

The impact of domestication process on eggshell microstructure in *Gallus gallus*, *Anser anser* and *Anas platyrhynchos*

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Abstract: The domestication process has had a substantial and varied impact on animals in terms of anatomy, physiology, and behaviour. Poultry species are particularly important for humans, with the most significant being chickens, geese, and ducks. However, it is not well understood whether, or to what extent selection influences the eggshell structure compared to wild ancestors. In the present study, we compared eggshells from three species: Red junglefowl and its four domesticated forms: Green-legged partridge, Lohmann Brown, Ko-Shamo, meat type breeder (Cobb 500); Greylag goose, and two domesticated breeds: Bilgoraj goose and White Koluda goose, as well as Mallard duck and domesticated crossbred KhO-01. The analyses revealed significant differences in the eggshell structure both between species ($P < 0.001$) and between breeds ($P < 0.001$ for chickens and geese, and $P = 0.039$ for ducks). Domesticated forms tend to have fewer mammillary knobs per mm^2 ($P = 0.004$), which were larger ($P < 0.001$) and they show the smaller coverage of the mammillary knobs ($P < 0.001$). Analyses showed significant correlations of the body mass and egg size with eggshell characteristics ($P < 0.001$). Considering that domesticated forms are usually larger, it cannot be conclusively determined whether changes in structure result from the domestication process itself or are physiologically linked to body mass and egg size. The relatively high similarity between the eggs of the wild ancestor and the Ko-Shamo breed, which exhibits substantial morphological changes but has a body mass similar to that of the Red junglefowl, supports this interpretation.

Keywords: chicken; duck; egg; goose; SEM

Domesticated animals differ from their ancestors. Some changes result from planned selection carried out by humans, e.g. increase in egg production (Kozak 2019), increase in body size (Kerje et al. 2003), behavioural changes (Ericsson et al. 2014), while others seem to be a side-effect like decrease of the brain size (Mehlhorn and Rehkamper 2013).

At the early stages of domestication, significant differences in the biology of the species may arise, but when humans start intensive selection, they are more probable (Tixier-Boichard et al. 2011).

Man has domesticated relatively few species of animals, including birds. The most important are chickens (*Gallus gallus*), geese (*Anser anser* and

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Anser cygnoides), ducks (*Anas platyrhynchos* and *Cairina moschata*) and turkeys (*Meleagris gallopavo*). Among the first three species, geese have undergone the relatively least changes in the process of domestication, but some changes can be seen in external appearance, reproductive traits and behaviour. Geese have increased their body weight, reach sexual maturity earlier and exhibit higher prolificacy. According to Kozak (2019), egg production of goose breeds has increased 600–1 000% compared to the ancestor. Similar changes were found in another important poultry species – the chicken. As it was domesticated 6 000–10 000 years ago (Tixier-Boichard et al. 2011), the changes may be most noticeable. Chickens are distributed throughout the world resulting from the creation of many breeds adapted to local environmental conditions, ~100 standard breeds (with ~200 varieties) and an equal number of bantam (miniature) breeds (Delany 2004). This has led to substantial variation in body size and shape, plumage, comb morphology, etc. Commercial stocks, however, are the most numerous due to their economic importance. Their growth rates (broiler-type birds) and reproductive capacity (laying hens) are unusual compared to unselected breeds, and the wild ancestor. Features favoured by humans usually have a negative selective value in the wild, but in captivity, they persist due to protection from predators and assisted reproduction.

The process of domestication has affected genetic diversity (Granevitze et al. 2007; Berthouly et al. 2009) and it has been related to many reproduction issues (Burrows and Quinn 1939; Mohan et al. 2018). However, little is known about egg quality in domesticated breeds compared to ancestors. Nutrition has a great impact on the egg chemical composition, but genetic factors play a role as well (Surai et al. 2001). It has been proved that the concentrations of antioxidants are higher in eggs of wild birds than in domesticated forms (Speake et al. 1999; Surai et al. 2001), and in commercial duck breeds, the ratio of arachidonic to docosahexaenoic acid is higher than in undomesticated forms. The authors speculated that this difference was influenced not only by captive feeding, but also by the domestication process itself (Speake et al. 2002). Other studies on the domesticated species helmeted guinea fowl (*Numida meleagris*) have shown significant differences in eggshell porosity. It was found to correlate with incubation length,

which can be translated into the hatch window in incubators. In production, the aim is to narrow the hatch window, as chicks that hatch too early or too late are of poorer quality. This can lead to the unintentional selection of birds whose eggs possess specific characteristics (Damaziak et al. 2023). Domestication also affects shell pigmentation, as exemplified by quails (*Coturnix japonica*), which include lines of birds that lay eggs with celadon, red, and white shells (Tsudzuki 2008). Therefore, our hypothesis was that the genetic selection by humans focused on increasing productivity, which would alter eggshell structure, partly due to the absence of natural selection pressures, such as predators and environmental conditions in nests. These changes will also vary between breeds due to their history and the purpose why they were bred. Our aim was to examine and describe these differences and to analyse the potential underlying causes. We focused primarily on the innermost mammillary layer, which is crucial for embryo development, and we also measured the thickness of the middle palisade layer and the outermost crystalline layer, treating them together due to poor visibility of the boundaries between them.

MATERIAL AND METHODS

Material collection and preparation. Eggs were collected from three species, i.e. Red junglefowl (RJ) and its domesticated breeds: Green-legged partridge (GP), Lohmann Brown (LB), Ko-Shamo (KS) and meat-type breeder Cobb 500; Greylag geese (GG) and two domesticated breeds – White Koluda (WK) and Bilgoraj geese (BG), and Mallard ducks and KhO-01 ducks (hybrids of Khaki Campbell with Orpington Fauve). From each wild and domesticated form, eleven proper-looking eggs (size, shape, pigmentation) were randomly selected. Eggs were collected from different females to avoid pseudoreplication. Eggs from domesticated birds came from individuals that were kept in the aviaries of Wrocław University of Environmental and Life Sciences, while from ancestors they were obtained from Wrocław Zoological Garden (RJ), Zoological Garden Lubin (GG), and game breeding centre in Grodzisko (Mallard). All individuals were fed as required by the species and breed, and they were kept with permanent access to outdoor paddocks. Eggs were not incubated, thus they did not contain

any developing embryos that would affect the mamillary layer structure of the eggshell.

Eggs were measured (maximum length and breadth) with an electronic calliper and based on these measurements, the eggshell surface area was calculated according to the formula developed by Narushin (2005): $S = (3.155 - 0.0136L + 0.0115B)LB$, where L is the egg length and B is the maximum egg breadth. Egg content was removed and the eggshell was cleaned under running water. From the equatorial region of each egg, one fragment of the eggshell of about 1 cm² in size was taken. To remove organic debris, the chosen fragment was put in boiling 5% sodium hydroxide solution for five minutes (Silyn-Roberts 1983). After that, eggshell pieces were rinsed thoroughly in distilled water and dried at 25 °C for 48 hours.

Scanning electron microscope analysis. Samples were coated with gold using a Scancoat Six Sputter Coater with a 300-s program (Scancoat Six Edwards; HHV Ltd., Crawley, UK). These prepared eggshells were analysed under microscope SEM, EM, Evo LS 15 (Zeiss, Oberkochen, Germany). The inner

eggshell layer was analysed at 150× magnification, cross-sections under 45–150× magnification (depending on the thickness of the eggshell, enough to make the individual layers visible). Thinner eggshells (e.g. RJ and KS) had to be viewed at a higher magnification than the thicker eggshells (e.g. goose eggs). Three photos of the inner surface and two photos of a cross-section of each egg were taken.

The obtained microphotographs were processed using Fiji, an extended version of ImageJ (ImageJ 1.53n; Wayne Rasband, National Institute of Health, USA). The number of knobs per mm² was counted in the mamillary layer within the inner surface of the eggshells. Furthermore, the area of single knobs was measured and the percent coverage with knobs of the inner eggshell was determined using the Weka trainable segmentation plugin (Figure 1). The cross-section microphotographs were used to determine the thickness of individual layers (mamillary and palisade plus crystalline layer) (Figure 2).

Statistical analyses. The Shapiro–Wilk *W* test was used to evaluate the distribution normality

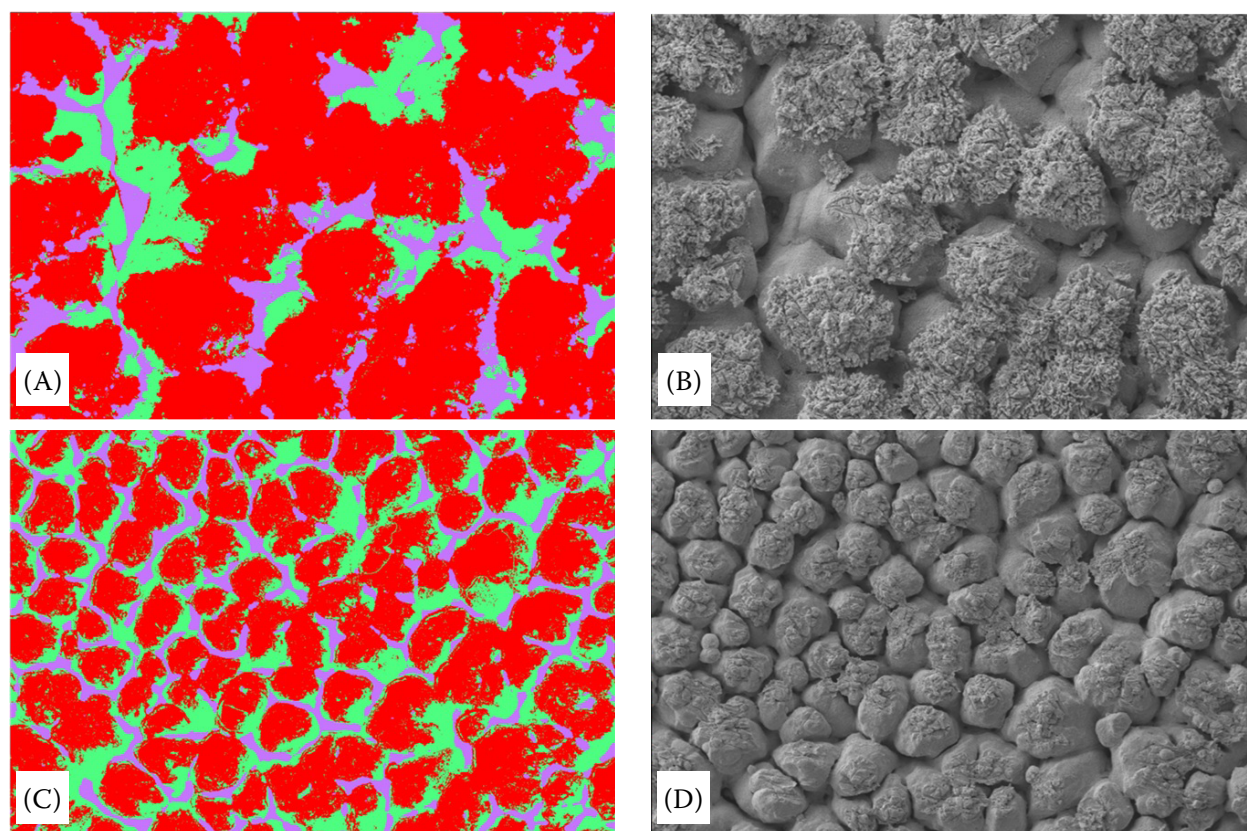


Figure 1. Sample images after processing in Weka trainable segmentation plugin (A,C) and before (B,D) Red colour marks knobs surface, green – side of the knobs, violet – spaces between knobs. (A,B) *Gallus gallus*, Lohmann Brown; (C,D) *Anser anser*, Greylag goose

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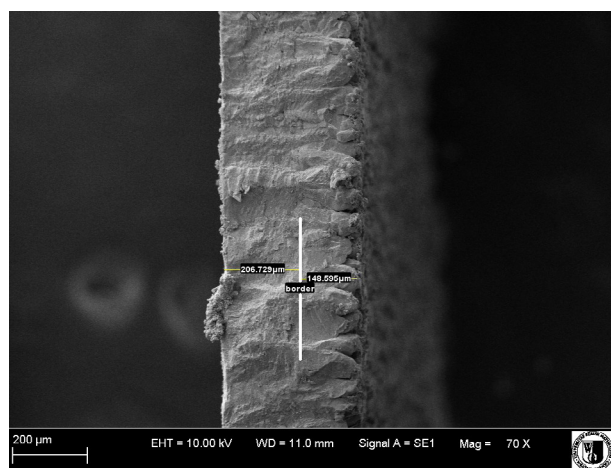


Figure 2. Layer measurements

On the right mammillary layer with knobs is visible. On the left palisade and crystalline layer are visible. The white vertical line marks the boundary of the layers

of dependent variables in each group and Levene's test was employed to examine the homogeneity of variance between them. One-way ANOVA, or Welch test in the case of non-homogeneous variances, with post-hoc Tukey's HSD test were used to test differences in eggshell structure features between species and breeds/lines. Additionally, two-way ANOVA with 'species' and 'domestication' independent variables (factors) including the interaction effect ('species' × 'domestication') was applied with post-hoc Tukey's HSD test to demonstrate the simultaneous effect of those two factors on dependent variables. Spearman's (ρ) correlations and Spearman's partial correlations were used to investigate the relationships of eggshell structure features with each other, mean female body mass for breed (as a factor that significantly differentiates the studied breeds) and calculated eggshell surface. All statistical analyses were conducted in Statistica v13.3 software (1984–2017 TIBCO Software Inc, Palo Alto, California, USA) and JASP (JASP Team v0.17.2, 2023). Statistical significance was set at $P < 0.05$.

RESULTS

Mammillary layer. There were significant differences in the structure of the mammillary layer between the studied species [Electronic Supplementary Materials (ESM) Figures S1–S3]. The number of knobs per mm^2 differed between

wild ancestors. *Gallus gallus* had the highest number of knobs, while *Anser anser* the lowest ($P < 0.001$; $F_{2,96} = 70.50$). The area of the knobs also differed between species: *Gallus gallus* had the smallest knobs, while *Anser anser* the largest ($P < 0.001$; $F_{2,178.8} = 501.38$). The knob percent coverage differed significantly between species: the highest was observed in *Gallus gallus*, intermediate in *Anser anser*, and the lowest in *Anas platyrhynchos* ($P < 0.001$; $F_{2,96} = 26.88$).

Further analyses revealed differences between ancestors and domesticated forms (Table 1). In most cases, ancestors had more knobs. In *Gallus gallus*, both RJ and KS had more knobs per mm^2 than the other breeds ($P < 0.001$; $F_{4,160} = 21.20$). In *Anser anser*, GG and BG had more knobs than WK ($P < 0.001$; $F_{2,96} = 10.64$), and in *Anas platyrhynchos*, the Mallard had more knobs than KhO-01 ($P < 0.039$; $F_{1,64} = 4.43$). The area of the knobs was also smaller in ancestor forms and some smaller breeds. In *Gallus gallus* ($P < 0.001$; $F_{4,296.4} = 308.53$) the smallest knobs were found in KS followed by RJ. In *Anser anser* ancestor and BG had smaller knobs than WK ($P < 0.039$; $F_{2,186.4} = 10.66$), and Mallard had smaller knobs than its domesticated form ($P < 0.001$; $F_{1,115.2} = 510.27$). The knob coverage was higher in RJ and KS than for the other chicken breeds ($P < 0.001$; $F_{4,160} = 24.69$). Similarly, in geese, both the ancestor and BG had a higher coverage of knobs ($P < 0.001$; $F_{2,96} = 20.20$). Only in ducks, no statistically significant difference was found ($P = 0.851$; $F_{1,64} = 0.04$).

'Species' as well as 'domestication' factors revealed a significant impact on the knob area ($P < 0.001$). The interaction effect ('species' × 'domestication') was also statistically significant ($P < 0.001$). The strongest effect was observed for the 'species' factor (partial $\eta^2 = 0.25$). Tukey's HSD test showed that the ancestor and domesticated breeds of *Anser anser* did not differ significantly from each other. Also, the ancestor of *Anas platyrhynchos* and *Gallus gallus* did not reveal any significant difference from each other. The greatest knob area in domesticated forms was found in *Anser anser*, then in *Anas platyrhynchos* and the smallest area was observed in *Gallus gallus*; in the case of ancestors the trend was the same except for *Gallus gallus*, which had the greater knob area in this case than *Anas platyrhynchos*. 'Species' and 'domestication' factors had a significant influence on the number of knobs per mm^2 but no in-

Table 1. Single knob area, mean number of knobs/mm² and knobs coverage of the inner surface for domesticated forms and their ancestors

Species	Breed	Mean knob area (μm ²) (<i>n</i> -measures)	Mean <i>n</i> knobs/mm ²	Coverage inner surface with knobs (%)
<i>Gallus gallus</i>	Red junglefowl	2 633 ^c (<i>n</i> = 489)	214.9 ^b (<i>n</i> = 33)	71.9 ^a (<i>n</i> = 33)
	Green-legged partridge	3 111 ^a (<i>n</i> = 572)	174.9 ^c (<i>n</i> = 33)	67.5 ^b (<i>n</i> = 33)
	Lohmann Brown	3 815 ^b (<i>n</i> = 566)	177.7 ^c (<i>n</i> = 33)	61.2 ^c (<i>n</i> = 33)
	Ko-Shamo	2 121 ^d (<i>n</i> = 695)	260.2 ^a (<i>n</i> = 33)	70.5 ^{ab} (<i>n</i> = 33)
	Cobb 500	3 021 ^a (<i>n</i> = 647)	213.2 ^b (<i>n</i> = 33)	63.9 ^c (<i>n</i> = 33)
<i>F</i> test (<i>P</i> -value)		343.6 (<0.001)	12.5 (<0.001)	24.4 (<0.001)
<i>Anser anser</i>	Greylag goose	4 624 ^b (<i>n</i> = 705)	114.4 ^a (<i>n</i> = 33)	67.3 ^a (<i>n</i> = 33)
	Bilgoraj goose	4 593 ^b (<i>n</i> = 609)	113.4 ^a (<i>n</i> = 33)	67.7 ^a (<i>n</i> = 33)
	White Koluda goose	4 968 ^a (<i>n</i> = 553)	96.8 ^a (<i>n</i> = 33)	59.6 ^b (<i>n</i> = 33)
<i>F</i> test (<i>P</i> -value)		10.8 (<0.001)	2.1 (ns)	9.4 (<0.001)
<i>Anas platyrhynchos</i>	Mallard duck	2 571 ^b (<i>n</i> = 597)	173.8 ^a (<i>n</i> = 33)	62.0 (<i>n</i> = 33)
	KhO-01	4 253 ^a (<i>n</i> = 557)	155.2 ^b (<i>n</i> = 33)	61.7 (<i>n</i> = 33)
<i>F</i> test (<i>P</i> -value)		494.9 (<0.001)	5.3 (0.027)	0.2 (0.640)

Tested using post-hoc Tukey HSD; ^{a-c}Means significantly different at the 5% level are identified by different letters within each column; *P*-values always refer to comparisons within species

ns = not significant

teraction effect of those two factors was found. The strongest effect was observed for the 'species' factor again (partial $\eta^2 = 0.46$). Ancestors of all species had a higher number of knobs. The highest number of knobs was observed for *Gallus gallus*, then for *Anas platyrhynchos* and the smallest for *Anser anser*. The effects of the 'species', 'domestication' as well as 'species' $\times$ 'domestication' (interaction effect) factors were observed in the knob coverage of the inner surface. The strongest effect was found for the 'species' factor (partial $\eta^2 = 0.13$). The highest values of the knob coverage of the inner surface were observed for *Gallus gallus*, then for *Anser anser* and the lowest for *Anas platyrhynchos*. In the case of the first two above-mentioned species ancestors showed higher values of the knob coverage of the inner surface but in *Anas platyrhynchos* no significant difference was found (significant interaction effect). All these results are presented in Figure 3 and in ESM Table S1.

Correlations of mammillary layer structure with egg and female traits. Within *Gallus gallus*, where we analysed eggs from ancestor and four domesticated forms, we examined the correlations between female body weight, egg characteristics and eggshell structure. Female body weight was positively correlated with the knob area ($P < 0.001$;

$\rho = 0.367$) and negatively correlated with both the number of knobs per mm² ($P < 0.001$; $\rho = -0.327$) and coverage of knobs ($P < 0.001$; $\rho = -0.369$). For egg traits: length, breadth, weight, and eggshell surface, there was found a positive correlation with the knob area ($P < 0.001$; $\rho = 0.378$) and a negative correlations with the number of knobs ($P = 0.004$; $\rho = -0.221$) and knob coverage ($P < 0.001$; $\rho = -0.547$) (Figure 4). Additionally, correlations were observed among the structural parameters themselves: the number of knobs was negatively correlated with their area ($P < 0.001$; $\rho = -0.596$), but positively correlated with the knob coverage ($P = 0.001$; $\rho = 0.263$). Knob area and coverage were negatively correlated ($P = 0.001$; $\rho = -0.258$).

We also tested whether relationships between egg size as determined by the eggshell surface area and eggshell structural features were consistent across species. There was a significant correlation between knob and eggshell area ($P < 0.001$; $\rho = 0.646$). Partial correlations of the knob area with the eggshell area excluding the effect of knob number and knob coverage were also significant ($P < 0.001$; $\rho = 0.450$). Similarly, significant partial correlations of the eggshell surface were revealed for the number of knobs ($P < 0.001$; $\rho = -0.692$) and knob coverage ($P < 0.001$; $\rho = -0.306$).

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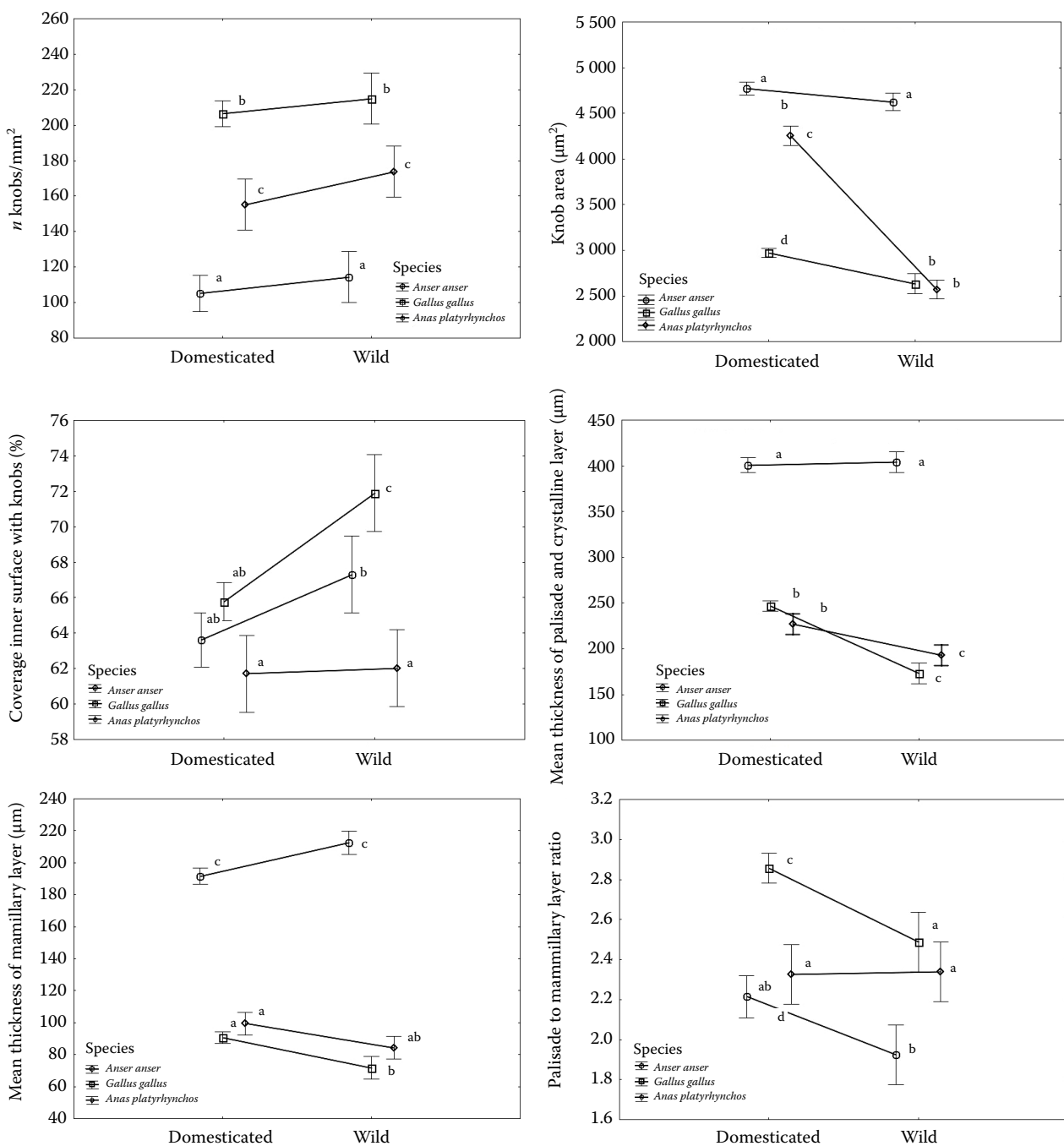


Figure 3. Tukey's post-hoc test results for two-way ANOVA for eggshell features (*n* knobs per mm², knob area, coverage of inner surface with knobs, mean thickness of palisade and crystalline layer, mean thickness of mamillary layer, palisade to mamillary layer ratio) in domesticated forms and their ancestors

a–d) Different letters mean significant difference in post-hoc Tukey's HSD test ($P < 0.001$)

Eggshell thickness and palisade to mamillary layer ratio. Comparing ancestors, the thickest eggshells were observed in Greylag goose, intermediate in the Mallard, and thinnest in the Red junglefowl ($P < 0.001$, $F_{2,177} = 818.87$). The palisade to mamillary layer ratio was comparable in chick-

en and duck, but lower than in goose ($P < 0.001$, $F_{2,177} = 28.71$).

Within *Gallus gallus*, significant differences were found in total eggshell thickness ($P < 0.001$, $F_{4,295} = 58.23$), as well as in the palisade to mamillary layer ratio ($P < 0.001$; $F_{4,295} = 21.07$). KS had the

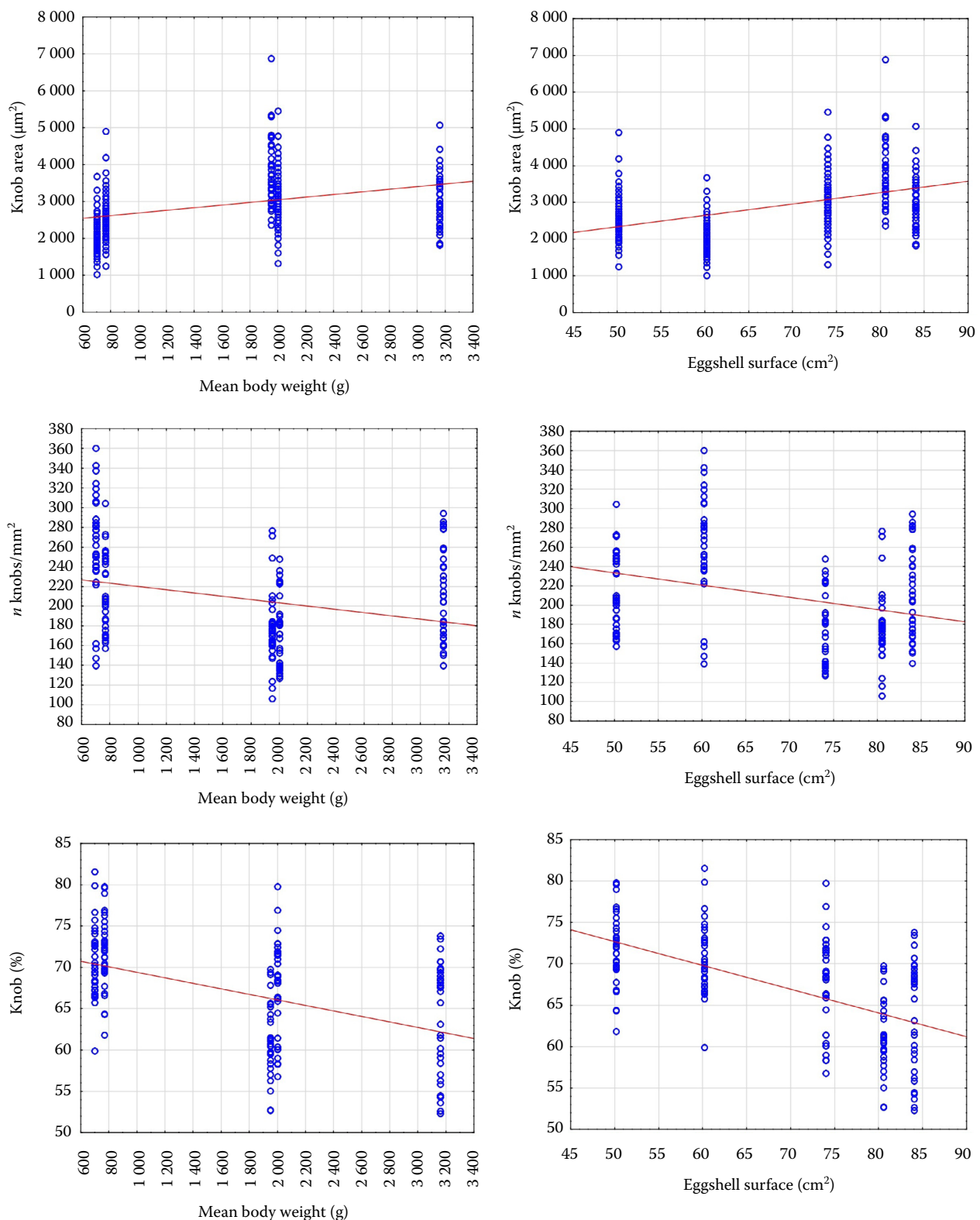


Figure 4. Correlations of body weight and eggshell surface with eggshell structure traits

highest layer ratio. RJ, as well as GP and Cobb 500 were most similar in the thickness of layers. In geese, significant differences were also observed in egg-

shell thickness ($P = 0.006$, $F_{2,177} = 5.33$) and layer ratio ($P < 0.001$; $F_{2,177} = 10.99$). The ancestor had the lowest layer ratio while WK had the highest ratio.

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Table 2. Mean thickness of the palisade and crystalline layer, mammillary layer and their ratio for domesticated forms and their ancestors

Species	Breed	Mean thickness of mammillary layer (μm)	Mean thickness of palisade and crystalline layer (μm)	Palisade to mamillary layer ratio
<i>Gallus gallus</i>	Red junglefowl (<i>n</i> = 60)	71.8 ^b	172.9 ^b	2.5 ^c
	Green-legged partridge (<i>n</i> = 60)	93.4 ^a	232.0 ^a	2.6 ^c
	Lohmann Brown (<i>n</i> = 60)	103.9 ^a	286.6 ^c	2.9 ^b
	Ko-Shamo (<i>n</i> = 60)	71.1 ^b	227.8 ^a	3.4 ^a
	Cobb 500 (<i>n</i> = 60)	93.9 ^a	241.6 ^a	2.6 ^{bc}
<i>F</i> test (<i>P</i> -value)		81.3 (<0.001)	36.5 (<0.001)	15.5 (<0.001)
<i>Anser anser</i>	Greylag goose (<i>n</i> = 60)	212.5 ^b	404.2 ^{ab}	1.9 ^a
	Bilgoraj goose (<i>n</i> = 60)	194.2 ^{ab}	383.6 ^b	2.0 ^a
	White Koluda goose (<i>n</i> = 60)	188.8 ^a	417.7 ^a	2.4 ^b
<i>F</i> test (<i>P</i> -value)		11.2 (<0.001)	7.2 (0.001)	8.4 (<0.001)
<i>Anas platyrhynchos</i>	Mallard duck (<i>n</i> = 60)	84.3 ^a	192.8 ^a	2.3
	KhO-01 (<i>n</i> = 60)	99.4 ^b	226.6 ^b	2.3
<i>F</i> test (<i>P</i> -value)		38.6 (<0.001)	25.0 (<0.001)	0.0 (0.875)

Tested using post-hoc Tukey HSD; ^{a-c}Means significantly different at the 5% level are identified by different letters within each column; *P*-values always refer to comparisons within species

In ducks, there were differences in eggshell thickness ($P < 0.001$, $F_{1,118} = 47.02$) but not in the layer thickness ratio ($P = 0.070$; $F_{1,118} = 0.02$) (Table 2). 'Species', 'domestication' as well as 'species' × 'domestication' (interaction effect) had a significant impact on the mean thickness of palisade and crystalline layers. The greatest effect was observed for the 'species' factor (partial $\eta^2 = 0.77$). The highest values of the thickness of palisade and crystalline layers were found for *Anser anser* but no significant difference between its ancestors and domesticated breeds was noticed. In the case of *Gallus gallus* and *Anas platyrhynchos* domesticated breeds had greater thickness of palisade and crystalline layers than ancestors (significant interaction effect). The 'species' factor had a significant strong influence on the mammillary layer values (partial $\eta^2 = 0.75$) but no significant effect of the 'domestication' factor was found. Also 'species' × 'domestication' (interaction effect) was significant. The highest values of the mammillary layer were found in *Anser anser*, then in *Anas platyrhynchos* while *Gallus gallus* revealed the smallest values. For *Anser anser* ancestors the individuals showed higher values than the domesticated ones but for *Gallus gallus* as well as *Anas platyrhynchos* the effect was opposite (significant interaction effect). 'Species' and 'domestication' fac-

tors had a significant effect on the palisade to mammillary layer ratio. Also in 'species' × 'domestication' (interaction effect) a significant impact was found. The strongest effect was observed for the 'species' factor (partial $\eta^2 = 0.14$). The highest values of this ratio were found for *Gallus gallus*, then for *Anas platyrhynchos*, and *Anser anser* species showed the lowest ratio. For *Gallus gallus* and *Anser anser* the domesticated forms revealed higher values of the ratio of palisade and crystalline layer to mammillary layer but in *Anas platyrhynchos* no significant difference was found (significant interaction effect). All these results are presented in Figure 3 and in ESM Table S1.

DISCUSSION

The general eggshell structure is consistent across avian taxa. It is composed of three layers; the innermost mammillary layer in the form of knobs, the middle palisade layer, and the outermost crystalline layer, which is usually covered with a thin cuticle (Becking 1975). However, in the photos taken, the border between the palisade and crystalline layers was invisible or so poorly visible that we could not distinguish it.

It is known that the eggshell structure varies among birds, however the potential impact of domestication on the eggshell structure within species has not been investigated. Some breeds may lay better quality eggshells than others: e.g. Fayoumi eggshells had a thicker palisade layer and a thinner mammillary layer than Dandarawi, which also possesses a higher number of type B bodies (round formations with no contribution to the palisade layer). These differences translate into eggshell durability (Fathi et al. 2007). It shows that breeds have a different genetic potential for producing eggshells with good structural formation. In view of the wide range of changes that occur during domestication, changes in the eggshell are likely. While early-occurring embryo developmental events seem to be highly evolutionarily conserved, later stages of ontogeny differ between breeds (Nunez-Leon et al. 2021). In wild birds, reproductive success will be a main selection factor, whereas in domesticated forms, emphasis may be placed on eggshell strength or eggshell pigmentation in some cases. Van Toledo et al. (1982) analysed eggshells from two White Leghorn lines – one selected for weak shells and the other for strong eggshells. The authors found the reduced average cross-sectional diameter of mammillary knobs, which resulted in a higher density of knobs per unit area in low eggshell strength line chickens. Thus, selection can influence the eggshell potentially contributing to breeding success, chick quality, and their development. Meat type birds have faster growth not only postnatally, but also during embryonic development. Consequently, their musculoskeletal system must be well developed. Therefore, it was expected that the mammillary layer would be more developed in meat type birds than in other breeds, with greater coverage of mammillary knobs, which serve as calcium source (Bingol et al. 2016). While the number of knobs was comparable to the wild ancestor, the percentage of knob coverage was lower than in most other breeds or lines. Since meat type birds are particularly susceptible to damage to the musculoskeletal apparatus due to their high body weight and rapid growth, the observed eggshell structure could negatively affect skeletal calcification at the time of hatching. Based on the study comparing the mammillary layer structure in Galliformes and Anseriformes, which indicated that the greater coverage of knobs in Galliformes is likely related to differences in ossification patterns (Rosenberger and Pytlak 2024), it would be appropriate to com-

pare the development of the skeletal system among breeds and relate it to eggshell structure. Currently, it is unclear how conservative ossification patterns are among poultry breeds and how closely they are linked to eggshell structure. Higher knob coverage may be associated with greater calcium availability, which is essential for embryonic development. As higher coverage of knobs is related to the greater availability of calcium that is necessary for the development of the embryo, and chicks of undomesticated birds are more prone to predation, therefore they need to be more agile. Among domesticated forms Lohmann Brown had the largest knobs but the lower total coverage of knobs, supporting previous observations that such eggshells may be more durable (Van Toledo et al. 1982). Laying hens have been selected for eggshell strength, but this selection may also have occurred in meat type birds possibly at the expense of calcium availability for the embryo. However, further research needs to be conducted to confirm this. Interestingly, the eggs of the ancestor closely resembled those of the heavily human-modified KS. Two hypotheses may explain this similarity. The first relates to the body size and consequently to the egg size. It is possible that physiological constraints underlie these similarities. The second explanation relates to KS selection, which was not conducted on production traits, but on behaviour and conformation. KS individuals are also characterized by a relatively low laying rate, comparable to RJ.

It is known that the eggshell structure may have an impact on hatchability, what was proven by Olkowski et al. (2015), who found that eggshells with a low-density eggshell matrix were three times more likely to be infected by pathogens. Understanding the factors influencing hatchability success can help in planning selective breeding programs aimed at improving the profitability of poultry production. Other research (Nascimento et al. 1992; Liao et al. 2013) has shown that the thickness of the mammillary layer was correlated positively with hatchability. Because chicken breeds exhibit greater variability than waterfowl (including body size and breeding purpose), the greatest variation in the eggshell microstructure was expected among chickens. In geese and ducks, there was also a trend that the wild ancestors had more knobs that were larger.

Domesticated breeds are usually bigger than their wild ancestors, they also lay bigger eggs which typi-

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cally have thicker eggshells (Birchard and Deeming 2009). It is possible that it is the size of the egg that affects the eggshell structure, as indicated by the correlations found in our study. Thinner eggshells have smaller knobs that may be more densely packed. This would follow from the observations that the coverage of knobs in chickens was most similar between RJ, whose average body weight is 485–1 050 kg (McGowan and Kirwan 2020), and KS with an average body weight around 750 g. The knob area followed a similar pattern: Cobb 500 eggs had relatively large knobs, while KS had the smallest. In ducks, where the domesticated form weighs around 1 900 g and the ancestor up to 1 320 g (Drilling et al. 2020), some significant differences were also observed. In geese, however, no significant differences were observed between the ancestor and the BG. This may be related to the age of the birds the eggs were obtained from. BG eggs came from birds in the first year of life, and young individuals tend to lay smaller eggs (Robertson et al. 2006). This would support the hypothesis that egg size is related to eggshell structure. Most of our considerations are limited to the structure of the mammillary layer because it has been shown to be constant within the species and breed and line. The thicknesses of the layers were more variable and the results difficult to interpret, likely due to strong individual and environmental influences on eggshell thickness.

Conducted research indicates a relationship between domestication and the eggshell structure. However, the underlying cause remains unclear – whether it results from selection for productivity traits or from the increased body size of domesticated breeds. Further research is needed to clarify this issue.

CONCLUSION

Aside from interspecific diversity in eggshell structure, there exists intraspecific diversity between wild and domesticated forms. Ancestors typically have more and smaller mammillary knobs, resulting in their greater coverage of the eggshell inner layer. This may be a result of the domestication process itself, but also a consequence of increased body size and related egg size in domesticated forms. Selective breeding for certain traits can influence eggshell structure, so can environmental

factors. However, given the significant differences observed between the studied birds, the influence of breed/line cannot be overlooked, as they may be associated with predispositions towards forming eggshells of particular structures.

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Conflict of interest

The authors declare no conflict of interest.

REFERENCES

- Becking JH. The ultrastructure of the Asian eggshell. *Ibis*. 1975 Apr;117(2):143-51.
- Berthouly C, Leroy G, Nhu Van T, Hoang Thanh H, Bed'Hom B, Trong Nguyen B, Vu Chi C, Monicat F, Tixier-Boichard M, Verrier E, Maillard JJ, Rognon X. Genetic analysis of local Vietnamese chickens provides evidence of gene flow from wild to domestic populations. *BMC Genet*. 2009 Jan 9;10:1.
- Bingol SA, Deprem T, Karadag Sari E, Koral Tasci S, Aslan S. Comparison between goose (*Anser anser*) and chicken (*Gallus gallus domesticus*) eggshells during embryonic development by scanning electron microscopy. *Kafkas Univ Vet Fak Derg*. 2016 Jul;22(6):937-43.
- Birchard GE, Deeming DC. Avian eggshell thickness: Scaling and maximum body mass in birds. *J Zool*. 2009 Aug; 279(1):95-101.
- Burrows WH, Quinn JP. Artificial insemination of chickens and turkeys. Washington (DC): US Department of Agriculture; 1939. p. 1-13.
- Damaziak K, Marzec A, Riedel J, Wojcik W, Pstrokonski P, Szudrowicz H, Gozdowski D. Effect of pearl guinea fowl eggshell ultrastructure and microstructure on keets hatchability. *Poult Sci*. 2023 Jul;102(7):102733.
- Tsudzuki M. Mutations of Japanese quail (*Coturnix japonica*) and recent advances of molecular genetics for this species. *J Poult Sci*. 2008;45(3):159-79.
- Delany ME. Genetic variants for chick biology research: From breeds to mutants. *Mech Dev*. 2004 Sep;121(9): 1169-77.

- Drilling N, Titman RD, McKinney F. Mallard (*Anas platyrhynchos*). In: Billerman SM, Keeney BK, Rodewald PG, Schulenberg TS, editors. *Birds of the world*. Ithaca (NY): Cornell Lab of Ornithology; 2020.
- Ericsson M, Fallahsharoudi A, Bergquist J, Kushnir MM, Jensen P. Domestication effects on behavioural and hormonal responses to acute stress in chickens. *Physiol Behav*. 2014 Jun 22;133:161-9.
- Fathi MM, Zein El-Dein A, El-Safty AS, Radwan LM. Using scanning electron microscopy to detect the ultrastructural variations in eggshell quality of Fayoumi and Dandarawi chicken breeds. *Int J Poult Sci*. 2007;6(4):236-41.
- Granevitze Z, Hillel J, Chen GH, Cuc NTK, Feldman M, Eding H, Weigend S. Genetic diversity within chicken populations from different continents and management histories. *Anim Genet*. 2007 Dec;38(6):576-83.
- Kerje S, Carlborg O, Jacobsson L, Schutz K, Hartmann C, Jensen P, Andersson L. The twofold difference in adult size between the red junglefowl and White Leghorn chickens is largely explained by a limited number of QTLs. *Anim Genet*. 2003 Aug;34(4):264-74.
- Kozak J. Variations of geese under domestication. *Worlds Poult Sci J*. 2019 Mar;75(2):247-60.
- Liao B, Qiao HG, Zhao XY, Bao M, Liu L, Zheng CW, Li CF, Ning ZH. Influence of eggshell ultrastructural organization on hatchability. *Poult Sci*. 2013 Aug;92(8):2236-9.
- McGowan PJK, Kirwan GM. Red junglefowl (*Gallus gallus*). In: Billerman SM, Keeney BK, Rodewald PG, Schulenberg TS, editors. *Birds of the world*. Ithaca (NY): Cornell Lab of Ornithology; 2020.
- Mehlhorn J, Rehkamper G. Some remarks on bird brain and behavior under the constraints of domestication. *ISRN Evol Biol*. 2013;2013:460580.
- Mohan J, Sharma SK, Kolluri G, Dhama K. History of artificial insemination in poultry, its components and significance. *Worlds Poult Sci J*. 2018 Jun;74(3):475-88.
- Narushin VG. Egg geometry calculation using the measurements of length and breadth. *Poult Sci*. 2005 Mar;84(3):482-4.
- Nascimento VP, Cranstoun S, Solomon SE. Relationship between shell structure and movement of *Salmonella enteritidis* across the eggshell wall. *Br Poult Sci*. 1992 Aug;33(1):37-48.
- Nunez-Leon D, Cordero GA, Schlindwein X, Jensen P, Stoeckli E, Sanchez-Villagra MR, Werneburg I. Shifts in growth, but not differentiation, foreshadow the formation of exaggerated forms under chicken domestication. *Proc R Soc B*. 2021 Jun 30;288(1953):20210392.
- Olkowski AA, Nain S, Laarveld B, Wojnarowicz C. Changes in eggshell structure and predisposition of broilers to health problems: Is there a common pathophysiology? *Br Poult Sci*. 2015 Dec;56(2):267-74.
- Robertson HA, Colbourne RM, Nelson A, Westbrooke IM. At what age should brown kiwi (*Apteryx mantelli*) eggs be collected for artificial incubation? *Notornis*. 2006;53(2):231-4.
- Rosenberger J, Pytlak K. Comparison of the eggshell mamillary microstructure of Galliformes and Anseriformes. *J Zool*. 2024 Jan;322(4):340-50.
- Silyn-Roberts H. The pore geometry and structure of the eggshell of the North Island brown kiwi, *Apteryx australis mantelli*. *J Microsc*. 1983 Apr;130(1):23-36.
- Speake BK, Surai PF, Bortolotti GR. Fatty acid profiles of yolk lipids of five species of wild ducks (*Anatidae*) differing in dietary preference. *J Zool*. 2002 Feb;257(4):533-8.
- Speake BK, Surai PF, Noble RC, Beer JV, Wood NAR. Differences in egg lipid and antioxidant composition between wild and captive pheasants and geese. *Comp Biochem Physiol B*. 1999 Sep;124(1):101-7.
- Surai PF, Bortolotti GR, Fidgett AL, Blount JD, Speake BK. Effects of piscivory on the fatty acid profiles and antioxidants of avian yolk. *J Zool*. 2001 Oct;255(3):305-12.
- Tixier-Boichard M, Bed'Hom B, Rognon X. Chicken domestication: From archeology to genomics. *C R Biol*. 2011 Apr;334(4):197-204.
- Van Toledo B, Parsons AH, Combs GF. Role of ultrastructure in determining eggshell strength. *Poult Sci*. 1982 Mar;61(3):569-72.

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