



UNIWERSYTET  
MIKOŁAJA KOPERNIKA  
W TORUNIU

Wydział Nauk Biologicznych  
i Weterynaryjnych

dr hab. Michał S. Wojciechowski, prof. UMK  
Katedra Zoologii i Ekologii Kręgowców  
Uniwersytet Mikołaja Kopernika  
ul. Lwowska 1  
87-100 Toruń

Toruń, 2023-08-23

Acceptance report of a Ph.D. thesis by Mgr. Maciej Działo "A berry a day keeps the costs away? The physiological effects of dietary anthocyanins in a migratory songbird, European starling (*Sturnus vulgaris*)"

The following assessment was prepared based on the documentation presented by the Research Discipline Council of Biological Sciences of the Jagiellonian University in Kraków. The thesis and the research described therein were done by Mgr. Maciej Działo, supervised by Dr. Hab. Ulf Bauchinger and Dr. Edyta Sadowska. The thesis has a typical structure and is composed of the following chapters: General Introduction providing the extensive overview of the state of the art and introducing research questions and hypotheses, Chapter 2 describing three investigations aiming to answer the research question, and General Discussion of the results of all studies, drawing conclusions and highlighting potential future directions stemming from the reported research. The detailed results are presented in six tables and eight figures. The list of references contains XXX positions of relevant literature. Additionally the thesis contains the list of papers published and conference presentations given by Mgr. Maciej Działo. The author of the dissertation was supported by the funds from the project "Jagiellonian Interdisciplinary Ph.D. Programme" and from the grant awarded to Dr. Hab. Ulf Bauchinger by the National Science Centre in Poland.

Because the thesis was prepared in English, I also prepared the assessment in English. The final conclusion of the assessments is presented also in Polish.

**In my opinion, the reviewed Ph.D. thesis by Maciej Działo meets the criteria pursuant to art. 13 of Act of 14 March 2003 The Law on academic degrees and**

**academic titles as well as degrees and titles in the field of art (Journal of Laws of 2003, item 65, as amended).**

The studies reported in the thesis aimed to answer three main questions:

1. How do dietary anthocyanins influence oxidative status and aerobic metabolism?
2. Can dietary anthocyanins promote phenotypic adjustments that support the current or future metabolic needs of an individual (e.g. those associated with seasonal challenges)?
3. What is the link between the size and metabolism of a cell and what is the relationship between the metabolic rate at cellular and whole organismal levels?

To answer these questions, the Author used European starlings (*Sturnus vulgaris*) as a model species for his laboratory experiments. Animals were kept in captivity and were fed the diet supplemented with the addition of elderberry fruit extract which is rich in dietary anthocyanins. Here I would like to complement a very clear and straightforward design of the experiments and their detailed description. The results of the studies reported in the thesis provide novel information on the potential role of dietary switches during animal life. Seasonal changes in diet composition are observed among many taxa, yet their role is still not fully understood. As suggested by the results of the studies done by Mgr. Działo, the addition of anthocyanins present in fruits may have important consequences for animal health and performance, particularly during energy-demanding activities, like molt or migration (in the case of birds).

The results of the presented studies give a quite solid answer to the questions asked. The most important conclusions may be summarized in the following points:

1. Indeed, anthocyanins facilitate antioxidative defense by increasing non-enzymatic antioxidant capacity, decreasing oxidative damage markers, and sparing endogenous antioxidants such as uric acid.
2. Basal metabolic rate and metabolism at the cellular level in birds supplemented with anthocyanins were markedly reduced. This may indicate important consequences for birds during energy-demanding stages of their life
3. Dietary supplementation with anthocyanins affects hematological variables, which may result in improved oxygen delivery to the tissues.
4. Anthocyanins facilitate cognitive and cardiovascular functions by increasing brain and heart mass. This corresponds well with the improved functioning of the oxygen delivery system

Finally, as stated by the Author, this study provides “the first empirical evidence that cell size is inversely related to cellular metabolism and can also indirectly influence whole-organism metabolic rate through its cascading effects on cellular metabolic rate and body mass.”

I do not have many critical points to the thesis. The thesis by Mgr. Działo was one of the best I reviewed so far. It was clearly written, and well presented to the reader.

There are however several points that were unclear to me. First of all, the Author calculates the parameter called the total surface area of the erythrocytes. It is calculated by multiplying the surface area:volume ratio of the red blood cells by the red blood cell count. Either I do not understand it, or something is wrong here. How the surface area to volume ratio multiplied by the number of cells can provide the measure of the total surface area of the erythrocytes? How surface area can be expressed as a ratio of  $\mu\text{m}^2/\mu\text{m}^3$ ? I do not understand this metric or its biological meaning. It would be some kind of the total ratio of surface area to volume of all erythrocytes. But, still, from the physical or biological point of view, it makes no sense. The total surface area should be calculated by multiplying the area of a single erythrocyte by the red blood cell count. I would be grateful for the explanation. Especially since there are several metrics dependent on the total surface area of the erythrocytes, based on which the Author draws conclusions important for the study.

The second issue, I would like to ask about, is the Author's opinion on the biological importance of the expression of metabolic rate per unit of body mass. If I understand correctly, the Author used the proper way of accounting for body mass in the analyses of basal metabolic rate, which is using body mass as a covariate. However, at the outset of Chapter 2.3 it states: “Over the past decades, multiple studies have demonstrated that larger species or individuals have lower metabolic rates per unit of mass than smaller ones, and suggested that this allometry should hold for different size scales of biological organization. Intuitively the size of a cell should influence its physiological function, yet there is scant experimental evidence to support this prediction.” These sentences confused me. As was shown in several studies, the metabolic rate:body mass ratio (so-called mass-specific metabolic rate) is a methodological error. Two organisms that differ in body mass may have the same metabolic rate, yet because of the difference in the denominator, the mass-specific metabolic rate will be lower in the larger species. However, as stated by McNab, animals do not live on the “per unit of body mass basis.” Were the analyses in

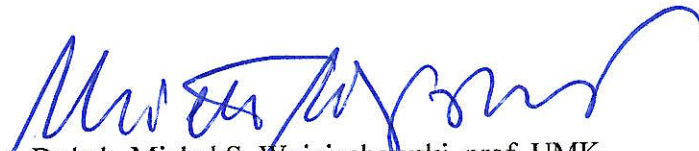
Chapter 2.3 done for the mass-specific metabolic rates? I would be very grateful for clarification and the Author's opinion.

It was also unclear to me, whether the analyses of the factors affecting basal metabolic rate in starlings (Chapter 2.3) were done for the diurnal or the nocturnal basal metabolic rate? I would like to ask whether the results of the path-analyses differed for these phases of the day?

In summary, I would like to express my very positive opinion about the assessed doctoral thesis of Mgr. Maciej Działo. The thesis fulfills all the legal requirements for the doctoral thesis and thus **I request that the Research Discipline Council of Biological Sciences of the Jagiellonian University in Kraków accepts Maciej Działo for further stages of doctoral proceedings in the field of exact and biological sciences, in the discipline of biological sciences.** Because of the high value of the thesis and results presented therein, as well as because of the other achievements of Mgr. Maciej Działo (as presented in the thesis), **I request that the thesis is accepted with distinctions.**

**Ja, niżej podpisany stwierdzam, że recenzowana rozprawa doktorska mgr. Macieja Działo spełnia warunki określone w art. 13.1 Ustawy z dnia 14 marca 2003 r. o stopniach naukowych i tytule naukowym oraz o stopniach i tytule w zakresie sztuki (Dz.U. nr 65 poz. 595 z późn. zmianami) i wnioskuję do Rady Dyscypliny Nauki biologiczne Uniwersytetu Jagiellońskiego w Krakowie o dopuszczenie mgr. Macieja Działo do dalszych etapów przewodu doktorskiego.**

**Biorąc pod uwagę wysoką wartość ocenianej rozprawy oraz inne osiągnięcia Doktoranta zaprezentowane w rozprawie, wnioskuję o wyróżnienie rozprawy doktorskiej mrg. Macieja Działo.**



Dr hab. Michał S. Wojciechowski, prof. UMK