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A descriptive study of keel bone fractures in hens and roosters from four non-commercial laying breeds housed in a live poultry gene preservation bank

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Simple Summary: The keel bone in birds is an extension of the sternum. Fractures to the keel are common in modern laying hen breeds. Several of the proposed causal mechanisms behind KBF are linked to selection for efficient production. It is therefore of interest to explore whether less selected breeds have a lower occurrence of keel bone fractures compared to reports from highly selected, modern laying hen breeds. Thus, the aim of the current study was to investigate keel bones of hens from four non-commercial layer breeds at two different ages; 30 and 75 weeks of age housed in furnished cages, using a portable x-ray equipment. The results from this descriptive study indicate a low prevalence of keel bone fractures in hens at both ages in all four breeds. No fractures were observed in the examined roosters. The overall low numbers of fractures indicate that genetic factors may be involved and, thus, that selective breeding may help to reduce the susceptibility to keel bone fractures. Finally, this study highlights the importance of poultry conservation to secure existing genetic diversity, which may be an important resource in future selection schemes.

Abstract: The presence of keel bone fractures (KBF) in laying hens has been documented and discussed by several authors, nevertheless the causative factors behind KBF remain uncertain. High prevalence of KBF have been reported in all commercial production systems, in different genetic lines and at different ages. Several of the proposed causal mechanisms behind KBF are linked to selection for efficient production. It is therefore of interest to explore whether less selected breeds have a lower occurrence of keel bone fractures compared to reports from highly selected, modern laying hen breeds. Thus, the aim of the current study was to investigate keel bones of hens from four non-commercial layer breeds at two different ages; 30 and 75 weeks of age housed in furnished cages, using a portable x-ray equipment. The results from this descriptive study indicate a low prevalence of KBF at both ages in all four breeds, with only five KBF detected in 213 x-ray pictures. Of these, four of the KBF were observed in the most genetically selected breed, with an early onset of lay. None of the roosters examined exhibited KBFs. The overall low numbers of KBF found indicate that genetic factors may be involved in KBF and, thus, that selective breeding may help to reduce the susceptibility to KBF. Finally, this study highlights the importance of poultry conservation to secure genetic diversity, which may be an important resource in future selection schemes.

Keywords: keel bone fracture; laying hen; animal welfare; poultry welfare; gene preservation

1. Introduction

Bones have two major functions in avian species: as a reservoir for calcium and phosphorous and as a support for the musculature [1]. The bone quality of modern laying hens has been a topic for scientific research since the 1950s, focusing mainly on osteoporosis [3-6]. A growing concern affecting avian bones is fractures to the keel bone [7-9], which has gained increasingly more attention the last decade.

Keel bone fracture (KBF) has been defined as fragmentation, shearing or bending of the keel bone [9]. The prevalence of KBF in modern laying hens in commercial production systems is alarmingly high, reported higher than 80 % in several studies [10-13]. Several fractures of the keel in the same bird is not uncommon [14]. In contrast, a recent pilot study of the ancestor of modern layers; the red jungle fowl (*Gallus gallus gallus*) found a much lower prevalence [15]. KBF have been found to a varying degree in all major commercial production systems; barren and furnished cages, non-cage systems and organic production [12, 31]. The prevalence is found to be higher in loose housed systems, compared to cage-systems [10,28,32,33]. Studies investigating the occurrence of KBF in roosters are generally lacking, but one study reported no cases of KBF in the included roosters [16]. Welfare implications of KBFs include the likely associated pain [17-19], reduced mobility [20-22] and altered affective state [23]. The high prevalence, along with the impact on affected animals, makes KBF one of the biggest welfare challenges faced by the laying hen industry today [24].

Despite extensive scientific effort over the last decade, the aetiology of KBF is still not clear. It seems likely that KBF is a multifactorial disorder [25,26]. Several risk factors have been suggested: trauma and fractures due to high impact collision with the elements in the housing system [27], selection for increased egg production [28], hen age [29], osteoporosis [16,30], early onset of lay [31,32], and late ossification of the keel [14,26]. Keel bone investigation in different strains and lines of modern layer hybrids, indicate that the genetic lines differ in the prevalence of KBF [7,8,13,16,33]. The cause of these differences between hybrids is uncertain. Besides one investigation of keel bones from the red jungle fowl [15], reports on KBF prevalence in non-commercial laying hen breeds are lacking.

Different assessment methods can be used to evaluate keel bones. Palpation is the most common method used in live hens [29]. Palpation relies on detection of the callus formed during fracture healing [34]. However, callus takes some time to develop; it is estimated that healing time for keel bone fractures in laying hens is six weeks [24]. New fractures, fractures with little callus formation or small fractures may be difficult to palpate leading to a low accuracy of a diagnostic procedure consisting of palpation only [29,35-37]. Furthermore, mobile fracture sites will create more periosteal callus formation than fracture sites with less mobility [38]. Thus, palpation might result in a larger underestimation of KBF occurrence in caged hens compared to loose-housed birds with more activity [14]. To accurately determine the prevalence of KBF, dissection or radiographs are considered the most reliable methods [29,36].

Several of the proposed causal mechanisms behind KBF are linked to selection for efficient production. It is therefore of interest to explore whether less selected breeds have a lower occurrence of keel bone fractures compared to reports from highly selected, modern laying hen breeds. Thus, the aim of the current study was to investigate keel bones of hens from four non-commercial layer breeds at two different ages; 30 and 75 weeks of age housed in furnished cages, using a portable x-ray equipment. Furthermore, we wanted to examine the keel bones of roosters from the same breeds and ages in the same holding.

2. Materials and Methods

This descriptive study included four different laying hen breeds (Table 1). Birds of all breeds were hatched, reared and housed at the Norwegian live poultry gene preservation bank at Hvam Agricultural College, Norway. These breeds and lines have, since 1995, been maintained by a rotational mating scheme with approximately 23 families per line [39]. The birds were raised in cages (120x49x54 cm, length x width x height) furnished with perches, nests and a dust bathing area

(Modell T8, Victorsson Poultry AB, Sweden). Housing was identical during both rearing and production. Each cage housed one rooster and 6 hens.

Table 1. Laying hen breeds and characteristics.

Breed	**Classification**	**Origin[1]**	**Onset of lay, in weeks[2]**
Icelandic landrace	Egg layer	The native breed of Iceland, originating from Old Norwegian Jadar[3]. Not cultivated for specific characteristics	22
NorBrid 8	Egg layer	The paternal line of the last Norwegian, commercial layer hybrid, NorBrid 87. Descends from Red Rhode Island[5]	15
Minorca	Egg layer	Developed in England from imported Castilian fowl	22
Roko	Egg layer	The oldest existing purebred line in Norway. Originated from White Leghorn[4]	16

[1] Information on origin is based on literature review; [2] Personal communication from Mette Nafstad Bjerkestrand, the Norwegian live poultry gene preservation bank, Hvam Agricultural College; [3] Data from Lyimo et al 2014 [1]; [4] Data from Brekke et al 2017 [2].

Birds were radiographed at two different time points and ages; 30 and 75 weeks of age (WOA). At 30 WOA 112 birds from 16 cages, were examined: 96 hens and 16 roosters (Table 2). At 75 WOA 101 birds were examined: 85 hens and 16 roosters (Table 2). Several hens were sold between the two visits; therefore only 55 of the original 96 hens radiographed at 30 WOA could be examined at 75 WOA. Therefore 30 new hens from the same four breeds were included at the second investigation.

The non-anaesthetized birds were gently held upside down by a grip in both legs, inducing immobility. The left side of the bird was facing the digital flat panel detector and the keel bone was at a right angle. Digital radiographs were taken using a portable radiograph unit (Konica Minolta, Aero DR NS3543 mobil) with images obtained using a Poskom Vet20-BT. The x-ray handler was a member of the EU KeelBoneDamage COST Action Group and has received training in keel bone palpation from the group. In addition, training in x-ray handling and evaluation was received from Medivet Scandinavian AB, Ängelholm, Sweden. Images were taken with 50.0 kV, 2mAs and a focus-film distance of 100 cm. All radiographic images were scored by the same person for the absence (0) or presence of one or more (1) keel bone fractures.

This study comprised non-invasive radiographic examination of keel bones of laying hens and roosters. Therefore, approval by an ethics committee for animal experiments was not required according to Norwegian legislation [3].

3. Results

In the present study, a total of 126 hens were radiographed, of which 55 were radiographed twice; at both 30 and 75 WOA. In total, 16 roosters were radiographed, all 16 at both occasions. Altogether resulting in 213 radiographs. At 30 WOA, three hens were classified with KBF; two (8%) of the 24 hens from the Norbrid 8 breed and one (4%) of the 24 hens of the Minorca breed (Table 2). Both hens had a single fracture in the caudal third of the keel bone. The Minorca hen with fracture at 30 WOA had been sold and could not be investigated a second time at 75 WOA. At 75 WOA, two hens were classified with KBF; both from the Norbid 8 breed. One of these had multiple fractures affecting both the middle and the caudal part of the keel. The other had a single fracture in the caudal third of the keel; this bird was diagnosed with a fracture at both 30 and 75 WOA. No fractures were observed in the roosters at any age.

Table 2. Overall keel bone findings in the four breeds and males/females (n=213).

Breed	**Examination, 30 weeks of age**				**Examination, 75 weeks of age**			
	Females	Females with fractures		Roosters*	Females	Females with fractures[1]		Roosters*
	n	*n*	%	*n*	*n*	*n*	%	*n*
Icelandic landrace	24	0	0	4	19	0	0	4
Norbrid 8	24	2	8.3	4	20	2	10	4
Minorca	24	1	4.2	4	29	0	0	4
Roko	24	0	0	4	17	0	0	4
Total	96	3	3.1	16	85	2	2.4	16

*No fractures were detected in any of the roosters; [1] One of the Norbrid 8 birds with fracture at 75 WOA was also diagnosed with a fracture at 30 WOA

All fractures were located to the middle or caudal part of the keel bone. Figure 1 shows an x-ray picture of an unfractured keel bone, 30 WOA. Figure 2a shows a fractured keel bone (indicated by a red arrow) from a laying hen, 30 WOA. Figure 2 b shows a keel bone with multiple fractures (indicated by red arrows), at 75 WOA.

Figure 1. Normal, unfractured keel bone, 75 weeks of age

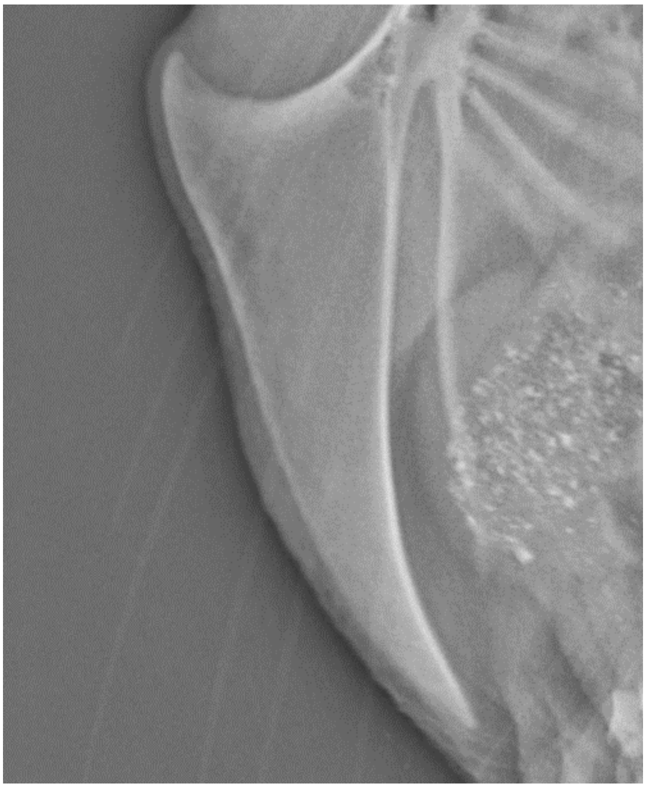

Figure 2a. A keel bone with one fracture, 30 weeks of age.

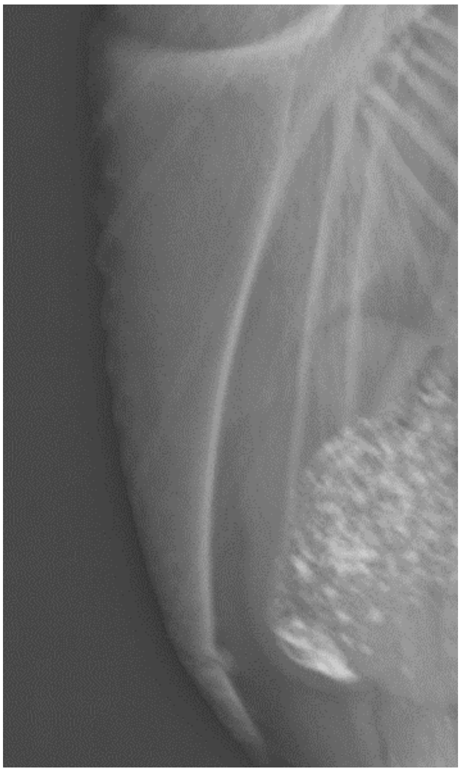

Figure 2b. A keel bone with multiple fractures, age 75 weeks of age.

4. Discussion

This study used portable radiography to examine keel bones of hens and roosters from four non-commercial layer breeds at 30 and 75 weeks of age in order to explore whether less selected breeds have a lower occurrence of keel bone fractures compared to reports from highly selected, modern laying hen breeds. Overall, the prevalence of KBF was low among all the breeds investigated. The low occurrence stands in contrast to published results from modern layer breeds, ranging from 30-97 % [12,13,31]. None of the investigated birds from the breeds Islandic landrace and Roko exhibited keel bone fractures, a result in accordance with findings in the red jungle fowl, which is the ancestor to all laying hen breeds [15]. In the Minorca breed one animal exhibited a fracture. The highest number of KBF was found in the breed Norbrid 8, with 2 KBF out of 24 (8.3%) examined birds at 30 WOA and 2 KBF out of 20 examined birds (10%) at 75 WOA. The Norbrid 8 is the most modern and selected of the four breeds included in the study. This breed was used as the male line in the Norwegian commercial laying hen breeding program until 1994, when it was replaced by international laying hen breeds. An association between breed and KBF occurrence has been found in previous studies reporting various prevalence of KBF in different strains and lines of modern layer breeds [6,7,12,13,29]. The prevalence of KBFs in the present study was low at both 30 and 75 WOA. This contrasts with several studies where the prevalence of KBF increase significantly with age [16,32,43,44]. However, the strength of the association between hen age and susceptibility to KBF may vary by strain and line [29], which may explain the current results. It must be noted that comparison of KBF occurrence across studies can be challenging due to the sensitivity of different assessment methods [29]. Still, the differences between lines and breeds may indicate that selection has affected the susceptibility to develop KBF. Including robustness towards KBF development in multi-trait selection program could be an important preventive measure to reduce the occurrence of KBF in layers, and thus improve laying hen welfare. A weakness of the present study is the low number of birds per breed, and the high number of birds lost to follow-up. To assess the incidence of KBF during the entire production period, large scale longitudinal studies is needed.

All hens in this study lived in identical enriched cages, a housing form that has been assumed to have fewer KBFs than loose house systems like aviaries [29]. This assumption fits well with the overall low occurrence observed in the present study. However, it does not explain the differences between the breeds. Hens originating from cage-free systems have more pronounced callus formation versus no or minimal callus formation observed in caged hens [14]. This is due to fracture sites with less mobility accumulates less callus, and therefore such fractures are harder to palpate [38]. This implies that KBFs in caged hens may have been underestimated in previous studies based on palpation. To avoid this inaccuracy the current study used radiography to investigate the keel bones. All the fractured keel bones had fracture sites dorsally in the caudal third of the keel. This anatomical location is in accordance with findings by both Thøfner et al. [14] and Bauer et al. [45]. Fractures in this location are difficult to detect by palpation, hence, these fractures contribute to the low accuracy and reliability of palpation [35]. This is of particular importance when comparing results from different studies, especially based on palpation. Future studies in the same breeds housed in different housing systems are needed to assess how housing system affects KBF-prevalence in these breeds.

Age of first egg (AOF) varied from 15 to 22 weeks in the breeds in this study. The breed with the earliest AOF coincided with the breed with highest prevalence of KBF. This finding is in agreement with Andersson et al (2017) who found early egg numbers to be associated with KBF in modern layer lines [29,32], where AOF is typically around 16 WOA. However, Roko also had an early AOF, without any KBF. The design of the current study does not allow for causations. Future studies are needed to investigate the effect of production traits like AOF, hen weight and egg weight on hen level.

Another aim in the current study was to investigate keel bone of roosters. The examination revealed that none of the roosters displayed any keel bone fracture, neither at 30 nor at 74 WOA. This is in accordance with Fleming et al (2004), one of the few studies that previously have investigated keel bones from roosters [13]. It is also in accordance with findings in red jungle fowl roosters [15]. The lack of KBF in male specimens supports the speculation that KBF is linked to egg laying [26].

5. Conclusions

In the current study portable radiography was used to investigate keel bones of hens and roosters from four pure breed non-commercial layer breeds at 30 and 75 weeks of age. The findings indicate a low prevalence of KBF in the laying hens. Of the five KBF found in the 213 birds, four were from the same breed, which is the most selected and efficient breed in the study. None of the roosters examined exhibited a KBF. The results may indicate that selective breeding could reduce the susceptibility to keel bone fractures. Furthermore, the results from this study highlight the importance of poultry conservation to secure genetic diversity, which may be a genetic resource in future production and selection efforts.

Author Contributions: Conceptualization, K.E.K and G.V.; methodology, K.E.K., G.V., T.B.H., I.T. and R.O.M.; software, K.E.K., G.V. and T.B.H.; validation, K.E.K., G.V and J.P.C., formal analysis, I.T.; investigation, K.E.K., G.V., T.B.H and R.O.M.; resources, K.E.K., G.V., T.B.H., I.T., R.O.M and J.P.C.; writing—original draft preparation, K.E.K.; writing—review and editing, K.E.K., G.V., T.B.H., I.T., R.O.M and J.P.C.; visualization, K.E.K and G.V.; supervision, K.E.K.; project administration, G.V and K.E.K.; funding acquisition, G.V and K.E.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Norwegian Research Council, NFR project number 309159.

Acknowledgments: This article is based upon investigation of historical layer breeds preserved at the Norwegian live poultry gene preservation bank at Hvam Agricultural College, Norway. The authors sincerely appreciate the important work Hvam does to preserve these breeds. Mette Nafstad and the students are acknowledged for valuable practical help during the experiment.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Tully, J.T. Basic avian bone growth and healing. The veterinary clinics of North America. Exotic animal practice 2002, 5, 23-30.
2. Webster, A. Welfare implications of avian osteoporosis. Poultry science 2004, 83, 184-192.
3. Whitehead, C.; Fleming, R. Osteoporosis in cage layers. Poultry science 2000, 79, 1033-1041.
4. Urist, M.R.; DEUTSCH, N.M. Osteoporosis in the laying hen. Endocrinology 1960, 66, 377-391.
5. Wilson, S.; Duff, S.; Whitehead, C. Effects of age, sex and housing on the trabecular bone of kaying strain domestic fowl. Research in veterinary science 1992, 53, 52-58.
6. Whitehead, C. Overview of bone biology in the egg-laying hen. Poultry science 2004, 83, 193-199.
7. Käppeli, S.; Gebhardt-Henrich, S.; Fröhlich, E.; Pfulg, A.; Stoffel, M.H. Prevalence of keel bone deformities in swiss laying hens. British poultry science 2011, 52, 531-536.
8. Candelotto, L.; Stratmann, A.; Gebhardt-Henrich, S.G.; Rufener, C.; van de Braak, T.; Toscano, M.J. Susceptibility to keel bone fractures in laying hens and the role of genetic variation. Poultry science 2017, 96, 3517-3528.
9. Casey-Trott, T.; Heerkens, J.; Petrik, M.; Regmi, P.; Schrader, L.; Toscano, M.J.; Widowski, T. Methods for assessment of keel bone damage in poultry. Poultry science 2015, 94, 2339-2350.
10. Wilkins, L.; McKinstry, J.; Avery, N.; Knowles, T.; Brown, S.; Tarlton, J.; Nicol, C. Influence of housing system and design on bone strength and keel bone fractures in laying hens. Veterinary Record 2011, vetrecd4831.
11. Heerkens, J.; Delezie, E.; Rodenburg, T.B.; Kempen, I.; Zoons, J.; Ampe, B.; Tuyttens, F. Risk factors associated with keel bone and foot pad disorders in laying hens housed in aviary systems. Poultry science 2016, 95, 482-488.
12. Rodenburg, T.; Tuyttens, F.; De Reu, K.; Herman, L.; Zoons, J.; Sonck, B. Welfare assessment of laying hens in furnished cages and non-cage systems: An on-farm comparison. Animal Welfare 2008, 17, 363-373.
13. Heerkens, J.; Delezie, E.; Ampe, B.; Rodenburg, T.; Tuyttens, F. Ramps and hybrid effects on keel bone and foot pad disorders in modified aviaries for laying hens. Poultry science 2016, 95, 2479-2488.
14. Thøfner, I.; Hougen, H.P.; Villa, C.; Lynnerup, N.; Christensen, J.P. Pathological characterization of keel bone fractures in laying hens does not support external trauma as the underlying cause. PLOS ONE 2020, 15, e0229735.
15. Kittelsen, K.E.J., P.; Christensen, J.P.; Toftaker, I.; Moe, R.O.; Vasdal, G., . Prevalence of keel bone damage in red jungle fowls (gallus gallus)-a pilot study. Animals, 2020, 10.9:1655
16. Fleming, R.; McCormack, H.; McTeir, L.; Whitehead, C. Incidence, pathology and prevention of keel bone deformities in the laying hen. British poultry science 2004, 45, 320-330.
17. Nasr, M.A.; Nicol, C.J.; Murrell, J.C. Do laying hens with keel bone fractures experience pain? PLoS One 2012, 7, e42420.
18. Nasr, M.; Murrell, J.; Nicol, C. The effect of keel fractures on egg production, feed and water consumption in individual laying hens. British poultry science 2013, 54, 165-170.
19. Nasr, M.; Browne, W.; Caplen, G.; Hothersall, B.; Murrell, J.; Nicol, C. Positive affective state induced by opioid analgesia in laying hens with bone fractures. Applied animal behaviour science 2013, 147, 127-131.
20. Richards, G.; Wilkins, L.; Knowles, T.; Booth, F.; Toscano, M.; Nicol, C.; Brown, S. Pop hole use by hens with different keel fracture status monitored throughout the laying period. Veterinary Record 2012.
21. Rufener, C.; Baur, S.; Stratmann, A.; Toscano, M.J. Keel bone fractures affect egg laying performance but not egg quality in laying hens housed in a commercial aviary system. Poultry science 2019, 98, 1589-1600.
22. Rentsch, A.K.; Rufener, C.B.; Spadavecchia, C.; Stratmann, A.; Toscano, M.J. Laying hen's mobility is impaired by keel bone fractures and does not improve with paracetamol treatment. Applied animal behaviour science 2019, 216, 19-25.
23. Armstrong, E.; Rufener, C.; Toscano, M.; Eastham, J.; Guy, J.; Sandilands, V.; Boswell, T.; Smulders, T. Keel bone fractures induce a depressive-like state in laying hens. Scientific Reports 2020, 10, 1-14.
24. FAWC. An open letter to great britain governments: Keel bone fracture in laying hens. Farm Animal Welfare Council London, UK: 2013.
25. Harlander-Matauschek, A.; Rodenburg, T.; Sandilands, V.; Tobalske, B.; Toscano, M.J. Causes of keel bone damage and their solutions in laying hens. World's Poultry Science Journal 2015, 71, 461-472.
26. Toscano, M.J.; Dunn, I.C.; Christensen, J.-P.; Petow, S.; Kittelsen, K.; Ulrich, R. Explanations for keel bone fractures in laying hens: Are there explanations in addition to elevated egg production? Poultry Science 2020.
27. Sandilands, V.; Moinard, C.; Sparks, N. Providing laying hens with perches: Fulfilling behavioural needs but causing injury? British poultry science 2009, 50, 395-406.
28. Eusemann, B.K.; Patt, A.; Schrader, L.; Weigend, S.; Thöne-Reineke, C.; Petow, S. The role of egg production in the etiology of keel bone damage in laying hens. Frontiers in Veterinary Science 2020, 7, 81.

29. Rufener, C.; Makagon, M.M. Keel bone fractures in laying hens: A systematic review of prevalence across age, housing systems, and strains. Journal of Animal Science 2020, 98, S36-S51.
30. Council, F.A.W. Opinion on osteoporosis and bone fractures in laying hens. London, UK 2010.
31. Gebhardt-Henrich, S.G.; Fröhlich, E.K. Early onset of laying and bumblefoot favor keel bone fractures. Animals 2015, 5, 1192-1206.
32. Andersson, B. Genetic aspects of keel bone deformities and fractures determined by palpation in laying hens. Lohmann Information 2017, 51, 36-41.
33. Eusemann, B.K.; Baulain, U.; Schrader, L.; Thöne-Reineke, C.; Patt, A.; Petow, S. Radiographic examination of keel bone damage in living laying hens of different strains kept in two housing systems. PloS one 2018, 13.
34. Wilkins, L.; Brown, S.; Zimmerman, P.; Leeb, C.; Nicol, C. Investigation of palpation as a method for determining the prevalence of keel and furculum damage in laying hens. Veterinary Record 2004, 155, 547-549.
35. Buijs, S.; Heerkens, J.; Ampe, B.; Delezie, E.; Rodenburg, T.; Tuyttens, F. Assessing keel bone damage in laying hens by palpation: Effects of assessor experience on accuracy, inter-rater agreement and intra-rater consistency. Poultry science 2019, 98, 514-521.
36. Tracy, L.M.; Temple, S.M.; Bennett, D.C.; Sprayberry, K.A.; Makagon, M.M.; Blatchford, R.A. The reliability and accuracy of palpation, radiography, and sonography for the detection of keel bone damage. Animals 2019, 9, 894.
37. Gebhardt-Henrich, S.G.; Rufener, C.; Stratmann, A. Improving intra-and inter-observer repeatability and accuracy of keel bone assessment by training with radiographs. Poultry science 2019, 98, 5234-5240.
38. Cornell, C.N.; Lane, J.M. Newest factors in fracture healing. Clinical orthopaedics and related research 1992, 297-311.
39. Groeneveld L.F., M., U., Groeneveld, E., Sæther,N. & Berg, P. . Inbreeding in native norwegian poultry breeds, with partially unknown maternal pedigree. 66th Annual Meeting of the European Federation of Animal Science, Warsaw, Poland. 2015.
40. Lyimo, C.; Weigend, A.; Msoffe, P.; Eding, H.; Simianer, H.; Weigend, S. Global diversity and genetic contributions of chicken populations from a frican, a sian and e uropean regions. Animal genetics 2014, 45, 836-848.
41. Brekke, C. Genetic diversity in five chicken lines from the norwegian live poultry gene bank. Norwegian University of Life Sciences, Ås, 2017.
42. Forskrift om bruk av dyr i forsøk. Landbruks- og matdepartementet, Ed. 2015.
43. Habig, C.; Distl, O. Evaluation of bone strength, keel bone status, plumage condition and egg quality of two layer lines kept in small group housing systems. British poultry science 2013, 54, 413-424.
44. Rørvang, M.; Hinrichsen, L.; Riber, A. Welfare of layers housed in small furnished cages on danish commercial farms: The condition of keel bone, feet, plumage and skin. British poultry science 2019, 60, 1-7.
45. Baur, S.; Rufener, C.; Toscano, M.J.; Geissbühler, U. Radiographic evaluation of keel bone damage in laying hens—morphologic and temporal observations in a longitudinal study. Frontiers in Veterinary Science 2020, 7.