



Faculty of Agriculture  
University of Banja Luka



**XIII INTERNATIONAL SYMPOSIUM ON  
AGRICULTURAL SCIENCES**



**BOOK OF PROCEEDINGS**

27-30 May 2024, Trebinje, Bosnia and Herzegovina

ISSN 2831-1248

XIII International Symposium on Agricultural Sciences "AgroReS 2024"

27-30 May 2024, Trebinje, Bosnia and Herzegovina

**BOOK OF PROCEEDINGS**

---

**Publisher**

University of Banja Luka  
Faculty of Agriculture  
University City, Bulevar vojvode Petra Bojovića 1A  
78000 Banja Luka, Republic of Srpska, Bosnia and Herzegovina

**Editor in Chief**

Boris Pašalić

**Technical Editor**

Marinko Vekić

**Edition**

Electronic edition

Available at: <https://agrores.agro.unibl.org/en/proceedings/>

CIP - Каталогизacija у публикацији  
Народна и универзитетска библиотека  
Републике Српске, Бања Лука

631(082)(0.034.2)

**INTERNATIONAL Symposium on Agricultural Sciences (13 ;  
Trebinje ; 2024)**

Book of Proceedings [Електронски извор] / XIII International  
Symposium on Agricultural Sciences "AgroReS 2024", 27-30 May, 2024,  
Trebinje, Bosnia and Herzegovina ; [editor in chief Boris Pašalić]. - Onlajn izd. -  
El. zbornik. - Banja Luka : Faculty of Agriculture = Poljoprivredni fakultet,  
2024. - Ilustr. - (AgroRes, ISSN 2831-1248)

Sistemski zahtjevi: Nisu navedeni. - Dostupno i  
na: <https://agrores.agro.unibl.org/en/proceedings/>. - Nasl. sa nasl. ekrana. -  
Opis izvora dana 23.5.2024. - El. publikacija u PDF formatu opsega 287 str. -  
Bibliografija uz radove

ISBN 978-99938-93-99-8

COBISS.RS-ID 140613121

## **Effect of rearing system and genotype of laying hens on the breaking strength of tibia and femur**

Simeon Rakonjac<sup>1</sup>✉, Snežana Bogosavljević-Bošković<sup>1</sup>, Vladimir Dosković<sup>1</sup>,  
Miloš Lukić<sup>2</sup>, Zdenka Škrbić<sup>2</sup>, Veselin Petričević<sup>2</sup>, Milun D. Petrović<sup>1</sup>

<sup>1</sup> *University of Kragujevac, Faculty of Agronomy, Čačak, Serbia*

<sup>2</sup> *Institute for Animal Husbandry, Belgrade-Zemun, Serbia*

✉ simcepb@yahoo.com

### **Abstract**

The aim of this study was to determine the effect of the rearing system and the genotype of laying hens on the breaking strength of the tibia and femur. The experiment was conducted in a 2x2 factorial design with two laying hen genotypes (commercial hybrid Isa Brown and New Hampshire dual-purpose breed) and two rearing systems (floor and organic). At the end of the one-year production cycle, six birds per group (24 hens in total) were randomly selected and slaughtered. To examine the quality of the bones, the femur and tibia were removed from each slaughtered laying hen. The breaking strength was measured by a three-point bending test with the IPNIS device. The results show that the rearing system had no significant effect on the breaking strength of the femur and tibia. On the other hand, genotype had a significant effect on femur and tibia breaking strength, such that the New Hampshire hens had better bone quality than Isa Brown hens. Importantly, there was a significant interaction between rearing system and genotype on tibia breaking strength - in the floor rearing system, the New Hampshire genotype had significantly higher breaking strength than the Isa Brown genotype, while the difference that occurred in the organic system between the genotypes studied was not statistically confirmed.

**Key words:** bone quality, laying hens, rearing systems, genotype

---

### **INTRODUCTION**

Osteoporosis and bone fractures, and poor bone quality in general, are a serious problem for the welfare of laying hens, with both genetics and the environment, such as the housing system, making a significant contribution to bone strength. Over the course of their lives, laying hens experience a progressive weakening of bone structure and an increasing risk of bone fractures (Johansson et al., 2023). Osteoporosis in laying hens is associated with the loss of minerals from the bones during the laying period, resulting in weaker bones and more frequent fractures (Bishop et al., 2010). When birds have bone-related problems, egg production decreases, feed intake increases and mortality increases (Riber et al., 2018). Mašić and Pavlovski (1994), studying the papers of some authors from the USA in the 1960s, note that the incidence of bone fractures in laying hens removed from cages was so common that some slaughterhouses even refused to slaughter them, and some studies estimated the incidence of bone fractures in caged laying hens at 11-26% (Budgell & Silversides, 2004; Sandilands et al., 2005) and an additional 25% during removal from cages (Sandilands et al., 2005). The Farm Animal Welfare Council (2010) found in its research in Great Britain that around 250 000 laying hens die from osteoporosis each year. This estimate excludes mortality during depopulation and transport to the slaughterhouse.

The causes of bone fractures are likely to be multifactorial and are influenced by age, diet, genetic predisposition, restricted movement, lack of exercise and other factors (Toscano et al., 2018). Fleming et al. (2006) found that environment has a secondary effect on bone status after genotype, and

Sandinlands et al. (2005) determined that over 70% of bone fractures in laying hens are influenced by the rearing system and genotype. Laying hens selected for egg production are more susceptible to osteoporosis due to a negative calcium balance, which is due to the high demand for calcium during eggshell formation (Kim et al., 2012). Bone quality is closely related to egg production and egg quality, with a negative correlation observed between egg production, eggshell thickness and bone breaking strength (Leyendecker et al., 2001; Kim et al., 2005). Elaroussi et al. (1994) found that hens absorb 28 to 30 times more calcium via the eggshell during the production cycle than they have in their bodies. For this reason, Rayan et al. (2020) state that a major physiological challenge for modern laying hens is to produce large quantities of eggs while maintaining the strength of their skeleton. In this context, Hocking et al. (2003) concluded that this trait can be strongly influenced by egg production intensity indirectly via genotype. They proved this by comparing autochthonous breeds and commercial hybrids and found that at the end of the laying period, commercial hybrids with a higher laying intensity had a significantly lower bone fracture strength, so that there was also a more intensive mobilization of calcium from the bones for the formation of the eggshell. Alternative rearing systems not only improve animal welfare, but also have a positive effect on bone strength (Regmi et al., 2015). Rearing systems that allow more movement promote bone development, while systems that restrict movement lead to bone loss as the bone adapts to the load (Rodriguez-Navarro et al., 2018). On the other hand, systems that encourage movement may also increase the risk of fracture, for example due to an accidental fall from height or a collision (Hester et al., 2013). Freire et al. (2003) conclude that bone strength is better when more space is available to the individual, and Michel and Huonnic (2003) that the ability to fly during production time reduces the risk of fracture. Leyendecker et al. (2005) found that increased space, the presence of perches and sand for bathing increased the strength of the humerus, probably because behaviors such as wing stretching, flying and bathing in sand have a positive effect on the mechanical properties of the bones.

## MATERIAL AND METHODS

The experiment was conducted in a 2x2 factorial design with two laying hen genotypes (commercial hybrid Isa Brown and New Hampshire dual-purpose breed) and two rearing systems (floor and organic). 30 birds were housed per group.

The stocking density in the floor rearing system was 2.5 birds/m<sup>2</sup>. The feeding program was designed according to the requirements of laying hens in conventional rearing (Table 1).

The organic groups had the same stocking density as the floor groups in the barns, but each hen had about 5 m<sup>2</sup> of outdoor pasture. The feeding program was designed according to organic farming regulations, without the addition of synthetic amino acids, vitamins and minerals and using mainly organically produced components (Table 1). Feed and drinking water were available *ad libitum*.

Table 1. Chemical composition of complete feed mixtures for the feeding of laying hens

Chemical composition	Floor system	Organic system
Dry matter	88.38	89.82
Crude protein	16.79	16.82
Fat	5.15	4.31
Cellulose	4.82	4.29
Ash	12.52	12.68
BEM	49.10	51.90
Ca	3.72	3.43
Total P	0.71	0.81
Na	0.17	0.18
Lysine	0.79	0.80
Methionine + cysteine	0.68	0.48
Methabolizable energy	11.5 MJ	11.3 MJ

At the end of the one-year production cycle, six birds per group (24 laying hens in total) were randomly selected. After a fasting period of 12 hours, the selected hens were slaughtered.

To examine the quality of the bones, the femur and tibia were removed from each slaughtered laying hen. The breaking force was measured by a three-point bending test using the IPNIS device, with the distance between the supports being 40 mm (Mašić & Pavlovski, 1994).

Bones quality data were analyzed by ANOVA and LSD test (Stat Soft Inc Statistica for Windows, Version 7.0., 2006).

## RESULTS AND DISCUSSION

The quality of the bones of laying hens is an important parameter for assessing the welfare of reared hens. Apart from this, possible bone fractures can also affect the quality of the carcass and pose a major problem for the processing industry.

Table 2. Bone breaking strength of laying hens

	Femur (N)	Tibia (N)
Rearing system		
Floor	50.65±20.81	51.44±24.40
Organic	47.00±14.37	45.66±14.08
Genotype		
Isa Brown	36.36 <sup>b</sup> ±9.38	36.06 <sup>b</sup> ±13.07
New Hampshire	61.30 <sup>a</sup> ±14.82	61.05 <sup>a</sup> ±17.32
Rearing system x Genotype		
Floor	Isa Brown	35.91 <sup>b</sup> ±11.27
	New Hampshire	65.39 <sup>a</sup> ±17.44
Organic	Isa Brown	36.80 <sup>b</sup> ±8.13
	New Hampshire	57.21 <sup>a</sup> ±11.77
ANOVA		
Rearing system	ns	ns
Genotype	*	*
Rearing system x Genotype	ns	*

a-b Values within column with no common superscript are significantly different ( $p < 0.05$ ).

\* $p < 0.05$ , ns - not significant.

Table 2 shows the bone breaking strength of the femur and tibia of the laying hens studied. The rearing system had no significant influence on the values of these parameters ( $p > 0.05$ ). Similar results were obtained by Fu et al. (2022), who found no significant difference in the femur breaking strength of the aviary and cage-reared laying hens (270.43 N : 263.27 N,  $p > 0.05$ ), and Mašić and Pavlovski (1994), who found no difference in the breaking strength of the femur (26.32 kg : 32.12 kg) and tibia (28.72 kg : 31.00 kg) between floor and free-range laying hens. On the other hand, Qiaoxian et al. (2020) determined a significant effect of the rearing system on tibia breaking strength between caged and floor reared Taihang hens ( $p < 0.05$ ), while the differences in this parameter were not significant for femur breaking strength ( $p > 0.05$ ). Similar results that the effect of the rearing system of laying hens had a significant effect on the femur and not on the tibia were also reported by Ross (2021). Slightly different results were published by Lolli et al. (2013), who determined significant differences ( $p < 0.05$ ) in bone breaking strength in the Hy-Line Brown hybrid: for the tibiotarsus (floor 146 N, organic 171 N) and humerus (floor 182 N, organic 211 N) and Sharma et al. (2022) for tibia breaking strength between free-range hens (26.47 KgF) and conventional cage-reared hens (25.05 KgF). Dedousi et. al. (2022) determined a significant effect of the rearing system on the strength of the keel bones.

In both bones examined, the genotype had a significant effect on the breaking strength, so that the New Hampshire hens had a higher breaking strength than the Isa Brown hybrids ( $p < 0.05$ ), which is a consequence of the greater robustness of the bones of the New Hampshire hens compared to the Isa Brown genotype and the lower laying intensity of the dual-purpose breed compared to the hybrid. Johansson et al. (2023) also established a positive relationship between body weight and tibia breaking strength, explaining about 10% of the variance in tibia breaking strength by body weight. Results consistent with these were also published by Hocking et al. (2003) who, when comparing autochthonous breeds and commercial hybrids, determined significantly lower breaking strength in commercial hybrids at the end of the production period, which they explained by the greater egg production of these birds, so that there was also greater mobilization of calcium from the bones to form the eggshell. A significant effect of the genotype on the breaking strength of the tibia and femur was determined by Kraus et al. (2022) on three genotypes - Czech golden spotted, White Leghorn and Dominant Partridge D300 hens - and by Sözcü et al. (2023) on four genotypes: Lohmann Brown, Lohmann White, Atak-S and Atabey in free range rearing system. A significant effect of genotype on tibia breaking strength was also found by Rayan et al. (2020), Ross (2021) and Sharma et al. (2022).

It is important to note that there was a significant interaction of the investigated factors in the breaking strength of the tibia ( $p < 0.05$ ). In fact, in the floor rearing system New Hampshire genotype had a significantly higher breaking strength compared to the Isa Brown laying hens ( $p < 0.05$ ), while the difference that occurred in the organic system between the genotypes studied was not statistically confirmed ( $p > 0.05$ ). The correlation between laying intensity and bone breaking strength can be explained by the interaction between rearing system and genotype that occurred in the study of tibia breaking strength. Namely, New Hampshire floor hens had a significantly lower laying intensity than organic hens (data not shown), which contributed to a weaker mobilization of calcium from the bones and therefore a significantly stronger bone strength compared to organic hens.

## CONCLUSION

From the results obtained, it can be concluded that the rearing system had no significant effect on the femur and tibia breaking strength. On the other hand, the genotype had a significant effect on femur and tibia breaking strength, so that the New Hampshire hens had better bone quality than Isa Brown hens. Importantly, there was a significant interaction between rearing system and genotype on the tibia breaking strength - in floor rearing system New Hampshire genotype had significantly higher breaking strength than the Isa Brown genotype, while the difference that occurred in the organic system between the genotypes studied was not statistically confirmed.

## ACKNOWLEDGEMENT

This study was funded by the Ministry of Science, Technological Development and Innovation of Republic of Serbia, No. 451-03-66/2024-03/200088 and No. 451-03-66/2024-03/ 200022.

## REFERENCES

- Bishop, S.C., Axford, R.F.E., Nicolas, F.W. & Owen, J.B. (2010). Breeding for disease resistance in farm animals. Cab International, p.340.
- Budgell, K.L. & Silversides, F.G. (2004). Bone breakage in three strains of end-of-lay hens. *Canadian Journal of Animal Science*, 84(4), 745-747.
- Dedousi, A., Đukić Stojčić, M. & Sossidou, E. (2020). Effects of housing systems on keel bone damage and egg quality of laying hens. *Veterinary Research Forum*, 11(4), 299-304.
- Elaroussi, A.E., Forte, L.R., Eber, S.L. & Bieller, H.V. (1994). Calcium homeostasis in the laying hen. 1. Age and dietary calcium effects. *Poultry Science*, 73(10), 1581-89.
- Farm Animal Welfare Council (2010). Opinion on Osteoporosis and Bone Fractures in Laying Hens.

- Fleming, R.H., McCormack, H.A. McTeir, L. & Whitehead, C.C. (2006). Relationships between genetic, environmental and nutritional factors influencing osteoporosis in laying hens. *British Poultry Science*, 47, 742-755.
- Freire, L.J., Wilkins, F., Short, F. & Nicol, C.J. (2003). Behaviour and welfare of individual laying hens in a non-cage system. *British Poultry Science*, 44, 22-29.
- Fu, Y., Wang, J., Schroyen, M., Chen, G., Zhang, H.-J., Wu, S.-G., Li, B.-M. & Qi, G.-H. (2022). Effects of rearing systems on the eggshell quality, bone parameters and expression of genes related to bone remodeling in aged laying hens. *Frontiers in Physiology*, 13, 962330.
- Hester, P., Enneking, S., Haley, B., Cheng, H.W., Einstein, M. & Rubin, D. (2013). The effect of perch availability during pullet rearing and egg laying on musculoskeletal health of caged White Leghorn hens. *Poultry Science*, 92, 1972-1980.
- Hocking, P.M., Brain, M., Channing, L.E., Fleming, R. & Willson, S. (2003). Genetic variation for egg production, egg quality, and bone strength in selected and traditional breeds of laying fowl. *British Poultry Science*, 44, 365-373.
- Johnsson, M., Wall, H., Lopes Pinto, F.A., Fleming, R.H., McCormack, H.A., Benavides-Reyes, C., Dominguez-Gasca, N., Sanchez-Rodriguez, E., Dunn, I.C., Rodriguez-Navarro, A.B., Kindmark, A. & de Koning, D.J. (2023). Genetics of tibia bone properties of crossbred commercial laying hens in different housing systems. *G3 - Genes, Genomes, Genetics*, 13(2), jkac302.
- Kim, W.K., Bloomfield, S.A., Sugiyama, T. & Ricke, S.C. (2012). Concepts and methods for understanding bone metabolism in laying hens. *Worlds Poultry Science Journal*, 68, 71-82.
- Kim, W.K., Donalson, L.M., Herrera, P.L., Kubena, F., Nisbet, D.J. & Ricke, S.C. (2005). Comparisons of molting diets on skeletal quality and eggshell parameters in hens at the end of the second egg-laying cycle. *Poultry Science*, 84, 522-527.
- Kraus, A., Krunt, O., Zita, L., Vejvodova, K. & Drabek, O. (2022). Laying hens under smallholder conditions: laying performance, growth and bone quality of tibia and femur including essential elements. *Poultry Science*, 101, 101927.
- Leyendecker, M., Hamann, H., Hartung, J., Kamphues, J., Neumann, U., Surie, C. & Distl, O. (2005). Keeping laying hens in furnished cages and an aviary housing system enhances their bone stability. *British Poultry Science*, 46(5), 536-544.
- Leyendecker, M., Hamann, H., Hartung, J., Kamphues, J., Ring, C., Glunder, G., Ahlers, C., Sander, I., Neumann, U. & Distl, O. (2001). Analysis of genotype-environment interactions between layer lines and housing systems for performance traits, egg quality and bone breaking strength – 1st communication: performance traits. *Zuchtungskunde*, 73, 290-307.
- Lolli, A., Hidalgo, C., Alampresse, C., Ferrante, V. & Rossi, M. (2013). Layer performances, eggshell characteristics and bone strength in three different housing systems. *Biotechnology in Animal Husbandry*, 29(4), 591-606.
- Mašić, B. & Pavlovski, Z. (1994). Mala jata kokoši nosilja u različitim sistemima držanja. Naučni institut za stočarstvo, Beograd-Zemun.
- Michel, V. & Huonnic, D. (2003). A comparison of welfare, health and production performance of laying hens reared in cages or in aviaries. *British Poultry Science*, 44(5), 775-776.
- Qiaoxian, Y., Hui, C., Yingjue, X., Chenxuan, H., Jianzhong, X., Rongyan, Z., Lijun, X., Han, W. & Ye, C. (2020). Effect of housing system and age on products and bone properties of Taihang chickens. *Poultry Science*, 99(3), 1341-1348.
- Rayan, G.N., El-Attar, A.H. & Fathi, M.M. (2020). Eggshell and bone quality in two different genetic groups of aged layer breeders. *Brazilian Journal of Poultry Science*, 22(2), 001-008.
- Regmi, P., Deland, T.S., Steibel, J.P., Robison, C.I., Haut, R.C., Orth, M.W., et al. (2015). Effect of rearing environment on bone growth of pullets. *Poultry Science*, 94, 502-511.
- Riber, A.B., Van de Weerd, H.A., De Jong, I.C. & Steinfeldt, S. (2018). Review of environmental enrichment for broiler chickens. *Poultry Science*, 97, 378-396.

- Rodriguez-Navarro, A., McCormack, H., Fleming, R., Alvarez-Lloret, P., Romero-Pastor, J., Dominguez-Gasca, N., Prozorov, T. & Dunn, I. (2018). Influence of physical activity on tibial bone material properties in laying hens. *Journal of Structural Biology*, 201, 36-45.
- Ross, E. (2021). The effect of early rearing environment on musculoskeletal traits and proximate composition in laying hen pullets. Master Thesis. The University of Guelph.
- Sandilands, V., Sparks, N., Wilson, S. & Nevison, I. (2005). Laying hens at depopulation: the impact of the production system on bird welfare. *British Poultry Abstract*, 1, 23-24.
- Sharma, M.K., White, D., Chen, C., Kim, W.K. & Adhikari, P. (2021). Effects of the housing environment and laying hen strain on tibia and femur bone properties of different laying phases of Hy-Line hens. *Poultry Science*, 100(3), 100933.
- Sözcü, A., Ipek, A. & Gündüz, M. (2023). Comparison of laying performance, egg quality and bone characteristics of commercial and Türk laying hen genotypes kept in a free-range system. *Kafkas Universitesi Veteriner Fakültesi Dergisi*, 29(5), 437-444.
- Stat Soft Inc Statistica for Windows (2006). Version 7.0. Computer program manual Tulsa.
- Toscano, M., Booth, F., Richards, G., Brown, S., Karcher, D. & Tarlton, J. (2018). Modeling collisions in laying hens as a tool to identify causative factors for keel bone fractures and means to reduce their occurrence and severity. *PLoS One*, 13, e0200025.