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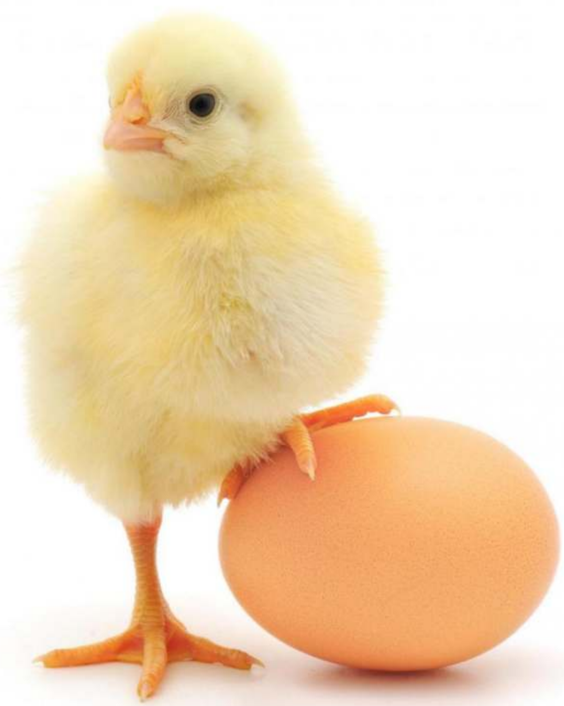

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Influence of Supplemental Dietary Poultry Fat on the Blood Characteristics of Commercial Layers Inoculated Before or at the Onset of Lay with F-Strain *Mycoplasma gallisepticum*[1,2]

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Abstract: Effects of F-strain *Mycoplasma gallisepticum* (FMG) inoculation and 1.5% supplemental dietary Poultry Fat (PF) on the blood characteristics of commercial layers between 24 and 58 wk of age were investigated. Sham and FMG inoculations were administered at 12 (before lay) and 22 (early in lay) wk of age and dietary treatments [Basal Control Diets (BC) and basal control diets with 1.5% supplemental PF (BCPF)] were initiated at 20 wk of age. In birds inoculated at 12 wk of age (sham or FMG), total plasma protein and serum cholesterol concentrations were increased when the BCPF rather than the BC diet was fed and in birds fed, BC diets, inoculation at 22 rather than at 12 wk of age (sham or FMG) increased total plasma protein and serum cholesterol concentrations. At wk 34, serum triglyceride concentrations were reduced in birds inoculated with FMG (12 or 22 wk of age) in comparison to sham-inoculated controls. Furthermore, at wk 34, serum calcium concentrations were greater in birds that had been sham-inoculated on wk 22 in comparison to those inoculated with FMG at the same time or to those that were sham-or FMG-inoculated on wk 12. Conversely, on wk 58, serum calcium concentrations were greater in birds that had been inoculated with FMG at 22 wk in comparison to those that had been sham-inoculated at 12 or 22 wk of age. In conclusion, serum calcium concentrations may be decreased during peak egg production by a prelay inoculation procedure or the specific inoculation of FMG at the onset of lay; whereas calcium levels may become elevated late in lay by an FMG inoculation at lay onset. Also, while the inoculation of FMG may reduce serum triglyceride levels in layers during peak egg production, 1.5% supplemental dietary PF may interact with the age (12 or 22 wk) at which an inoculation (sham or FMG) is given to influence serum cholesterol and total plasma protein levels in layers.

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

INTRODUCTION

Due to its proven ability to displace more virulent field strains of *Mycoplasma gallisepticum* (MG) and thereby protect layers from field strain MG infections, the F-strain of MG (FMG) has predominantly been used in vaccine programs implemented on multi-age layer facilities (Carpenter *et al.*, 1981; Levisohn and Kleven, 1981; Mohammed *et al.*, 1987; Kleven, 1998). However, inoculation of pullets with FMG at 12 wk of age has been reported to lead to a delay in Egg Production (EP) and to a decrease in total EP (Burnham *et al.*, 2002). Furthermore, Branton *et al.* (1988) reported a significant reduction in EP subsequent to the inoculation of 45-wk-old commercial layers with FMG.

In a recent article companion to the current one (Park *et al.*, 2009a), it was reported that except for an increase in egg weight in birds inoculated with FMG at 12 or 22 wk of age, there were no coherent treatment effects on eggshell quality throughout lay. Total EP through 58 wk was also highest in birds that were inoculated with FMG at 22 wk, whereas an FMG inoculation at 12 or 22 wk increased subsequent feed consumption during the early and late stages of EP. In that study, it was also shown that supplementation of diets with 1.5% Poultry Fat (PF) beginning at 20 wk of age reduced subsequent feed consumption throughout lay in hens having experienced a prelay (12 wk) inoculation.

However, Park *et al.* (2009b) showed in another companion article that despite the interaction of 1.5% supplemental PF with the prelay inoculation of FMG on early (18-26 wk) layer performance noted in a previous report (Peebles *et al.*, 2003), the effects of a prelay FMG inoculation and 1.5% supplemental PF on the egg yolk characteristics of layers were independent of each other. This suggested that 1.5% supplemental PF was not effective in modulating the effects of an FMG inoculation at 12 wk of age on hen egg yolk characteristics between 24 and 58 wk of age and that the combined effects of PF supplementation and FMG inoculation on performance did not influence egg yolk characteristics.

Characterization studies of the blood from birds inoculated with FMG at 12 wk of age have been reported (Burnham *et al.*, 2003). However, the effects of supplemental dietary PF throughout lay in conjunction with FMG inoculations before and during lay on the blood characteristics of laying hens have not been investigated. Therefore, the objective of the current study was to determine the possible independent and interactive effects of 1.5% supplemental PF fed beginning at 20 wk of age with FMG inoculations at 12 or 22 wk of age on the blood characteristics of commercial laying hens.

MATERIALS AND METHODS

Bird management: Three trials were conducted using Hy-Line W-36 Single Comb White Leghorn pullets that were obtained at 1 d of age from a commercial source that was monitored and certified free of both MG and *Mycoplasma synoviae* (MS; USDA-APHIS-VS, 2003). All trials were conducted under an approved USDA animal care and use protocol. Until 12 wk of age, birds were raised, fed, vaccinated and tested for the presence of MG and MS 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 wing-banded and randomly assigned to individual cages in one of 2 enclosed and isolated ends of a caged layer facility according to inoculation treatment. Sham and FMG inoculations at 12 and 22 wk and *Mycoplasma* detection during lay were as described by Peebles *et al.* (2003) and Park *et al.* (2009a).

At 20 wk of age, 2 isocaloric and isonitrogenous treatment layer diets were randomly provided to birds within each end of the layer house. Each dietary treatment was assigned to birds belonging to each inoculation type (sham- or FMG-inoculated) and inoculation age (12 or 22 wk) treatment combination. A basal layer diet formulated at the USDA South Central Poultry Research Unit served as the control dietary treatment (BC; contained 0.5% PF); whereas the basal diet supplemented with 1.5% PF served as the other dietary treatment (BCPF; 2.0% total PF). In each trial, there were 3 replicate groups (10 birds per replicate group) for each inoculation type, inoculation age and dietary treatment combination. Feed and water were provided for *ad libitum* consumption and birds in each side of the house were watered, fed and ventilated separately. Descriptions of the diets fed to the layers are provided by Peebles *et al.* (2003) and Park *et al.* (2009a). All pullet and layer diets were formulated to meet or exceed NRC (1994) specifications.

Data collection: In each trial, 2 tagged hens per replicate isolation unit 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 whole blood hematocrit (HCT) and plasma total protein (PP), serum cholesterol (SCHOL), serum triglycerides (STRIG) and serum calcium (SCA) concentrations.

Analysis of blood and serum constituents: Hematocrit was expressed as percentage blood packed 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 µL 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 µL 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 completely randomized experimental design, with trial as a block, was employed. The data of all 3 trials were pooled and then analyzed together. Therefore, the results from each trial were not reported independently but were reported over all 3 trials. Trial was considered as a random effect. Individual sample data within each replicate unit were averaged prior to analysis and all data were subjected to a repeated measures analysis. Least-squares means were compared in the event of significant global effects (Steel and Torrie, 1980). Global effects and differences among least-squares means were considered significant at $p \leq 0.05$. All data were analyzed using the MIXED procedure of SAS software (SAS Institute, 2003).

RESULTS AND DISCUSSION

There were significant age main effects for HCT ($p \leq 0.0007$), PP ($p \leq 0.0001$) and SCHOL ($p \leq 0.0001$). These data are provided in Table 1. Hematocrit was observed to decrease between wk 24 and 34, increase between wk 44 and 50 and decrease again between wk 50 and 58; whereas PP and SCHOL increased between wk 24 and 34 and again between wk 44 and 50. Age-related changes in HCT, PP and SCHOL in layers have also been previously documented by Burnham *et al.* (2003).

Branton *et al.* (1997) observed no chronic effect of an inoculation of FMG given at 6 wk of age on packed cell volume or plasma protein values in layers between 66 and 70 wk of age. However, Burnham *et al.* (2003) noted a significant increase in HCT at 20 wk, increases in PP and STRIG at 22 wk and later respective decreases in PP and STRIG at 52 and 54 wk of age in layers that had been inoculated prelay with FMG at 12 wk of age. The birds examined by Burnham *et al.* (2003) also exhibited a subsequent delay in onset of lay and a decrease in total EP (Burnham *et al.*, 2002). Boyd (1981) has

Table 1: Hematocrit (HCT) and concentrations of plasma total protein (PP) and serum cholesterol (SCHOL) at 24, 34, 44, 50 and 58 wk of age[1]

Week of age	HCT (%)	PP (g/dL)	SCHOL (mg/dL)
24	27.6[ab]	3.91[c]	167[d]
34	26.8[c]	5.06[ab]	194[bc]
44	26.8[c]	4.86[b]	187[c]
50	27.7[a]	5.44[a]	223[a]
58	27.2[bc]	5.09[ab]	212[ab]
Pooled SEM	0.82	0.216	37.1

[a-d]Means within column (parameter) with no common superscript differ significantly ($p \leq 0.05$).
[1]Blood samples from 2 birds within each of 72 replicate units were used for calculation of parameter means within each row (wk)

Table 2: Total Plasma Protein (PP) and serum cholesterol (SCHOL) concentrations in birds inoculated at 12 and 22 wk of age and fed basal control diets (BC) and basal control diets supplemented with 1.5 % poultry fat (BCPF)[1]

	PP[2] (g/dL)		SCHOL[3] (mg/dL)	
Age of inoculation	12	22	12	22
Diet				
BC	4.57[b]	4.93[a]	177[b]	203[a]
BCPF	5.07[a]	4.92[ab]	208[a]	199[ab]

[a,b]Means within parameter with no common superscript differ significantly ($p \leq 0.05$).
[1]Blood samples from 2 birds within each of 90 replicate units were used for calculation of means.
[2]SEM based on pooled estimate of variance = 0.208
[3]SEM based on pooled estimate of variance = 37.3

documented that HCT and PP commonly increase during dehydration, with PP showing the greater response. Furthermore, Warriss *et al.* (1997) concluded more specifically that increases in PP are indicative of dehydration in broilers. Therefore, increases in HCT and PP between 8 and 10 wk postchallenge in layers, as reported by Burnham *et al.* (2003), may be suggestive of an acute dehydration response to an F-strain inoculation.

In the current study, blood and swab sample tests showed that control hens remained MG-free; whereas systemic infections were confirmed in FMG-inoculated hens throughout lay. Nevertheless, only STRIG and SCA were influenced by the specific inoculation of FMG. Hematocrit and PP, as well as SCHOL, were not influenced by an inoculation of FMG at 12 wk. Therefore, the current results do not confirm earlier reports of a possible acute dehydration effect in layers in response to a prelay FMG inoculation. Furthermore, HCT and PP were not subsequently affected by an FMG inoculation at wk 22 and unlike the results of Burnham *et al.* (2002), the earlier reported performance of the layers used in the present study were not found to be adversely affected by the inoculation of FMG at 12 or 22 wk of age (Park *et al.*, 2009a). Although HCT and PP did not change in response to the specific inoculation of FMG, the age at which an inoculation (sham or FMG) is given did have an

Table 3: Serum triglyceride concentrations for sham- and FMG-inoculated birds at 24, 34, 44, 50 and 58 wk of age[1,2]

	Sham	FMG
Type of inoculation	(mg/dL)	
Week of age		
24	5,549	6,080
34	6,319[a]	4,909[b]
44	6,173	6,763
50	5,963	6,018
58	6,454	7,234

[a,b]Means among treatments within a row (wk of age) with no common superscript differ ($p \leq 0.05$)
[1]Blood samples from 2 birds within each of 36 replicate units were used for calculation of the means of each treatment at each wk.
[2]SEM based on pooled estimate of variance = 2,175.5

influence on PP. It has been suggested that relative differences in the stress levels of the wk 12 and 22 inoculations in the birds may be the basis for their differential effects on various blood parameters including PP (Peebles *et al.*, 2007).

The influence of age of inoculation on PP was indicated through a significant ($p \leq 0.05$) age of inoculation by dietary treatment interaction on PP. The same significant ($p \leq 0.05$) interaction was also noted for SCHOL. These data are presented in Table 2. In birds inoculated at 12 wk of age (sham or FMG), 1.5% supplemental PF increased both PP and SCHOL and in birds fed BC diets without the addition of 1.5% supplemental PF, inoculation at 22 rather than at 12 wk of age (sham or FMG) increased both PP and SCHOL. Furthermore, in a study by Peebles *et al.* (2007), it was shown that PP at 34 wk of age was higher in birds that were inoculated with FMG at the onset of lay compared with those that were inoculated prelay. Taken together, these results would indicate that the use of 1.5% supplemental PF can modify the responses of PP and SCHOL to the age at which an inoculation procedure is applied to layers. In birds that are subjected to a prelay (12 wk of age) inoculation, supplementation may result in increases in PP and SCHOL, but at the same time, supplementation may eliminate an increase in both blood parameters that might otherwise occur when inoculations are given at 22 rather than at 12 wk of age.

The effects of various added dietary fat sources and levels, including 1.5% PF, on broiler breeder performance (Peebles *et al.*, 2000a) and eggshell quality (Peebles *et al.*, 2000b) have been examined and it was concluded that both were influenced by the source and level of fat. Peebles *et al.* (2003) demonstrated that 1.5% PF alleviated reductions in the early EP of layers due to the inoculation of FMG at 12 wk and Park *et al.* (2009a) examined the performance of the birds in the current study in response to the applied treatments and showed that the 1.5% PF dietary supplement reduced subsequent feed consumption throughout lay in birds that experienced an inoculation (sham or FMG) at 12 wk of age. However, it was further concluded by Park *et al.*

Table 4: Serum calcium concentrations at 24, 34, 44, 50 and 58 wk of age in birds that were sham- and FMG-inoculated at 12 and 22 wk of age[1,2]

Age of inoculation	Week of age 24: 12	24: 22	34: 12	34: 22	44: 12	44: 22	50: 12	50: 22	58: 12	58: 22
	(mg/dL)									
Type of inoculation										
Sham	45.3	48.4	34.7^{b}	49.8^{a}	41.2	38.1	40.1	35.3	37.7^{b}	37.9^{b}
FMG	44.8	46.5	40.4^{b}	37.1^{b}	43.4	44.1	38.1	38.4	44.8^{ab}	46.9^{a}

[a,b]Means within week of age with no common superscript differ significantly ($p \leq 0.05$).
[1]Blood samples from 2 birds within each of 18 replicate units were used for calculation of means.
[2]SEM based on pooled estimate of variance = 16.24

(2009b) in a later companion article that 1.5% supplemental PF was not effective in modulating the effects of a wk 12 FMG inoculation on the egg yolk characteristics of these same birds between 24 and 58 wk of age and that the combined effects of PF supplementation and FMG challenge on layer performance do not influence egg yolk characteristics. Reductions in feed consumption may, therefore, be associated with simultaneous increases in PP and SCHOL in response to 1.5% PF dietary supplementation in birds having experienced the stress of an inoculation procedure (sham or FMG) at 12 wk of age. Nevertheless, these results demonstrate that although 1.5% supplemental PF can modify the effects of age of inoculation on PP, SCHOL and feed consumption, supplementation does not influence the effects of a specific FMG challenge on the performance or yolk and blood characteristics of layers.

There was a significant ($p \leq 0.02$) bird age by type of inoculation interaction for STRIG (Table 3). At wk 34, STRIG was reduced in birds that had been inoculated with FMG (12 or 22 wk of age) in comparison to sham-inoculated controls. However, at wk 24, 44, 50 and 58, there were no significant effects on STRIG due to FMG inoculation. In previous work, Burnham *et al.* (2003) noted an increase in STRIG at wk 22 following an FMG challenge at wk 12. It was discussed that this 10 wk postinoculation increase in STRIG may be indicative of a compensatory response to an FMG challenge and more specifically to the colonization of FMG in the liver. Nevertheless, a decrease in STRIG occurred between 12 and 22 wk after an FMG challenge in the current study, whereas Burnham *et al.* (2003) noted a later decrease in STRIG 42 wk after the inoculation of FMG prelay (12 wk of age) or at 54 wk of age. This suggests that decreases in STRIG could occur between 12 and 42 wk after an FMG inoculation depending on the age (prelay or onset of lay) at which FMG is inoculated. It has been suggested that colonization of the liver by FMG may negatively affect liver metabolism (Sahu and Olson, 1976) and an elevation in STRIG is known to be a common response to the presence of infectious disease agents (Guyton and Hall, 1996). A reduction in yolk lipid concentration at 24 wk of age, as reported in a companion article for these same birds (Park *et al.*, 2009b), in response to the inoculation of FMG at 12 or 22 wk of age may be associated with the decrease in STRIG. Furthermore, through its activity in the liver, FMG may have a more chronic effect on STRIG than it has on either HCT or PP.

Lastly, there was also a significant ($p \leq 0.01$) bird age by age of inoculation by type of inoculation interaction for SCA (Table 4). At wk 34, SCA concentration was greater in birds that had been sham-inoculated on wk 22 in comparison to those inoculated with FMG at the same time or to those that were sham- or FMG-inoculated on wk 12. Conversely, on wk 58, SCA levels were greater in birds that had been inoculated with FMG at 22 wk in comparison to those that had been sham-inoculated at 12 or 22 wk of age. Park *et al.* (2009a) observed increases in the feed consumption and egg weight of the birds in this study at various ages between 29 and 58 wk after they had been inoculated with FMG (12 or 22 wk), without any consistently associated effects on eggshell quality. Therefore, changes in SCA at wk 34 and 58 after the inoculation of FMG at 12 or 22 wk were not related to those on feed consumption, egg weight, or eggshell quality. Apparently, the effects of an FMG challenge at either age on feed consumption did not consistently influence SCA and in turn the treatment effects on SCA were too transient or too low in magnitude to elicit a subsequent change in egg size or shell quality.

Conclusion: In conclusion, the use of 1.5% supplemental dietary PF may increase PP and SCHOL in association with a reduction in the feed consumption of commercial layers that have been subjected to the stress of a prelay inoculation procedure. Nevertheless, the use of 1.5% supplemental PF does not influence the effects that an FMG challenge has on the blood characteristics of laying hens.

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