsebnost aminokislin in maščobnih kisli, kot tudi vsebnost nekaterih elementov v sledovih. Povprečna velikost jajc THB je bila 29,9 × 39,5 mm, masa pa 20,7 g. Celotno jajce je bilo sestavljeno iz 12,5 % lupine, 46,9 % beljaka in 40,6 % rumenjaka. Indeks refrakcije beljaka je znašal 1,3341 in rumenjaka 1,5120. Beljak je vseboval 98,2 % vode, 0,9 % beljakovin, 0,7 % pepela in sledove maščobe. Rumenjak je vseboval 60,6 % vode, 21,0 % beljakovin, 14,2 % maščob in 4,0 % pepela. Vrednost pH beljaka je bila 8,8.
Najvišji delež maščobnih kislin v rumenjaku so predstavljale oleinska kislina, palmitinska kislina, palmitooleinska kislina in vakcenska kislina. V beljaku smo potrdili 18 različnih aminokislin. Najvišji delež le-teh so predstavljale glutaminska kislina, asparginska kislina, levcin, fenilalanin, lizin, glicin, serin, treonin in alanin.
Koncentracije elementov v sledovih v lupini so bile v naslednjem padajočem zaporedju: železo (Fe), nikelj (Ni), baker (Cu), mangan (Mn), kobalt (Co), cink (Zn), krom (Cr), arzen (As), selen (Se); v beljaku: Fe, Cu, Cr, Se, v rumenjaku pa: Fe, Zn, Cu, Mn, Se, Cr, Ni, Pb, Co.

Ključne besede: *Testudo hermanni*; jajca; kemična sestava; maščobne kisline; aminokisline; elementi v sledovih