MSc. thesis review

MSc. thesis (Diplomová práce): **Blood glucose concentration in Barn Swallow (*Hirundo rustica*): sources of variability and association with fitness**
Author: **Kamila Bendová**
Faculty of Science, University of South Bohemia, České Budějovice 2020

Reviewer: doc. RNDr. Michal Vinkler, Ph.D., Department of Zoology, Faculty of Science, Charles University

Annotation:

The diploma thesis by **Kamila Bendová** titled „ **Blood glucose concentration in Barn Swallow (*Hirundo rustica*): sources of variability and association with fitness**" comprises 57 pages. The main objective of the research was to describe factors influencing blood glucose levels as an important physiological parameter. Kamila Bendová performed her study in the Barn swallow, a classical avian model species for evolutionary ecology. The tasks were as follows: 1) to analyse variability in blood glucose concentration in free living Barn Swallows, 2) to assess repeatability of individual glycaemia during season and across seasons, 3) to identify sources of variability in blood glucose concentration, and 4) to examine the connection between blood glucose level and survival or life expectancy. The thesis is clear and well written, containing in total 109 references that are relevant and appropriately used for the result interpretation and discussion.

Formal aspects:

In my opinion, the structure of the thesis is suitable, providing a relatively brief, but meaningful introduction and leaving enough space for the discussion. The language is fine, I did not notice any important errors, misleading or confusing formulations. Perhaps, at some places I would suggest avoiding the use of plural (we) – in general for a thesis I recommend the use of singular (active) – after all, it is the student alone, who is responsible for the content of the thesis (see the signed declaration). The text was mostly easy to read and understand (except for some parts of the methods on which I comment below). Also the graphics are well prepared and containing proper captions and labels. The only possible shortcoming may be seen in the Annotation (but perhaps here the faculty conventions differ), where I would expect an abstract also revealing the most important results. In contrast, in this annotation only the aim is clarified, but no conclusion is provided. If the thesis is meant as a training material for scientific publication, then the summary/abstract should definitely provide also insight into the main results and conclusions.

Scientific content:

The study is a well performed scientific work that (after publication) will form a fine contribution to the avian wildlife physiology. (Assumingly mainly thanks to the input of the supervisor, who is an expert in his field) the study design is clear, taking into account most explanatory variables that are feasible to be investigated in the field. The sample size is respectable and so is the use of the advanced statistics. However, here I must admit that at certain parts my own expertise was too limited to see the benefit of certain operations: brief justification of the selected approaches would be helpful (although this was available for some parts). The results appear consistent and well discussed.

The main questions:

1) Introduction, page 4: what may be the reason why (in contrast to mammals) in birds the glucose levels in blood vary so dramatically? What could be the reasons for low or high glucose levels? (from 6.7 mmol/L in Mute Swan (*Cygnus olor*) to 26.9 mmol/L in Snowy Egret (*Egretta thula*) – if you asked me, I would not be able to think of any good reason why these two species in particular should vary so much). Have these interspecific comparisons been derived from measurements in higher numbers of individuals per species? Might there be any ecological variation/phenotypic plasticity that was not considered?
2) Methods, page 16: I am not sure how to interpret your investigation of the effect of stress-induced glucose dynamics that is possibly meant to validate the sampling latency effects. Since part of the observed changes likely represent outcome of a blood loss due to repeated bleeding rather than stress resulting from the capture itself, what do you think these results exactly tell about your data?
3) Methods, page 16: My main question concerns the relationship between daytime and food intake. In the Methods you mention "predefined time", but this is probably referring only to the sampling latency, not predefined time of individual bird capture. In your study, the time was recorded, but did you also somehow standardise the time of capture? From your introduction it appears clear that glucose levels depend on food intake and food intake (I suppose unknown and unstandardised in the wild animals) depends on daily periodicity and weather. Thus, the issue of time is crucial for estimating individual stability in the trait. I was surprised not to find any mention of this issue in the subchapter 3.4.3 "Repeatability". This variable appears only in the following subchapter 3.4.4 "Potential predictors". Could this explain the results mentioned in the subchapter 1.4 "Repeatability and variation explained by sampling date and year"? (in medical research, glucose levels would be evaluated only after overnight fasting) Could you get better results, if the glucose levels of each individual were taken e.g. by sampling on the nest during the night (standard time)? I am a bit surprised that the issue of food intake with respect to circadian biorhythms in glucose levels is not mentioned in the discussion (I would assume that the food intake is a more important parameter than e.g. light alone; I understand that in a wild-bird study it is not possible to control on that or follow its effects, but in the captive-bird research I expected this parameter was evaluated; some mention of the possible effect of food is given only with respect to seasonality on the page 39).
4) Methods, page 20: I suspect various weather conditions such as temperature, rainfall and relative humidity inter-correlated and dependent on date – although I see that their effects were extensively evaluated, did you somehow treat their correlation in your models? Honestly, I must admit that the statistics used is too advanced to my understanding. Therefore, I would like to ask the student for brief justification of the approach (why correlations among the variables were not tested before the data analysis?)
5) Results, page 33: what is the biological meaning of the third order polynomial effect of the blood sampling latency?
6) Discussion, page 42: is there any experimental evidence suggesting that birds may suffer from hyperglycaemia? The evidence from wild birds is valuable addition, but I would expect that the primary source of understanding will come from standardised experiments in captivity, e.g. in chickens.

Other specific issues (not necessary to be read during the defence)

- What may be the relationship between metabolic efficiency and metabolic rate? Can metabolic efficiency be predicted individually consistent and heritable?

- Cited references – this is a diploma thesis, so the criteria for literature cited are milder, but in scientific writing typically only research articles, reviews and monographs (i.e. resources strictly referencing other citation sources) are used – this is probably not completely true for Vácha 2016 or Encyclopaedia Britannica 2016. Since Vácha 2016 is probably a regular course, you could possibly use "F. Vácha 2016, pers. comm.", which is nonetheless rarely used, because it is not easy to check, what exactly was that what did F. Vácha tell you.
- Introduction, page 4: some less common terms, such as here, e.g., the "postprandial state" could be explained.
- Introduction, page 5: is the regulation of glucose levels by insulin and glucagon specific to mammals as indicated here? I would assume it conserved at least across amniotes, if not all vertebrates.
- Introduction, page 6: are the glucose levels size dependent? (explained later in the text, but should be highlighted already here)
- Introduction, page 6: the differences between birds and mammals mentioned in the last paragraph on this page do not really represent differences in glucose metabolism – birds and mammals are different in absolute levels, but the components of the glucose metabolism and their functional roles appear to me mostly the same after reading this text.
- Introduction, page 8: the last sentence lacks the reference. There are no positive associations reported between the glucose levels and body mass – why then inconsistent results?
- Methods: the models tested appear relatively complex and I may misunderstand their description in the text – a table listing the full models tested would be beneficial.
- Results, page 24: in the subchapter 1.4 I see two times "within season", but with different repeatability levels (r).
- Results, page 32: viewing Figure 9 I must ask whether the quadratic trend is significant even after excluding the two apparent outliers captured later than 10 hours after the sunrise.
- Results, page 33: "Individual repeatability during season was r = 0.34 and within season was r = 0.28." what is the difference between "during" and "within"?
- Results, page 33 Fig.10 and Discussion, page 38: looking at the graph and reading the discussion, I am not sure if it is not simply possible, that you overfitted your data by including the third order polynom, which is not biologically interpretable (just fits the data slightly better precisely because its complexity). What would be the physiological interpretation of the drop in glucose levels between 15 and 40 minutes after capture followed by the repeated increase?

Conclusion:
The potentially critical point raised above by no means decrease the value of this thesis, which I find both interesting and relevant. The student clearly manifested her research proficiency as well as capacity of scientific writing. Therefore, I feel convinced that the thesis fulfilled its intended purpose.

Evaluation:
For the above mentioned reasons I recommend the evaluation mark „výborně".

In Prague dne 11.1. 2021,