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Influence of Supplemental Dietary Poultry Fat, Phytase and 25-hydroxycholecalciferol on the Blood Characteristics of Commercial Layers Inoculated Before or at the Onset of Lay with F-strain *Mycoplasma gallisepticum*^{1,2}

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Abstract: The effects of 2 supplemental levels of dietary Poultry Fat (PF) and the combination of PF, phytase (PHY) and 25-hydroxycholecalciferol [25(OH)D] on the blood characteristics of commercial layers inoculated with F-strain *Mycoplasma gallisepticum* (FMG) were investigated in 2 trials. Sham and FMG inoculations were administered at 12 (before lay) and 22 (early in lay) wk and dietary treatments [Basal Control Diet (BCD); BCD with 0.75% supplemental PF (LPFD); BCD with 1.50% supplemental PF (HPFD); HPFD additionally supplemented with 0.013% PHY and 0.025% 25(OH)D] were initiated at 20 wk of age. Whole blood hematocrit and plasma protein, serum cholesterol, serum triglycerides and serum calcium concentrations were determined at 24, 34, 44, 50 and 58 wk of age. There were no treatment effects on any of the parameters examined except for serum triglyceride and calcium concentrations. Regardless of the age at which the layers were inoculated (12 or 22 wk), when compared with a sham-inoculation, an FMG inoculation reduced the serum concentrations of triglycerides at 24 wk of age and calcium at 34 wk of age, but subsequently increased serum calcium concentrations at 58 wk of age. In conclusion, inoculation with FMG before or early in lay resulted in decreases in serum triglycerides early in lay and respective decreases and increases in serum calcium concentrations during the peak and late periods of lay. Furthermore, the use of 1.50% supplemental dietary PF alone or in combination with supplementary PHY and 25(OH)D had no effect on the blood parameters investigated throughout lay in birds that did or did not receive FMG inoculations at 12 or 22 wk of age.

Key words: Blood, diet, F-strain *Mycoplasma gallisepticum*, inoculation, layer

INTRODUCTION

To protect commercial layer flocks against natural *Mycoplasma gallisepticum* (MG) infections, live MG vaccines produced from the F-strain of MG (FMG) have become available (Branton *et al.*, 1997). However, Burnham *et al.* (2002a) reported that subsequent to an inoculation of FMG at 12 wk of age, commercial layer flocks experienced a reduction in Egg Production (EP) and a 1 wk delay in initiation of lay. Ovarian and reproductive tract regression, onset of fatty liver hemorrhagic syndrome (Burnham *et al.*, 2002b), prepeak EP decreases in total yolk lipid and cholesterol concentrations (Burnham *et al.*, 2003b) and postpeak EP decreases in Serum Triglyceride (STRIG) and Plasma Protein (PP) concentrations (Burnham *et al.*, 2003a) also occurred in these same birds in response to the inoculation of FMG on wk 12.

Upon examining the effects of 1.50% supplemental dietary Poultry Fat (PF) on the blood characteristics of layers inoculated with FMG at 12 or 22 wk of age, Peebles *et al.* (2009a) found that a wk 12 inoculation procedure (sham or FMG) or an inoculation of FMG on

wk 22 reduced Serum Calcium Concentrations (SCA) during peak EP. Conversely, SCA was increased late in lay in response to an FMG inoculation given at 22 wk of age. Furthermore, the inoculation of FMG (12 or 22 wk of age) reduced STRIG during peak EP and Serum Cholesterol (SCHOL) and PP were influenced by an interaction between diet [Basal Control (BCD) and BCD supplemented with 1.50% PF (HPFD)] and the age at which inoculations were administered. In a separate study in which effects of the diet supplement combination of 0.025% phytase (PHY) and 25-hydroxycholecalciferol [25(OH)D] on the blood profiles of layers inoculated with FMG at 12 or 22 wk of age were examined, Peebles *et al.* (2007) found that PHY lowered whole blood Hematocrit (HCT) in all sham- and FMG-inoculated birds throughout lay and that PP at 34 wk of age was higher in birds inoculated (sham or FMG) prelay than proximate to the onset of lay.

The performance and egg characteristics of the birds in the current study have been reported in earlier companion articles. Park *et al.* (2010) noted that changes in the BW and feed consumption of these birds

subsequent to their intake of added dietary PF were influenced by their age and the age at which they were inoculated. Additionally, Peebles *et al.* (2010) concluded that when supplementary PHY and 25(OH)D was used in combination with added 1.50% PF in the diets of birds inoculated on wk 22, the PHY and 25(OH)D prevented an increase in percentage of yolk weight that occurred in response to diets supplemented only with 1.50% PF. In consideration of the aforementioned results, the potential interactive influences of supplementary PF and of PHY and 25(OH)D on the blood characteristics of layers inoculated with FMG were investigated in this study. Sham and FMG inoculations were administered prelay (12 wk of age) and proximate to lay onset (22 wk of age), 0.75 or 1.50% levels of supplemental PF were used in 2 diets and a diet supplemented with 1.50% PF in combination with PHY and 25(OH)D was used.

MATERIALS AND METHODS

General: In each of 2 trials, 1-d-old Single Comb White Leghorn pullets of a single genetic strain (Hy-line variety W-36) were obtained from a commercial hatchery certified free of MG and *Mycoplasma synoviae* (USDA-APHIS-VS, 2003) and both trials were subsequently conducted under an approved USDA animal care and use protocol. Details of pullet management, vaccination and tests for *Mycoplasma* species presence were as described by Peebles *et al.* (2003). At 12 and 22 wk of age in each trial, 120 sham- (control) and 120 FMG- (treated) inoculated birds were randomly assigned to individual cages in 1 of 2 enclosed and isolated ends of a caged layer facility according to inoculation treatment. Pullets treated with FMG were inoculated via eye drop in the right eye at either 12 or 22 wk of age with 0.04 mL of a 24-h broth culture of high-passage FMG (Park *et al.*, 2010). At 12 and 22 wk in both trials, titers of the inocula were similarly determined as described by Peebles *et al.* (2003) and were 1.0×10^{10} cfu/mL (Park *et al.*, 2010). Pullets designated as controls at both 12 and 22 wk were sham-inoculated via eye drop in the right eye at 12 wk of age with 0.04 mL of sterile Frey's broth medium. Further testing for the presence of MG in sham- and FMG-inoculated birds at wk 20 and 58 were as described by Park *et al.* (2010).

In both trials, 4 isocaloric and isonitrogenous treatment layer diets were randomly provided to birds within each end of the layer house beginning at 20 wk of age, with all dietary treatments assigned to birds belonging to each inoculation type (sham- or FMG-inoculated) and inoculation age (12 or 22 wk) treatment combination. One diet was a BCD (contained 0.5% total PF), a second diet was a BCD supplemented with 0.75% PF (LPFD; contained 1.25% total PF), a third diet was an HPFD (contained 2.00% total PF) and a fourth diet was an

HPFD additionally supplemented with PHY (0.013%) and 25(OH)D (premix, 0.025%). There were 3 replicate groups (10 birds per replicate group) for each inoculation type, inoculation age and dietary treatment combination. Layer management, as well as the ingredient descriptions and percentages and calculated and determined analyses of all 4 diets are provided by Park *et al.* (2010). All pullet and layer diets were formulated to meet or exceed National Research Council (1994) specifications.

Data collection: In each trial, 2 tagged hens per replicate group were bled from the left *cutanea ulnae* wing vein. Blood was drawn and harvested at the same time of day at 24, 34, 44, 50 and 58 wk of age. Variables measured in each trial were HCT, PP, SCHOL, STRIG and SCA.

Analyses of blood and serum constituents: Hematocrit was expressed as percentage packed blood cell (primarily red blood cell) volume and was determined through use of capillary tubes that were centrifuged in a micro-HCT centrifuge and then read with a microcapillary reader. Serum cholesterol and STRIG expressed in mg per dL and PP expressed in g per dL were determined by placing 10 μ L of serum or plasma for each test on test slides, which were analyzed on a Kodak Ektachem DT-60 analyzer (Eastman Kodak Co., Rochester, NY) as described by Latour *et al.* (1996). Similarly, SCA concentrations expressed in mg per dL were determined by placing 10 μ L of serum on a test slide which was analyzed on a Kodak Ektachem DTSC module analyzer (Eastman Kodak Co., Rochester, NY), according to procedures of Tietz (1986). Control analyses were performed to assure that each sample was in the appropriate test range for accurate analysis.

Statistical analysis: A randomized complete block experimental design with trial as the block was utilized. The data of both trials were pooled then analyzed together. Therefore, results from trials 1 and 2 were not reported independently but were reported over both trials. Individual sample data within each replicate group were averaged prior to data analysis. All data were subjected to a repeated measures analysis to assess the effects of diet, inoculation type and inoculation age over multiple age periods. In the analysis, diet, inoculation type, inoculation age and hen age were designated as fixed effects and trial as a random effect. Comparisons of means were by Fisher's (protected) lsd in the event of significant global effects (Steel and Torrie, 1980). All data were analyzed using the MIXED procedure of SAS software (SAS Institute, 2003). Global effects and means separations were considered significant at $p \leq 0.05$.

Table 1: Serum Triglyceride (STRIG) and Calcium (SCA) concentrations within type of inoculation (sham and FMG) across age of inoculation and dietary treatment at 24, 34, 44, 50 and 58 wk of hen age¹

Type of inoculation	STRIG ²		SCA ³	
	Sham	FMG	Sham	FMG
Weeks of age	----- (mg/dL) -----			
24	3.988 ^a	2.671 ^b	18.9	18.3
34	2.994	2.729	27.4 ^a	20.4 ^b
44	2.472	2.358	17.8	20.7
50	2.855	3.053	20.3	19.9
58	2.722	2.606	17.8 ^b	23.7 ^a

^{a,b}Means among type of inoculation for each parameter within a row (wk of age) with no common superscript differ ($p \leq 0.05$).

¹Blood samples from 2 birds within each of 48 replicate units were used for the calculation of the means of each treatment at each wk.

²SEM based on pooled estimate of variance = 327.6.

³SEM based on pooled estimate of variance = 5.91.

RESULTS AND DISCUSSION

There were no significant treatment effects due to diet, type of inoculation, or age of inoculation on any of the blood parameters investigated except for STRIG and SCA. There were significant hen age main effects for HCT ($p \leq 0.0001$), SCHOL ($p \leq 0.0001$) and PP ($p \leq 0.03$). However, because similar age effects on these same 3 parameters have been previously reported by Peebles *et al.* (2009a), these data are not again presented in this report. Serum triglyceride ($p \leq 0.005$) and calcium ($p \leq 0.03$) concentrations were affected by a significant inoculation type x hen age interaction (Table 1). In comparison to sham-inoculated controls, the STRIG of FMG-inoculated birds was lower at wk 24 and the SCA of FMG-inoculated birds was lower at 34 wk but was higher at 58 wk of age, regardless of the age at which the inoculation was administered (12 or 22 wk of age). In contrast to the current observations, Burnham *et al.* (2003a) observed that an FMG inoculation on wk 12 caused an early (wk 22) increase and a late (wk 54) decrease in STRIG during lay, but had no significant effect on the SCA of layers that were between 16 and 58 wk of age. Furthermore, Peebles *et al.* (2007) did not observe any effects of an FMG inoculation administered at either 12 or 22 wk of age on the STRIG or SCA of layers at any of the ages investigated, which included wk 34, 50 and 58.

However, Peebles *et al.* (2008) did note a significant rise in the SCA levels of hens at 47 wk of age after they had received an inoculation of the 6/85 strain of MG on wk 10 followed by an inoculation of FMG on wk 45 and Peebles *et al.* (2009b) noted that an inoculation of FMG on wk 22 led to an increase in SCA that same wk when preceded by an inoculation of the ts-11 strain of MG on wk 10. In addition, Peebles *et al.* (2009a) concluded that STRIG may be decreased during peak EP (34 wk of age) by an FMG inoculation (12 or 22 wk of age) and that SCA may

be decreased during peak EP by a prelay (12 wk of age) inoculation procedure or the specific inoculation of FMG proximate to the onset of lay (22 wk of age); whereas SCA may be increased late in lay (58 wk of age) by an FMG inoculation proximate to lay onset (22 wk of age). The results of the current study nevertheless confirm that the inoculation of FMG can lead to subsequent changes in the STRIG and SCA of layers, with differences in the specific effect and the age at which it is observed varying between flocks.

The dietary treatments imposed in this study did not directly affect any of the blood parameters examined. Furthermore, the diets did not modify the effects that FMG had on STRIG and SCA. Conversely, Peebles *et al.* (2009a) did note that 1.50% supplemental dietary PF increased PP and SCHOL in layers that were subjected to a wk 12 inoculation procedure (sham or FMG) and Peebles *et al.* (2007) observed decreases in the HCT of hens throughout lay in response to supplemental dietary PHY and 25(OH)D. Together, the results of this and the previous 2 studies suggest that the levels of various blood parameters and their subsequent changes in layers after an inoculation can be influenced by the use of PF, PHY and 25(OH)D as dietary supplements. However, the blood parameter that is affected, its specific response and the age period in which the response is observed may vary between flocks. Nevertheless, PP, SCHOL and HCT are the only parameters that have been documented in these reports as being affected by the 3 supplements (Peebles *et al.*, 2007; Peebles *et al.*, 2009a). The results of this particular study show that diet supplementation with 0.75 or 1.50% PF or 1.50% PF in combination with 0.013% PHY and 0.025% 25(OH)D exert no effect on any of the investigated blood parameters in birds that did or did not receive FMG inoculations at either 12 or 22 wk of age.

Upon examining the performance of the birds used in this current study in a companion report, Park *et al.* (2010) noted that without any additional effect from the supplemental combination of PHY and 25(OH)D, the 0.75 and 1.50% levels of supplemental PF increased the BW of hens during postpeak EP. It was also observed that 1.50% PF increased the BW and decreased the feed consumption of hens that had undergone an inoculation procedure on wk 22. In another companion report in which the egg characteristics of these birds were determined, Peebles *et al.* (2010) found that use of the supplemental combination of PHY and 25(OH)D counteracted the stimulatory effect that 1.50% PF had on percentage egg yolk weight. It could, therefore, be further concluded from the results of this current study, that the dietary effects on BW, feed consumption and percentage egg yolk weight noted in those earlier reports, are not mediated by changes in HCT, PP, SCHOL, STRIG, or SCA.

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