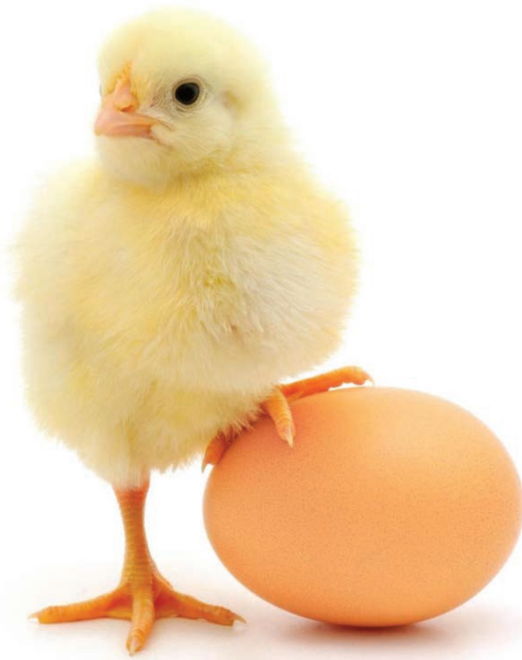
ISSN 1682-8356
ansinet.com/ijps



INTERNATIONAL JOURNAL OF POULTRY SCIENCE

Science Alert
scialert.net

ANSInet
an open access publisher
http://ansinet.com

OPEN ACCESS International Journal of Poultry Science
ISSN 1682-8356
DOI: 10.3923/ijps.2021.209.214

Research Article

Effects of Supplemental Dietary Phytase and 25-Hydroxycholecalciferol on Excreta Characteristics and Nutrient Content from Commercial Layers Inoculated Before or at the Onset of Lay with the F-Strain of *Mycoplasma gallisepticum*

[1]D.M. Miles, [2]S.L. Branton, [3]E.D. Peebles, [4]M.R. Burnham, [1]J.P. Brooks, [5]P.A. Moore, Jr.

[1]Genetics and Sustainable Agriculture Research, USDA-ARS, 810 Hwy, 12 East, Mississippi State, MS 39762, USA
[2]Poultry Research, USDA-ARS, Mississippi State, MS 39762, USA
[3]Department of Poultry Science, Mississippi State University, Mississippi State, MS 39762, USA
[4]Department of Science, Jones College, Ellisville, MS 39437, USA
[5]Poultry Production and Product Safety Research, USDA-ARS, Fayetteville, AR 72701, USA

Abstract

Background and Objective: To sustain commercial egg production, innovative dietary inputs, their interaction with disease control agents and effects on all aspects of the production system and bird health must be investigated. The objective of the current study was to ascertain the effects of F-strain *Mycoplasma gallisepticum* (FMG) inoculation in conjunction with supplemented diets on excreta characteristics and nutrient content as hens age. **Materials and Methods:** Commercial layer diets were supplemented with phytase and 25-hydroxycholecalciferol to determine the effects on excreta from 39-55 week of age for hens that were inoculated (or sham inoculated) with FMG at 12 week (prelay) or at 22 week (onset of laying cycle). Basal vs. supplemented diets, type of inoculation (FMG vs. sham) and age of inoculation were compared. In two trials, layer excreta were collected at 39, 43, 47, 51 and 55 week of age where moisture content, pH, N, C, Ca, K, Mg, Na, P, Cu, Fe, Mn and Zn concentrations were determined. Statistical assessments were performed using a mixed linear model to accommodate both the fixed- and random-effects parameters. **Results:** Fecal moisture, pH, total N, or total C were not affected by diet, inoculation age, or inoculation type. The results showed that fecal moisture content decreased (83-79%) while total N (35.8 to 54.6 g kg^{-1}), total C (317-339 g kg^{-1}), K (22.8-27.1 mg kg^{-1}) and Zn (552-794 mg kg^{-1}) generally increased with hen age. **Conclusion:** Of the studied parameters, only hen age affected these excreta characteristics. Integrators need not be concerned that phytase/25-hydroxycholecalciferol supplemented diets or FMG inoculation will incur manure handling changes relative to nutrient or mineral content.

Key words: Layer, excreta, phytase, F-strain *Mycoplasma gallisepticum*, 25-Hydroxycholecalciferol

Citation: D.M. Miles, S.L. Branton, E.D. Peebles, M.R. Burnham, J.P. Brooks, P.A. Moore, Jr., 2021. Effects of supplemental dietary phytase and 25-hydroxycholecalciferol on excreta characteristics and nutrient content from commercial layers inoculated before or at the onset of lay with the F-strain of *Mycoplasma gallisepticum*. Int. J. Poult. Sci., 20: 209-214.

Corresponding Author: D.M. Miles, Genetics and Sustainable Agriculture Research, USDA-ARS, 810 Hwy, 12 East, Mississippi State, MS 39762, USA Tel: 662-320-2024 Fax: 662-320-7569

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Mycoplasma gallisepticum (MG) is a significant avian bacterial pathogen causing severe production and economic losses to the poultry industry worldwide. Specifically, these impacts are reduced egg production, hatchability, poor feed conversion, carcass condemnation and reduced weight gain[1]. Evans *et al.*[1] covered the salient characteristics of this chronic respiratory disease in chickens and infectious sinusitis in turkeys. Means of MG control are currently limited to biosecurity and using live attenuated vaccines (LAVs) which are licensed for use in commercial egg layer flocks. Commercially available LAVS vary in the protection afforded and level of virulence associated with the LAV. The F strain of MG (FMG) is a popular LAV which is of low to moderate virulence but can protect against losses associated with MG field strain infections[2]. However, FMG can also negatively impact FMG-infected avian hosts although means responsible for these losses are not fully understood. Though FMG aids in minimizing production losses compared to the wild-type strains, researchers strive to elucidate the causes of reduced egg production and any interaction with production parameters. Unlike FMG, apathogenic strains of MG used in vaccines do not result in reduced egg production but have not been proven to displace the more virulent, wild-type infections[3].

Most of the available literature surrounding MG and layer manure focuses on MG-related transmissibility, survivability, biosecurity, hygiene and the proximity of backyard flocks to commercial facilities[4-7]. Here the focus was on fecal characteristics with supplemented diets in conjunction with FMG vaccination. Relative to the poultry diet, the phosphorus (P) in yellow dent corn is primarily phytate bound; phytase (PHY) enzyme added to poultry diets increases the digestibility of phytate bound P and can reduce needed inorganic P inputs[8]. In broiler chickens, Edwards *et al.*[9] found that 25-hydroxycholecalciferol (25-D3) produced maximum bone ash with two-thirds the input of dietary Ca. Further, when phytase (PHY) was present, broilers had greater Ca retention and greater body weight[10]. Again, for broiler chickens, relative to PHY inclusion in diets, the literature[11] demonstrated improved performance, especially at higher rates (>500 FTU kg^{-1}) and that inclusion is common. For these reasons, Peebles *et al.*[2,12,13] examined the effects of a PHY and 25-D3 supplemented diet in combination with FMG inoculations given before onset of lay (12 week) and at onset of lay (22 week) on mortality, body weight and blood parameters[2]; digestive and reproductive organ characteristics[12] and the performance characteristics[13] of commercial layer chickens.

Briefly, the major findings of those companion studies[2,12,13] include the following. Peebles *et al.*[2] noted decreased blood hematocrit values for the PHY, 25-D3 supplemented diet (across hen age, inoculation type and age of inoculation) along with a decreased body weight in sham-inoculated birds (across bird age and age of inoculation). However, FMG inoculation alleviated that decrease in body weight. Also, total plasma protein at 34 week of age was greater for hens inoculated at onset of lay (22 week) vs prelay (12 week). In the second companion report, Peebles *et al.*[12] discussed digestive and reproductive organ characteristics of hens at 58 week of age and found that the overall timing of inoculation affected the reproductive organs of the bird but FMG inoculation specifically affected digestive organs. Without consideration of inoculation type or age at inoculation, the supplemented diet affected both the reproductive and digestive organs (percentage infundibulum length and isthmus weight increased but duodenum length decreased). In the final companion article, Peebles *et al.*[13] reported the performance characteristics of layers and showed no treatment effects of any kind on total egg production, egg weight, or any internal egg or eggshell characteristics.

The objective of this investigation was to determine the fecal characteristics (moisture content and pH), nutrient content (total N and total C) and mineral composition (Ca, K, Mg, Na, P, Cu, Fe, Mn and Zn) of excreta from commercial layers from 39-55 week of age for hens that were inoculated with F-strain *Mycoplasma gallisepticum* (or sham inoculated) at 12 week of age (prelay) or at 22 week of age (onset of laying cycle), while comparing a basal diet to a diet supplemented with PHY and 25-D3. The aim was achieved through analysis of more than 200 manure samples for the above given 13 parameters. The expanse of the data lends itself for comparison to broiler litter characterization, providing a resource for animal agriculture, production system modeling and national inventories.

MATERIALS AND METHODS

Bird management: An approved USDA Animal Care and Use protocol was used in all trials. In the pullet stage prior to the onset of treatments, Hy-Line W-36 chickens were vaccinated, reared on basal starter and grower diets[2] and tested for *Mycoplasma gallisepticum* and *Mycoplasma synoviae.* The specificity of these tests, materials and procedures have been fully described by Peebles *et al.*[2] No medications were given during any of the trials and all diets were created to meet or surpass NRC[14] requirements.

In each of two trials, birds (n =240) were individually and randomly placed into cages in a commercial layer facility, divided into isolated ends for sham-inoculated control (n = 120) and FMG-inoculated birds (n = 120). Birds were sham-inoculated or inoculated at 12 or 22 week of age in each end of the facility. Birds that were inoculated for FMG at 22 week of age remained in the control end until that time. The experimental diets, a basal diet or the basal diet supplemented with PHY (0.025%; 600 FTU phytase units kg^{-1} of diet; BASF, Corp., Florham Park, NJ) and 25-D3 (diluted premix, 0.025%; pure crystalline, 34.5 ug kg^{-1} of diet; Hoffman-La Roche Inc., Parsippany, NJ), began at 20 week of age and continued through 58 week of age. There were three replicates for each treatment combination (diet and age of inoculation) where each replicate was comprised of 10 individually caged birds. Dietary formulations, particulars of housing and inoculation methods were provided by Peebles *et al.*[2]

Data collection: At 39, 43, 47, 51 and 55 week of age, fecal samples were collected by placing 0.61 $\times$ 0.91 m stainless steel trays under the middle portion of the cages belonging to each replicate group. Trays were placed on Monday of each sampling age and the collection period was 48 hours. Upon collection, fecal samples were scraped off each collection tray into an aluminum pan for obtaining total wet weight. Pans were placed in pre-labeled plastic bags and frozen at -20°C until analysis. Samples were thawed overnight before moisture content was determined by loss in weight after oven drying manure (85°C for 96 h); pH was found using a deionized H_2O-to-manure ratio of 5:1. Total N and C were ascertained via combustion analysis (Max CN analyzer, Elementar Americas, Inc., Mt. Laurel, NJ, U.S.).

In trial 1 only and for each replication and sampling date, the manure mineral content (Ca, K, Mg, Na, P, Cu, Fe, Mn and Zn) was determined via inductively coupled plasma (ICP) by first dry ashing a 1 g fine-ground sample in a ceramic crucible at 500 C for 4 h. Dissolution of the ash followed in 1 mL of 6 M HCl for 1 h. A second dissolution for an additional hour was processed in 40 mL of 0.125 M H_2SO_4 and 0.05 M HCl double acid solution. The solution within the crucible was filtered through Whatman no. 2 paper. The filtrate was then analyzed by ICP for mineral content.

Statistical analysis: A randomized complete block experimental design was utilized with trial as block. Data was pooled from the two trials and analyzed together except for mineral content that was only measured in trial 1. The treatment structure was a split-plot where the whole-plot factor was inoculation type and the sub-plot factors were diet and age of inoculation. Fecal moisture content, pH, total N, total C, Ca, K, Mg, Na, P, Cu, Fe, Mn and Zn at four-week intervals for 39-55 week of age were analyzed as repeated measures because the same experimental units were used for each observation. Trial was a random effect and diet-basal vs. basal supplemented with PHY and 25-D3; inoculation age-12 vs. 22 week and inoculation type-sham vs. FMG treatments were fixed effects. The MIXED procedure of SAS[15] was used to conduct the analyses of the main and interactive effects of the treatments with significant differences based on $p \leq 0.05$.

RESULTS AND DISCUSSION

Table 1 shows the fecal moisture content, pH, total N and total C at 39, 43, 47, 51 and 55 weeks of age. The studied factors (PHY and 25-D3 supplemented vs. basal diet), age of inoculation (12 or 22 week) and type of inoculation (sham vs. FMG) had no significant main or interactive effects on the fecal characteristics and nutrient contents examined. Only hen age affected the measured parameters. Fecal moisture content overall decreased with age. Similar moisture contents among all the samples at 39, 43 and 47 week were 83.3, 82.3 and

Table 1: Layer excreta moisture content, pH, total N and total C concentrations at 39, 43, 47, 51 and 55 weeks of age for birds inoculated or sham inoculated with F-strain *Mycoplasma gallisepticum*

	Hen age (week)				
	39	43	47	51	55
Moisture (%)	83.3^{a*}	82.3^{a}	82.8^{a}	80.8^{b}	79.0^{b}
	(0.598)**	(0.598)	(0.598)	(0.613)	(0.603)
pH	6.92^{ab}	6.90^{ab}	6.81^{b}	6.90^{ab}	6.99^{a}
	(0.057)	(0.074)	(0.057)	(0.058)	(0.057)
N (g kg^{-1})	36.5^{c}	35.8^{c}	38.3^{c}	43.8^{b}	54.6^{a}
	(1.69)	(1.69)	(1.69)	(1.75)	(1.71)
C (g kg^{-1})	323^{ab}	317^{b}	339^{a}	335^{a}	334^{a}
	(5.89)	(5.89)	(5.89)	(6.10)	(5.96)

*Letters a-c denote significant differences within each row based on $p \leq 0.05$. **Numbers in parenthesis below each mean represent standard error. Nutrient concentrations are on dry weight basis

Table 2: Mineral content of layer excreta at 39, 43, 47, 51 and 55 weeks of age for birds inoculated or sham inoculated with F-strain *Mycoplasma gallisepticum*

	Hen age (week)				
	39	43	47	51	55
Ca (g kg^{-1})	54.4	69.0	55.5	49.9	57.7
	(3.21)**	(3.11)	(3.11)	(3.30)	(3.21)
K (g kg^{-1})	24.8^{bc}	22.8^{c}	25.5^{ab}	27.1^{a}	26.3^{a}
	(0.77)	(0.77)	(0.77)	(0.81)	(0.79)
Mg (g kg^{-1})	5.88	5.46	5.67	6.58	6.55
	(0.097)	(0.097)	(0.097)	(0.103)	(0.100)
Na (g kg^{-1})	5.74	5.44	5.72	8.07	7.66
	(0.20)	(0.20)	(0.20)	(0.21)	(0.20)
P (g kg^{-1})	24.1	23.3	23.7	23.5	22.3
	(0.44)	(0.44)	(0.44)	(0.47)	(0.46)
Cu (mg kg^{-1})	26.7^{c}	28.7^{bc}	33.5^{a}	30.1^{b}	29.9^{b}
	(0.98)	(0.98)	(0.98)	(1.04)	(1.01)
Fe (mg kg^{-1})	1128	1312	1178	1083	1173
	(22.1)	(22.1)	(22.1)	(23.4)	(22.7)
Mn (mg kg^{-1})	305	353	341	274	282
	(4.70)	(4.70)	(4.70)	(4.98)	(4.84)
Zn (mg kg^{-1})	552^{b}	711^{a}	713^{a}	740^{a}	794^{a}
	(32.7)	(32.7)	(32.7)	(32.7)	(32.8)

*Letters a-c denote significant differences within each row based on $p \leq 0.05$. **Numbers in parenthesis below each mean represent standard error. Mineral concentrations are on dry weight basis

82.8% respectively. At 51 and 55 week, moisture content was lower than the previous measurements being 80.8 and 79.0% but was not significantly different from each other. Manure pH was least at 47 week (6.81) and greatest at 55 week (6.99) and was similar among the remaining samples at the other measurement dates. Total N in the fecal samples increased overall with hen age. The samples were similar at 39, 43 and 47 week of age, having values of 36.5, 35.8 and 38.3 g kg^{-1} N. Values increased to 43.8 g kg^{-1} N at 51 week and further increased to 54.6 g kg^{-1} N at 55 week of age. The results for total C in the manure trended higher for the later 3 sampling ages. At 47, 51 and 55 week, total C did not appear different among the samples and ranged from 334-339 g kg^{-1}. At 43 week, total C was the lowest at 317 g kg^{-1} but was not different from the previous time of sampling at 39 week, when total C was 323 g kg^{-1} (Table 1). Also, total C at 39 week was not statistically different among the samples at 47, 51 and 55 week of age.

Mineral contents of the layer excreta is reported in Table 2 for hens at 39, 43, 47, 51 and 55 week of age. There was a diet by hen age interaction for Ca ($p = 0.003$), Mg ($p = 0.0266$), Na ($p = 0.0002$), P ($p = 0.0029$) and Fe ($p = 0.0227$). For Mn, there was a diet by inoculation type interaction ($p = 0.0116$). Because of these interactions and that only one trial measured the mineral contents of the layer manure, further discussion of these minerals is presented within the various concentration ranges. The range of each concentration (±standard deviation) in the manure was: Ca (57.3±7.1 g kg^{-1}); Mg (6±0.5 g kg^{-1}); Na (6.5±1.2 g kg^{-1}); P (23.4±0.7 g kg^{-1}); Fe (1223±136 mg kg^{-1}) and Mn (311±35 mg kg^{-1}). For K, Cu and Zn, hen age as a main effect was noted but the trends were not consistent. Over the entire measurement period, the range of K was 25.3±1.6 g kg^{-1}. At 51 and 55 week of age, K content of the manure was the highest, which was followed by that at 47 week. However, the concentration of K at 39 week was not different from 47 week. The lowest concentration of K was discovered at 43 week (22.8 g kg^{-1}) but the value was not statistically different from the previous value at 39 week. The excretion of Cu exhibited a peak at the middle age of 47 week but was lower at the prior two sampling dates (39 and 43 week). A mid-range excretion level was exhibited during the last two sampling dates (51 and 55 week). The overall range for Cu in layer excreta was 29.8±2.5 mg kg^{-1}. Finally, the trend for Zn in the manure samples was again different, where the lowest value (552 mg kg^{-1}) was observed at 39 week. Zn levels in the remaining samples increased as hens aged and were in the range of 711-794 mg kg^{-1}. The overall average Zn in layer excreta was 702±90 mg kg^{-1}.

The above data shows significant changes in excreta characteristics and nutrient content as hens age. Layer hen manure found in the literature was presented as characteristics and quantity removed from the houses. Chastain *et al*.[16] reported two management scenarios: high-rise deep-pit (HRDP) stored on an unpaved surface with duration of storage not reported and under cage scraped (UCS) stored on a paved surface that removed manure every two days. The HRDP had moisture content (47%) and when converted to consistent units, total N (17 g kg^{-1}), Ca (38 g kg^{-1}), Mg (2.9 g kg^{-1}), Na (1.7 g kg^{-1}), Cu (29 mg kg^{-1})

and Zn (175 mg kg^{-1})[16]. The UCS had moisture content (65%), total N (14 g kg^{-1}), Ca (21 g kg^{-1}), Mg (2.8 g kg^{-1}), Na (1.4 g kg^{-1}), Cu (17 mg kg^{-1}) and Zn (155 mg kg^{-1})[16]. Both the HRDP and the UCS reported approximately half of the total N and lower mineral content as compared to the current study. This may be due to different dietary inputs and the means by which the elements were analyzed (i.e. dry vs wet weight basis), both of which are unknown.

Manure is excreted as moist but dries out over time. The HRDP[16] had the least moisture content at 47%, which may be due to the duration of storage and storage on an unpaved surface. By comparison, Lorimor and Xin[17] reported 41% manure moisture for high-rise layer manure collected monthly for a year but that scraper systems in Iowa had lower moisture (29.8%). In contrast, the UCS[16] that had 65% moisture content was stored on a paved ally and scraped every two days, which is the same as the 48 h collection period (in the present study). However, the UCS had roughly 15% less moisture content in the manure than the moisture observed in the present study. That may be due to sampling and excess moisture runoff on the ally surface, whereas, the current study captured the entirety of the manure and moisture content on pans for the 48 h collection period. Lower fecal moisture is important for lowering ammonia volatilization[18] and to control fly and odor in commercial facilities. The current study demonstrated a decrease in fecal moisture as the hens aged but the manure handling facility would be of paramount importance. A good review of dietary factors that do increase fecal moisture was reported by Francesch and Brufau[19] for the interested reader.

Because of the vast differences between the production systems of layers and broiler chickens, the global concerns are to manage resources effectively. There is a need to develop accurate production models, making it interesting to compare the excreta characteristics between the two systems. Miles *et al.*[20] reported the mineral composition of litter in U.S. commercial broiler houses. It was found that 23.7$\pm$8.6 g kg^{-1} Ca existed in broiler litter, which is approximately half of the Ca reported in the present study for layer hens. It was due to the importance of Ca in egg quality and production. Broilers produced more K (35.4$\pm$9.1 g kg^{-1}), Mg (8.1$\pm$2.4 g kg^{-1}), Na (10.9$\pm$3.3 g kg^{-1}), Cu (730$\pm$360 mg kg^{-1}) and Mn (730$\pm$216 mg kg^{-1}) but less P (16.6$\pm$4.6 g kg^{-1}) and Zn (550$\pm$150 mg kg^{-1}) in their excreta than layers. However, Fe (1120$\pm$560 mg kg^{-1}) concentrations in broiler and layer excreta were similar. The basis for these differences lie in the dietary needs of broilers in reaching maturity within a few weeks compared to the long-term maintenance requirements of layers in the production facility, which is approximately 10 times longer.

The FMG vaccination during the pullet period have variable effects on layer performance. Branton *et al.*[21] observed that a 10 week inoculation had no effect on reproductive performance and egg production of hens but Peebles *et al.*[22] reported that an FMG inoculation at 12 week of age significantly reduced egg production between 18-26 week of age. Peebles *et al.*[22] also reported that 1.5% supplemental dietary poultry fat alleviated reductions in early egg production due to an FMG inoculation at 12 week of age. These results indicated the potential benefits of dietary intervention strategies to control negative effects of FMG inoculation given during the pullet period.

More recently, Peebles *et al.*[2,12,13] conducted the studies in which the same protocol and birds were used, and it was reported that there were no significant differences among the inoculation age, inoculation type, or dietary treatments for mortality, reproductive organ histopathological lesion scores, serum cholesterol and Ca concentrations. Peebles *et al.*[13] reported that the diet supplemented with PHY and 25-D3 did not affect layer performance or interact with FMG inoculation and FMG did not have an overall effect on total egg production. Similarly, a recent Brazilian study found little effect on egg quality whether a double (at 8 and 12 weeks) or single (at 8 weeks) ocular FMG vaccination was administered[23].

CONCLUSION

Hen age is not considered as the factor for mineral excretion of Ca, Mg, Na, P, Fe, and Mn because of significant interactions with the experimental treatments. Further studies would be required to resolve significant changes in excreta concentrations of minerals. Although trends were not entirely consistent, in general, the highest concentrations of K, Cu and Zn occurred for the older hens at 47, 51 and 55 week of age. Fecal moisture generally decreased with age while total N and total C increased. The lack of effect of the primary treatment factors suggests that layer hens were not negatively impacted by the imposed dietary or FMG inoculation treatments.

SIGNIFICANCE STATEMENT

This study discovered the effect of hen age on manure characteristics that can be beneficial for flock management and waste handling. Moreover, it provided a data resource for national manure inventories. The study will help the researcher to uncover the critical areas of excreta changes relative to PHY and 25-D3 supplemented diets with FMG inoculation that many researchers were not able to explore. Thus, a new theory on layer MG, dietary and manure management may be arrived at.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the efforts of Rhonda Cornelius in collecting, processing and analyzing the very large number of fecal samples.

REFERENCES

1. Evans, J.D., S.A. Leigh, S.L. Branton, S.D. Collier, G.T. Pharr and S.M.D. Bearson, 2005. *Mycoplasma gallisepticum*: Current and developing means to control the avian pathogen. J. Applied Poult. Res., 14: 757-763.
2. Peebles, E.D., S.L. Branton, M.R. Burnham, S.K. Whitmarsh and P.D. Gerard, 2007. Effects of supplemental dietary phytase and 25-hydroxycholecalciferol on the blood characteristics of commercial layers inoculated before or at the onset of lay with the F-strain of *Mycoplasma gallisepticum*. Poult. Sci., 86: 768-774.
3. Jacob, R., S.L. Branton, J.D. Evans, S.A. Leigh and E.D. Peebles, 2014. Effects of live and killed vaccines against *Mycoplasma gallisepticum* on the performance characteristics of commercial layer chickens. Poult. Sci., 93: 1403-1409.
4. Mohammed, H.O., 1990. A multivariate indexing system for hygiene in relation to the risk of *Mycoplasma gallisepticum* infection in chickens. Preventive Vet. Med., 9: 75-83.
5. Halvorson, D.A., 2011. Biosecurity on a multiple-age egg production complex: A 15-year experience. Avian Dis., 55: 139-142.
6. Adell, E., S. Calvet, A. Pérez-Bonilla, A. Jiménez-Belenguer, J. García, J. Herrera and M. Cambra-López, 2015. Air disinfection in laying hen houses: Effect on airborne microorganisms with focus on *Mycoplasma gallisepticum*. Biosyst. Eng., 129: 315-323.
7. Derksen, T., R. Lampron, R. Hauck, M. Pitesky and R.A. Gallardo, 2018. Biosecurity assessment and seroprevalence of respiratory diseases in backyard poultry flocks located close to and far from commercial premises. Avian Dis., 62: 1-5.
8. Huff, W.E., P.A. Moore Junior, P.W. Waldroup, A.L. Waldroup and J.M. Balog *et al.*, 1998. Effect of dietary phytase and high available phosphorus corn on broiler chicken performance. Poult. Sci., 77: 1899-1904.
9. Edwards, H.M., M.A. Elliot and S. Sooncharernying, 1992. Effect of dietary calcium on tibial dyschondroplasia. interaction with light, cholecalciferol, 1,25-dihydroxycholecalciferol, protein and synthetic zeolite. Poult. Sci., 71: 2041-2055.
10. Edwards, Jr. H.M., 1993. Dietary 1, 25-dihydroxycholecalciferol supplementation increases natural phytate phosphorous utilization in chickens. J. Nutr., 123: 567-577.
11. Sharma, N.K., M. Choct, S. Wu, R. Smillie and N. Morgan *et al.*, 2016. Performance, litter quality and gaseous odour emissions of broilers fed phytase supplemented diets. Anim. Nutr., 2: 288-295.
12. Peebles, E.D., S.L. Branton, M.R. Burnham, S.K. Whitmarsh and P.D. Gerard, 2007. Effects of supplemental dietary phytase and 25-hydroxycholecalciferol on the digestive and reproductive organ characteristics of commercial layers inoculated before or at the onset of lay with the f-strain of *Mycoplasma gallisepticum*. Poult. Sci., 86: 1805-1809.
13. Peebles, E.D., S.L. Branton, M.R. Burnham, S.K. Whitmarsh and P.D. Gerard, 2008. Effects of supplemental dietary phytase and 25-hydroxycho-lecalciferol on the performance characteristics of commercial layers inoculated before or at the onset of lay with the F-strain of *Mycoplasma gallisepticum*. Poult. Sci., 87: 598-601.
14. NRC., 1994. Nutrient Requirements of Poultry. 9th Edn., National Academy Press, Washington, DC., USA., ISBN-13: 9780309048927, Pages: 155.
15. SAS., 2003. SAS System for Microsoft Windows, Version 9.1 (TSM1). SAS Institute Inc., Cary, NC., USA.
16. Chastain, J.P., J.J. Camberato and P. Skewes, 2002. Poultry Manure Production & Nutrient Content. In: Poultry Training Manual, Smith, B., B. Beer, J.P. Chastain, J. Helm and S. Henry *et al*, (Eds.). Clemson University, Clemson, South Carolina, Pages: 17.
17. Lorimar, J.C. and H. Xin, 1999. Manure production and nutrient concentrations from high-rise layer houses. Applied Eng. Agric., 15: 337-340.
18. Elliott, H.A. and N.E. Collins, 1982. Factors affecting ammonia release in broiler houses. Trans. ASAE, 25: 413-424.
19. Francesch, M. and J. Brufau, 2004. Nutritional factors affecting excreta/litter moisture and quality. Worlds Poult. Sci. J., 60: 64-75.
20. Miles, D.M., J.P. Brooks and P.A. Moore, 2018. Mineral composition of litter in commercial broiler houses. Int. J. Poult. Sci., 17: 85-91.
21. Branton, S.L., B.D. Lott, J.D. May, W.R. Maslin, C.R. Boyle and G.T. Pharr, 1997. The effects of F-strain *Mycoplasma gallisepticum*, *Mycoplasma synoviae* and the dual infection in commercial layer chickens over a 44-week laying cycle when challenged before beginning of lay. I. Egg production and selected egg quality parameters. Avian Dis., 41: 832-837.
22. Peebles, E.D., S.L. Branton, M.R. Burnham and P.D. Gerard, 2003. Influences of supplemental dietary poultry fat and F-strain *Mycoplasma gallisepticum* infection on the early performance of commercial egg laying hens. Poult. Sci., 82: 596-602.
23. Machado, L.D., F.F. d Santos, C.K. Togashi, D.L.D. Abreu and J.C. Pimentel *et al.*, 2017. Egg quality in laying hens exposed to *Mycoplasma gallisepticum* f-strain attenuated vaccine. Poult. Sci., 96: 976-979.