

GROWTH PERFORMANCE, TIBIAL BONE MORPHOMETRICS, COPPER AND ZINC STATUS IN ASEEL CHICKEN UNDER DIFFERENT SYSTEMS OF REARING – A FIELD STUDY

P. Murali¹, P. Vasanthakumar², A. Natarajan³, A. Balasubramaniam⁴ and T. Vasanthakumar⁵

^{1&2}Department of Animal Nutrition, Veterinary College and Research Institute, Namakkal, India.

³Animal Feed Analytical and Quality Assurance Laboratory, Namakkal.

⁴Department of Veterinary Microbiology, Veterinary College and Research Institute, Namakkal.

⁵Department of Poultry Science, Veterinary College and Research Institute, Namakkal.

Tamil Nadu Veterinary and Animal Sciences University (TANUVAS).

¹Corresponding author's E-mail: drpvknkl@gmail.com

ABSTRACT

The present study was conducted to evaluate the growth performance, bone morphometrics, copper and zinc profile of body tissues in Aseel chicken reared under different systems of rearing. A total of 72 Aseel birds were selected at marketing age from 18 farms, of which 24 birds each from intensive (3 months), semi-intensive (6 months) and backyard system (9 months) of rearing. Feed and water samples from each farm were collected to assess the copper and zinc levels. The selected birds were weighed and slaughtered to collect bone and liver samples for copper and zinc analysis. The data collected were analyzed following completely randomized design. copper and zinc levels in feed and water to birds in intensive system, semi-intensive system and backyard system of rearing were 27.39, 15.16, 7.41 and 147.33, 84.19, 28.98 ppm and 0.92, 0.48, 0.49 and 1.07, 0.97, 0.72 ppm, respectively. The body weight of birds (both male and female) was significantly ($P<0.01$) higher in the intensive system as compared to semi-intensive and backyard system of rearing. Tibial bone length, bone weight and seedor index were significantly ($P<0.05$) higher in birds reared under intensive and semi-intensive systems, whereas no significant difference was noticed in robustness index, circumference and relative bone weight among the birds reared under different systems. Both zinc and copper content of liver and bone were significantly ($P<0.01$) higher in the birds grown under intensive system of rearing when compared to semi-intensive and backyard system of rearing. The present study revealed that the growth performance, bone morphometric indices, copper and zinc status in liver and tibial bone were significantly higher in Aseel birds grown under intensive system as compared to semi-intensive and backyard system which might be due to feeding with a concentrate feed containing higher (27.39 ppm copper and 147.33 ppm zinc) levels of these minerals.

Key words: Aseel chicken, growth performance, system of rearing, copper and zinc status.

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0>)

Published first online December 28, 2025

Published final February 28, 2026

INTRODUCTION

Rural poultry population contributes over 40 per cent of total poultry production in India. Recently, there has been a growing fascination among farmers towards native chicken breeds due to their resilience, capacity to flourish in challenging environments, and the appealing taste and flavour of their eggs and meat. Among these native breeds, Aseel is renowned for its endurance, aggressiveness, dignified stride, and determined fighting capabilities (Rajkumar *et al.* 2021). Among the minor nutrients, trace minerals such as zinc and copper are crucial in maintaining the health and productivity of birds. Zinc is a component of over 200 metalloenzymes, making it vital for numerous physiological processes especially for supporting bone and tissue development

and ensuring proper functioning of immune system. Zinc deficiency causes poor growth due to improper nucleic acid synthesis. In chickens, it leads to slow leg development, short, thickened legs, expanded hocks, and frizzled feathers. High dietary zinc may reduce chick growth and cause mortality (Mondal *et al.*, 2010). Copper plays a critical role as a co-factor in various enzymes which are essential for maintaining proper bodily functions such as bone formation, Fe transport, and as a component in ceruloplasmin, cytochrome C oxidase and haemoglobin synthesis (El-Husseiny *et al.* 2012). Copper deficiency inhibited bone growth and strength, affected reproduction, feather pigmentation and body weight. Excess copper supplementation depressed growth, feed efficiency, and caused gizzard and liver damage (Shen *et al.*, 2022).

The requirement of copper and zinc in poultry feeds are available in Bureau of Indian Standards or the nutrient specifications prescribed by the chicken breeders for their broiler and layer strains and not for native chicken breeds like Aseel. As the research work on copper and zinc requirements for Aseel chicken are very limited in India. The present study was conducted to assess the status of copper and zinc in the body tissues, tibial bone morphometric indices and growth performance of Aseel chicken under different systems of rearing at farmer's field.

MATERIALS AND METHODS

The study was conducted in the north-eastern and north-western agro-climatic zones of Tamil Nadu in India. Forty-eight farms were randomly surveyed based on feeding practices and rearing systems (intensive, semi-intensive and backyard) followed to rear Aseel chicken from which 18 farms (six farms each from intensive, semi-intensive, and backyard systems of rearing) were chosen for this study. The flock size varied 300 - 5000 under intensive, 100 - 300 birds under semi-intensive and 20 - 150 birds under backyard system of rearing.

The birds reared under the intensive system were fed with commercial poultry feeds *ad libitum* whereas in semi-intensive system, the birds were offered 40 - 70g/day/bird with commercial feeds diluted with grains (maize/rice) or brans (rice/wheat bran) with grains *viz* maize and rice at a ratio of 50 kg concentrate feed to 5 to 15 kg of grains or brans. However, the birds under backyard system were predominantly fed only with locally available grains such as maize and rice at the rate of 25 - 50 g/day/bird along with access to free range grazing.

The feeds and feed ingredients used for feeding Aseel chicken in different system of rearing were analysed as per the protocols of AOAC (2019) in Animal Feed Analytical and Quality Assurance Laboratory (AFAQAL), Namakkal for its proximate composition (moisture, crude protein, crude fiber, ether extract and total ash), calcium, total phosphorus, copper and zinc content and are presented in Table 1 and 2.

Growth performance and slaughter study: A total of 72 birds, twenty-four birds (twelve male and twelve female) each from intensive, semi-intensive, and backyard systems of rearing were selected at three months of age (Intensive system), six months of age (Semi-intensive system) and nine months of age (Backyard system) marketing age. The birds were selected at the stage of being ready for sale as meat. The birds were collected from different rearing systems to evaluate feed-based variations. In addition, six numbers of feed and water samples were collected from each system of rearing. The birds (n=72) were weighed and

sacrificed to collect samples of tibia bone and liver for morphometric and mineral analysis.

Measurement of bone osteomorphometry: Tibia bone length and weight of birds (n=72) were measured in fat-free dried samples using a digital calliper and digital weighing balance to the nearest 0.0001 g accuracy. The Seedor Index (SI), was calculated by dividing the bone weight (g) by its length (mm) as reported by Seedor *et al.*, (1991). Likewise, the robusticity index (RI) expressed as mm/g was calculated by dividing the tibia proximal length (mm) by the cubic root of dried tibial bone weight (g) as reported by Reisenfeld (1972).

Estimation of copper and zinc in liver and tibia bone: The dried tibia bone and liver samples of all birds were triturated and 0.5 g of the sample was wet digested on a hot plate using diacid (2:1 v/v of nitric: perchloric acid). After complete digestion, the solution was transferred to a 50 ml volumetric flask filtering through Whatman filter paper No.42 and the volume was adjusted to 50 ml (Palma *et al.*, 2015). The concentration of copper and zinc in tibia bone and liver was analysed using Atomic Absorption Spectroscopy (Perkin Elmer PinAAcle 900T) at a wavelength of 213.8 nm for Zn and 324.8 nm for Cu) at AFAQAL, Veterinary College and Research Institute, Namakkal.

Statistical analyses: The data were collected and analysed by analysis of variance technique under completely randomized design (Snedecor and Cochran, 1989). The Least Significant Difference (LSD) test was performed to assess the significance of differences among different system of rearing ($P \leq 0.05$). The analysis was carried out with the SPSS Version 20.

Ethical committee approval: Institutional Animal Ethics Committee, Veterinary College and Research Institute, Namakkal approved the protocol and to conduct field study (IAEC project proposal No. 01/IAEC/VCRI-NKL/2023).

RESULTS AND DISCUSSION

Proximate composition of diets: The crude protein, ether extract, total phosphorus and salt levels in diet (Table 1) were significantly ($P < 0.01$) higher in intensive system of rearing as compared to semi-intensive and backyard systems of rearing. However, no significant difference was noticed in moisture, crude fibre, total ash, acid insoluble ash and calcium levels. Higher crude protein and fat were noticed in feed fed to birds reared under intensive system of rearing. Conversely, the birds reared under semi-intensive system were fed with commercial diets mixed with grains or brans and backyard system, where birds were solely fed with grains had lower levels of crude protein and crude fat.

Table: 1. Chemical composition of diets fed to Aseel chicken under different systems of rearing

System of rearing	Moisture (%)	Crude Protein (%)	Crude Fibre (%)	Ether extract (%)	Total Ash (%)	Acid insoluble ash (%)	Calcium (%)	Total Phosphorus (%)	Salt (%)
Intensive system	9.90 ± 0.32	17.24 ^a ± 1.32	3.80 ± 0.52	4.11 ^a ± 0.41	6.12 ± 0.73	1.35 ± 0.28	0.83 ± 0.09	0.76 ^a ± 0.08	0.44 ^a ± 0.05
Semi-Intensive system	10.04 ± 0.38	12.71 ^b ± 1.57	2.73 ± 0.90	2.06 ^b ± 0.66	5.34 ± 2.80	1.12 ± 0.50	1.25 ± 0.86	0.47 ^b ± 0.15	0.26 ^b ± 0.09
Backyard farming	10.93 ± 0.72	9.22 ^c ± 0.05	1.39 ± 0.59	0.95 ^c ± 0.20	1.41 ± 0.36	0.58 ± 0.30	0.13 ± 0.02	0.19 ^c ± 0.01	0.10 ^c ± 0.04
F value	1.21	11.53	3.04	11.90	2.24	1.12	1.28	8.10	9.14
P value	0.32	0.001	0.07	0.001	0.14	0.35	0.30	0.001	0.001

Each value is a mean of six observations

^{abc}Means in each column bearing different superscripts vary significantly

Copper and zinc levels in feed and water: Significant difference ($P < 0.01$) was noticed on feed copper and zinc levels in different systems of rearing (Table 2), with highest level in intensive system followed by semi-intensive system and backyard system. Whereas, no significant difference was noticed in copper and zinc content in water offered to birds under different systems

of rearing. The elevated concentration of copper and zinc in the diet fed to the birds reared under intensive system might be due to the supplementation of trace minerals in commercial poultry feeds. The reduced level in the diet of the backyard system was due to low concentration of copper and zinc present in grains (Yadav *et al.* 2018).

Table: 2. Copper and zinc level in feed and water fed to Aseel chicken under different systems of rearing

Rearing system	Sample size (n)	Feed		Water	
		Zinc (ppm)	Copper (ppm)	Zinc (ppm)	Copper (ppm)
Intensive system*	6	147.33 ^a ± 8.19	27.39 ^a ± 2.29	1.07 ± 0.18	0.92 ± 0.24
Semi-intensive system#	6	84.19 ^b ± 26.99	15.16 ^b ± 3.28	0.97 ± 0.14	0.48 ± 0.12
Backyard farming###	6	28.98 ^c ± 1.62	7.41 ^c ± 1.34	0.72 ± 0.27	0.49 ± 0.19
	F	13.17	17.04	0.73	1.63
	P value	0.001	<0.0001	0.49	0.72

^{abc}Means in each column bearing different superscripts vary significantly ($P \leq 0.05$)

*Birds fed with commercial concentrate feed

#Birds fed with concentrate feed diluted at the ratio of 50 kg feed with 5 – 15 kg grains

###Birds fed with grains and had access to free range grazing

Growth performance: The body weight of male and female Aseel chicken at marketing age is presented in Fig.1. The body weight of Aseel chicken significantly ($P < 0.01$) varied under different systems of rearing. Highest male and female body weights were recorded in intensive system of rearing followed by birds reared under semi-intensive and backyard system.

Similar to present findings, Gondwe and Wollny (2005) observed better growth performance in local chickens fed with concentrate feeds, reared under intensive deep litter system up to 14 weeks of age. Similarly, Sheikh *et al.* (2021) found better growth performance in indigenous, Vanaraja and Crossbred chickens reared under intensive system than the birds reared under the scavenging system for 52 weeks.

Improved body weight observed in intensively reared birds is likely due to the provision of nutritionally balanced diets, particularly those containing adequate levels of essential trace minerals such as copper and zinc. Zinc functions as a vital cofactor for numerous enzymes, including DNA and RNA polymerases, which are essential for cellular growth and proliferation. In addition, both copper and zinc support the metabolism of lipids, carbohydrates, and proteins, thereby enhancing nutrient utilization and promoting overall growth performance (Tomaszewska *et al.*, 2017). In contrast, birds reared under semi-intensive systems tend to exhibit lower body weights, likely due to their dependence on diluted concentrate feeds and partial scavenging, which may not fulfill their nutritional requirements (Sanka and

Mbaga, 2014). Similarly, free-range birds demonstrate reduced growth performance, potentially due to increased energy expenditure from greater locomotor activity during scavenging, along with consumption of grain-based diets that are often deficient in key trace minerals, particularly Cu and Zn.

The marketing age under intensive, semi-intensive, and backyard systems of rearing were three, six and nine months, respectively. In this study, the observed effects were diet-dependent, with no influence from the housing system. Generally, higher nutrient density in feed

promotes growth in fast-growing broilers, but this may not directly apply to Aseel chickens due to their distinct metabolic and physiological traits. This study evaluated growth performance, copper and zinc bioavailability and tissue deposition in Aseels under field conditions, as Cu and Zn levels are closely linked to growth and bone mineralization. Results indicated that higher Cu and Zn levels were associated with improved growth and bone mineral density, highlighting the need for breed-specific mineral supplementation and feed formulation.

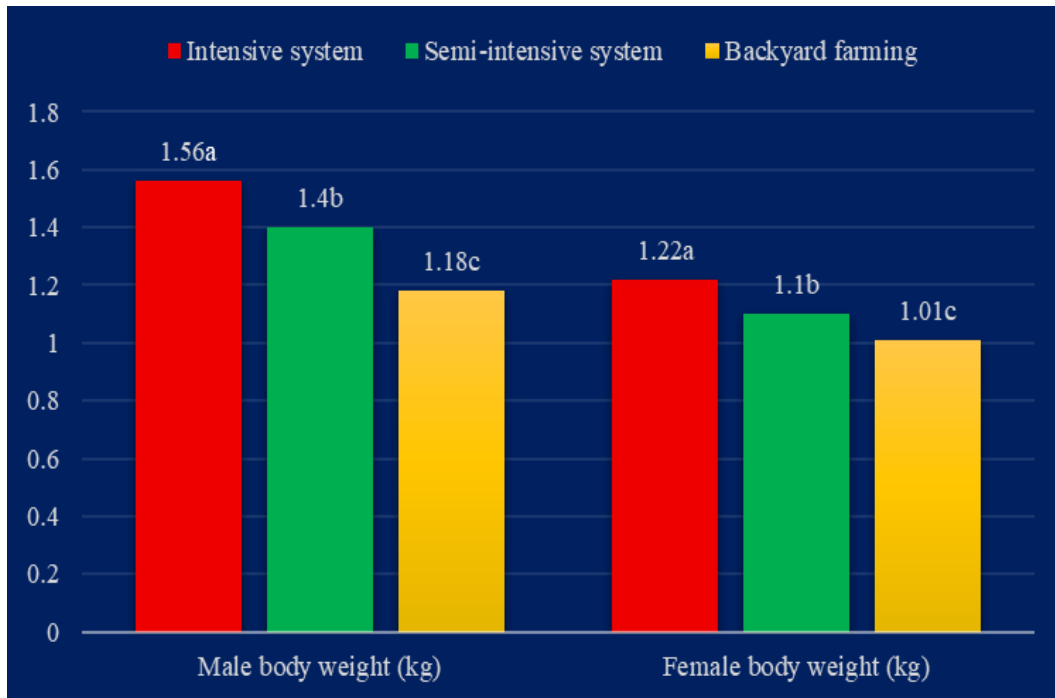


Fig: 1. Body weight (kg) of Aseel chick ken at marketing age in intensive (three months), semi-intensive (six months) and backyard system (nine months) of rearing

Bone osteomorphometry: The data on bone morphometric indices are presented in Table: 3. Tibial bone length, weight and Seedor index in Aseel chicken was significantly ($P < 0.05$) higher in intensive system compared to backyard system of rearing. However, no significant difference was noticed between intensive and semi-intensive system. While, tibial bone circumference, robusticity index, and relative bone weight values remain unaffected across different rearing systems. The present findings agreed with the findings of El-Husseiny *et al.* (2012) who stated that tibial bone weight and Seedor index were high in broilers at 100 ppm zinc and 16 ppm copper diet than 50 ppm zinc and 8 ppm copper. Similarly, Seedor index and tibial bone weight were significantly improved by increased zinc concentration in broiler diet from 20 to 80 ppm (Azad *et al.* 2020). Bone status is mainly indicated by its weight, length, thickness.

As Seedor index is a simple index of bone density, higher the Seedor index, denser the bone (Monteagudo *et al.* 1997).

In the present study, the robusticity index values did not differ significantly ($P > 0.05$) among the treatment groups, indicating that bone geometry and overall shaft development were not markedly influenced by the different rearing conditions. This aligns with the interpretation that robusticity reflects structural proportions rather than true mineral content. Consistent with the present findings, Mabelebele *et al.* (2017) found no significant differences in the robusticity index between indigenous and broiler chickens under intensive rearing. However, a significantly higher Seedor index was observed in broilers, suggesting that while bone geometry remains stable, bone density can vary with feeding management practices.

Table: 3 Tibial bone morphometric parameters of Aseel chicken at market age across intensive (3 months), semi-intensive (6 months) and backyard (9 months) rearing systems.

System of rearing	Length (mm)	Circumference (mm)	Bone weight (g)	Seedor index (g/mm)	Robusticity index (mm/g)	Relative bone weight (%)
Intensive system	136.43 ^a ± 2.50	7.71 ± 0.13	10.39 ^a ± 0.54	75.60 ^a ± 3.13	6.30 ± 0.09	0.76 ± 0.03
Semi-intensive system	132.71 ^a ± 2.51	7.56 ± 0.18	9.59 ^{ab} ± 0.48	71.60 ^{ab} ± 2.73	6.29 ± 0.07	0.71 ± 0.03
Backyard farming	125.75 ^b ± 2.23	7.21 ± 0.17	8.25 ^b ± 0.48	64.73 ^b ± 2.92	6.29 ± 0.06	0.69 ± 0.03
F	5.00	2.32	4.56	3.50	0.01	1.05
P value	0.00	0.10	0.01	0.03	0.98	0.35

Each value is a mean of twenty-four observations

^{ab}Means in a column bearing different superscripts vary significantly

Copper and zinc status in liver and tibial bones: The levels of zinc and copper in liver and bone samples were significantly ($P < 0.01$) higher in intensive system of rearing when compared to semi-intensive and backyard systems of rearing in Aseel chicken. Whereas no significant difference was noticed between semi-intensive and backyard system of rearing on bone zinc, liver copper and bone copper levels, except liver zinc levels. The data on copper and zinc levels in liver and tibial bone are presented in Table 4. Similar to the present findings, Sunder *et al.* (2008) noted a linear increase in zinc retention ($P < 0.05$) in liver (117 to 143 ppm) and bone (251 to 482 ppm). Notably, the rate of zinc retention in bone exhibited a significance level with each incremental rise from 0, 20, 40 and 80 ppm.

Likewise, Liu *et al.* (2015) observed a linear increase in liver zinc level following supplementation with various zinc sources (zinc sulfate, zinc amino acid chelate, and zinc proteinate) at concentrations of 60, 120, and 180 mg/kg zinc in broiler diets. Similarly, Hamdi *et al.*, (2018) reported that copper supplementation in broiler diets elicited an improvement in the storage of copper in the tibia. Bone status serves as a prevalent parameter for gauging mineral sufficiency in poultry diets. Zinc plays a crucial role in fortifying bones. It contributes to bone tissue formation through alkaline phosphatase, collagenase, and aminoacyl tRNA synthetase. Inadequate dietary zinc leads to compromised bone formation, resulting in decreased bone density due to its pivotal involvement in protein synthesis (Shelton and Southern, 2007).

Table: 4 Liver and tibia bone copper and zinc concentrations in Aseel chicken at market age across intensive (3 months), semi-intensive (6 months) and backyard (9 months) rearing systems.

Systems of rearing	Zinc (ppm)		Copper (ppm)	
	Liver	Tibia bone	Liver	Tibia bone
Intensive system	58.27 ^a ± 2.02	365.31 ^a ± 12.88	33.05 ^a ± 1.42	9.13 ^a ± 0.39
Semi-intensive system	49.47 ^b ± 1.45	257.84 ^b ± 15.68	16.12 ^b ± 0.90	6.26 ^b ± 0.31
Backyard farming	41.88 ^c ± 1.42	222.98 ^b ± 8.47	15.96 ^b ± 0.48	5.55 ^b ± 0.30
F	24.44	34.13	93.69	30.92
P value	0.00	0.00	0.00	0.00

Each value is a mean of twenty-four observations

^{abc}Means in a column bearing different superscripts vary significantly

Junior *et al.* (2022) demonstrated a linear increase in copper content in both tibial bone and liver as copper supplementation levels increased from 0, 4, 8, and 16 ppm copper in broiler chicken diets. Similarly, Wen *et al.* (2019) reported an increase in liver copper concentration with copper supplementation at 10 and 20 ppm in broiler diets. Additionally, significant differences

were observed among treatments in liver copper concentrations when copper levels in the feed were incremented from 8.31 to 20.31 ppm in broiler diets (Soster *et al.*, 2023). The liver acts as the primary reservoir for copper, regulating its distribution across the body. When the liver reaches its copper storage limit, surplus copper is released into the bloodstream, leading

to accumulation in other organs (Santos *et al.* 2021). In several studies on broilers and layers, high levels of dietary copper and zinc have not consistently resulted in proportional increases in tissue deposition, as excess minerals beyond saturation levels are typically excreted. This highlights the importance of understanding mineral utilization efficiency. The present study emphasizes the relevance of assessing mineral deposition specifically in Aseel chickens, a breed with distinct physiological traits, to determine the levels of copper and zinc commonly supplied through field-level feeding practices. These findings provide valuable insights into optimizing mineral supplementation strategies for indigenous breeds.

In the present study, desi chickens were assessed at their respective marketing ages such as 9 months (backyard), 6 months (semi-intensive) and 3 months (intensive) representing typical field management and related differences in feed. This approach enables meaningful evaluation of bone morphometry and trace mineral status especially Cu and Zn under field-relevant conditions. Age is a key determinant of skeletal development, for instance, Pines *et al.* (1999) reported tibial ash content increasing from 51.2% at 7 weeks to 60.1% at 72 weeks, alongside higher pyridinoline cross-links, indicating enhanced mineralization. Rearing system also impacts mineral deposition due to variations in mobility and nutrient access. Regmi *et al.* (2017) found hens in aviary systems had greater tibia breaking strength (up to 200 N) and ash content (62.7%) than caged birds. Moreover, Huang *et al.* (2023) showed that Cu and Zn supplementation influenced tibia ash (39.5%–45.8%) and bone density, highlighting the role of diet in mineral incorporation. Together, these findings validate that differences in age and feeding regimes across systems significantly influence bone and mineral traits.

Conclusion: The Aseel chicken raised under intensive system provided with a concentrate feed containing 27.39 ppm copper and 147.33 ppm zinc exhibited better body weight, bone morphometric indices and increased deposition of copper and zinc in liver and tibia as compared to those fed under semi-intensive or backyard systems. These improvements were primarily attributed to the feeding pattern and mineral concentration in the diet, with no effect from the housing system. Consequently, feeding of birds with an optimal level of copper and zinc in their diets enhances the quality of tibia bone in Aseel chicken.

REFERENCES

- AOAC. (2019). Official Methods of Analysis, Association of Official Analytical Chemists, 19th Edn, Washington, D. C, USA.
- Azad, S. K., F. Shariatmadari, M. A. K. Torshizi and L. Chiba (2020). Comparative effect of zinc concentration and sources on growth performance, accumulation in tissues, tibia status, mineral excretion and immunity of broiler chickens. *Rev Bras Cienc Avic.* 22: 1 - 10. <https://doi.org/10.1590/1806-9061-2019-1245>
- El-Husseiny, O.M., S. M. Hashish, R. A. Ali, S. A. Arafa, L. D. Abd El-Samee and A. A. Olemly (2012). Effects of feeding organic zinc, manganese, and copper on broiler growth, carcass characteristics, bone quality, and mineral content in bone, liver and excreta. *Int. J. Poult. Sci.* 11: 368-377. <https://doi.org/10.3923/ijps.2012.368.377>
- Gondwe, T. N. and C. B. A. Wollny (2005). Evaluation of the Growth Potential of Local Chickens in Malawi. *Int. J. Poult. Sci.*, 4: 64 - 70. <https://doi.org/10.3923/ijps.2005.64.70>
- Hamdi, M., D. Sola, R. Franco, S. Durosoy, A. Romeo and J.F. Perez (2018). Including copper sulphate or dicopper oxide in the diet of broiler chickens affects performance and copper content in the liver. *Anim. Feed Sci. Technol.* 237: 89 - 97. <https://doi.org/10.1016/j.anifeedsci.2018.01.014>
- Huang, Y., T. He, X. Yang, W. Liu, Q. Wang, Y. Liu and C. Yang (2023). Effect of dietary copper and zinc hydroxychloride on bone development and mineral deposition in broilers. *Poult. Sci.* 102(5): 102567.
- Junior, F. H. C., D. L. da Silva, B. R. de Carvalho, H. C. de Oliveira, J. C. L. Muniz, W. J. Alves, J. E. Pettigrew, S. E. F. Guimaraes, G. S. Viana and M. I. Hannas (2022). Broiler responses to copper levels and sources: growth, tissue mineral content, antioxidant status and mRNA expression of genes involved in lipid and protein metabolism. *BMC Vet. Res.* 18: 223. <https://doi.org/10.1186/s12917-022-03286-5>
- Liu, Z.H., L. Lu, R.L. Wang, H.L. Lei, S.F. Li, L.Y. Zhang and X.G. Luo (2015). Effects of supplemental zinc source and level on antioxidant ability and fat metabolism-related enzymes of broilers. *Poult. Sci.* 94(11): 2686 - 2694. <https://doi.org/10.3382/ps/pev251>
- Mabelebele, M., D. Norris, N. A. Siwendu, J. W. Ngambi, O. J. Alabi and C. A. Mbajiorgu (2017). Bone morphometric parameters of the tibia and femur of indigenous and broiler chickens reared intensively. *Appl. Ecol. Environ. Res.* 15(4): 1387 - 1398. https://doi.org/10.15666/aecer/1504_13871398
- Mondal, S., S. Haldar, P. Saha and T. K. Ghosh, 2010. Metabolism and tissue distribution of trace elements in broiler chicken's fed diets containing deficient and plethoric levels of copper, manganese and zinc. *Biol. Trace Elem.*

- Res., 137: 190 - 205.
<https://doi.org/10.1007/s12011-009-8570-z>
- Monteagudo, M. D., E. R. Hernandez, C. Seco, J. Gonzales-Riola, M. Revilla, L. F. Villa and H. Rico (1997). Comparison of the bone robusticity index and bone weight/bone length index with the results of bone densitometry and bone histomorphometry in experimental studies. *Acta Anat.* 160: 195 - 199.
<https://doi.org/10.1159/000148011>
- Palma, M. N. N., G. C. Rocha, S. C. Valadares Filho and E. Detmann (2015). Evaluation of acid digestion procedures to estimate mineral contents in materials from animal trials. *Asian-Australas. J. Anim. Sci.* 28(11): 1624 - 1628.
<http://dx.doi.org/10.5713/ajas.15.0068>
- Pines, M., V. Knopov and O. Genina (1999). Age and strain-related differences in bone strength, mineralization, and collagen crosslinks in broiler breeders. *Poult. Sci.* 78(8): 1232 - 1238.
- Rajkumar, U., S. V. R. Rao, M. V. L. N. Raju and R. N. Chatterjee (2021). Backyard poultry farming for sustained production and enhanced nutritional and livelihood security with special reference to India: a review. *Trop Anim. Health. Prod.* 53: 176. <https://doi.org/10.1007/s11250-021-02621-6>
- Regmi, P., N. Nelson, R.C. Haut, M.W. Orth and D.M. Karcher (2017). Influence of age and housing systems on properties of tibia and humerus of Lohmann White hens. *Poult. Sci.* 96(10): 3755 - 3762. <https://doi.org/10.3382/ps/pex194>
- Reisenfeld, A (1972). Metatarsal robusticity in bipedal rats. *Am. J. Phys. Anthropol.* 40: 229 - 234.
<https://doi.org/10.1002/ajpa.1330360211>
- Sanka, Y. D and S. H. Mbaga 2014. Evaluation of Tanzanian local chicken reared under intensive and semi-intensive systems: I. Growth performance and carcass characteristics. *Livest. Res. Rural Dev.* 26(7): 1 - 7.
- Santos, T. S., K. V. Z. Augusto, Y. Han, M. M. P. Sartori, J. C. Denadai, C. T. Santos, N. C. Sobral, R. O. Roca and J. R. Sartori (2021). High levels of copper and zinc supplementation in broiler diets on growth performance, carcass traits and apparent ileal mineral absorption. *Br. Poult. Sci.* 62(4): 579 - 588.
<https://doi.org/10.1080/00071668.2021.1887453>
- Seedor, J., H. A. Quarruccio and D. D. Thompson (1991). The bisphosphonate alendronate inhibits bone loss due to ovariectomy in rats. *J. Bone Miner. Res.* 6: 339 - 346.
<https://doi.org/10.1002/jbmr.5650060405>
- Sheikh, I. U., N. Kalita and J. D. Mahanta (2021). Comparative performances of indigenous, Vanaraja and crossbred (PB2xIndigenous) chicken under different systems of rearing. *J. Pharmacogn. Phytochem.* 10(2): 966 - 970.
- Shelton, J. L and L. L. Southern (2007). Interactive effect of zinc, copper and manganese in diets for broilers. *Int. J. Poult. Sci.* 6: 466 - 469.
<https://doi.org/10.3923/ijps.2007.466.469>
- Shen, Q., Y. Qi, Y. Kong, H. Bao, Y. Wang, A. Dong, H. Wu and Y. Xu (2022). Advances in copper-based biomaterials with antibacterial and osteogenic properties for bone tissue engineering. *Front. Bioeng. Biotechnol.*, 9: 795425.
<https://doi.org/10.3389/fbioe.2021.795425>
- Snedecor, G. W and W. G. Cochran (1989). *Statistical Methods*, 8th Edn, Iowa State University Press, Ames, USA.
<https://doi.org/10.1017/S0021859600074104>
- Soster, P., S. L. Vieira, J. C. Feijo, W. E. Altevogt and G. B. Tormes (2023). Dietary phytase effects on copper requirements of broilers. *Front. Vet. Sci.* 10:1170488.
<https://doi.org/10.3389/fvets.2023.1170488>
- Sunder, G. S., A. K. Panda, N. C. S. Gopinath, S. Rao, V. Rama, M. V. L. N. Raju, M. R. Reddy and C. V. Kumar (2008). Effects of higher levels of zinc supplementation on performance, mineral availability and immune competence in broiler chickens. *J. Appl. Poult. Res.* 17: 79 - 86.
<https://doi.org/10.3382/japr.2007-00029>
- Tomaszewska, E., S. Muszynski, P. Dobrowolski, M. Kwiecien, A. Winiarska-Mieczan, I. Swietlicka and A. Wawrzyniak (2017). Effect of zinc level and source (zinc oxide vs. zinc glycine) on bone mechanical and geometric parameters, and histomorphology in male Ross 308 broiler chicken. *Braz. J. Poult. Sci.* 19(1): 159 - 170.
<https://doi.org/10.1590/1806-9061-2016-0285>
- Wen, A., S. Dai, X. Wu and Z. Cai (2019). Copper bioavailability, mineral utilization, and lipid metabolism in broilers. *Czech. J. Anim. Sci.* 64(12): 483 - 490.
<https://doi.org/10.17221/210/2019-CJAS>
- Yadav, S.P., V.K. Paswan, P. Singh, B.K. Bhinchhar and P.K. Gupta (2018). Chemical composition and mineral profile of concentrate feeds from dairy farms of Varanasi, India. *Asian J. Dairy and Food Res.* 37(3): 182 - 186.
<https://doi.org/10.18805/ajdfr.DR-1378>