Effects of a Prelay 6/85-Strain *Mycoplasma gallisepticum* Inoculation Alone or in Conjunction with Subsequent F-Strain *M. gallisepticum* Inoculations During Lay on the Internal Egg Characteristics of Commercial Egg-Laying Hens[1,2]

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ABSTRACT The effects of a prelay 6/85-strain *Mycoplasma gallisepticum* (6/85MG) inoculation alone or in conjunction with subsequent F-strain *M. gallisepticum* (FMG) inoculations during lay on the internal egg characteristics of commercial egg-laying hens were investigated. In the first 2 treatment groups, birds were sham inoculated or were inoculated with 6/85MG at 10 wk of age. In a third treatment group, birds were inoculated with 6/85MG at 10 wk in conjunction with a subsequent inoculation of FMG at 22 wk, and in a fourth treatment group, birds were inoculated with 6/85MG at 10 wk in conjunction with a subsequent inoculation of FMG at 45 wk. Percentage yolk weight, albumen weight, yolk moisture, and yolk lipid were determined at 24, 32, 43, 47, and 58 wk of hen age. The data from wk 24, 32, and 43 were analyzed separately from those at wk 47 and 58. Furthermore, yolk fatty acid profiles were determined at wk 58. The applied treatments affected yolk moisture and fatty acid profiles. Across wk 24, 32, and 43, yolk moisture content was higher in birds inoculated with 6/85MG at 10 wk and FMG at 22 wk when compared with control birds and those inoculated with 6/85MG at 10 wk alone. In addition, at wk 58, yolk palmitic, oleic, and linolenic acid concentrations were affected differently by treatment. A 22-wk FMG inoculation in birds previously inoculated with 6/85MG at 10 wk may increase yolk moisture content, and alterations in yolk palmitic, oleic, and linolenic acid levels with treatment may be manifested by disturbances in fatty acid synthesis.

Key words: 6/85-strain *Mycoplasma gallisepticum*, F-strain *Mycoplasma gallisepticum*, inoculation, laying hen, yolk

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INTRODUCTION

Vaccination programs are currently used to help protect commercial egg-laying flocks from field strains of *Mycoplasma gallisepticum* (**MG**). The F (**FMG**), ts11, and 6/85 (**6/85MG**) strains of MG are commonly available for use as live vaccines in the United States. Continuous use of FMG vaccines for replacement flocks in multiage commercial layer operations has been shown to protect flocks from field strain infections (Kleven et al., 1990). However, although vaccines are commercially available to help lower egg production (**EP**) losses in commercial layers, the vaccines themselves can cause drops in EP. The FMG strain is known to depress the EP of layers when inocula are administered at 12 or 22 wk (Peebles et al., 2008). Studies using 6/85MG vaccines have noted that they exhibit minimal virulence, rare to no transmissibility, and resistance to challenges by virulent MG strains (Evans and Hafez, 1992; Evans et al., 1992). Nevertheless, although 6/85MG is less pathogenic and disruptive to EP than FMG when administered prelay, FMG provides the layer with more protection against virulent field-strain MG infections than does 6/85MG (Kleven, 1998; Branton et al., 2002; Burnham et al., 2002).

Because layer operators may acquire flocks already inoculated with 6/85MG, but desire to continue using the FMG inoculation program familiar to them, vaccination regimens involving the subsequent inoculation of FMG during lay on a prelay 6/85MG inoculation may have importance for commercial application. In a companion article that examined the effects of 6/85MG inoculation alone at 10 wk of age or in conjunction with subsequent

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FMG inoculations at 22 or 45 wk of age on the performance of commercial egg-laying hens, Viscione et al. (2008) suggested that prelay 6/85MG inoculations may be a suitable substitute for prelay FMG inoculations, and subsequent FMG inoculations during lay on prelay 6/85MG inoculations may provide continual protection without eliciting any subsequent suppressive effects on performance. Branton et al. (2002) also concluded that vaccination of commercial layer chickens at 10 wk of age with 6/85MG does not detrimentally affect EP, egg size distribution, or ovary and oviduct function. Nevertheless, Burnham et al. (2003) noted that egg yolk composition in commercial layers changed in response to an FMG inoculation at 12 wk of age, and Peebles et al. (2006b) found that postpeak yolk total lipid and fatty acid contents were altered variably by the timing (10, 22, 45 wk of age) of an S6-strain MG inoculation. These noted effects of the above MG strains on yolk composition suggest that 6/85MG might also affect yolk composition. Because possible changes in internal egg content, including yolk composition, may influence egg nutritional value and consumer acceptance (Leeson and Caston, 1997; Herber-McNeill and Van Elswyk, 1998), the possible effects of 6/85MG on the internal characteristics of eggs would be of concern to the table egg industry. Therefore, the goal of this study was to investigate the effects of a prelay 6/85MG inoculation given in conjunction with an FMG inoculation during lay on the internal egg characteristics, particularly those of the yolk, of commercial layers.

MATERIALS AND METHODS

Pullet Housing and Management

Two individual consecutive trials were conducted for this study. In each of the 2 trials, 1,000 Hy-Line W-36 Single Comb White Leghorn pullets were obtained at 1 d of age from a commercial source that was monitored and certified free of MG and *Mycoplasma synoviae* (USDA-Animal and Plant Health Inspection Service-Veterinary Services, 2003). Both trials were conducted under an approved USDA Animal Care and Use protocol. In the pretreatment pullet period, chickens were raised on clean, dry litter in a 5.5×6.1 m section of a conventional poultry house with an initial flock density of 0.034 m^2/bird. Chicks were vaccinated at 10 d of age for infectious bursal disease via the drinking water. At 12 d and again at 4 wk of age, chicks were vaccinated for Newcastle disease and infectious bronchitis by the same route. At 9 wk of age in trial 1 and at 6 wk of age in trial 2, blood and choanal cleft swabs were collected from 10 randomly selected pullets to test for the presence of MG and *M. synoviae*. Further details of pretreatment pullet rearing and of blood and swab sample testing procedures are described by Peebles et al. (2006a).

At 10 wk of age in each trial, 11 pullets were randomly assigned to each of 16 negative pressure fiberglass biological isolation units (1.16 m^2/unit; total of 176 pullets). The temperatures inside each biological unit were maintained at 25°C. The units were located in a USDA poultry disease isolation facility described previously at the same USDA research laboratory (Branton and Simmons, 1992). All birds were wing-banded for purposes of identification and individual data collection.

Layer Housing and Management

At initiation of lay (18 wk of age), the number of birds in each treatment unit was reduced to 10 (total of 160 pullets), resulting in a bird density of 0.116 m^2/bird for the duration of each trial. In each trial, 4 negative-pressure isolation units were assigned to each of 4 treatments, and each treatment was represented by one row of the 4 units. In trial 2, the location of treatments within the isolation facility was different from that in trial 1 to ensure environmental randomization between trials. Beginning at 18 wk of age, the duration of the artificial lighting schedule was increased by 15 min/d until a cycle of 16 h 15 min of light per 7 h 45 min of dark was achieved in trial 1, and a cycle of 17 h 15 min of light per 6 h 45 min of dark was achieved in trial 2. These artificial lighting programs were maintained through the end of both trials.

Pullet and Layer Diets

For the duration of each trial, chickens had ad libitum access to feed and water. Diets in both trials were formulated according to the age of the birds and included the following: starter (0 to 6 wk), grower (7 to 12 wk), developer (13 to 18 wk), prelay (18 to 19 wk), and layer (20 to 60 wk). All diets were formulated to meet or exceed NRC (1994) recommendations. Ingredient percentages, and calculated and determined analyses of these diets were provided by Burnham et al. (2002). In both trials, CP and Lys percentages in the layer diet were adjusted according to the percentage of feed consumed per bird every 28 d until trial termination. No medication was administered during either trial.

6/85MG and FMG Inoculation

Four replicate isolation units were assigned to one of the following treatment groups on administration of treatment inocula: control birds received sham inoculations in the right eye with 0.04 mL of sterile Frey's broth medium (Frey et al., 1968) at 10 wk of age. A second treatment group of birds was inoculated with a 24-h broth culture of 6/85MG at 10 wk of age (6/85MG-10). In a third treatment group, birds were inoculated with 6/85MG at 10 wk and subsequently received an inoculation of FMG at 22 wk (6/85MG-10, FMG-22); and in a fourth treatment group, birds that were inoculated with 6/85MG at 10 wk received a subsequent inoculation of FMG at 45 wk (6/85MG-10, FMG-45). Of the 16 total units, 4 replicate units served as sham-inoculated controls throughout the study. Until wk 22, the replicate units ultimately assigned to the third and fourth treatment groups were included among those replicate units that had been assigned to the

second treatment (12 replicates in treatment 2), and until wk 45, the replicate units that were eventually assigned to the fourth treatment group were included among those replicate units that had been assigned to the second treatment (8 replicates in treatment 2). Beginning on wk 45, 4 replicate units belonged to each of the 4 treatment groups. *Mycoplasma gallisepticum* organisms were advanced after being received from S. H. Kleven (University of Georgia, Athens, GA) at the 212th passage. Titers of the inocula in each trial were as described by Viscione et al. (2008).

Mycoplasma Detection

At 58 wk of age in both trials, 3 randomly selected hens from each replicate unit in each of the 4 treatment groups (sham inoculated, inoculated with 6/85MG alone, or 6/85MG with subsequent FMG inoculations at 22 or 45 wk of age during lay) were bled from a wing vein and were swabbed to test for the presence of MG. Testing procedures at this time were the same as those for pullets.

Data Collection

Percentages of yolk weight (**PY**), albumen weight (**PA**), yolk moisture (**YM**), and yolk lipid (**YL**) were determined at wk 24, 32, 43, 47, and 58. Furthermore, yolk fatty acid profiles were determined at wk 58. Fatty acid profiles included determination of myristic, palmitic, palmitoleic, stearic, oleic, linoleic, linolenic, and arachidonic acid concentrations. For determination of the above-mentioned internal egg quality parameters, a total of at least 10 eggs were collected from each replicate unit for an accurate estimate of each parameter (Buss, 1984). If less than 10 eggs were collected on a given day, the rest were collected the following day of the same week. Nevertheless, determinations were made on the same day that eggs were collected. Relative egg yolk and albumen weights were expressed as percentages of total egg weight.

Quantification of Yolk Moisture and Lipid Content

For the determination of YM, fresh yolk samples (2 g) were dried according to the procedure of Peebles et al. (1999). Yolk moisture was calculated as the difference between the fresh and dry weight of the sample and was expressed as a percentage of fresh sample weight. For analysis of YL, lipid was extracted from a 3-g sample of fresh yolk according to the procedure described by Bligh and Dryer (1959), and as modified by Latour et al. (1998). Yolk lipid concentration was expressed in terms of dry lipid sample weight as a percentage of total fresh yolk sample weight.

Methyl Esterification of Yolk Lipids and Chromatographic Analysis of Yolk Contents

Duplicate lipid samples were methylated according to the procedure described by Morrison and Smith (1964). A Multi-Block system (Lab-Line Instruments Inc., Melrose Park, IL) was used to boil each sample in a test tube at 80 ± 0.5°C for 30 min. A 200-μL aliquot of the solution was placed in a 2-mL gas chromatography vial along with 400 μL of isooctane and sealed with a rubber-lined cap for further fatty acid analyses by gas chromatography. Fatty acid profiles of duplicate yolk lipid samples were determined with a 5890 A Series I gas chromatograph (Hewlett-Packard Co., Boise, ID) according to the procedure by Latour et al. (1998). Fatty acids were identified by comparing peak retention times against polyunsaturated fatty acids and rapeseed oil. The standards were injected periodically to ensure accurate measurement by the gas chromatograph. The individual fatty acids retained by the gas chromatograph were expressed as a percentage of the total fatty acid content of the fresh yolk sample.

Statistical Analysis

A completely randomized experimental design, with trial as a block, was used. Data from wk 22 through 44 (age interval 1) and from wk 45 through 58 (age interval 2) were analyzed separately. The data from both trials were pooled and analyzed together. Therefore, the results from both trials were not reported independently but were reported over both trials. Trial was considered as a random effect. Because all parameters were examined at multiple age periods in each age interval, all data within each age interval were subjected to a repeated measures analysis. In the first age interval, control birds and those having had 6/85MG-10 and 6/85MG-10, FMG-22 inoculations were compared. In the second age interval, the control; 6/85MG-10; 6/85MG-10, FMG-22; and 6/85MG-10, FMG-45 groups were compared. Individual sample data within each of these replicate units were averaged before 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 by using the MIXED procedure of SAS software (SAS Institute, 2003).

RESULTS AND DISCUSSION

All initial test results obtained from pullets were negative for MG and MS, and at the end of each trial (58 wk of age), tests verified systemic infections in 6/85MG- and FMG-inoculated hens. Conversely, sham-inoculated birds remained free of MG throughout the study. The 6/85MG- and FMG-inoculated hens exhibited no outward pathological symptoms.

There was no significant treatment main effect or age × treatment interaction for PY in interval 1 (wk 22 through 44). Nevertheless, there was a significant ($P \leq 0.005$) age main effect on PY in interval 1. Percentage egg yolk weight at wk 24, 32, and 43 was 24.3, 26.1, and 27.7%, respectively (pooled SEM = 0.52). Percentage yolk weight was significantly higher at wk 43 than that at wk 32, and PY at wk 32 was also higher than that at wk 24. There

Table 1. Summary of significant main effects for all affected parameters within age intervals 1 (wk 22 through 44) and 2 (wk 45 through 58)[1]

Parameter	Age interval 1	Age interval 2
Yolk weight (%)	Age ($P \leq 0.005$)	—
Yolk moisture (%)	Treatment ($P \leq 0.009$)	—
Yolk palmitic acid (%)	—	Treatment ($P \leq 0.02$)
Yolk oleic acid (%)	—	Treatment ($P \leq 0.03$)
Yolk linolenic acid (%)	—	Treatment ($P \leq 0.04$)

[1]No significant age × treatment interactions were found for any of the parameters in either age interval.

were no noted significant effects on PY in interval 2 (wk 45 through 58), percentage of albumen weight, or YL in age intervals 1 and 2, or on YM in interval 2. However, there was a significant treatment main effect ($P \leq 0.009$) on YM in interval 1. Egg yolk moisture concentration in the control; 6/85MG-10; and 6/85MG-10, FMG-22 treatment groups in age interval 1 (across wk 24, 32, and 43) was 48.2, 48.8, and 49.7%, respectively (pooled SEM = 0.46). Yolk moisture content was higher in birds from the 6/85MG-10, FMG-22 treatment group when compared with control birds and those inoculated with 6/85MG at 10 wk alone. No significant age main effect or age × treatment interaction was found for YM in interval 1. A summary of the significant effects noted for the above parameters is provided in Table 1.

Unlike the results of Burnham et al. (2003), who found no effect of a 12-wk FMG inoculation on YM, in this study YM was found to be significantly higher in the 6/85MG-10, FMG-22 treatment group compared with both the control and 6/85MG-10 treatment groups in interval 2. It is suggested that associated changes in YM reflect changes in the hydration status of the birds exposed to the inoculation regimens in this study. These data also indicate that the inoculation treatments used influenced the hydration status of the layers without affecting overall performance.

Significant treatment main effects at wk 58 (interval 2) were found only for yolk palmitic ($P \leq 0.02$), oleic ($P \leq 0.03$), and linolenic ($P \leq 0.04$) acid concentrations (Table 2). Palmitic acid concentrations were higher in the 6/85MG-10, FMG-22 inoculation group compared with the 6/85MG-10 and 6/5MG-10, FMG-45 treatment groups, with the control group intermediate. However, oleic acid concentrations were higher in the 6/85MG-10 group compared with the 6/85MG-10, FMG-22 and control groups, with the 6/85MG-10, FMG-45 treatment group intermediate. Linolenic acid concentrations in control birds were higher than those in the 6/85MG-10 birds, with the 6/85MG-10, FMG-45 group intermediate. Furthermore, the 6/85MG-10, FMG-45 birds had a higher yolk linolenic acid concentration than did the 6/85MG-10, FMG-22 group, with the 6/85MG-10 group intermediate. A summary of the significant effects noted for the above parameters is provided in Table 1.

In a report by Burnham et al. (2003), in which egg yolk composition was altered by 12-wk FMG inoculations, it

Table 2. Yolk palmitic, oleic, and linolenic acid concentrations (% of total yolk fatty acids) in control, 6/85-strain *Mycoplasma gallisepticum* (6/85MG) at 10 wk (6/85MG-10), 6/85MG at 10 wk and F-strain *M. gallisepticum* (FMG) at 22 wk (6/85MG-10, FMG-22), and 6/85MG at 10 wk and FMG at 45 wk (6/85MG-10, FMG-45) treatment groups at 58 wk of age (age interval 2)[1]

Treatment	Palmitic acid	Oleic acid	Linolenic acid
	(%)		
Control	31.7[ab]	31.2[b]	0.150[a]
6/85MG-10	31.3[b]	32.2[a]	0.113[bc]
6/85MG-10, FMG-22	32.1[a]	31.0[b]	0.097[c]
6/85MG-10, FMG-45	31.4[b]	31.6[ab]	0.138[ab]
SEM	0.28	0.43	0.0226

[a–c]Means among column with no common superscript differ ($P \leq 0.05$).

[1]n = 4 replicate units used for calculation of means within each treatment group.

was concluded that the alterations in EP were associated with colonization of the liver by FMG, with subsequent functional disturbances in the metabolism of liver lipids and the deposition of circulating lipids in the ovarian follicles. Furthermore, Burnham et al. (2003) more specifically reported that yolk total lipid and cholesterol were decreased; linoleic, stearic, and arachidonic acid concentrations were increased; and yolk myristic, palmitoleic, and oleic acid concentrations were decreased by 12-wk FMG inoculations.

Similar to the results of Burnham et al. (2003), treatment effects on the yolk fatty acid profile were found in this study. However, specific changes in the fatty acid profile of the yolk were different in response to treatment. The yolk concentrations of palmitic, oleic, and linolenic acids were affected by the 10-wk 6/85MG inoculation and the subsequent FMG inoculations during lay. Taken together, these results suggest that the inoculation regimen used in this study may have affected fatty acid elongation and desaturation processes in the liver endoplasmic reticula of the hens. Included in fatty acid synthesis, palmitic acid is elongated and desaturated to form oleic acid. Oleic acid then undergoes further desaturation to form linolenic acid (Lehninger, 1975). Palmitic acid concentrations were significantly higher in 6/85MG-10, FMG-22 birds compared with 6/85MG-10 and 6/85MG-10, FMG-45 birds, with control birds intermediate. Oleic acid concentrations were significantly lower for the 6/85MG-10, FMG-22 and control birds when compared with the 6/85MG-10 birds, with the 6/85MG-10, FMG-45 birds intermediate. Linolenic acid concentrations were higher in control birds when compared with the 6/85MG-10 and 6/85MG-10, FMG-22 treatment groups, with the 6/85MG-10, FMG-45 group being intermediate.

In general, the 6/85MG-10 inoculation may have blocked or interfered with the desaturation of oleic acid to linolenic acid. A depression in the desaturation process without a concomitant increase in oleic acid may have likewise occurred when the 6/85MG-10 inoculation was given in conjunction with the subsequent FMG inoculation at 22 wk. In addition, the subsequent FMG inocula-

tion at 22 wk in birds given a prelay 6/85MG inoculation at 10 wk may have interfered with the elongation of palmitic to oleic acid, leading to an associated increase in palmitic acid in the 6/85MG-10, FMG-22-treated birds. Using the same birds under the same inoculation regimens as in this study, Viscione et al. (2008) noted no treatment effects on layer performance. Therefore, as for YM, these fatty acid differences were unrelated to performance.

In conclusion, changes in layer yolk fatty acid profiles in response to prelay 6/85MG inoculation alone or in conjunction with FMG inoculations during lay may be manifested by disturbances in fatty acid synthesis, and a 22-wk FMG inoculation in hens previously inoculated with 6/85MG at 10 wk may increase their YM content.

REFERENCES

Bligh, E. G., and W. J. Dryer. 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37:911–917.

Branton, S. L., S. M. D. Bearson, B. Bearson, B. D. Lott, W. R. Maslin, S. D. Collier, G. T. Pharr, and D. L. Boykin. 2002. The effects of 6/85 live *Mycoplasma gallisepticum* vaccine in commercial layer hens over a 43-week laying cycle on egg production, selected egg quality parameters, and egg size distribution when challenged before beginning of lay. Avian Dis. 46:423–428.

Branton, S. L., and J. D. Simmons. 1992. Design of a poultry disease isolation facility with programmable environmental control. Appl. Eng. Agric. 8:695–699.

Burnham, M. R., S. L. Branton, E. D. Peebles, B. D. Lott, and P. D. Gerard. 2002. Effects of F-strain *Mycoplasma gallisepticum* inoculation at twelve weeks of age on performance and egg characteristics of commercial egg laying hens. Poult. Sci. 81:1478–1485.

Burnham, M. R., E. D. Peebles, S. L. Branton, and S. K. Whitmarsh. 2003. Effects of F-strain *Mycoplasma gallisepticum* inoculation at twelve weeks of age on the egg yolk composition in commercial egg laying hens. Poult. Sci. 82:577–584.

Buss, E. G. 1984. Useful and nonuseful measurements in the genetic selection for improvement of eggshell quality. Pages 12–41 in Proc. 33rd Annu. Natl. Breeder's Roundtable, St. Louis, MO. Poult. Breed. Am., Tucker, GA.

Evans, R. D., and Y. S. Hafez. 1992. Evaluation of a *Mycoplasma gallisepticum* strain exhibiting reduced virulence for prevention and control of poultry mycoplasmosis. Avian Dis. 36:197–201.

Evans, R. D., Y. S. Hafez, and C. S. Schreurs. 1992. Demonstration of the genetic stability of a *Mycoplasma gallisepticum* strain following in vivo passage. Avian Dis. 36:554–560.

Frey, M. C., R. P. Hanson, and D. P. Anderson. 1968. A medium for the isolation of avian *Mycoplasma.* Am. J. Vet. Res. 29:2164–2171.

Herber-McNeill, S. M., and M. E. Van Elswyk. 1998. Dietary marine algae maintains egg consumer acceptability while enhancing yolk color. Poult. Sci. 77:493–496.

Kleven, S. H. 1998. Mycoplasmas in the etiology of multifactorial respiratory disease. Poult. Sci. 77:1146–1149.

Kleven, S. H., M. I. Khan, and R. Yamamoto. 1990. Fingerprinting of *Mycoplasma gallisepticum* strains isolated from multiple-age layers vaccinated with live F-strain. Avian Dis. 34:984–990.

Latour, M. A., E. D. Peebles, S. M. Doyle, T. M. Smith, and C. R. Boyle. 1998. Broiler breeder age and dietary fat influence the yolk fatty acid profiles of fresh eggs and newly hatched chicks. Poult. Sci. 77:47–53.

Leeson, S., and L. J. Caston. 1997. A problem with characteristics of the thin albumen in laying hens. Poult. Sci. 76:1332–1336.

Lehninger, A. C. 1975. The Biosynthesis of Lipids. 2nd ed. Pages 659–691 in Biochemistry. Worth Publ. Inc. New York, NY.

Morrison, W. R., and L. W. Smith. 1964. Preparation of fatty acyl esters and dimethylacetals from lipids with boron fluoride-methanol. J. Lipid Res. 5:600–608.

NRC. 1994. Nutrient Requirements of Poultry. 9th rev. ed. Natl. Acad. Press, Washington, DC.

Peebles, E. D., E. Y. Basenko, S. L. Branton, S. K. Whitmarsh, and P. D. Gerard. 2006a. Effects of S6-strain *Mycoplasma gallisepticum* inoculation at ten, twenty-two, or forty-five weeks of age on the blood characteristics of commercial egg-laying hens. Poult. Sci. 85:2012–2018.

Peebles, E. D., E. Y. Basenko, S. L. Branton, S. K. Whitmarsh, D. V. Maurice, and P. D. Gerard. 2006b. Effects of S6-strain *Mycoplasma gallisepticum* inoculation at ten, twenty-two, or forty-five weeks of age on the egg yolk composition of commercial egg-laying hens. Poult. Sci. 85:1502–1508.

Peebles, E. D., S. L. Branton, M. R. Burnham, S. K. Whitmarsh, and P. D. Gerard. 2008. Effects of supplemental dietary phytase and 25-hydroxycholecalciferol 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.

Peebles, E. D., L. Li, S. Miller, T. Pansky, S. Whitmarsh, M. A. Latour, and P. D. Gerard. 1999. Embryo and yolk compositional relationships in broiler hatching eggs during incubation. Poult. Sci. 78:1435–1442.

SAS Institute. 2003. SAS Proprietary Software Release 9.1. SAS Inst. Inc., Carey, NC.

Steel, R. G. D., and J. H. Torrie. 1980. Principles and Procedures of Statistics: A Biometrical Approach. 2nd ed. McGraw-Hill, New York, NY.

USDA-Animal and Plant Health Inspection Service-Veterinary Services. 2003. National poultry improvement plan and auxiliary provisions. Fed. Regist. 68:28169–28175.

Viscione, K. A., S. L. Branton, A. M. Vance, P. D. Gerard, S. K. Whitmarsh, and E. D. Peebles. 2008. Effects of 6/85-strain *Mycoplasma gallisepticum* inoculation alone at ten weeks of age or in conjunction with F-strain *Mycoplasma gallisepticum* inoculation overlays at twenty-two or forty-five weeks of age on the performance of commercial egg-laying hens. Poult. Sci. 87:588–593.