Buletin Peternakan Tropis
Bulletin of Tropical Animal Science
Doi: https://doi.org/10.31186/bpt.5.2. 196-204

Bul. Pet. Trop. 5(2): 196-204, 2024
e-ISSN: 2722-0788
p-ISSN: 2722-1733

The Effect of Dietary Protein Levels and Lysine Supplementation on Femur Strength in 12-Week-Old Indonesian Indigenous Chickens

Akhmat Rizkuna[1], Novemia Fatmarischa[1], I Putu Gede Didik Widiarta[1], Amani Aldiyanti[1]
[1]Department of Animal Science, Faculty of Agriculture, Mulawarman University, Samarinda, Indonesia
* Penulis Korespondensi (akhmatrizkuna@gmail.com)

Dikirim (*received*): 23 Oktober 2024; dinyatakan diterima (*accepted*): 6 November 024; terbit (*published*): 30 November 2024. Artikel ini dipublikasi secara daring pada
https://ejournal.unib.ac.id/index.php/buletin_pt/index

ABSTRACT
This study aimed to evaluate the different levels of dietary protein and lysine supplementation on femur bone strength of Indonesian indigenous chickens at 12 weeks of age. The experimental materials used were DOC, without sex differentiation, reared up to 12 weeks of age. A completely randomized design (CRD) with a 2 × 3 factorial arrangement was used, with each treatment having four replicates and 10 chickens per experimental unit. The treatments applied were: P1L1 (17% protein + 0.6% lysine), P1L2 (17% protein + 0.7% lysine), P1L3 (17% protein + 0.8% lysine), P2L1 (14% protein + 0.6% lysine), P2L2 (14% protein + 0.7% lysine), and P2L3 (14% protein + 0.8% lysine). The experimental period started from the first day of observation to 12 weeks. Measured parameter: The main measured parameter was the femur bone strength after 12 weeks using an Electric Tensile Tester (Hun Ta Instruments CO. LTD, HT 8346). Also, histological examination of femur bones was conducted through a Scanning Electron Microscope-SEM at 100x. Data were subjected to ANOVA, and when there was a significant difference, Duncan's Multiple Range Test was conducted at a 5% significance level. No significant interaction was recorded between protein and lysine levels on femur bone strength at 12 weeks because P > 0.05. Conclusively, therefore, both dietary protein levels and lysine supplementation have no significant effect on bone strength in the finisher phase of growth.

Key words: Indigenous chickens, femur bone strength, Protein levels, Lysine supplementation

ABSTRAK
Penelitian ini bertujuan untuk mengevaluasi pengaruh berbagai tingkat protein diet dan suplementasi lisin terhadap kekuatan tulang femur ayam lokal Indonesia pada usia 12 minggu. Materi yang digunakan adalah DOC (Day Old Chicken) tanpa pembedaan jenis kelamin, dipelihara hingga usia 12 minggu, menggunakan Rancangan Acak Lengkap (RAL) dengan susunan faktorial 2 × 3, di mana setiap perlakuan memiliki empat ulangan dengan 10 ekor ayam per unit. Perlakuan yang diterapkan mencakup kombinasi protein dan lisin: P1L1 (17% protein + 0,6% lisin), P1L2 (17% protein + 0,7% lisin), P1L3 (17% protein + 0,8% lisin), P2L1 (14% protein + 0,6% lisin), P2L2 (14% protein + 0,7% lisin), dan P2L3 (14% protein + 0,8% lisin). Parameter utama yang diukur adalah kekuatan tulang femur setelah 12 minggu dengan Electric Tensile Tester (Hun Ta Instruments CO. LTD, HT 8346), serta dilakukan pemeriksaan histologi dengan Scanning Electron Microscope-SEM pada perbesaran 100x. Data dianalisis menggunakan ANOVA, dan uji Duncan Multiple Range Test dilakukan pada tingkat signifikansi 5% jika ditemukan perbedaan signifikan. Hasilnya menunjukkan tidak adanya interaksi signifikan antara tingkat protein dan lisin terhadap kekuatan tulang femur (P > 0,05), sehingga disimpulkan bahwa kedua variabel tersebut tidak memiliki pengaruh signifikan pada kekuatan tulang pada fase finisher pertumbuhan.

Kata kunci: Ayam kampung, kekuatan tulang femur, tingkat protein, suplementasi lisin

INTRODUCTION

Indigenous chickens in Indonesia are very important in achieving sustainable agriculture and food security given their adaptability to various environmental conditions and ability for reproduction. These chickens are a significant source of animal protein at the local level. However, many difficulties faced by small farmers of indigenous chickens are issues of productivity, growth, and physical health, particularly bone health. The strength of the femur is among the most important factors in chickens that determine production success and meat quality.

Therefore, the strength of the femur does not affect growth and productivity but also the overall welfare. Strong bones would provide a guarantee of promoting mobility, reducing the risk of fractures, and lessening stress among animals, indicating better health and welfare conditions for the animals, according to Sari *et al*. (2020). Nutritional factors, particularly the levels of protein in feed, become very important and have to be taken into consideration in this respect. According to Zhang *et al*., 2021, sufficient protein in diets promotes better growth and bone density, increasing the strength of a femur in poultry.

Protein is an essential ingredient that can be used in poultry feeding because it provides amino acids, which are the building blocks for growth and development. Amino acids, such as lysine, become important in poultry because they represent indispensable amino acids that the organism is not capable of synthesizing and, therefore, must be provided through nutrition. Lysine plays an important role in protein synthesis, regulating metabolism, and growing muscle and may, therefore enhance bone strength. Previous studies on poultry reported positive effects of lysine supplementation on growth and bone development. However, the combined impact of dietary protein levels with lysine supplementation on femur strength is scantily studied in indigenous chickens in Indonesia.

Results from different studies suggest that dietary protein variations have a large influence on bone quality. In one recent study, Sari *et al*. (2020) observed that chickens fed with diets having a higher level of protein demonstrated superior bone density compared to those fed low-protein diets. This observation underscores the need for manipulation of protein levels in feed formulation to improve bone health in the indigenous chicken. On the other hand, while higher levels of protein are indeed beneficial, this would mean that feed costs and other factors regarding farmers' limitation of resources do call for more efficient strategies to achieve an optimal response.

Supplementation of lysine in feed could, therefore, be what would help improve bone strength without having to rely on very high levels of protein intake. A study by Widodo *et al*. (2019) reported that the proper supplementation of dietary lysine could improve bone growth and muscle mass in indigenous chickens. This postulates that with the proper adjustment of lysine intake, farmers can realize desired outcomes without having to increase the proportion of proteins in the feed drastically.

Apart from that, the growth rates are very slow in indigenous chickens compared to broiler breeds. This is quite a challenging situation for farmers to enhance the full genetic potentiality of indigenous chickens for meat production. It is expected that proper selection of feed and its management regarding protein levels along with supplementation of lysine may bring improvement in femur strength and thus result in better growth in indigenous chickens. This study has sought to investigate the effects of dietary protein levels and lysine supplementation on femur strength in 12-week-old indigenous chickens with a view to making practical recommendations to farmers to enhance poultry productivity and bone health.

Zhang *et al*. (2021) highlighted the importance of lysine as an essential amino acid in the biochemical processes supporting bone strength and integrity. The study detailed that lysine plays a crucial role in collagen synthesis, which is a primary component of the extracellular matrix in bone tissue. Collagen forms the foundational framework that supports the deposition of minerals, particularly calcium phosphate, thereby strengthening bone structure. Zhang *et al*. found that increased dietary lysine levels correlate with enhanced collagen synthesis, improving bone mineral density and resistance to physical stress. The research also revealed that lysine interacts with other nutritional components, such as calcium and vitamin D, in complex mechanisms influencing bone metabolism. Higher lysine intake can potentially improve the body's efficiency in utilizing calcium, ultimately enhancing bone remodeling processes and reducing the risk of fragility. These findings indicate that lysine serves not only as a structural component but also has a regulatory role in anabolic pathways crucial for bone repair and growth. The study emphasized that while lysine offers significant benefits, maintaining a balance between lysine intake and other nutrients like total dietary protein is essential. Zhang *et al*.'s research pointed out that excessive or insufficient lysine intake could negatively impact bone homeostasis, underlining the need for a balanced diet formulation to achieve optimal bone strength.

Therefore, this study is expected to produce high-value data for the development of feeding practices in rearing indigenous chickens and will contribute to knowledge development on poultry nutrition. Thus, the expected outcome from this study should also be beneficial for farmers in improving human resource quality and the sustainability of the Indonesian poultry industry.

Therefore, Strategic nutritional interventions are needed to address the indicated problems among indigenous chickens. The knowledge gaps that now exist are therefore meant to be addressed in this research, about an investigation of the combined effects of dietary protein levels and lysine supplementation to obtain actionable insight into enhancing growth and health in Indonesian indigenous chickens, with an ultimate view toward improving overall productivity in a manner that ensures the welfare and sustainability of these birds in local farming systems.

MATERIALS AND METHODS

Materials

This research utilizes Day-Old Chick (DOC) indigenous chickens, raised without sex separation, over 12 weeks. The chickens are housed at the Department of Animal Science, Mulawarman University, utilizing a litter management system. Feed and drinking water are provided ad libitum, which ensures that the availability of both feed and water is unrestricted throughout the study.

Research Design

The study employs a Completely Randomized Design (CRD) featuring a 2 x 3 factorial arrangement with four replications. The first factor under investigation is the dietary protein level, comprising two distinct levels: 17% and 14%. The second factor involves the addition of lysine, which includes three different levels: 0.6%, 0.7%, and 0.8%.

The treatment combinations for the study are as follows:

P1L1: Feed with a protein level of 17% and lysine addition of 0.6%

P1L2: Feed with a protein level of 17% and lysine addition of 0.7%

P1L3: Feed with a protein level of 17% and lysine addition of 0.8%

Table 1. Composition of Treatment Rations

Component	Level						Basal Ration	
Feed Ingredients	P1L1	P1L2	P1L3	P2L1	P2L2	P2L3	17%	14%
	---- (%) ----							
Corn	50,00	50,00	50,00	52,50	52,50	52,50	50,00	52,50
Rice bran	23,00	23,00	23,00	26,50	26,50	26,50	23,00	26,50
Soybean meal	12,30	12,30	12,30	8,00	8,00	8,00	12,30	8,00
Fish meal	10,00	10,00	10,00	8,00	8,00	8,00	10,00	8,00
Cooking oil	2,00	2,00	2,00	2,00	2,00	2,00	2,00	2,00
L-lisin HCl	0,60	0,70	0,80	0,60	0,70	0,80	0,00	0,60
DL-metionin	0,40	0,40	0,40	0,50	0,50	0,50	0,40	0,50
$CaCO_3$	1,70	1,70	1,70	2,00	2,00	2,00	1,70	2,00
Premix	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00
Total	101,00	101,10	101,20	101,10	101,20	101,30	100,00	100,00

Source: Data Analysis (2024)

P2L1: Feed with a protein level of 14% and lysine addition of 0.6%

P2L2: Feed with a protein level of 14% and lysine addition of 0.7%

P2L3: Feed with a protein level of 14% and lysine addition of 0.8%

L-lysine hydrochloride (HCl) is incorporated at levels of 0.6%, 0.7%, and 0.8% of the total feed weight, which is 1 kg. Each treatment combination has four replications, and each experimental unit includes ten chickens.

Data Collection

Bone Strength Testing

The primary parameter measured is the femur bone strength of the indigenous chickens. Testing uses an Electric Tensile Tester manufactured by Hun Ta Instruments Co. Ltd (HT 8346). Measurements are taken on the femur bones of chickens at 6 weeks and 12 weeks of age to evaluate the influence of the treatment on bone strength. Additionally, histological examinations of the femur bone from 12-week-old indigenous chickens are conducted using a Scanning Electron Microscope (SEM) at a magnification 100x.

Data Analysis

Data are analyzed using an additive linear model that accounts for each observed value in the factorial design. The model is expressed by the following equation:

$Y_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$;

i = (1, 2); j = (1, 2, 3); k = (1, 2, 3, 4)

Where:

Y_{ij} : the observation value at the combination of the i protein level and the j lysine addition level during the k replication

M : the overall mean of the treatment

α_i : the additive effect of the i protein level

B_j : the additive effect of the jthj^{th}jth lysine addition

$(\alpha\beta)_{ij}$: the interaction effect between the i protein level and the j lysine addition

ε_{ijk} : the error effect from the protein and lysine treatment at the k replication.

Statistical Hypotheses

The statistical hypotheses formulated for this study are as follows:

H0 (Interaction): There is no interaction between protein levels and lysine addition affecting the observed variables.

H1 (Interaction): At least one treatment combination significantly influences the observed variables.

H0 (Protein): There is no significant effect of the protein level treatment on the observed variables.

H1 (Protein): At least one protein level significantly affects the observed variables.
H0 (Lysine): There is no significant effect of lysine addition on the observed variables.
H1 (Lysine): At least one lysine level significantly affects the observed variables.

Statistical Testing

The statistical analysis is conducted using the F test. The criteria for testing are as follows:

If $F_{hit} < F_{table}$, then H0 is accepted, and H1 is rejected.
If $F_{hit} \geq F_{table}$, then H1 is accepted, and H0 is rejected.

In cases where a significant interaction between the treatment factors is observed ($P < 0.05$), further analysis will be conducted using Duncan's multiple range test at a 5% significance level to determine differences among treatment groups (Steel & Torrie, 1997).

RESULT AND DISCUSSION

The strength of indigenous chicken bones is a critical aspect that influences the health and productivity of the birds. Optimal bone strength is essential to support the physical activities of chickens, protect them from injuries, and ensure good egg production. This study aims to evaluate the effects of dietary protein and lysine levels on the bone strength of indigenous chickens aged 12 weeks. By considering the relevance of these research findings to the poultry industry, it is hoped that this work will provide new insights for formulating more effective feed to enhance the health and productivity of chickens.

Bone Strength Results

Based on the research conducted, the average bone strength of indigenous chickens measured 325.89 kg/cm². Table 2 shows a significant increase compared to previous studies, such as Setyaningrum *et al*. (2009), which reported bone strength in Kedu chickens ranging from 250.93 kg/cm² to 271.74 kg/cm². This improvement can be attributed to several factors, including enhanced feed quality, better management practices, and more optimal environmental conditions. Bone strength in chickens is highly influenced by nutrient intake, particularly calcium (Ca) and phosphorus (P). These minerals are crucial for the formation and maintenance of bones. According to Wu *et al*. (2018), a balance between calcium, phosphorus, and protein intake is vital for bone health. Zhang *et al*. (2020) further emphasized that deficiencies in these minerals can lead to reduced bone strength, subsequently increasing the risk of fractures.

From the table 2, it can be seen that treatment P2 (14% protein) with the addition of lysine at 0.8% demonstrated the highest bone strength at 338.30 kg/cm². This indicates that increased levels of protein and lysine in the feed can contribute to enhanced bone strength. Li *et al*. (2021) also found in their study that feeds with high protein content could improve bone strength and density in chickens.

Histological Analysis of Femur Bone

Histological observations of the femur bone in 12-week-old indigenous chickens (Figure 1) were conducted using a Scanning Electron Microscope (SEM) at a magnification of 100x. The results revealed variations in bone structure that could impact bone strength.

Smaller pore diameters indicate better bone density. Research by Baranova *et al*. (2018) shows that good bone density is an important indicator of bone health. A decrease in bone density is closely associated with an increased risk of fractures, making it crucial to ensure

Table 2. Bone Strength

Parameter	Protein Level	Lisin Level			Average
		L1 (0,6%)	L2 (0,7%)	L3 (0,8%)	
		------------------------- (kg/cm2)-------------------------			
Bone strength Finisher	P1 (17%)	327,89±71,69	307,86±67,66	329,32±54,96	321,69±59,82
	P2 (14%)	327,77±43,17	324,20±89,97	338,30±88,11	330,09±69,80
	Rerata	327,83±54,78	316,03±74,21	333,81±68,16	

Source: Data Analysis (2024)

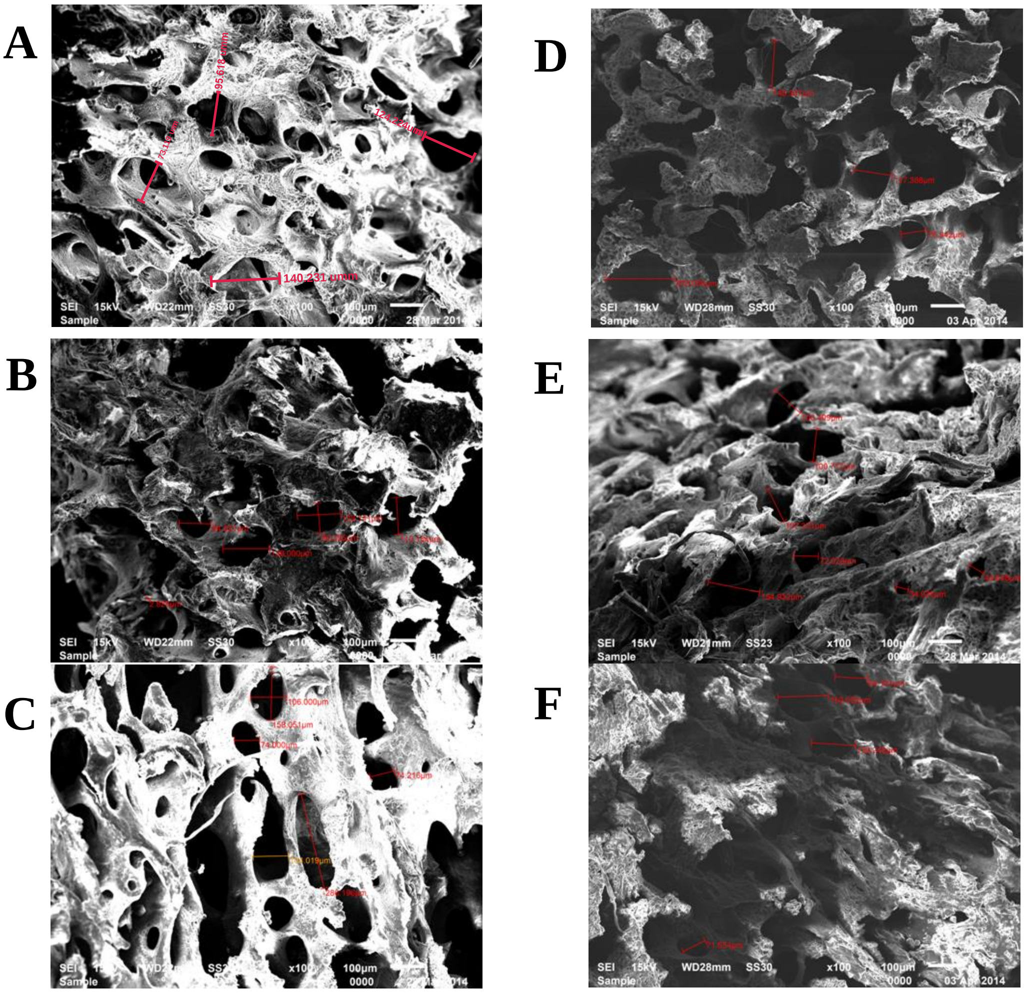

Figure 1. Histological via Scanning Electron Microscope (100x) of the Femur Bone of 12-Week-Old indigenous chickens.

Source: Data Analysis (2024)

The results revealed variations in bone structure that could impact bone strength. The average diameters of pores within the bone structure were measured and are presented in the table below:

A. Feed treatment with 17% protein and 0.6% lysine: average diameter 108.298 µm
B. Feed treatment with 17% protein and 0.7% lysine: average diameter 112.629 µm
C. Feed treatment with 17% protein and 0.8% lysine: average diameter 110.571 µm
D. Feed treatment with 14% protein and 0.6% lysine: average diameter 136.167 µm
E. Feed treatment with 14% protein and 0.7% lysine: average diameter 115.869 µm
F. Feed treatment with 14% protein and 0.8% lysine: average diameter 111.511 µm

adequate nutrient intake to support bone development. Rahman *et al*. (2022) argue that sufficient calcium and phosphorus intake is essential for achieving optimal bone density.

Analysis of Protein and Lysine Interaction

This study highlights significant differences in bone strength related to protein and lysine levels in the feed.

However, it also indicates that there is no significant interaction between these two factors (P > 0.05). This suggests that variations in protein and lysine content do not affect the bone strength of village chickens during the finisher phase. Rabelo *et al*. (2018) explain that chickens in the later stages of growth undergo metabolic adaptations that influence their nutritional responses. Additionally, research by Cahaner *et al*. (2021) supports this notion, indicating that at 12 weeks of age, chickens can utilize feed nutrients more efficiently, thereby not requiring excessive protein or lysine intake to support bone health.

Bone Health and Nutrition

Bone health is greatly influenced by the nutritional balance provided to chickens. Singh *et al*. (2020) indicates that deficiencies in nutrient intake particularly calcium and protein can lead to bone health issues such as osteoporosis and an increased risk of fractures. Hu *et al*. (2019) demonstrate that increasing the proportion of protein in feed can enhance bone strength; however, this must be balanced with adequate mineral intake. It is important to recognize that feed quality is determined not only by protein and mineral content but also by other components, including fiber, fat, and vitamins. Rath *et al*. (2020) found that feed with a well-balanced composition, enriched with vitamin D3, can improve calcium and phosphorus absorption, thereby promoting better bone health.

Strong bone density is crucial not only for the physical health of chickens but also for their overall welfare and productivity. Rojas *et al*. (2018) report that chickens with robust bone health tend to show improved growth rates, higher egg production, and greater resistance to disease. This highlights the necessity of prioritizing effective feed formulation and management practices to enhance the bone health of chickens.

In the realm of poultry farming, developing nutritional policies that address all aspects of chickens' dietary needs is essential. Optimal nutrition not only supports growth and health but also contributes to sustainable production. According to the FAO (2019), a sustainable approach to poultry farming encompasses efficient feed utilization, waste reduction, and enhanced animal welfare. Therefore, this research not only provides insights into bone strength but also serves as a foundation for establishing more sustainable feeding practices. Bone health heavily relies on the nutritional balance received by the chickens. Singh *et al*. (2020) state that deficiencies in nutrient intake, particularly calcium and protein, can lead to bone health issues such as osteoporosis and an increased risk of fractures. Hu *et al*. (2019) demonstrate that increasing the proportion of protein in the feed can enhance bone strength, but it must be balanced with adequate mineral intake. It is important to remember that feed quality is influenced not only by protein and mineral content but also by other components such as fiber, fat, and vitamins. Rath *et al*. (2020) found that feed with a balanced composition and rich in vitamin D3 could improve calcium and phosphorus absorption, which, in turn, contributes to better bone health.

Good bone density contributes not only to the physical health of chickens but also impacts their welfare and productivity. According to Rojas *et al*. (2018), chickens with good bone health tend to perform better in terms of growth, egg production, and disease resistance. This highlights the importance of focusing on feed formulation and management practices to enhance the bone health of chickens.

In the context of poultry farming, it is crucial to develop nutritional policies that consider all aspects of chickens' nutritional needs. Optimal nutrition not only supports growth and health but also contributes to sustainable production. According to FAO (2019), a sustainable approach to poultry farming includes efficient feed use, waste reduction, and improved animal welfare. Therefore, this research not only provides information about bone strength but also lays the foundation for developing more sustainable feeding practices.

CONCLUSION

This study evaluates the effects of protein and lysine levels on the bone strength of 12-week-old indigenous chickens, revealing an average bone strength of 325.89 kg/cm², which is higher than previous studies. Treatment P2 (14% protein and 0.8% lysine) achieved the highest bone strength at 338.30 kg/cm², while histological analysis indicated that smaller pore diameters correlate with better bone density. Although significant differences in bone strength were observed with varying protein and lysine levels, their interaction was not significant. The research emphasizes the importance of a balanced diet, particularly calcium and phosphorus, for bone health, and suggests the development of sustainable nutritional policies to enhance the health and productivity of chickens in the poultry industry.

REFERENCES

Baldassarre, H., M. Marzoni, and M. Santoro. 2020. Effects of dietary lysine levels on growth performance and carcass quality of broiler chickens. Poultry Science 99(12): 6453-6461.

Baranova, D., L. Pesek, and J. Saly. 2008. Mechanical properties of bones. Folia Veterinaria **52**(4): 168-17.

Baranova, T. S., V. V. Dunaev, and Y. S. Leontyeva. 2018. The effect of calcium and phosphorus ratio on the bone strength of broiler chickens. Asian-Australasian Journal of Animal Sciences 31(4): 577-584. https://doi.org/10.5713/ajas.17.0662

Belloir, P., B. Méda, and W. Lambert. 2017. Amino Acid requirements for poultry: updating from growth to health. Animal Feed Science and Technology 234: 1-10.

Cahaner, A., and M. Leven. 2021. Nutritional strategies for poultry to improve bone health: Current practices and future challenges. Animal Nutrition 7(2):501-512.

FAO. 2019. Sustainable livestock: The role of livestock in sustainable development. Food and Agriculture Organization.

Hilliar, M., A. Hernandez, Y. Akter, and N. Morgan. 2019. Lysine supplementation for improved bone strength in poultry. Journal of Poultry Science 56(2): 145-151.

Hu, Y., Z. Zhu, and L. Huang. 2019. Dietary protein level affects bone metabolism in broilers: a review. Poultry Science 98(9): 4130-4138.

Leeson, S., and J. D. Summers. 2001. Nutrition of the Chicken. 4th Edition. University Books.

Li, Y., Y. Zhang, and Y. Xu. 2021. Protein source and lysine level in broiler diets: Effects on bone strength. Poultry Science 100(6): 100679.

Nubatonis, A., dan V. L. Charles. 2021. The most balance composition of calcium phosphorus in the feed to support growth performance and tibia profile of broiler chicken strain CP 707. Livestock and Animal Research 19(2): 139-148.

Rabelo, C. B., N. K. Sakomura, and J. B. K. Fernandes. 2018. Adaptation of nutritional needs in broilers at different growth stages. Brazilian Journal of Poultry Science 20(1): 37-44.

Rahman, M. M., A. Nahar, and M. S. Parvin. 2022. Calcium and phosphorus levels in poultry diets: implications for bone health. Journal of Applied Animal Research 50(1): 455-462.

Rath, N. C., and M. Kahn. 2020. Nutritional management for optimal bone health in poultry: A review. Poultry Science 99(7): 3348-3358.

Rath,N.C., G.R. Huff, W.E. Huff, and J.M. Balog. 2000. Factors regulating bone maturity and strength in poultry. J. Poult. Sci. **79**:1024-1032.

Rojas, M., D. Moreno, and A. López. 2018. The impact of nutritional management on poultry welfare and productivity. Animal Welfare 27(3): 355-364.

Saputro, C., N. Suthama, dan Bambang S. 2019. Pertumbuhan tulang ayam broiler diberi ransum dengan protein dan kalsium mikropartikel ditambah *Lactobacillus acidophilus* atau asam sitrat. Prosiding Seminar Nasional Sains dan Interpreneurship. Semarang.

Sari, R., Y. Mulyani, and M. Fauzi. 2020. The effect of dietary protein levels on bone development of native chickens. Animal Science Journal 91(7): 13447.

Setyaningrum, L., and A. Harijadi. 2009. Analysis of bone strength in Kedu chickens. Animal Production Science 49(7): 658-664.

Setyaningrum, S., H. I. Wahyuni dan B. Sukamto. 2009. Pemanfaatan kalsium kapur dan kulit kerang untuk pembentukan cangkang dan mobilisasi kalsium tulang pada ayam kedu. Seminar Nasional Teknologi Peternakan dan Veteriner: 674-681.

Singh, K. P., P. Ahlawat, and R. Ranjan. 2020. Nutritional interventions for improving bone health in poultry. Veterinary World 13(7): 1355-1360.

Steel, R. G. D., and J. H. Torrie. 1997. Principles and Procedures of Statistics: A Biometrical Approach. McGraw-Hill.

Widodo, T., R. M. Sari, and E. Hartono. 2019. Nutritional strategies to improve bone health in indigenous chickens. Journal of Animal Science and Technology 61(1): 25-32.

Wu, G. 2014. Amino Acids: Biochemistry and Nutrition. CRC Press.

Wu, X., F. Xu, and Z. Zhang. 2018. The effects of dietary calcium and phosphorus on bone health in poultry. Poultry Science 97(6): 2117-2124.

Zhang, H., D. Li, and H. Zhang. 2021. The role of protein intake in the development of skeletal strength in poultry: A review. Journal of Poultry Science 58(3): 227-235.

Zhang, L., H. Liu, and J. Zhang. 2020. Bone health in poultry: The role of dietary factors and management practices. Frontiers in Veterinary Science 7: 99.