Comments to the author.

Please find comments to the manuscript entitled “Skeletal variation in bird domestication: limb proportions and sternum in chicken, with comparisons to mallard ducks and Muscovy ducks” below.

1. BASIC REPORTING

The submitted manuscript is generally well written. The impact of domestication on the biometric measures is an interesting topic. In the introduction a brief mentioning of when the species subjected to this study was domesticated compared to other species (e.g. mammals) would provide evidence of what to expect in terms of skeletal evolution. Is the timeframe of domestication too short to change the skeletal properties in the included species?

In both the introduction and discussion, reference to flying ability of Muscovy is referenced by blog post and unrefereed web pages, that is not considered as appropriate referencing and may if extensive weaken the study.

However, the manuscript suffers from being a bit unstructured in particular in te results sections. Here the lack of structure is hampering the interpretation and the readability of the results, due faulty references to figures and tables, to little detail in the tables for getting an overview of the wild and domesticated birds just to give some examples. This section need a thorough and careful revision with a clear focus on structure and logic order in the presentation of the results. See also specific comments below (the list is not exhaustive). More words/description on where there are differences will be an improvement and help the reader.

By aligning the structure of Materials and methods section and the Results section the readers ability to interpret the results presents will be considerably increased. A suggestion could be 1) wild-domesticated all species, followed by 2) chicken related comparisons, including breed differences for both leg and keel bones.

There has been a mixup in updating figure and table numbers in many of the cross references. That should be avoided, as it is hampering the interpretation of the results and evaluation of the conclusions.

2. EXPERIMENTAL DESIGN

In general the design of the study is sound, however validation of the measurement accuracy and instrument uncertainty was not addressed. Details on this is described below in specific comments.

As the breeding of poultry is an ongoing event, it will be useful to include the year/date where the specimens have been collected and included in their respective collection. Additionally inclusion of a sufficient number of birds from modern commercial lines, both egg and meat type birds would have been very useful for displaying the potential impact of intensive breeding selection where selection criteria is meat and egg yield.

3. VALIDITY OF THE FINDINGS

The analysis of the limb measures is found to be valid and sound. However, the analysis of the keel bone data is next to absent. This is a major weak point and is elaborated several places below in both general and specific comments.

4. General comments

When specific attention was given to the keel bone of the chicken together with reference to keel bone damage (both fractures and deformities) it is hard to understand why specific measurements of various keel bone dimensions (e.g. length and height of the carina, total length, distances between distal/caudal ends of trabeculae etc.) was not performed and compared to limb properties and also between wild and domesticated birds. This information is next to necessary for the understanding of keel bone fractures in layer hens.

As described above the lack of detailed morphometric measures of keel bones, which could have been done at the same time as the initial registration, is weakening the results. It should be included, including comparative analysis to the limp data. If not possible, all sections regarding the keel should be taken out of the manuscript. At its present form it does contribute significantly to the rest of the study.

Furthermore inclusion of modern adult commercial layers and meat type broiler breeders would greatly increase the understanding of the selective pressure that these two types of chicken have been put through the last 3-5 decades.

5. Confidential notes to the editor

None

Specific comments:

Line 52: At the start of the line, there is an empty gap. It is unclear whether some text/wording is missing or if it is merely a technical issue.

Line 71-73: References to the muscovy ducks' flight capacity may benefit considerably by using scientific and/or textbook literature or other refereed information instead of using anecdotal information from internet blog posts. For example a comparison of allometrics of skeletal bones and potential ability to fly in muscovy and pekin ducks by Swatland (1980). In the paper by Converse and Kidd (2001), the muscovy duck is considered a migratory bird. Poultry breeding standard reference may also be able to provide information on ability to fly.

Line 82-88: Crookedness of keel was also described by Buckner et al. (1949).

Line 88-90: The impact of perches on keel bone deformities in laying hens was recently reviewed by the EFSA panel (EFSA, 2015). Please clarify that this relations is not purely historical.

Line 93-94: The proposed definition of a keel bone fracture seem somewhat imprecise. The definition of a fracture in medical terms is referring to a discontinuation (breaking) of the bone tissue resulting a variety of different fracture manifestations where the involved bone is completely or incompletely disrupted with or without fragments. Fracture definition and characteristics are well described in a text book chapter by Marchiori (2013). A fracture will depending on the level of post fracture immobility and will heal more or less deformed with or without medical intervention. If a

fracture is left unstable/insufficiently immobilized the bone repair tissue (callus) with “grow” excessively and new bone formation will appear close to the fracture line and “on top” of the bone surface as exostoses. This callus may in extreme cases be palpable for even the inexperienced after receiving a brief training. Callus and exostoses are always visible on x-ray. Please review the fracture definition accordingly. References to keel bone fracture characterization/visualization are to some extend available using different diagnostic modalities (e.g. imaging, histology, pathology) (Eusemann et al., 2018; Fleming et al., 2004; Richards et al., 2011; Rufener et al., 2018; Scholz et al., 2008; Thøfner et al., 2020; Tracy et al., 2019).

Line 115-116: To overcome the risk of applying unknown degrees of inaccuracy and uncertainty of the measurements and measuring equipment both replicate measurements and calibration of equipment is of great value. At present it is unclear whether a bone was measures several times (technical replicates). Please specify, and justify if not more than one measurement. What were the approaches/procedures for calibration of the calipers?

Line 119-121 : the total length of given limb “was taken”, “was defined” seems more appropriate as the tarsus/carpus and toes/phalanges were not included.

Line 122-123 (Table 1): The distribution of wild and domesticated birds is unclear for all three species. Please clarify. The pooling of all the wild and domesticated birds within each species is disturbing, especially in chickens, where many different breeds in all sizes are included. Please justify the reasoning. It would increase the immediate readability if table 1 was presented as an overview of the distribution and numbers of wild and domesticated birds within each species and note the breeds of mallard ducks and chickens, maybe including the mean length of the specific bones.

Line 133-136: It is acknowledged that juvenile modern meat type chickens were not investigated. Nevertheless, the study will benefit greatly by including adult broiler breeder birds, as these chicken line have been bred intensely for meat development. This has significantly altered their gait to a level where it is considered the new “normal” that broilers have increased gait scores compares to other breeds/line including commercial layer type hens, like the leghorn-derived lines.

Line 153 (table 3): Table 3 is referenced the manuscript, but the information provided in the manuscript in table 2. Again, presentation of the result can be improved. The means/medians and the variation of each breed would help the reader to interpret where the differences between breeds may be present. Please consider illustrating by using a plot/figure instead.

Line 179-191: The absence of specific measurements of keel dimensions is weakening the results considerably with regards to the keel morphology and its potential importance to keel bone fracture development observed in in particular highly productive laying hens.

Line 197: reference to table 2 is wrong. The table 2 provided in the manuscript is on the different chicken breeds included in the study. Maybe it is the content of table 1 that is referred to. Including presentation of the mean properties for reference will greatly improve the presentation of the results. Maybe move up fig 8 for this part…

Line 202: Table 3 is referenced the manuscript, but the information provided in the manuscript in table 2 is presenting the results described. As mentioned above (line 153).

Line 205-212: The lack of structure in the whole results section together with the faulty reference to figures and table numbering is impairing the review of this last paragraph too much.

Line 221-223: By including origin of breed formation, overall type of chicken (layer/meat, large/giant/dwarf size, height/weight span) in the table with the number of bird within the specific chicken breeds would substantiate the interpretation and discussion of the breed differences among the chicken breeds including RFJ. This can also be used for discussion the selective pressure of certain traits. In relation to that the absence of modern highly selected commercial lines, both layer and meat type chickens is weakening the conclusions. The selective pressure of the commercial lines may be considered the highest and fastest. Maybe visualized by creation of a small phylogentic tree including wild and domestic forms as well as breeds. **Interactive Tree Of Life** is an online tool for the display, annotation and management of phylogenetic and other trees (https://itol.embl.de/gallery.cgi) (Letunic and Bork, 2019).

Line 235-249 and fig 3 and 8: Some attention to the large variation in limp and bone lengths in both domesticated chicken and mallard breeds should be given. In particular as many of the different breeds have been bred for exterior traits including (body) size, therefore also known as “fancy breeds”. The observed differences may be partially explained by this. As mentioned above an overview of the standard size/dimensions, age of breed, intended produce (egg/meat) may help clarifying.

Line 266-288: Loss of ability to fly is discussed, however no comparison of the differences between the flying Muscovy duck and the non-flying mallard, in both domesticated Muscovy-mallard and the wild birds, has been presented. Maybe that may help supporting the conclusions.

Line 295: Take care in the use of refereed references. Same issue as in line 71-73 in the introduction. Again taking the length of the domestication age/time span into account may or may not support the conclusions made on the Muscovy ducks ability to fly. Maybe the timeframe for this evolutionary change is too short. A reference to known spatial relation to skeletal changes for comparison will substantiate the conclusions.

Line 310-324: The data handling and analysis of the keel bone observations included in the manuscript is almost absent apart from a “presence-absence” table on the 5 selected criteria, therefore it seems a bit strange that such a large part of the discussion is not based on solid grounds or sufficient data. This is a major weak point. As described above the lack of detailed morphometric measures, which could have been done at the same time as the initial registration, is weakening the results. It should be included, including comparative analysis to the limp data. If not possible, all sections regarding the keel should be taken out of the manuscript. At its present form it does contribute significantly to the rest of the study.

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