# Short Communication

# Effect of calcium, phosphorus premix with synergistic herbs supplementation in improving overall performance, carcass quality and tibial mineralization in broiler chickens

N. V. Jadhav, Vivek M. Patil, Shrikant Kulkarni, Pradeep Swamy, Siddalingswamy Hiremath, K. Ravikanth, Adarsh Choudhary and Shivi Maini

• Received: June 3, 2016 • Revised: July 6, 2016 • Accepted: July 9, 2016 • Published Online: July 19, 2016

# AFFILIATIONS

- N. V. Jadhav
- Vivek M. Patil
- Shrikant Kulkarni
- Pradeep Swamy

• Siddalingswamy Hiremath College of Veterinary & Animal Sciences, KVAFSU, Bidar, Karnataka, India.

- K. Ravikanth
- Adarsh Choudhary
- Shivi Maini

R&D Team, Ayurvet Limited, Baddi, India.

# CORRESPONDENCE

Adarsh Choudhary R&D Team, Ayurvet Limited, Baddi, India. E-mail: adarsh66677(@gmail.com

# ABSTRACT

**Objective:** Efficacy of AV/CSP/29 premix (Ca, P supplement with Vit. A & D3 along with synergistic herbs) (M/S Ayurvet Ltd.) was evaluated in commercial broiler chicks.

**Materials and methods:** A total of 144 day-old chicks were randomly divided into three groups. Group  $T_0$  (n=48) control group, supplemented with basal diet without any additional source of calcium (Ca) and phosphorus (P). Group  $T_1$  (n=48) was administered with AV/CSP/29 premix at 500 gm/ton of feed and Group  $T_2$  birds (n=48) were supplemented with Brand A at 500 gm/ton of feed for 0 to 42 days. Parameters *viz.*, growth, performance, serum biochemical and carcass quality traits were evaluated.

**Results:** More gain in body weight was observed in the AV/CSP/29 premix supplemented group ( $T_1$ ) birds as compared to other groups. Similarly, FCR was improved in the AV/CSP/29 premix supplemented group ( $T_1$ ) birds. Blood Ca and P concentrations were comparatively improved in AV/CSP/29 premix supplemented birds. Parameters *viz.*, carcass yield, dressing percentage, tibial mineralization, water holding capacity and extract release volume were also found to be better in AV/CSP/29 premix supplemented group ( $T_1$ ) birds.

**Conclusion:** The results from the current study suggest that feeding of AV/CSP/29 premix to broiler chickens may have a beneficial effect on bone quality and in improving overall performance.

# **KEYWORDS**

Carcass quality, Growth, Tibial mineralization

How to cite: Jadhav NV, Patil VM, Kulkarni S, Swamy P, Hiremath S, Ravikanth K, Choudhary A, Maini S (2016). Effect of calcium, phosphorus premix with synergistic herbs supplementation in improving overall performance, carcass quality and tibial mineralization in broiler chickens. Journal of Advanced Veterinary and Animal Research, 3(3): 268-273.



Vol 3 No 3, Pages 268-273.

September 2016



# INTRODUCTION

Metabolic and structural function of Ca and P in bone and eggshell formation is essential in poultry production (Pelicia et al., 2009). Calcium also plays significant role in metabolism, blood clotting, enzyme activation, neuromuscular function, muscle contraction, cell adhesion, and intracellular signaling (Weglarz and Angel, 2013). Poor mineralization has been associated with increased risk of fractures (Blake and Fogelman, 2002). Bone breakage and associated infections contribute to mortality, low productivity, and carcass condemnations (Rath et al., 2004). Inadequate level of Phosphorus results in loss of skeletal integrity and loss of appetite. This results in subnormal growth in young birds and weight loss in older birds (Narcy et al., 2009; Venalainen et al., 2006). Overall deficiency or excess of Ca and P interferes with homeostasis of each other, results in depressed growth rate and bone mineralization (Shafey et al., 1990; Hurwitz et al., 1995). Vitamin D is involved in various physiological processes, including the absorption of calcium and phosphorus, bone mineralization and mobilization (Sanotra et al., 2002; Garcia et al., 2013). Similarly deficiency of vitamin A seriously affect growth rate, feed utilization, development of bone, movements, vision, reproduction, resistance against diseases, and mortality in poultry birds (Bhuiyan et al., 2004). Many herbs viz., Moringa oleifera, Lepidium sativum are rich source of calcium and have role in calcium and phosphorus utilization (Olson and Carlquist, 2001; Juma, 2007). Therefore the present study was undertaken to evaluate efficacy of supplementation of AV/CSP/29 premix (Ca, P supplement with Vit. A & D3 along with synergistic herbs) in improving g performance, carcass quality and tibial mineralization.

# MATERIALS AND METHODS

The trial was conducted at the College of Veterinary Sciences and Animal Husbandry, Bidar, Karnataka, India. The trial duration was 6 weeks with identical management conditions for all the groups. A total of 144 day-old commercial broiler chicks were divided into 3 groups having 48 birds comprising of 3 replicates of 16 birds in each group. Group T<sub>0</sub> birds were fed with standard basal diet (commercial feed) without any supplementation and Group T<sub>1</sub> birds were supplemented with AV/CSP/29 premix at 500 gm/ton of feed (M/S Ayurvet Ltd.). AV/CSP/29 premix is acalcium, phosphorus supplement with Vitamin A & D3 along with synergistic herbs viz., Cisssus quadrangularis, Lepidium sativum, Uraria picta, Zingiber officinale etc. Group T2 birds were supplemented with Brand A dosed at 500 mg/ton of feed from 0-42 days. To check the efficacy of product supplementation the

parameters viz. body weight, body weight gain, feed intake, FCR, health status, carcass quality traits, and biochemical parameters were studied. The results were analyzed statistically (Snedecor and Cochran, 1991).

# RESULTS

## Body weight (gm)

At the beginning of the trial there was no significant difference in body weight of birds in all the groups. The mean initial body weight in Group T<sub>0</sub>, Group T<sub>1</sub> and Group T<sub>2</sub> was 45.3 gm, 45.4 gm and 45.6 gm, respectively (Table 1). Second week after supplementation the body weight (gm) was significantly (P<0.05) more in AV/CSP/29 premix supplemented Group T<sub>1</sub> birds (460.2) as compared to Group T2 (441.4) and Group  $T_0$ (430.1) birds. At the end of 6th week the mean body weight was non-significantly more in AV/CSP/29 premix supplemented Group T1 birds (2203.4) as compared to Brand A supplemented Group T<sub>2</sub> birds (2137.5) but was significantly (P < 0.05) more than control Group T<sub>0</sub> birds (2089.3). The mean weekly body weight gain did not vary significantly among all the groups on 6th week. At 6th week, the mean weekly body weight gain was higher in AV/CSP/29 premix supplemented Group T<sub>1</sub> (436 gm) followed by Group  $T_2$  (414.6 gm) and Group  $T_0$  (402.5 gm) (Table 1).

# Feed consumption (gm) and Feed conversion ratio (FCR)

Cumulative feed consumption and feed conversion ratio did not vary significantly among different groups during the entire experimental period. The cumulative feed consumption was highest in the AV/CSP/29 premix supplemented Group T<sub>1</sub> (3944.1 gm) in comparison to Group T<sub>2</sub> (3868.9 gm) and Group T<sub>0</sub> (3802.5 gm), but AV/CSP/29 premix supplemented Group T<sub>1</sub> had the best cumulative FCR *i.e.*, 1.79 as compared to the other groups (T<sub>0</sub>-1.82, T<sub>1</sub>-1.81) (**Table 2**).

#### **Biochemical parameters**

There was no significant difference in Biochemical parameters in all groups. However, there is considerable increase in serum calcium and phosphorous concentration in AV/CSP/29 premix supplemented Group  $T_1$  (8.23 mg/dL and 4.58 mg/dL, respectively) followed by Group  $T_2$  (8.15 mg/dL and 4.28 mg/dL, respectively) and Group  $T_0$  (8.12 mg/dL and 4.41 mg/dL, respectively) (**Table 3**). Similarly, Total protein (g/dL) and Albumin (gm/dL) concentration was also better in AV/CSP/29 premix supplemented Group  $T_1$ 

Age	Group	Group T <sub>0</sub>		Group T <sub>1</sub>		Group T <sub>2</sub>	
(Weeks)	Body weight (gm)	BWG (gm)	Body weight (gm)	BWG (gm)	Body weight (gm)	BWG (gm)	
0	45.3ª	-	45.4ª	-	45.6ª	-	
	$\pm 0.43$		$\pm 0.51$		$\pm 0.44$		
1	173.3ª	128.1ª	180.2ª	135.7 <sup>b</sup>	178.6ª	132.3 <sup>ab</sup>	
	± 2.12	$\pm 2.04$	± 1.91	± 1.77	$\pm 2.02$	± 1.62	
2	430.1	256.8ª	460.2 <sup>b</sup>	278.9 <sup>b</sup>	441.4 <sup>ab</sup>	268.8 <sup>b</sup>	
	a± 5.13	$\pm 4.02$	± 5.31	± 3.98	± 5.29	± 3.55	
3	789.9ª	359.1ª	840.1 <sup>b</sup>	372.9 <sup>b</sup>	809.9 <sup>a</sup>	367.7 <sup>ab</sup>	
	± 9.31	± 5.84	± 9.04	$\pm 4.56$	± 10.09	± 4.75	
4	1217.7	431.7ª	1287.9 <sup>b</sup>	447.6ª	1265.6 <sup>b</sup>	454.9 <sup>b</sup>	
	<sup>a</sup> ± 14.94	± 15.41	± 15.13	±13.73	$\pm 18.07$	±12.02	
5	1688.2ª	471.6ª	1767.0 <sup>b</sup>	479.5ª	1724.7 <sup>b</sup>	460.3ª	
	± 25.27	± 19.39	± 31.22	±15.64	$\pm 29.09$	$\pm 17.20$	
6	2089.3ª	402.5ª	2203.4 <sup>b</sup>	436.0ª	2137.5 <sup>b</sup>	414.6ª	
	± 33.47	± 21.34	$\pm 39.58$	$\pm 22.85$	±36.02	$\pm 20.97$	

Table 1. Mean weekly body weight and body weight gain (BWG) in broiler flocks

Means within different superscript varied significantly (P<0.05)

Table 2. Cumulative Feed Consumption (gm) and Cumulative Feed Consumption Ratio of experimental broilers

Age	Group T <sub>0</sub>		Group T <sub>1</sub>		Group T <sub>2</sub>	
(Weeks)	Cumulative Feed Cumulative Feed		Cumulative	Cumulative Feed	Cumulative Feed	Cumulative Feed
	Consumption	Consumption	Feed	Consumption	Consumption	Consumption Ratio
	(gm)	Ratio	Consumption	Ratio		
1	161.2	0.93	167.6	0.93	164.3	0.92
2	481.7	1.12	520.0	1.13	494.4	1.12
3	1098.0	1.39	1134.1	1.35	1117.7	1.38
4	1866.9	1.56	1944.7	1.51	1949.0	1.54
5	2937.5	1.74	3039.2	1.72	3001.0	1.74
6	3802.5	1.82	3944.1	1.79	3868.9	1.81

#### Table 3. Blood biochemical parameters of broiler flocks

Groups	Calcium (mg/dL)	Phosphorus (mg/dL)	Total protein (g/dL)	Albumin (g/dL)	Alkaline phosphatase (U/L)
$T_0$	8.12±0.16	4.41±0.11	$3.23 \pm 0.18$	$1.58 \pm 0.09$	156.72±5.12
$T_1$	8.23±0.11	$4.58 \pm 0.10$	3.64±0.12	$1.65 \pm 0.13$	$152.92 \pm 3.97$
$T_2$	8.15±0.15	$4.28 \pm 0.09$	$3.52 \pm 0.12$	$1.42 \pm 0.10$	149.68±4.22

#### Table 4. Serum immunoglobulin on 3rd and 5th week

Groups	Serum Immunoglobulin (g/dL)				
	Week 3	Week 5			
$T_0$	$0.78^{a}\pm0.08$	$0.92^{a}\pm0.06$			
$T_1$	$1.05^{b}\pm0.09$	$1.20^{b}\pm0.10$			
$T_2$	$1.09^{b} \pm 0.08$	$1.19^{b} \pm 0.07$			

Means within different superscript varied significantly (P<0.05)

(3.64 and 1.65, respectively) followed by Group  $T_2$  (3.52 and 1.42, respectively) and Group  $T_0$  (3.23 and 1.58, respectively). Serum alkaline phosphatase activity remained unaltered (**Table 3**).

#### Serum immunoglobulin

Serum immunoglobulin concentration was significantly (P<0.05) higher in both treatment groups as compared to control group on 3<sup>rd</sup> and 5<sup>th</sup> week. At 5<sup>th</sup> week serum immunoglobulin concentration in AV/CSP/29 premix

supplemented Group  $T_1$  (1.20 g/dL) and Brand A supplemented Group  $T_2$  (1.19 g/dL) was significantly (*P*<0.05) higher in comparison to control Group  $T_0$  (0.92 g/dL) (**Table 4**).

#### Pathology

Of the 48 birds in each group, there was only one mortality in the Group  $T_0$  in the  $2^{nd}$  week. Post-mortem examination revealed that the death was due to unspecified causes.

#### **Carcass Quality**

Highest carcass yield and dressing percentage was noticed in AV/CSP/29 premix supplemented Group  $T_1$  (1590.6 gm and 72.2%, respectively) followed by Brand A supplemented Group  $T_2$  (1474 gm and 69%, respectively) and unsupplemented control; Group  $T_0$  (1460 gm and

Group	Carcass yield	Dressing	Pectoral muscle	Muscle pH		ERV (mL/100	Sensory
	(gm)	%	weight	Before rigor	After rigor	gm)	evaluation
			(gm)	mortis	mortis		(max. 10)
$T_0$	$1460.8 \pm 30.67$	65.1±1.54	484.5±6.96	6.61±0.20	5.92±0.19	7.0	25.62±0.55
$T_1$	$1590.6 \pm 34.59$	$72.2 \pm 1.47$	$577.0 \pm 8.02$	6.62±0.13	5.82±0.15	8.7	32.61±0.73
$T_2$	1474.4±37.34	$69.0 \pm 1.44$	$519.5 \pm 7.78$	$6.65 \pm 0.24$	$5.89 \pm 0.27$	8.0	29.56±0.38

Table 5. Carcass quality traits of experimental broilers

Table 6. Effect of supplementation on Tibialmineralization in broilers

Traits	Treatment Groups					
	$T_0$	$T_1$	$T_2$			
Weight (gm)	10.81±0.44	$14.29 \pm 0.27$	12.92±0.59			
Length (mm	85.67±1.45	$101.52 \pm 1.31$	91.73±1.30			
Width (mm)	13.48±0.13	$16.26 \pm 0.20$	$14.88 \pm 0.17$			

65.1%, respectively). Pectoral muscle weight (g) was also maximum in AV/CSP/29 premix supplemented Group T<sub>1</sub> (577 gm) followed by Group T<sub>2</sub> (519.5 gm) and Group T<sub>0</sub> (484.5 gm). Muscle pH values varied none significantly in all groups (**Table 5**). In AV/CSP/29 premix supplemented Group T<sub>1</sub>, Extract release volume (ERV) (32.61 mL/100 gm) was found to be better than other Groups i.e. from Group T<sub>2</sub> (29.56 mL/100 gm) and control Group T<sub>0</sub> (25.62 mL/100 gm). Meat quality of chicken is stressed by consumers which refer to the sensory attributes of chicken product. The overall score for sensory quality of chicken product from AV/CSP/29 premix supplemented Group T<sub>1</sub> (8.7) was better than Brand B supplemented Group T<sub>2</sub> (8) and control Group T<sub>0</sub> (7) (**Table 5**).

#### **Tibial mineralization**

Characteristic relationship existed between growth performance and the development of the tibia. After AV/CSP/29 premix supplementation to Group T1, there is characteristic increase in tibial weight, length and width (14.29 gm, 101.52 mm and 16.26 mm, respectively) (**Table 6**) as compared to Brand A supplemented Group T<sub>2</sub> (12.92 gm, 91.73 mm, 14.88 mm, respectively) and control Group T<sub>0</sub> (10.81 gm, 85.67 mm, 13.48 mm, respectively).

#### Economics

The economic benefit analysis revealed that cost of production (Rs./kg) of broilers with AV/CSP/29 premix supplementation was economical (65.41) in comparison to cost of production of broilers supplemented with Brand A (66.17) and control group (67.18).

## DISCUSSION

Nutrition has a vital role in broiler production. Deficiency of calcium cause skeletal abnormalities, and phosphorus plays a critical role in cellular metabolism, as a part of the energy currency of the cell, in cellular regulatory mechanism, and in bone (85% of P is in bone) (Musilova et al., 2014). Supplementation of calcium and phosphorus has substantial effects on weight gain (Bintvihok and Kositcharoenkul, 2006) that's why after AV/CSP/29 premix (Ca, P supplement with Vitamin A & D3 along with synergistic herbs) supplementation to Group T1 birds showed increase in body weight and body weight gain. 70% of the total cost of production is because of feed cost (Waller, 2007), increase in weight gain after AV/CSP/29 premix supplementation will helps to reduce the production cost. Feed conversion ratio (FCR) is measure of how well a bird converts feed intake into live weight. AV/CSP/29 premix ingredient herb Zingiber officinale has role in improvement of FCR (Herawati, 2006; Herawati, 2010). The ways for exploring methodologies for enhancing bioavailability of minerals especially calcium and phosphorus is a strategy to reduce the poultry feed cost. Serum calcium and phosphorous concentration increased on AV/CSP/29 premix supplementation. AV/CSP/29 premix ingredient herbs viz., Cissus quadrangularis, Lepidium sativum and Uraria picta (Singh et al., 2011; Mohamed et al., 2013; Saxena et al., 2014) have synergistic effect on calcium and phosphorus utilization. Blood plasma proteins have significance role in homeostasis maintenance (Filipović et al., 2007). An improvement in total protein (g/dL) concentration in AV/CSP/29 premix supplemented Group T1 birds might act as the source of amino acids for synthesis of tissue proteins, and lead to better performance. Serum Alkaline phosphates (ALP) levels have been used as diagnostic indicators in bone and liver disease (Iyngkaran et al., 1995). Unaltered serum alkaline phosphates activity in the treatment groups indicates that none of the herbal products under study had any detrimental side effects on hepatic functions in broilers. Serum immunoglobulin levels are routinely determined in clinical practice to check the Humoral immune status (Gonzalez-Quintela et al., 2008). Serum immunoglobulin concentration increased on 3rd and 5th week after AV/CSP/29 premix supplementation to Group T<sub>1</sub>, might be because of its constituent Vitamin A. Researchers showed that after Vitamin А supplementation significant increase in Immunoglobulin (Moghaddam and Emadi, 2014). High carcass yield,

dressing percentage and improved tibial mineralization was observed in AV/CSP/29 premix supplemented Group  $T_1$ . This improvement may be attributed to the synergistic calcium and phosphorus absorption enhancing ability of the AV/CSP/29 premix ingredient herbs viz., Cissus quadrangularis, Lepidium sativum and Uraria picta (Jaiswal et al., 2004). Meat of good organoleptic quality with a relatively low bacterial count releases large volumes of extract, whereas meat of poor organoleptic quality with larger numbers of bacteria releases progressively less extract. Extract release volume (ERV) was more in meat from AV/CSP/29 premix supplemented Group T<sub>1</sub> birds. Extract release volume decreases with progress of spoilage (Jav and Hollingshed, <u>1990</u>). The high ERV value of Group  $T_1$  meat depicts that after AV/CSP/29 premix supplementation, the organoleptic quality of meat is enhanced. Sensory quality of meat was increased in AV/CSP/29 premix supplemented Group T<sub>1</sub>. Sensory attributes of foods are often used to evaluate food eating quality and serve as references during the selection of foods (Chumngoen and Tan, 2015). Thus, in this way AV/CSP/29 premix supplementation enhance the consumer preference.

# CONCLUSION

Incorporation of AV/CSP/29 premix in broiler feed improved the growth and overall performance of broilers, making broiler rearing comparatively more profitable. Tibial mineralization improved after AV/CSP/29 premix supplementation. AV/CSP/29 premix supplementation led to better humoral immunity and no adverse effect on other serum biochemical parameters.

# **CONFLICT OF INTEREST**

The authors declare that they have no conflict of interest with any other people or organizations in any financial or personal relationship.

# ACKNOWLEDGEMENT

Financial support, samples provided by Ayurvet Limited, Baddi, H.P., India and research facility provided by College of Veterinary Science, KVAFSU, Bidar is gratefully acknowledged.

# REFERENCES

Bhuiyan AR, Lauridsen C, Howlider AR, Jakobsen K (2004). Importance of vitamin A supplementation for performance of Sonali chickens under smallholder farm conditions in a tropical climate. Livestock Research and Rural Development, 16: 83

- Bintvihok A, Kositcharoenkul S (2006). Effect of dietary calcium propionate on performance, hepatic enzyme activities and aflatoxin residues in broilers fed a diet containing low levels of aflatoxin B1. Toxicon, 47: 41-46.
- Blake GM, Fogelman I (2002). Methods and clinical issues in bone densitometry and quantitative ultrasonometry. In: Principles of Bone Biology (Bilezikian JP, Raisz LG, Rodan GA, Edn.). Academic Press; pp 1573-1585.
- Chumngoen W, Tan FJ (2015). Relationships between descriptive sensory attributes and physicochemical analysis of broiler and Taiwan native chicken breast meat. Asian-Australasian Journal of Animal Sciences, 28: 1028-1037.
- Filipović N, Stojevic Z, Milinkovic-Tur S, Beer Ljubic B, Zdelar-Tuk M (2007). Changes in concentration and fractions of blood serum proteins of chickens during fattening. Veterinarski Arhiv, 77: 319-326.
- Garcia AFQM, Murakami AE, Duarte CRA, Rojas ICO, Picoli KP, Puzotti MM (2013). Use of Vitamin D3 and Its metabolites in broiler chicken feed on performance, bone parameters and meat quality. Asian-Australasian Journal of Animal Sciences, 26: 408-415.
- Gonzalez-Quintela A, Alende R, Gude F, Campos J, Rey J, Meijide LM, Fernandez-Merino C, Vidal C. (2008). Serum levels of immunoglobulins (IgG, IgA, IgM) in a general adult population and their relationship with alcohol consumption, smoking and common metabolic abnormalities. Clinical Experimental Immunology, 151: 42-50.
- Herawati (2006). Effect of red ginger (*Zingiber officinale Rosc*) phytobiotic addition to the broiler performance and blood profile. Pengaruh Penambahan Fitobiotik Jahe Merah., 14: 173-142.
- Herawati (2010). The effect of feeding red ginger as phytobiotic on body weight gain, feed conversion and internal organs condition of broiler. International Journal of Poultry Science, 9: 963-967.
- Hurwitz S, Plavnik I, Shapiro A, Wax E, Talpaz H, Bar A (1995). Calcium metabolism and requirements of chickens are affected by growth. Journal of Nutrition, 125: 2679-2686.
- Iyngkaran N, Yadav M, Boey CG (1995). Mucosal and serum alkaline phosphatase activities in milk allergy. Medical Journal of Malaysia, 50: 21-24.
- Jaiswal S, Singh SV, Singh B, Singh HN (2004). Plants used for tissue healing of animals. Natural Product Radiance, 3: 284-292.
- Jay JM, Hollingshed AM (1990). Two methods for determining extract release volume (erv) of fresh and spoiled beef and poultry meats. Journal of Food Science, 55: 1475-1476.

- Juma Ab (2007). The effects of *Lepidium sativum* seeds on fracture-induced healing in rabbits. Medscape General Medicine, 9: 23.
- Moghaddam HS, Emadi M (2014). The effect of threonine and vitamin A on immune system in broiler chickens. International Journal of Advanced Biological and Biomedical Research, 2: 756-763.
- Mohamed FE, Almalki AL, Hussein HK, Khan JA (2013). Synergistic antiosteoporotic effect of *Lepidium sativum* and alendronate in glucocorticoid-induced osteoporosis in wistar rats. African Journal of Traditional, Complementary and Alternative Medicines, 10: 267-273.
- Musilova A, Lichovníkova M, Przywarova A (2014). Effect of exogenous phytase on egg quality in laying hens. Acta Fytotechnica et Zootechnica, 17: 79-83.
- Narcy A, Letourneau-montminy MP, Magnin M, Lescoat P, Jondreville C, Sauvant D, Nys Y (2009).
  Phosphorus utilization in broilers. In: Proceedings of the 17th European Symposium on Poultry Nutrition. Edinburgh (UK); pp 1-20.
- Olson ME, Carlquist S (2001). Stem and root anatomical correlations with life form diversity, ecology, and systematics in Moringa (Moringaceae). Botanical Journal of the Linnean Society, 135: 315-348.
- Pelicia K, Garcia EA, Faitarone ABG, Silva AP, Berto DA, Molino AB, Vercese (2009). Calcium and available phosphorus levels for laying hens in second production cycle. Revista Brasileira de Ciência Avícola, 11: 39-49.
- Rath NC, Huff GR, Huff WE, Balog JM (2000). Factors regulating bone maturity and strength in poultry. Poultry Science, 79: 1024-1032.

- Sanotra GS, Damkjer Lund J, Vestergaard KS (2002). Influence of light-dark schedules and stocking density on behaviour, risk of leg problems and occurrence of chronic fear in broilers. British Poultry Science, 43: 344-354.
- Saxena HO, Soni A, Mohammad N, Choubey SK (2014). Phytochemical screening and elemental analysis in different plant parts of *Uraria picta* Desv.: A Dashmul species. Journal of Chemical and Pharmaceutical Research, 6: 756-760.
- Shafey TM, McDonald MW, Pym RA (1990). Effects of dietary calcium, available phosphorus and vitamin D on growth rate, food utilisation, plasma and bone constituents and calcium and phosphorus retention of commercial broiler strains. British Poultry Science, 31: 587-602.
- Singh V, Singh N, Pal US, Dhasmana S, Mohammad S, Singh N (2011). Clinical evaluation of *Cissus quadrangularis* and *Moringa oleifera* and osteoseal as osteogenic agents in mandibular fracture. National Journal of Maxillofacial Surgery, 2: 132-136.
- Snedecor GW, Cochran WG (1991). Statistical methods, 8th Edn., New York: Wiley; pp 1-503.
- Venalainen E, Valaja J, Jalava T (2006). Effects of dietary metabolisable energy calcium and phosphorus on bone mineralisation leg weakness and performance of broiler chickens. British Poultry Science, 47: 301-310.
- Waller A (2007). Take a fresh look at broiler nutrition. Poultry International, 9: 12-13.
- Weglarz MP, Angel R (2013). Calcium and phosphorus metabolism in broilers: Effect of homeostatic mechanism on calcium and phosphorus digestibility. Journal of Applied Poultry Research, 22: 609-627.

\*\*\*\*