

PhD Thesis Acceptance Report

Research Discipline Council of Biological Sciences

Jagiellonian University in Kraków

Candidate's name and surname: Elisavet Zagkle

PhD Thesis Title: Avian energy use and its link to oxidative stress

Thesis Supervisor: Dr. hab. Ulf Bauchinger

Assistant Supervisor / Second Supervisor / Co-supervisor (if applicable): Dr. Edyta Teresa Sadowska

Reviewer: Dr. hab. Małgorzata Jefimow, professor of the Nicolaus Copernicus University

THESIS EVALUATION

1. **Scientific merit of the thesis**

a. Originality of the research (25-200 words):

The thesis consists of three main chapters composed of original work that represents an interesting advancement in the field of avian energetics and oxidative stress. The Author is focused on the link between oxidative stress and the aerobic demands in birds. The Author asks three important questions based on the assumption that energy allocated into reproduction decreases the energy available for self-maintenance including antioxidant potential. Although the idea of a trade-off between reproduction and self-maintenance is not new, the Author was able to present new and interesting results. With two different experiments (described in Chapter 2) done in the field on free-living great tits *Parus major*, or under controlled laboratory conditions on captive zebra finches *Taeniopygia guttata*, the Author tested the hypothesis that during reproduction period oxidative stress may act as both, a cost and a constraint. It is also noteworthy that the heat dissipation limit hypothesis was tested in zebra finches under controlled laboratory conditions in a two-factor factorial experimental design. In this part of the experiment, oxidative stress was measured in the same individuals at three different stages of reproduction what gives original and unique results on within-individual changes. I also appreciate the field study on great tits as such work is always time and energy consuming and sometimes may be really challenging.

b. Scientific merit of the chapters / articles (25-200 words):

In the first study, done in the field on great tits, it was found that brood size manipulation affected daily energy expenditure (DEE) without an effect on basal metabolic rate (BMR). Such result indicates independent model of correlation between DEE and BMR *sensu* Careau and Garland (2012). While BMR was not correlated with oxidative stress, females with higher DEE had higher oxidative stress. Also, nestlings from the enlarged broods developed slower than control ones. The Author made valid conclusion that the slower development resulted from increased sibling competition within the brood and energetic limitations of their mothers.

In the second study zebra finches were acclimated to different ambient temperatures, with or without additional feather clipping, to test the heat dissipation limit (HDL) hypothesis. This very well designed study brought new and interesting results, which supported HDL hypothesis in captive birds, in relation to reproduction but not to oxidative stress. The Author concluded that

the ability to dissipate more heat allowed females to invest more energy in reproduction without constraining self-maintenance. Both, feather clipping and lower ambient temperature resulted in better reproductive output. The results on oxidative stress are not unequivocal. On the one hand, oxidative damage before reproduction was slightly higher at higher temperature but on the other hand, the difference in oxidative stress between birds kept in different temperatures did not reach the level of significance. The Author correctly concluded that it might result from the higher heat production due to increased foraging cost with concurrent lower capacity of heat dissipation than at lower ambient temperature. Similar explanation was offered for lower reproductive output in this group.

2. **Substantial merit of the thesis**

(ability to introduce the research topic and clarity of research hypotheses, the choice of research methods and statistical tools for data analysis, presentation and critical analysis of the research data, the ability to discuss research data and the theoretical background, clarity and quality of the conclusions) (25-200 words):

The submitted thesis presents investigations on the link between oxidative stress and energy use in two species of birds. The thesis starts with a General Introduction and provides the literature review on the life-history trade-offs, the relation between DEE and BMR, and the link between reproduction and oxidative stress. This chapter is very consistent and ends with three General Research Questions. The Author used standard research methods (open-flow respirometry, colorimetric assays for oxidative status biomarkers in plasma, morphological measurements), but blood samples for DEE measurements using the doubly-labelled water method were sent and measured by a different laboratory. It is very important that both arms of oxidative balance were measured. Statistical methods are correct. The hypothesis that "oxidative stress acts both as a cost and a constraint along the lifespan of the organisms, representing a physiological mechanism mediating energy trade-offs between reproductive-related traits and soma-maintenance" could be more detailed as the Author tested it only before, during, and just after reproductive period, not the entire lifespan. Furthermore, it is not clear which results are considered as the support for the hypothesis that oxidative stress is a constraint. All results, both on great tits and zebra finches, indicate that oxidative stress is a cost of higher energy expenditure. Only the ability to dissipate heat can be regarded as a constraint, especially that feather clipping and ambient temperature did not affect oxidative stress. Introduction and General discussion are well written and concise. These chapters indicate the deep theoretical knowledge on the studied problem and the very good ability of critical thinking. Critical approach to the results indicates that the Author is aware of the complexity of data and limitations resulting from experimental procedure (*e.g.* timing of blood sample collection, blood vs. tissue oxidative damage) and small sample size in the first experiment. The conclusions are based on the results and valid. Future perspectives take into account the current global climate change.

3. **Layout and register**

(layout, register and the clarity of the language, the quality of the visual material etc.) (25-200 words):

The thesis fulfils the formal requirements on good level. The dissertation consist of three main chapters: General Introduction, Experimental Part with separate Summary for each experiment, and General Discussion followed by Major Findings and Conclusion and References list. It also contains Summary in English and in Polish, and List of Figures. The use of different fonts helps the reader in better orientation in the text. The structure of the thesis conforms to principles of scientific thesis. However, with such structure the Author did not avoid several unnecessary repetitions. The author used appropriate number of bibliography sources and quoted them correctly in the thesis. Throughout the whole manuscript writing is clear, but there is several

missing information (see Critical notes). There are also few editing and punctuation errors. I also have to mention that some figures (Figs. 17-22) are not large enough to be easy readable. The figures could be better arranged and they could have better quality.

4. Critical notes

There are several inconsistencies and understatements in the description of field experiments. First of all, there is no data on the environmental conditions. I also did not find any criteria for nest boxes selection. Since 275 boxes were regularly checked why the average clutch size was calculated only for 74 nests? How the experimental boxes were selected? According to the dots on the map (Fig. 4) there were 10 control boxes and 12 enlarged ones (Reviewer is always meticulous), but stats results on the number of chicks on day 15 post-hatching indicate equal number of nests in both groups (n=10). The same numbers appear in the description of statistical analyses but they do not correspond to numbers in Table 2. Thus, it is not clear for me which data were analysed or which data and why were excluded from analyses. Some technical details on brood size manipulation are also missing. How many hatchlings were added to the nest and whether all of them were accepted? According to the above-mentioned stats there were ~9 chicks in control and ~12 chicks in the enlarged nests. What was the intended clutch size in the enlarged nests? I also wonder whether feeding intensity was observed? Based on the results on development of nestling, the question arises whether it was affected by thermal microenvironment, namely whether brood enlargement could increase ambient temperature inside the nest, resulting in slower development? The other possibility could be that higher ambient temperature could force females to leave the nest more frequently to protect themselves from overheating. Such speculation would be supported by the results of the second study. Moreover, longer time spent by females outside the nest could contribute to the slower development of nestlings. Other questions that remain open are: to what extent ambient conditions (temperature, humidity, rain, wind, etc.), directly or indirectly, affected nestling development, and whether they could also affect oxidative stress in females? These questions require discussion as the study was done in the field. I also wonder how to explain the negative correlation between nestling mass and female DEE (Fig. 11)?

In the second part of the experimental study I miss information on the differences, if any, in nests size. It would be easy to estimate because nest material was provided every day until birds stopped using it. I wonder why the Author has not decided to measure BMR and DEE to compare results of laboratory results done on zebra finches, and field studies on great tits. Although in different species but it still would be valuable. Also, why the results on RMR (Fig. 16) are presented in $\text{mlO}_2 \cdot \text{min}^{-1}$ while the Author calculated it in Watts? Furthermore, based on the repeated measurements of oxidative status the Author concluded that the decreased antioxidant capacity at the end of reproduction supports the hypothesis that reproduction is costly in terms of oxidative stress. This interpretation does not convince me. If uric acid took over a role of non-enzymatic antioxidant capacity (OXY) then the measure of oxidative stress ($\text{dROMs}/\text{OXY} \times 1000$) would not be indicative of stress. Note, that the dROMs did not change between day 13. and day 35. The continuous decrease of female body mass during rearing may indicate higher protein turnover contributing to the changes in uric acid concentration. Consistently, the largest difference in uric acid concentration between feather-clipped and unclipped birds at low temperature may suggest different substrates utilization. It would be great if the Author could extract data on respiratory exchange rate to look for the potential differences in protein oxidation between experimental groups. I am also a little bit confused with the scientific name of zebra finches. According to the International Ornithological Congress World Bird List the zebra finches (*Taeniopygia guttata*) is now divided into two species: Sunda zebra finch (*T. guttata*) and Australian zebra finch (*T. castanotis*). Which one was used in the experiments? I also wonder if there were any differences in the development of brood patch, if it is present, between experimental groups.

Finally, although I am confident that the Author had permit from Local Ethical Committee to do these experiment, but this information is missing in the thesis.

5. **Final grade** (justification 25-200 words):

The present thesis fulfil all criteria for a PhD Dissertation. The topic of thesis is current and relevant in the context of up-to-date research in the field of avian energetics and oxidative stress. This thesis is an original work providing new data on the physiology of free-living and captive birds. The results are well presented and discussed at a high scientific level. Despite several critical notes I state that the thesis is well written, and conclusions are well supported by the obtained results. The experiments on zebra finches were published recently and I hope to see the manuscript on great tits as soon as possible.

I, hereby, declare that the reviewed PhD thesis by Elisavet Zagkle meets the criteria pursuant to art. 187 of Act of 20 July 2018 The Law on Higher Education and Science (Journal of Laws of 2018, item 1668, as amended) and request that the Research Discipline Council of Biological Sciences of the Jagiellonian University in Kraków accepts Elisavet Zagkle for further stages of doctoral proceedings in the field of exact and biological sciences, in the discipline of biological sciences.


YES

I, hereby, request that the thesis is accepted with distinctions. Justification (25-200 words)

NO

The thesis is a part of the still up-to-date topic of the relationship between reproduction and oxidative stress. It should be noted that the existing literature data on this subject are ambiguous and often contradictory. Although the experimental part required huge effort, the dissertation has several flaws, especially due to the lack of presentation of certain data, methodological understatements and graphic design.

11-08-2022
date


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Reviewer's signature