



## Influence of herbal calcium supplements on nutrient utilisation, serum mineral concentration and leg health in commercial layers reared under the cage and deep litter systems

Krishna Daida<sup>1</sup>, S. Parashuramulu<sup>2\*</sup>, A. Jayasri<sup>3</sup> and Shreya Gupta<sup>4</sup>

<sup>1</sup>Department of Poultry Science, College of Veterinary Science, Korutla, PVNRTVU, <sup>2</sup>Department of Animal Nutrition, College of Veterinary Science, Korutla, PVNRTVU, <sup>3</sup>Department of Biochemistry, College of Veterinary Science, Hyderabad, <sup>4</sup>Clinical, Ayurvet Limited, Katha, Solan dist, Himachal Pradesh

**Citation:** Daida, K., Parashuramulu, S., Jayasri, A. and Gupta, S. 2024. Influence of herbal calcium supplements on nutrient utilisation, serum mineral concentration and leg health in commercial layers reared under the cage and deep litter systems. *J. Vet. Anim. Sci.* **55** (4):693-697

Received: 27.05.2024

Accepted: 10.09.2024

Published: 31.12.2024

### Abstract

*This study aimed to evaluate the herbal calcium supplements in commercial layers under cage and deep litter systems of rearing. A total of 240 White Leghorn layers (Bovans) were distributed randomly into 4 different treatments with 4 replicates having 15 birds in each replicate. The basal diet, which consisted of corn and soybean meal, was prepared as a control and fed to birds under cage (T1) and deep litter (T3) system. The other experimental diets (T2 & T4) were prepared by supplementing herbal calcium supplement (Ayucal D @ 9 g/60 birds/day, Ayurvet Limited, India) as a top-up supplement by mixing uniformly in the basal diet for 10 days in every month of the experimental period and fed to birds under cage (T2) and deep litter system (T4). Remaining 20 days every month, the birds in T2 and T4 were fed with a basal diet without herbal supplement Ayucal D supplementation and systems of rearing did not influence the nutrient utilisation of dry matter, crude protein, ether extract, total ash, metabolisable energy, bone strength and serum mineral concentration. However, the utilisation of calcium and phosphorus improved significantly. The leg health measure scores also significantly improved ( $P < 0.001$ ) in Ayucal D-supplemented birds. Thus, overall results indicated that supplementation of Ayucal D to layer birds reared under cage and deep litter systems improved the nutrient utilisation of dietary calcium and phosphorus without affecting the utilisation of proximate nutrients and energy and thereby improved leg health.*

**Keywords:** Ayucal-D, bone strength, cage, deep litter, layers, nutrients

Calcium (Ca) is one of the essential nutrients required for egg production and eggshell quality of laying hens. Even though several studies have been conducted on the effect of feeding different Ca and phosphorus (P) levels during early, mid or total laying stages, only limited information is available on herbal Ca supplements to diets of commercial layers reared under both cage and deep litter system. Ayucal D (Ayurvet Limited, India) premix is a Ca and P supplement enriched with synergistic herbs. This polyherbal formulation is meant to improve the utilisation of Ca and P. Previous studies on Ayucal liquid and Ayucal premix indicated improved egg specific gravity, eggshell thickness and egg weight, and breaking strength and they were correlated with the activity of herbal constituents of the products owing Ca and P mineralization properties, responsible for enhancing the bio-availability of Ca and P in gizzard (Sharma *et al.*, 2009).

\*Corresponding author: [spramnutri@gmail.com](mailto:spramnutri@gmail.com) Ph. 9491882504

Copyright: © 2024 Daida *et al.* This is an open access article distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Similarly significant increase in serum Ca and P and also improved weight, and length width of the tibia recorded in broiler birds offered Ayucal liquid (Jadhav *et al.*, 2010). The improvement in tibial mineralization was attributed to synergistic herbs present in the Ayucal liquid which could improve mineral absorption (Ashan *et al.*, 1989, Jadhav *et al.*, 2010). The herbal compounds of Ayucal liquid namely *Cissus quadrangularis*, *Lepidium sativum*, *Terminalia arjun* and *Uraria picta* were scientifically proven to increase Ca and P mineralization and influence their mobilization from the skeletal system to maintain the serum Ca and P levels (Ashan *et al.*, 1989, Deka *et al.*, 1994; Prasad *et al.*, 1965). Further, the use of phyto-genic feed additives or herbal plants has recently received much attention as an alternative to traditional feed supplements. However, studies on herbal Ca and P supplementations in layer birds reared under different systems of rearing appear negligible. Thus, the current investigation was made to study the effect of dietary herbal Ca and P supplementation to commercial layers reared under cage and deep litter systems on nutrient utilisation, serum mineral concentration and leg health measures.

## Materials and methods

This study aimed to evaluate the herbal Ca supplements in commercial layers under cage and deep litter systems. During this experiment, 240 commercial layers (*Bovans*) were randomly distributed to 4 experimental groups with 4 replicates per group and each replicate had 15 birds. The birds of the first two groups (T1 & T2) were kept under cage system, while birds of the other two groups (T3 & T4) were kept under a deep-litter system. In both systems of rearing the birds were kept under standard hygienic conditions throughout the experiment. Replicate groups were offered the respective diets *ad libitum*. Clean and fresh drinking water was provided *ad libitum* daily.

A corn soya diet was prepared as a basal diet (Table 1) and fed to birds under cage (T1) and deep litter (T3) system. The remaining two experimental diets were supplemented with herbal Ca supplement (Ayucal D @ 9g/60 birds/day) as a top-up supplement by mixing uniformly in the feed for 10 days every month and fed to birds under cage (T2) and deep litter system (T4).

A metabolic trial was conducted on birds at 62 weeks of age for 3 days as per the standard procedure to estimate nutrient retention. The nutrient content of feed and excreta samples was determined according to AOAC (2005).

At the end of the experiment, blood was collected from one bird from each replicate aseptically from the wing vein of birds using sterilised needles and vacutainers. Serum was collected by centrifuging the blood at 3000 rpm for 10 minutes and transferred to 1.0 mL Eppendorf tubes, which were stored at -20°C for estimation of serum

minerals. The serum Ca and P concentrations were estimated using a semiautomatic biochemical analyzer. Serum Ca was estimated by calorimetric estimation in serum using the O-cresolphthalein complexone (OCPC) method using a commercial kit of M/s Excel Diagnostic Pvt. Ltd and serum inorganic P was estimated using the Molybdate UV method using a commercial kit of GenX Phosphorus-ML.

At the end of the experiment, three birds from each replicate were slaughtered humanely and the tibia bone was separated by removing muscles. Bone weight was recorded using digital weighing balance, bone thickness was measured by using a screw gauge and bone density was recorded by using density balance (Scale-Tec ASTM D 792) with 0.0001 g/cm<sup>3</sup> accuracy.

Leg health measure scores were recorded at the end of the experiment for five birds in each replicate. The scores were awarded according to the scorecard and procedures given by Dawkins *et al.*, (2004) and the

**Table 1.** Ingredients and nutrient composition of basal diet

S. No	Ingredients	Quantity (kg)
1	Maize	622.00
2	Soyabean meal 45%	235.00
3	Deoiled Rice bran	16.00
4	Dicalcium Phosphate	14.00
5	Shell grit	104.00
6	Salt	3.200
7	DL Meth	1.500
8	Trace Minerals	1.000
9	Vitamin Premix	0.500
10	Choline chloride 75%	0.500
11	Toxin Binder	1.000
12	Sodium Bicarbonate	1.500
<b>TOTAL</b>		<b>1000</b>
<b>Nutrient composition</b>		
S. No	Nutrient	
1	M.E (Kcal/kg)	2628
2	Crude Protein (%)	16.5
3	Lysine (%)	0.81
4	Methionine (%)	0.40
5	Calcium (%)	4.04
6	Available phosphorus (%)	0.38

\* Trace mineral provided per kg diet: Manganese -120mg, Zinc -80mg, Iron -25mg, Copper -10mg, Iodine -1mg and Selenium -0.1mg.

\*\* Vitamin premix provided per kg diet: Vitamin A 200000 IU, Vitamin B<sub>2</sub> -25 mg, Vitamin D<sub>3</sub> - 3000IU, Vitamin K -2mg, Riboflavin-25mg, Vitamin B<sub>1</sub> - 1mg, Vitamin B<sub>6</sub> - 2mg, Vitamin B<sub>12</sub> - 40mg and Niacin - 15mg.

percentage of birds under each score was calculated for each treatment.

### Statistical analysis

The data was subjected to one-way analysis of variance in the SPSS software package (version 12, SPSS, Chicago, IL, USA). Means from each replicate were considered as units for statistical analysis. Analysis of variance was used to compare the effect of dietary variation on different dependent variables (Snedecor and Cochran, 1989). The differences between the means were tested by significance using Duncan's multiple range test (Duncan, 1955).

### Results and discussion

Nutrient retention (Table 2) of dry matter, crude protein, ether extract, total ash and metabolizable energy (ME) was not affected by dietary supplementation of Ayucal D to birds reared under both systems. Calcium retention significantly improved in Ayucal D-supplemented groups in both systems of rearing. The P retention among birds fed with or without Ayucal D supplement reared under the cage system was comparable. While Ayucal D supplementation in deep-litter-reared birds significantly improved P retention. The improved ( $P < 0.05$ ) retention of Ca and P in the Ayucal D supplemented group could be due to the presence of synergistic herbs (*Cissus quadrangularis*, *Lepidium sativum*, *Terminalia arjuna* and *Uraria picta*) having potentiating effect on Ca and P utilisation (Pradeep *et al.*, 2016). In line with present findings many reports on the effect of *Cissus quadrangularis* (Bhagath *et al.*, 2009, Jaiswal *et al.*, 2004, Sikarwar *et al.*, 2008 and Unnati, 2011), *Lepidium sativum* (Juma, 2007, Yogesh *et al.*, 2011, Divanji *et al.*, 2012 and Elhal *et al.*, 2013) and *Uraria picta* (Singh *et al.*, 2011) on Ca metabolism and bone mineralisation may substantiate present findings.

Serum Ca and P concentrations (Table 3) were not affected by dietary supplementation of Ayucal D to birds reared under both systems and were comparable among all treatment groups. On the contrary, Jadhav *et*

*al.* (2010) found notable improvement ( $P < 0.05$ ) in serum Ca and P concentrations of broilers supplemented with Ayucal liquid through the drinking water. In the present study, the bone parameters studied *viz.*, bone weight, bone density and bone thickness (Table 4) were unaffected by Ayucal D supplementation in birds reared under both systems. Whereas, Jadhav *et al.* (2010) found significant improvement ( $P < 0.05$ ) in tibia mineralization in broilers supplemented with Ayucal liquid through drinking water. Similarly, Sharma *et al.* (2009) also reported improved shell mineralisation properties in layers supplemented with Ayucal Liquid through drinking water and Ayucal premix through the feed. The improved bioavailability of Ca and P observed in previous studies was due to the presence of synergistic herbs in the Ayucal premix and liquid (Jadhav *et al.*, 2010; Sharma *et al.*, 2009) which improved the utilisation of Ca and P (Ashan *et al.*, 1989). Contrary to previous findings, no effect of Ayucal D on bone mineralisation and bioavailability in this study could be due to feeding this formulation for a shorter duration. Unlike in previous studies, the polyherbal formulation was fed for 10 days a month at a dose of 9 g/60 birds/day, while it was fed throughout the experimental period in the previous experiments.

Leg health measured in terms of per cent of birds falling under different leg health measure scores *viz.*, Gait Score, Hock Score, Pad Score, Angle in, Angle out and Rotation indicated that the Ayucal D supplementation to birds reared under both cage and deep litter systems improved ( $P < 0.001$ ) the scores of leg health measures compared to unsupplemented groups (Table 5). Significantly enhanced leg health scores recorded in Ayucal D-supplemented birds were well attributed to improved ( $P < 0.05$ ) utilisation of dietary Ca and P observed in the present study. The positive response of Ayucal D on leg health could be due to the potentiating effect of polyherbal compounds present in Ayucal D on bone mineralisation (Pradeep *et al.*, 2016).

### Conclusions

The overall study indicated that dietary

**Table 2.** Effect of dietary supplementation of herbal calcium on nutrient retention in White Leghorn layers at 62 weeks of age

Diet	Nutrient Retention (%)						
	Dry Matter	Crude protein	Ether extract	Total Ash	Calcium	Phosphorus	ME
Cage + Basal diet	70.94	71.26	68.99	40.03	44.39 <sup>b</sup>	30.25 <sup>b</sup>	75.27
Cage + Ayucal D	71.50	70.19	71.67	41.40	58.43 <sup>a</sup>	29.81 <sup>b</sup>	70.66
Deep litter + Basal diet	71.37	71.18	71.36	43.03	49.17 <sup>b</sup>	31.76 <sup>b</sup>	66.64
Deep Litter + Ayucal D	71.21	70.38	72.20	41.46	49.93 <sup>a</sup>	40.04 <sup>a</sup>	68.12
<b>SEM</b>	<b>0.735</b>	<b>0.904</b>	<b>0.768</b>	<b>1.020</b>	<b>1.726</b>	<b>1.231</b>	<b>1.384</b>
<b>P value</b>	<b>0.995</b>	<b>0.970</b>	<b>0.489</b>	<b>0.804</b>	<b>0.023</b>	<b>0.003</b>	<b>0.127</b>

<sup>a,b</sup>values with different superscripts in a column differ significantly ( $P < 0.05$ )

**Table 3.** Effect of dietary supplementation of herbal calcium on the serum calcium and phosphorus in White Leghorn layers

Diet	Serum Ca (mg/dl)			Serum P (mg/dl)		
	Period 1 (54-57 wks)	Period 2 (58-61 wks)	Overall (54-61 wks)	Period 1 (54-57 wks)	Period 2 (58-61 wks)	Overall (54-61 wks)
Cage + Basal diet	15.06	14.79	14.94	4.700	4.664	4.688
Cage + Ayucal D	14.48	14.26	14.36	4.698	4.875	4.800
Deep litter + Basal diet	14.71	15.54	15.14	5.039	5.305	5.175
Deep Litter + Ayucal D	15.19	15.53	15.36	4.449	4.531	4.488
<b>SEM</b>	<b>0.479</b>	<b>0.381</b>	<b>0.418</b>	<b>0.365</b>	<b>0.354</b>	<b>0.353</b>
<b>P value</b>	<b>0.957</b>	<b>0.591</b>	<b>0.866</b>	<b>0.959</b>	<b>0.888</b>	<b>0.926</b>

**Table 4.** Effect of dietary supplementation of herbal calcium on the Tibial bone parameters in White Leghorn layers

Diet	Tibial bone weight (g)	Tibial bone density (kg/cm <sup>3</sup> )	Tibial bone Thickness (mm)
Cage + Basal diet	5.264	1.227	1.222
Cage + Ayucal D	5.033	1.191	1.316
Deep litter + Basal diet	5.209	1.231	1.173
Deep Litter + Ayucal D	5.330	1.219	1.261
<b>SEM</b>	<b>0.128</b>	<b>0.010</b>	<b>0.068</b>
<b>P value</b>	<b>0.880</b>	<b>0.551</b>	<b>0.912</b>

**Table 5.** Effect of dietary supplementation of herbal calcium on leg health measure score

Leg health measures	Score	Cage + Basal diet	Cage + Ayucal D	Deep litter + Basal diet	Deep Litter + Ayucal D	SEM	P value
Gait Score	0 (%)	0.00 <sup>b</sup>	100.0 <sup>a</sup>	0.00 <sup>b</sup>	100.0 <sup>a</sup>	12.91	S
	1 (%)	100.0 <sup>a</sup>	0.00 <sup>b</sup>	100.0 <sup>a</sup>	0.00 <sup>b</sup>	12.91	S
	2 (%)	0.00	0.00	0.00	0.00	0.00	NS
Hock Score	0 (%)	0.00 <sup>b</sup>	100.0 <sup>a</sup>	0.00 <sup>b</sup>	100.0 <sup>a</sup>	12.91	S
	1 (%)	100.0 <sup>a</sup>	0.00 <sup>c</sup>	63.00 <sup>b</sup>	0.00 <sup>c</sup>	11.105	S
	2 (%)	0.00 <sup>b</sup>	0.00 <sup>b</sup>	37.00 <sup>a</sup>	0.00 <sup>b</sup>	4.279	S
Pad Score	0 (%)	37.00 <sup>c</sup>	74.75 <sup>b</sup>	0.00 <sup>d</sup>	100.0 <sup>a</sup>	9.902	S
	1 (%)	63.00 <sup>b</sup>	25.25 <sup>c</sup>	100.0 <sup>a</sup>	0.00 <sup>d</sup>	9.902	S
	2 (%)	0.00	0.00	0.00	0.00	0.00	NS
Angle in	0 (%)	0.00 <sup>d</sup>	100.0 <sup>a</sup>	12.50 <sup>c</sup>	75.25 <sup>b</sup>	10.86	S
	1 (%)	75.25 <sup>a</sup>	0.00 <sup>c</sup>	75.00 <sup>a</sup>	24.75 <sup>b</sup>	8.574	S
	2 (%)	24.75 <sup>a</sup>	0.00 <sup>c</sup>	12.50 <sup>b</sup>	0.00 <sup>c</sup>	2.849	S
Angle out	0 (%)	25.00 <sup>b</sup>	100.0 <sup>a</sup>	12.50 <sup>c</sup>	100.0 <sup>a</sup>	10.61	S
	1 (%)	74.75 <sup>b</sup>	0.00 <sup>c</sup>	87.50 <sup>a</sup>	0.00 <sup>c</sup>	10.60	S
	2 (%)	0.00	0.00	0.00	0.00	0.00	NS
Rotation	0 (%)	0.00 <sup>b</sup>	100.0 <sup>a</sup>	0.00 <sup>b</sup>	100.0 <sup>a</sup>	12.91	S
	1 (%)	100.0 <sup>a</sup>	0.00 <sup>b</sup>	100.0 <sup>a</sup>	0.00 <sup>b</sup>	12.91	S
	2 (%)	0.00	0.00	0.00	0.00	0.00	NS

<sup>abcd</sup> values with different superscripts in a row differ significantly (P<0.001)

supplementation of Ayucal D (@9g/60 birds/day) for a shorter duration (10 days in a month) to layer birds reared under cage and deep litter systems improved the utilisation of dietary Ca and P without affecting the utilisation of proximate nutrients and energy. Further, Ayucal D also improved the leg health of the layer birds.

#### Acknowledgements

The authors are thankful to Ayurvet Limited, India for providing the support received during this research work.

**Conflict of interest**

The authors declare that they have no conflict of interest.

**References**

- Ashan, S.K., Tariq, M., Ageel, M. Al-yaha, M.A. and Shah, A.H. 1989. Studies on some herbal drugs used in fracture healing. *Int. J. Crude Drug Res.* **27**: 235-239.
- AOAC 2005. *Official Methods of Analysis* (18<sup>th</sup> Ed.). Association of Official Analytical Chemists International, Gaithersburg, USA.
- Bhagath, K. P., Kumar, M.R., Bhat, M., Rao. S., Gopalan, K.N., Mallikarjuna, R. C., Soubhagya R. N. and Manjunatha, S.M. 2009. Petroleum ether extract of *Cissus quadrangularis* (Linn.) enhances bone marrow mesenchymal stem cell proliferation and facilitates osteoblastogenesis. *Clinics (Sao Paulo)*. **64**(10): 993-998.
- Dawkins, M.S., Donnelly. C.A. and Jones, T.A. 2004. Chicken welfare is more influenced by housing conditions than by stocking density. *Nature*. **427**(22): 342-344
- Deka, D.K., Lahon, I.C., Saikia, J. and Mukti, A. 1994. Effect of *Cissus quadrangularis* in accelerating healing process of experimentally fractured radius-ulna of dog: a preliminary study. *Ind. J. Pharm.* **26**: 44-45.
- Duncan, D. B. 1955. Multiple range and multiple F tests. *Biometrics*. **11**(1): 1-42.
- Divanji M., Viswanatha, G.L., Nagesh, S., Vishal Jain. and Shivaprasad, H.N. (2012). Ethnopharmacology of *Lepidium Sativum* Linn (Brassicaceae): A Review. *Int. J. Phytotherapy Res.* **2**(1): 1-7.
- Elshal, M.F., Almalki, A.L., Hussein, H.K. and Khan, J.A. 2013. Synergistic antiosteoporotic effect of *Lepidium sativum* and alendronate in glucocorticoid-induced osteoporosis in Wistar rats. *Afr. J. Trad. Compl. Altern. Med.* **10**(5): 267-73.
- Jadhav, N.V., Awati, B., Kumar, P., Kartikesh, S.M., Ravikanth, K., Maini, S. and Sood, D. 2010. Role of Ayucal Liquid in improving overall performance and tibial mineralisation in broilers. *Vet. World*. **3**(9): 424-426.
- Jaiswal, S., Singh, S.V., Singh, B. and Singh, H.N. 2004. Plants used for tissue healing of animals. *Nat. Prod. Radiance*. **3**(4): 284-292.
- Juma, A. 2007. The Effects of *Lepidium sativum* seeds on fracture induced healing in Rabbits. *Med. Gen. Med.* **9**(2): 23-29.
- Nascimento, G.R., Murakami, A.E.I., Guerra, A.F.Q.M.I., Ospinas-Rojas, I.C.I., Ferreira M. F.Z.I. and Fanhani, J.C.I. 2014. Effect of different vitamin D sources and calcium levels in the diet of layers in the second laying cycle. *Braz. J. Poul. Sci.* **16**(2): 37-42.
- Olobatoke, R.Y. and Mulugeta, S.D. 2011. Effect of dietary garlic powder on layer performance, fecal bacterial load, and egg quality. *Poul. Sci.* **90**: 665-670
- Pradeep K.N.V., Jadhav, B., Awati., Ravikant., Mani, S. and Adarsh. 2016. Potentiating activity of herbs along with calcium and phosphorous to improve the meat quality parameters in broilers. *Int. J. Sci. Env. Tech.* **5**(3): 1066-1074.
- Prasad, G., Sankaran, P.S. and Deshpande, P.J. 1965. Studies on fracture healing by using radioactive P-32 and Ca-45 under the influence of *Uraria picta*. *Ind. J. Med. Res.* **53**: 645-650.
- Romanoff, A.L. and Romanoff, A.J. 1963. *The Avian egg*. John Wiley and Sons Co., New York.
- Singh, S., Singh, H.N., Gangwar, A.K., Devi K. S., Niyogi D. and Waghaye J.Y. 2011. Effect of *Uraria picta* on bone healing in rabbits: a radiographic, angiographic and histopathological study. *Ind. J. Vet. Surg.* **32**(2): 107-110.
- Sikarwar, R.L.S., Pathak, B. and Jaiswal, A. 2008. Some unique ethnomedicinal perceptions of tribal communities of Chitrakoot, Madhya Pradesh. *Ind. J. Trad. Knowl.* **7**(4): 613-617.
- Sharma, R.K., Ravikanth, K., Maini, S., Rekhe, D.S. and Rastogi, S.K. 2009. Influence of calcium and phosphorus supplements with synergistic herbs on egg shell quality in late layers. *Vet. World*. **6**(2): 231-233
- Snedecor, G.W. and Cochran, W.G. 1980. *Statistical Methods*. Oxford and IBH. Publishing Company, New Delhi. 534p.
- Unnati, S. 2011. *Cissus quadrangularis* I: phytochemicals, traditional uses and pharmacological activities- a review. *Int. J. Pharm. Pharm. Sci.* **3**(4): 41-44
- Yogesh, C.Y., Avijeet, J., Srivastava, D.N. and Anurekha J. 2011. Fracture healing activity of ethanolic extract of *Lepidium sativum* L. seeds in an internally fixed rats' femoral osteotomy model. *Int. J. Pharm. Pharm. Sci.* **3**(2): 193-197. ■