Effects of F-strain *Mycoplasma gallisepticum* Inoculation at Twelve Weeks of Age on Performance and Egg Characteristics of Commercial Egg-Laying Hens[1,2]

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ABSTRACT The effects of F-strain *Mycoplasma gallisepticum* (FMG) inoculation during the pullet period on the subsequent performance and egg characteristics of commercial Single Combed White Leghorn hens were evaluated. In two trials, BW, feed consumption, egg production (EP), egg weight, egg size class, relative eggshell water vapor conductance, and relative percentages of eggshell, yolk and albumen weights were determined through approximately 60 wk of age. In each trial, pullets at 12 wk of age were randomly assigned to negative pressure biological isolation units. Birds in one-half of the total units were inoculated with FMG, and the other half were sham-inoculated with sterile media. In both trials, onset of lay was delayed approximately 1 wk in layers inoculated with FMG. Control birds that had not been previously inoculated with FMG laid their first egg at 18 wk of age, while birds that had been previously inoculated with FMG laid their first egg at 19 wk of age. In Trial 1, FMG-inoculated hens laid significantly fewer total eggs, which became apparent at each week after Week 42. In Trial 2, a numerical decrease in total EP occurred, and the percentage of undersized eggs laid by FMG-inoculated birds was significantly lower at 19 wk of age but was higher at 20 and 21 wk when compared to controls. Mortality was not significantly different between the treatments in either trial. These data demonstrate that when birds are housed in isolation facilities and inoculated with FMG at 12 wk of age, onset of lay is delayed. These data also suggest that FMG may lead to delays in undersize EP and decreases in total EP. However, because significant FMG effects on these parameters were observed in only one trial, additional studies may be necessary to verify these effects.

(*Key words*: albumen, egg production, *Mycoplasma gallisepticum*, shell, yolk)

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INTRODUCTION

Mycoplasma gallisepticum (MG) is a pathogenic organism that can infect (Kreig and Holt, 1984) and cause problems primarily within the respiratory tract of laying hens (Ley and Yoder, 1978; Branton et al., 1984). Chronic respiratory disease, airsacculitis, air sac infection, and pleuropneumonia are commonly used synonymous names associated with MG infection (Yoder, 1978). It has been speculated that infection can also spread through the blood from the hen's respiratory tract to the oviduct, causing reduced egg production (EP) and poor egg quality (Yoder and Hofstad, 1964; Domermuth et al., 1967; Patterson, 1994). Feed consumption, BW, and EP have been reported to be reduced in MG-infected birds by Mohammed et al. (1987). A reduction in feed consumption of layer hens may alter the essential dietary components necessary to sustain adequate egg formation and EP.

The table egg industry experiences financial losses primarily attributable to decreased EP from naturally infected layers (Carpenter et al., 1981; Mohammed et al., 1987; Stadelman, 1988). Other losses experienced by egg producers include poor feed conversion and increased medication costs (Patterson, 1994). Although most commercial poultry flocks in the United States are raised free of MG via strategic biosecurity and monitoring pro-

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Abbreviation Key: EP = egg production; EW = egg weight; FA = fluorescent antibody; FMG = F-strain of *Mycoplasma gallisepticum*; HI = hemagglutination-inhibition; MG = *Mycoplasma gallisepticum*; MS = *Mycoplasma synoviae*; PAW = percentage albumen weight; PSW = percentage shell weight; PYW = percentage yolk weight; RG = relative eggshell water vapor conductance; SPA = serum plate agglutination.

grams, they are still at risk of infection. In spite of intensive eradication programs, the frequency of outbreaks in layer facilities has increased over recent years and susceptible birds can be readily infected by this organism (Kerr and Olson, 1964; Kleven, 1981; Branton et al., 1984; Yoder, 1991). Once a bird is infected with MG, it is generally considered chronically infected for life (Brown et al., 1995).

During the past several years, vaccination of commercial layers with live MG vaccine produced from F-strain (FMG) of low to moderate virulence has become available to protect flocks against natural MG infections (Branton et al., 1997). The vaccine strain displaces natural field strain infections and has a low rate of bird-to-bird transmission (Levisohn and Kleven, 1981; Kleven et al., 1990). Once vaccinated, the birds remain permanent carriers of FMG (Brown et al., 1995). Live vaccines are effective in minimizing EP losses if administered to commercial layers before exposure to more virulent field strains of MG (Luginbuhl et al., 1976). Inoculations with FMG between 8 and 18 wk of age allow a pullet to receive a mild infection and recover before coming into EP (Yoder et al., 1984). Layers vaccinated with FMG will produce more eggs than unvaccinated hens naturally infected with MG, while MG clean flocks have been reported to lay more eggs than either FMG-vaccinated or field-strain MG-infected hens (Carpenter et al., 1981; Mohammed et al., 1987).

There is a scarcity of information characterizing egg production and egg characteristics in commercial layers inoculated between 8 and 18 wk of age with FMG. Therefore, the present study was designed to determine EP, feed utilization, and a comprehensive profile of various egg-quality parameters in commercial layers inoculated with FMG at 12 wk of age.

MATERIALS AND METHODS

Pullet Housing and Management

In each of two trials, one thousand 1-d-old pullets of a single genetic strain were obtained from a commercial source that was monitored and certified free for MG and *M. synoviae* (MS) (National Poultry Improvement Plan and Auxiliary Provisions, 1995). Chickens 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, chickens were also vaccinated for Newcastle Disease and infectious bronchitis by the same route. At 5 wk of age, 10 randomly selected pullets were bled from the left *cutanea ulnea* wing vein and tested for antibodies to MG and MS using both the serum plate agglutination (SPA) and the hemagglutination-inhibition (HI) tests (Yoder, 1975). At the same time, swabs were collected from the choanal cleft (Branton et al., 1984) and placed into tubes containing Frey's broth medium (Frey et al., 1968) supplemented with an additional 0.15 mg thallium acetate and 10^6 IU penicillin-G/mL. Tubes were incubated at 37 C for 30 d or until a phenol red indicator reaction occurred in the media. A sample from those that changed color was then inoculated onto Frey's-based (Papageorgiou medium) agar and incubated at 37 C. Colonies with morphology suggestive of *Mycoplasma* species were examined by an agar plate fluorescent antibody (FA) method (Baas and Jasper, 1972) that used direct labeling of colonies stained with anti-FMG polyclonal antibodies produced in rabbits and labeled with fluorescein isothiocyanate (Kleven, 1981).

Up until the pullets were 12 wk of age, they were placed on clean dry litter in a 5.5 × 6.1 m section of a conventional house resulting in an initial flock density of 0.034 m^2/bird. A daily artificial lighting schedule followed a 13h L:11h D cycle. One 75-W incandescent light bulb was used to illuminate each 8.4 m^2 of floor space, providing a calculated intensity at bird level of 35.5 lx. Feed and water were provided for ad libitum consumption in each trial. Ingredient percentages and dietary analyses of the basal starter and grower diets used in both trials are provided in Table 1. All diets were formulated to meet or exceed National Research Council (1994) specifications. No medication was administered during the interval of either trial.

At 12 wk of age, 11 pullets were randomly selected and placed in each of 8 (Trial 1; total of 88 pullets) or 16 (Trial 2; total of 176 pullets) negative pressure fiberglass biological isolation units (1.16 m^2). The units were housed in a previously described poultry disease isolation facility (Branton and Simmons, 1992). Hen numbers were reduced to 10 per unit at point-of-lay (18 wk of age) so that bird density was 0.116 m^2/bird for the duration of each trial. In each trial, half of the total number of isolation units contained FMG-free control birds, whereas the other half contained FMG-inoculated birds. There were four replicate units per treatment in Trial 1 and eight replicate units per treatment in Trial 2. Beginning at 18 wk of age, the artificial lighting schedule was increased 15 min/day until a 16 h 15 min L:7 h 45 min D cycle was achieved. Chickens were maintained on that schedule through the remainder of the experiments. Ingredient percentages and dietary analyses of the basal developer, pre-lay, and layer diets used in both trials are also provided in Table 1. In both trials at 26 and 54 wk of age, quadruplicate feed samples per lot of mixed feed were analyzed for moisture, ash, CP, crude fat, and crude fiber. All determined analyses were performed according to the methods of the Association of Official Analytical Chemists (1980) and averaged for each of the two trials at each time period. Available protein and lysine percentages in the layer diet were adjusted according to the percentage of feed consumed per bird every 28 d until trial termination (54 wk in Trial 1 and 60 wk in Trial 2).

FMG Inoculation

In each trial, pullets treated with FMG were inoculated via eye drop in the right eye at 12 wk of age with 0.04 mL of a 24-h broth culture of high-passage FMG (99th

TABLE 1. Ingredient percentages and calculated and determined analyses of pullet and layer diets

	Starter	Grower	Developer	Prelay	Layer[1]					
Age (week)	0–6	6–12	12–18	18–20	20	28	32	36	40	44–60
	(%)									
Ingredients										
Corn, 8.6%	64.51	73.63	72.24	61.36	58.12	64.94	68.38	71.33	63.38	70.47
Soybean meal, 48%	30.97	22.09	17.17	19.13	27.74	23.16	20.35	17.44	24.49	18.29
Wheat middlings, 15%	7.83	7.83	6.39	11.67	0.00	0.00	0.00	0.00	0.00	0.00
Vitamin premix[2,3]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
DL-methionine[4]	0.15	0.11	0.10	0.13	0.22	0.16	0.12	0.08	0.17	0.09
Dicalcium phosphate[5]	2.08	1.99	1.92	1.68	2.00	1.81	1.81	1.81	1.81	1.81
Limestone[6]	1.06	0.95	0.98	4.82	9.25	8.66	8.06	8.06	8.71	8.06
Sodium chloride[7]	0.48	0.47	0.47	0.47	0.53	0.53	0.53	0.53	0.53	0.53
Poultry fat	0.50	0.50	0.50	0.50	1.90	0.50	0.50	0.50	0.65	0.50
Dietary analyses										
CP, calculated	20.50	17.00	15.50	16.34	18.09	16.40	15.31	14.12	16.95	14.47
CP, determined	ND[8]	ND	ND	ND	18.70	ND	ND	ND	ND	14.55
Crude fiber, calculated	2.29	2.24	2.55	2.76	2.17	2.21	2.21	2.20	2.21	2.20
Crude fiber, determined	ND	ND	ND	ND	3.75	ND	ND	ND	ND	2.70
Crude fat, calculated	3.22	3.52	3.68	3.48	4.27	3.12	3.23	3.32	3.22	3.30
Crude fat, determined	ND	ND	ND	ND	4.00	ND	ND	ND	ND	2.85
Ash, determined	ND	ND	ND	ND	13.25	ND	ND	ND	ND	17.50
Moisture, determined	ND	ND	ND	ND	11.35	ND	ND	ND	ND	11.30
ME, calculated kcal/kg	3,000	3,101	3,051	2,819	2,819	2,828	2,879	2,910	2,819	2,901
Available phosphorus, calculated	0.43	0.42	0.42	0.38	0.37	0.33	0.34	0.34	0.34	0.34
Calcium, calculated	0.88	0.82	0.82	2.25	4.00	3.73	3.50	3.50	3.75	3.50
Lysine, calculated	1.10	0.85	0.73	0.80	0.97	0.85	0.77	0.69	0.88	0.71
Methionine, calculated	0.50	0.42	0.38	0.41	0.52	0.44	0.40	0.35	0.47	0.36
Methionine + cystine, calculated	0.81	0.68	0.61	0.65	0.80	0.70	0.63	0.56	0.73	0.58
Potassium, calculated	0.81	0.66	0.55	0.56	0.72	0.65	0.61	0.56	0.67	0.57
Sodium, calculated	0.20	0.20	0.20	0.20	0.21	0.21	0.21	0.21	0.21	0.21
Tryptophan, calculated	0.28	0.23	0.20	0.22	0.25	0.22	0.20	0.19	0.23	0.19
Xanthophyll, calculated	6.45	7.36	7.22	6.14	5.81	6.49	6.84	7.13	6.34	7.05

[1]Available protein and lysine percentages in the layer diet were adjusted as needed according to the percentage of feed consumed per bird every 28 d until trial termination.

[2]Vitamin premix provided per kilogram of diet: vitamin A, 7,710 IU; cholecalciferol, 2,202 IU; vitamin E, 10 IU; menadione, 0.88 mg; vitamin B_{12}, 0.01 mg; choline, 380 mg; riboflavin, 5 mg; niacin, 33 mg; pantothenic acid, 9 mg; thiamine, 1 mg; folic acid, 0.6 mg; biotin, 0.06 mg; pyridoxine, 0.9 mg; ethoxyquin, 0.03 g.

[3]Trace minerals provided in vitamin premix: manganese, 2.2%; zinc, 2.0%; iron, 1.1%; copper, 1,400 ppm; iodine, 200 ppm; and selenium, 40 ppm.

[4]Manufactured by Degussa Corp., Ridgeland Park, NJ.

[5]Manufactured by IMC-Agrico Feed Ingredients, Bannockburn, IL.

[6]Manufactured by Franklin Industrial Minerals, Nashville, TN.

[7]Manufactured by Cargill Incorporated, Minneapolis, MN.

[8]Not determined.

passage above the unknown passage level) provided by S. H. Kleven[4]. Inoculum titers were 5.0×10^6 and 1.0×10^5 cfu/mL in Trials 1 and 2, respectively. Similarly, pullets designated as controls 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.

Mycoplasma Detection

In each trial at 20 wk, and again at 54 wk in Trial 1 and 58 wk of age in Trial 2, one randomly selected hen from each of four FMG-free control and FMG-treated isolation units was bled and swabbed. Each of these samples were tested for the presence of *Mycoplasma* species as previously described for pullets.

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Data Collection

Individual BW of all hens in each unit were recorded at 12, 16, 20, 22, 24, 28, 30, 32, 36, 40, 44, 48, 52, and 54 wk of age in both trials. In Trial 2, BW was recorded as in Trial 1, but additionally at 34, 46, and 58 wk of age. Commensurate with the production of the first egg (18 wk of age) in control hens in both trials, eggs from control and treatment groups were collected daily until trial termination at 54 wk (Trial 1) and 60 wk (Trial 2). Egg production data for FMG-clean and FMG-inoculated hens in each trial were expressed as percentage hen-day production. Ten eggs per pen were weighed at 22, 24, 28, 30, 32, 36, 40, 44, 48, and 52 wk of age in both trials. In addition to egg weight (EW) being determined for the same weeks in Trial 2 as in Trial 1, in Trial 2 eggs were also weighed at 34, 46, and 58 wk of age. Egg size frequency distribution was determined by converting EW in grams to ounces and categorizing these as undersized, peewee,

TABLE 2. Determined fatty acid analyses of layer diets at 26 and 54 wk of age in Trials 1 and 2

Determined fatty acid analyses	26 wk		54 wk	
	Trial 1	Trial 2	Trial 1	Trial 2
	(%)			
Myristic (C14:0)	0.0	0.0	0.9	0.6
Palmitic (C16:0)	17.8	17.9	17.0	15.4
Palmitoleic (C16:1)	3.8	3.9	1.8	1.7
Stearic (C18:0)	4.3	3.3	3.8	4.2
Oleic (C18:1)	33.1	31.6	28.2	32.0
Linoleic (C18:2)	38.3	42.3	45.3	43.4
Linolenic (C18:3)	1.1	1.0	1.3	1.3

small, medium, large, extra-large, or jumbo sizes in accordance with the Agricultural Marketing Service of the United States Department of Agriculture (1996). During those same weeks listed above for EW, eggs were subsequently broken out to determine percentage yolk (PYW), albumen (PAW), and eggshell (PSW) weights and were expressed as percentages of EW. Eggshell moisture was removed according to the procedure of Brake et al. (1984). As specified by Peebles et al. (1998), at least 10 eggs per replicate pen were gathered for measurement of relative eggshell water vapor conductance (RG). These eggs were collected separately but at the same time as those used for the other egg-quality determinations. Measurement of RG was as described by Peebles et al. (1994).

In both trials at 26 and 54 wk of age, one fecal sample from each of two replicate units belonging to each treatment was randomly selected and analyzed for moisture, ash, CP, crude fat, crude fiber, and fatty acid content. Also, quadruplicate feed samples per lot of mixed feed were analyzed for fatty acid content. Determined fatty acid analyses of the layer diets are provided in Table 2. Between 42 and 58 wk in Trial 2, two randomly selected units (four total) in both the control and treatment groups were used in a pilot study for the determination of feed consumption (g/bird per d) and feed conversion (g feed intake/g eggs produced). Total feed consumed and numbers of eggs produced per unit were recorded each week to derive feed consumption and conversion.

Statistical Analysis

A completely randomized experimental design was utilized. Body weight, EP, EW, egg size, PSW, PYW, PAW, RG, feed consumption and conversion, and fecal contents were subjected to a repeated measures analysis where the same experimental units were observed over an extended time period. Individual sample data within each replicate unit were averaged prior to analysis. Least-squares means were compared in the event of significant global effects (Steel and Torrie, 1980; Petersen, 1985; Freund and Wilson, 1997). All data were analyzed using the MIXED Procedure of SAS, Version 8 (1996). Statements of significance were based on $P \leq 0.05$ unless otherwise stated.

RESULTS

In both trials, all initial mycoplasmal cultures as well as SPA and HI test results obtained from 5-wk-old pullets were negative for MG and MS. Control serum samples obtained at 20 wk of age in each trial and also at 54 wk (Trial 1) and 58 wk (Trial 2) were SPA and HI negative for MG, while the same tests were positive for MG in the FMG-inoculated hens. Hens were considered FMG-free when they exhibited no detectable HI titers. All FMG-inoculated hens had HI titers ≥ 1:80. Similarly, FA culture results for swabs obtained at 20 wk of age in each trial and also at 54 wk (Trial 1) and 58 wk (Trial 2) were negative for *Mycoplasma* species growth for four out of four FMG-free hens tested, while growth was evident for four out of four FMG-inoculated hens tested. Mortality was not significantly different between FMG-free and FMG-inoculated hens in either trial.

There was a main effect ($P \leq 0.0001$) due to layer hen age for BW in Trials 1 and 2 (Table 3). In general, birds in each trial grew as expected over the experimental periods, averaging 1,520 g at 54 wk in Trial 1 and 1,565 g at 58 wk in Trial 2. Treatment × age interactions and FMG treatment main effects were not significant; however, treatment means in Trial 1 were 1,381 and 1,388 g, and in Trial 2 were 1,346 and 1,367 g, for FMG-clean and FMG-infected birds, respectively. Layer hen age main effects were also observed in Trial 2 for EP ($P \leq 0.0001$), feed consumption ($P \leq 0.03$), and feed conversion ($P \leq 0.0001$). In Trial 2, at 22 (prepeak), 32 (peak), and 60 wk of age (study termination), EP was 61.6 ± 0.5, 80.4 ± 0.5, and 28.2 ± 0.5 percentage, respectively. Although no significant treatment × age interaction was observed for EP in Trial 2, weekly EP for both treatments are provided in

TABLE 3. Body weight (g) of laying hens at 12, 16, 20, 22, 24, 28, 30, 32, 34, 36, 40, 44, 46, 48, 52, 54, and 58 wk of age in Trials 1 and 2

Age (wk)	Trial 1[1]	Trial 2[1]
12	924.13^{j,2}	936.16^{j,2}
16	1160.41^{i}	1107.36^{i}
20	1321.11^{h}	1310.95^{h}
22	1364.91^{g}	ND
24	1375.26^{g}	1344.92^{g}
28	1409.90^{f}	1365.03^{f}
30	1408.39^{f}	ND
32	1426.25^{e}	ND
34	ND[3]	1425.73^{e}
36	1459.11^{d}	ND
40	1468.57^{d}	1468.84^{d}
44	1484.05^{c}	ND
46	ND	1510.81^{c}
48	1538.54^{a}	ND
52	1521.55^{b}	1526.47^{b}
54	1519.87^{b}	ND
58	ND	1565.17^{a}

[a-j]Means within trial among week of age with no common superscript differ significantly ($P \leq 0.05$).

[1]Based on pooled estimate of variance SEM = 14.5 in Trial 1 and 14.07 in Trial 2.

[2]n = 80 in Trial 1 and n = 160 in Trial 2.

[3]Not determined.

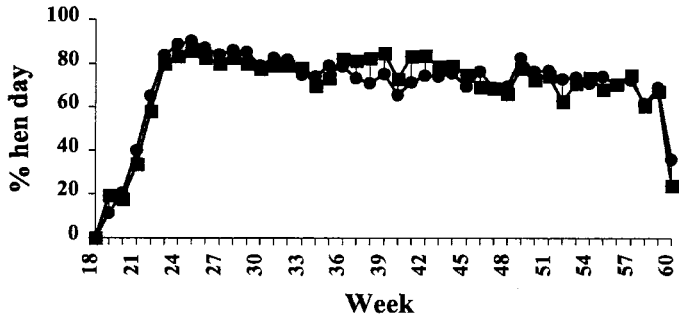

FIGURE 1. Weekly percentage hen day egg production at 18 to 60 wk of age for F-strain *Mycoplasma gallisepticum* (FMG)-free (●) vs. FMG-inoculated (■) layer hens in Trial 2 are significantly different ($P \leq 0.05$). Based on pooled estimate of variance SEM = 5.6.

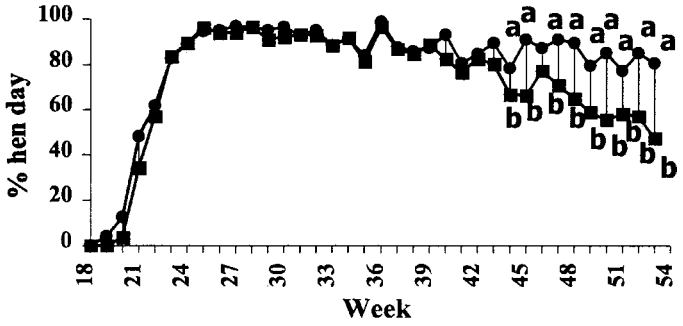

FIGURE 2. Weekly percentage hen day egg production at 18 to 54 wk of age for F-strain *Mycoplasma gallisepticum* (FMG)-free (●) vs. FMG-inoculated (■) layer hens in Trial 1. Symbols within a week having different letters are significantly different ($P \leq 0.05$). Based on pooled estimate of variance SEM = 4.72.

Figure 1. In Trial 2, at 42 and 58 wk, feed consumption (g/bird per day) was 99.5 ± 0.5 and 118.8 ± 0.5, respectively, and feed conversion (g feed intake/g eggs produced) was 2.35 ± 0.5 and 2.63 ± 0.5, respectively. In both trials, hen age main effects ($P \leq 0.0001$) were observed for EW. In Trial 1, at 22 (prepeak), 32 (peak), and 54 wk of age (study termination), EW was 44.8 ± 0.5, 54.1 ± 0.5, and 58.8 ± 0.5 g, respectively. In Trial 2, EW at 22 (prepeak), 32 (peak), and 60 (study termination) wk was 45.3 ± 0.5, 53.7 ± 0.5, and 59.1 ± 0.5 g, respectively. In Trial 1, a main effect ($P \leq 0.02$) due to layer hen age was observed for the percentage of undersized eggs that were laid. Those in the peewee, small, medium, large, and extra-large egg size categories were affected ($P \leq 0.0001$) by hen age in both trials. Furthermore, in both trials, hen age main effects ($P \leq 0.0001$) were observed for PAW, PYW, and RG, and in Trial 1, an age main effect ($P \leq 0.0001$) was observed for PSW. In Trial 1, main effects due to layer hen age were observed for fecal ash ($P \leq 0.02$), moisture ($P \leq 0.03$), fat ($P \leq 0.03$), fiber ($P \leq 0.04$), myristic acid ($P \leq 0.04$), and palmitoleic acid ($P \leq 0.05$) contents.

Initiation of lay was delayed 8 d in Trial 1 and 4 d in Trial 2 for FMG-inoculated hens in comparison to uninoculated controls. In Trial 1, there was a reduction ($P \leq 0.05$) in total number of eggs laid per hen due to FMG inoculation (Table 4). Although not significant ($P = 0.1$), total egg mass per hen was also reduced more than 1,000 g in Trial 1 due to FMG inoculation. However, in Trial 2 number of eggs and egg mass per hen were not affected by FMG inoculation (Table 4). In Trial 1, an age × FMG treatment interaction ($P \leq 0.003$) was observed for EP (Figure 2). A decline in EP of inoculated hens began at Week 44 so that EP became significantly less than that of uninoculated controls. This same comparative pattern of EP continued each week until the study was terminated at 54 wk of age. In Trial 2, there were ages by FMG treatment interactions for percentage undersized EP ($P \leq 0.0001$) and PSW ($P \leq 0.007$). Percentages of undersized eggs laid by FMG-inoculated birds were lower at 19 wk, but were higher at 20 and 21 wk when compared to controls (Table 5). Percentage eggshell weight at 24 and 34 wk of age was significantly higher in FMG-inoculated birds when compared to controls (Table 6). In Trial 1, there was an age by FMG treatment interaction ($P \leq 0.03$) for fecal stearic acid. The percentage of stearic acid in the feces of FMG-inoculated birds was significantly lower at 26 and 54 wk of age compared to controls. At 26 wk and 54 wk of age, fecal stearic acid was 7.8 and 4.2% in controls and 5.7 and 3.3% in FMG-inoculated hens, respectively (SEM = 0.59).

DISCUSSION

As described by Zander (1984), MG is already established on many multi-age farms, and transmission from mature hens to replacement pullets ensures its existence.

TABLE 4. Mean eggs per hen and egg mass per hen from F-strain *Mycoplasma gallisepticum* (FMG)-free and FMG-inoculated hens throughout lay in Trials 1 and 2

	Trial 1[1]		Trial 2[2]	
Treatment	Eggs/hen	Egg mass/hen (g)	Eggs/hen	Egg mass/hen (g)
FMG-free	197[a]	10,800	192	10,565
FMG-inoculated	178[b]	9,775	190	10,500

[a,b]Means within trial and parameter among treatment groups with no common superscript differ significantly ($P \leq 0.05$).

[1]n = 80, and based on pooled estimate of variance SEM = 5.27 for eggs/hen and SEM = 490 for egg mass/hen.

[2]n = 160, and based on pooled estimate of variance SEM = 12.05 for eggs/hen and SEM = 1,060 for egg mass/hen.

TABLE 5. Percentage undersized eggs in F-strain *Mycoplasma gallisepticum* (FMG)-free and FMG-inoculated hens at 19, 20, and 21 wk of age in Trial 2[1]

	Undersized eggs	
Age (wk)	FMG-free	FMG-inoculated
	(%)	
19	6.25[a,2]	0.00[b]
20	2.80[b]	13.64[a]
21	0.78[b]	4.10[a]

[a,b]Means within week of age among treatment groups with no common superscript differ significantly ($P \leq 0.05$).

[1]Based on pooled estimate of variance SEM = 1.16.

[2]n = 80.

TABLE 6. Shell weight as a percentage of fresh egg weight in F-strain *Mycoplasma gallisepticum* (FMG)-free and FMG-inoculated hens at 22, 24, 28, 34, 40, 46, 52, and 58 wk of age in Trial 2[1]

Age (week)	FMG-free	FMG-inoculated
	(%)	
22	8.92[2]	9.02
24	8.45[b]	9.21[a]
28	8.96	9.15
34	8.56[b]	8.97[a]
40	8.74	8.95
46	8.71	8.51
52	8.59	8.37
58	8.57	8.70

[a,b]Means within age among treatment group with no common superscript differ significantly ($P \leq 0.05$).

[1]Based on pooled estimate of variance SEM = 0.13.

[2]n = 40.

Unfortunately, since it is often impractical to totally depopulate and disinfect a large multi-age farm, the best solution in this case may be to establish an MG vaccination program (Bermudez and Kalbac, 1988). The development of immunity to mycoplasmas can protect animals, as vaccination for mycoplasma diseases in animals is commonly practiced (Barile, 1985) where the disease is endemic. These results from the current study indicate that there was no cross contamination between FMG-inoculated and FMG-clean birds. Zero cross contamination between FMG-inoculated and FMG-clean birds is indicative of a strategic biosecurity and sanitation program (Patterson, 1994). Mortality was negligible when FMG-inoculated birds were housed in temperature-regulated isolation units. Also, Branton and Deaton (1985) reported that although mortality may be negligible in adult flocks infected with FMG, there still can be a reduction in the number of birds in production.

At the beginning and end of both trials in this study, SPA tests from swabs and sera and HI sera tests, along with the FA tests verified systemic infections in FMG-inoculated birds. Conversely, sham-inoculated birds remained FMG-free throughout each trial. Age-related changes in EW and EP in the current study were similar to those in an earlier study of layer hens (Branton et al., 1997). Those authors found that inoculation with FMG at 10 wk of age did not affect EW or EP. Other authors have reported that EP, BW, and feed efficiency were reduced in flocks naturally infected with MG (Yoder, 1978, 1991; Mohammed et al., 1987). Variables such as PAW, PSW, PYW, and RG, along with feed consumption and conversion, and fecal analyses had not been previously explored until the present trials. All egg and eggshell quality parameters selected for this study were chosen to reflect the developmental process of eggs in chickens. Feed and fecal analyses provided additional dietary information and were similar in each trial.

In the existing MG literature, a delay in EP in MG-vaccinated or infected hens as compared to MG-free hens has not been reported. However, in both trials of this study, all birds inoculated with FMG at 12 wk of age laid their first egg approximately 1 wk after FMG-free controls.

Reduced EP and financial losses have been attributed to MG infection in layers (Mohammed et al., 1987; Stadelman, 1988). In Trial 1, FMG-inoculated birds had a significantly lower total EP, which was mainly due to significantly lower hen-day EP after 42 wk. Each control hen laid approximately 19 more eggs during the trial period than any FMG-inoculated hens. This has also been shown in laying flocks positive for field-strain MG where they produce as many as 16 fewer eggs per hen per year than MG-negative flocks (Carpenter et al., 1981). When the performance of FMG-vaccinated and MG-infected flocks are compared, FMG-vaccinated flocks will produce 7.0 more eggs per hen per year (Carpenter et al., 1981). In that same study, when uninfected flocks were compared to FMG-vaccinated flocks, the advantage decreased to 8.7 eggs per hen. However, Branton et al. (1997) reported that there was no difference in EP over a 45-wk laying period when FMG-free and FMG-inoculated hens were compared. Branton et al. (2000) also reported that there was no difference in EP when control and ts-11 vaccine-strain MG-inoculated hens were compared. Glisson and Kleven (1984) reported that all hens vaccinated with low-virulence MG at 16 or 20 wk of age were protected against EP drops seen in unvaccinated hens challenged with virulent MG. Other reports have described variable levels of protection against decreases in EP of hens vaccinated with low-virulence live MG (Truscott et al., 1974; Fabricant, 1977).

Past data indicate that FMG-vaccinated hens lay fewer large eggs than *Mycoplasma*-clean hens (Branton et al., 1999). Specifically, 43% of eggs laid in a 45-wk laying cycle are usually sized as large, and this phenomenon reportedly decreases by approximately 8% in FMG-vaccinated hens. Also, Branton et al. (2002) found a significant increase in jumbo-sized eggs laid by 6/85 strain MG-vaccinated hens as compared to control hens. The lower percentage of undersized eggs laid by FMG-inoculated hens at 19 wk and the higher percentage of similarly sized eggs laid at both 20 and 21 wk in the present study are indicative of a delay in EP in FMG-infected birds. As previously stated, initiation of lay in control hens began earlier than FMG-inoculated hens. Undersized eggs were

being laid by control hens until the beginning of lay occurred in FMG-inoculated hens. Concurrently, when treated birds began to lay undersized eggs, control hens shifted to a larger size category.

A lack of information on shell characteristics in MG-infected birds exists in the literature; however, in the present study relative PSW was significantly increased at 24 and 34 wk of age in FMG-inoculated birds. This may indicate that infected hens deposited relatively more calcium carbonate on eggs during peak production than those not infected with FMG. Conductance through eggshell pores provides a mechanism of chemical exchange with the environment (Tullett, 1984; Paganelli et al., 1987). Although, RG may be insignificant for infertile eggs produced from FMG-infected laying hens, it may be an important aspect in FMG-infected breeder and broiler breeder operations. This information may provide vital insights as to the effects of MG colonization in the oviduct.

Fatty acids are essential in the growth and performance of laying hens (Austic and Scott, 1997). These data indicate that FMG-inoculated birds have a significantly lower percentage of fecal stearic acid at 26 and 54 wk of age as compared to controls. Fecal stearic acid decreases at 26 wk of age and may be an indirect result of delayed onset of lay, while decreases at 54 wk of age may be an indirect result of lowered EP observed in the FMG-inoculated hens. Since nutritional balance is a key factor in layer-hen production, other variables that should be investigated to better understand the mechanisms behind the effects of FMG on layer performance should include digestive organ characteristics, along with those of the blood and reproductive tract.

Mycoplasma gallisepticum varies from being a subtle, low-level disease to an overt, severe disease, and variation of MG phenotypes and surface antigens may account for these different virulent patterns (Howard and Taylor, 1979). In addition, virulence of mycoplasmas appears to be related to the ability of the organisms to evade nonspecific defense mechanisms, and this feature rapidly declines through passage in artificial media (Davidson et al., 1988). Because environmental factors, contact with host cells, and other infectious agents influence the severity of mycoplasma infections, these isolation experiments are necessary to provide an understanding of the pathogenic processes associated with MG.

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REFERENCES

Association of Official Analytical Chemists Manual. 1980. Official Methods of Analysis. 14th ed. Association of Official Analytical Chemists, Washington, DC.

Austic, R. E., and M. L. Scott. 1997. Nutritional diseases/interactions between nutrition and infectious disease. Pages 47–80 in Diseases of Poultry. 10th ed. M. S. Hofstad, H. J. Barnes, B. W. Calnek, W. M. Reid, and H. W. Yoder, Jr., ed. Iowa State University Press, Ames, IA.

Baas, E. J., and D. E. Jasper. 1972. Agar block technique for identification of Mycoplasmas by use of fluorescent antibody. Appl. Microbiol. 23:1097–1100.

Barile, M. F. 1985. Immunization against mycoplasma infections. Pages 451–492 in The Mycoplasmas. S. Razin and M. F. Barile, ed. Academic Press, New York.

Bermudez, A. J., and M. Kalbac. 1988. Control of *Mycoplasma gallisepticum* infection in commercial layers: A field study. JAVMA. 192:1783.

Brake, J., J. F. Ort, T. A. Carter, and W. R. Campbell. 1984. Effect of insect growth regulator CGA-72662 (Larvadex) on broiler breeder production, hatchability, and subsequent chick performance. Poult. Sci. 63:910–916.

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* vaccination 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. W. Deaton. 1985. Egg production, egg weight, eggshell strength, and mortality in three strains of commercial layers vaccinated with F-strain *Mycoplasma gallisepticum*. Avian Dis. 29:832–837.

Branton, S. L., H. Gerlach, and S. H. Kleven. 1984. *Mycoplasma gallisepticum* isolation in layers. Poult. Sci. 63:1917–1919.

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.

Branton, S. L., B. D. Lott, J. D. May, W. R. Maslin, G. T. Pharr, S. D. Bearson, S. D. Collier, and D. L. Boykin, 2000. The effects of ts-11 strain *Mycoplasma gallisepticum* vaccination in commercial layers on egg production and selected egg quality parameters. Avian Dis. 44:618–623.

Branton, S. L., B. D. Lott, J. D. May, W. R. Maslin, G. T. Pharr, J. E. Brown, and D. L. Boykin. 1999. The effects of F-strain *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, and the dual infection in commercial layer hens over a 44-week laying cycle when challenged before beginning of lay. II. Egg size distribution. Avian Dis. 43:326–330.

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.

Brown, J. E., S. L. Branton, and J. D. May. 1995. Effect of isolation and sanitation on the recovery of F-strain *Mycoplasma gallisepticum* from chronically infected hens held in biological isolation units. Avian Dis. 39:263–268.

Carpenter, T. E., E. T. Mallinson, K. F. Miller, R. F. Gentry, and L. D. Schwartz. 1981. Vaccination with F-strain *Mycoplasma gallisepticum* to reduce production losses in layer chickens. Avian Dis. 25:404–409.

Davidson, M. K., J. K. Davis, J. R. Linsey, and G. H. Cassell. 1988. Clearance of different strains of *Mycoplasma pulmonis* from the respiratory tract of C3H/HeN mice. Infect. Immun. 56:2163–2168.

Domermuth, C. H., W. B. Gross, and R. T. DuBose. 1967. Mycoplasmal salpingitis of chickens and turkeys. Avian Dis. 11:393–398.

Fabricant, J. 1977. *Mycoplasma gallisepticum* (MG) immunization of commercial laying hens. Pages 71–74 in Proc. 26th Western Poultry Dis. Conf., Univ. of Calif. Academic Press, Davis, CA.

Freund, R. J., and W. J. Wilson. 1997. Statistical Methods. Academic Press, San Diego, CA.

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.

Glisson, J. R., and S. H. Kleven. 1984. *Mycoplasma gallisepticum* vaccination: Effects on egg transmission and egg production. Avian Dis. 28:406–415.

Howard, C. J., and G. Taylor. 1979. Variation in the virulence of strains of *Mycoplasma pulmonis* related to susceptibility to killing by macrophages in vitro. J. Gen. Microbiol. 114:289–294.

Kerr, K. M., and N. O. Olson. 1964. Control of infectious synovitis. The effect of age of chickens on the susceptibility to three agents. Avian Dis. 8:256–263.

Kleven, S. H.. 1981. Transmissibility of the F-strain of *Mycoplasma gallisepticum* in leghorn chickens. Poult. Sci. 25:1005–1018.

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.

Kreig, N. R., and J. G. Holt. 1984. Mycoplasmas. Pages 740–793 in Bergey's Manual of Systematic Bacteriology. 9th ed. Vol. 1. Williams & Wilkins, Baltimore.

Levisohn, S., and S. H. Kleven. 1981. Vaccination of chickens with nonpathogenic *Mycoplasma gallisepticum* as a means for displacement of pathogenic strains. Isr. J. Med. Sci. 17:669–673.

Ley, H. L., and H. W. Yoder, Jr.. 1978. *Mycoplasma gallisepticum* infections. Pages 194–207 in Diseases of Poultry. 7th ed. M. S. Hofstad, B. W. Calnek, C. F. Helmboldt, W. M. Reid, and H. W. Yoder, Jr., ed. Iowa State University Press, Ames, IA.

Luginbuhl, R. E., M. E. Tourtellotte, and M. N. Frazier. 1976. *Mycoplasma gallisepticum*-control by immunization. Ann. N. Y. Acad. Sci. 143:234–238.

Mohammed, H. O., T. E. Carpenter, and R. Yamamoto. 1987. Economic impact of *Mycoplasma gallisepticum* and *M. synoviae* in commercial layer flocks. Avian Dis. 31:477–482.

National Poultry Improvement Plan and Auxiliary Provisions. 1995. Chapter 9 Code of Federal Regulations (9CFR). USDA-APHIS-VS, Conyers, GA.

National Research Council. 1994. Nutrient Requirements of Poultry. 9th Rev. ed. National Academy Press, Washington, DC.

Paganelli, C. V., A. Ar, and H. Rahn. 1987. Diffusion-induced convective gas flow through the pores of the eggshell. J. Exp. Zool. 1:173–180.

Patterson, P. H. 1994. Coping with *Mycoplasma gallisepticum*. Internews 7:1-3.

Peebles, E. D., E. H. Miller, C. R. Boyle, J. D. Brake, and M. A. Latour. 1994. Effects of dietary thiouracil on thyroid activity and eggshell quality in commercial layers. Poult. Sci. 73:1829–1837.

Peebles, E. D., T. Pansky, S. M. Doyle, C. R. Boyle, T. W. Smith, M. A. Latour, and P. D. Gerard. 1998. Effects of dietary fat and eggshell cuticle removal on egg water loss and embryo growth in broiler hatching eggs. Poult. Sci. 77:1522–1530.

Petersen, R. G. 1985. Design and Analysis of Experiments. Marcel Dekker, New York.

SAS Institute. 1996. SAS/STAT® Software: Changes and Enhancements through Release 6.11. SAS Institute, Inc., Cary, NC.

Stadelman, W. J. 1988. Economic impact of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* infection of a laying flock. Egg Ind. 94:12.

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

Truscott, R. B., A. E. Ferguson, and G. Speckmann. 1974. An infection in chickens with a strain of *Mycoplasma gallisepticum* of low virulence. Can. J. Comp. Med. 38:341–343.

Tullett, S. G. 1984. The porosity of avian eggshells. Comp. Biochem. Physiol. 78:5–13.

United States Department of Agriculture-Agricultural Marketing Service. 1996. Poultry Division. U. S. Quality Standards. AMS-625. USDA Washington, DC.

Yoder, H. W., Jr. 1975. Mycoplasmosis: Isolation and identification of avian pathogens. Pages 109–116 in American Assoc. of Avian Path. College Station, TX.

Yoder, H. W., Jr. 1978. *Mycoplasma gallisepticum* infections. Pages 236–250 in Diseases of Poultry. 7th ed. M. S. Hofstad, B. W. Calnek, C. F. Helmboldt, W. M. Reid, and H. W. Yoder, Jr., ed. Iowa State University Press, Ames, IA.

Yoder, H. W., Jr. 1991. Mycoplasmosis. Pages 198–212 in Diseases of Poultry. 9th ed. B. W. Calnek, H. J. Barnes, C. W. Beard, W. M. Reid, and H. W. Yoder, Jr., ed. Iowa State University Press, Ames, IA.

Yoder, H. W., Jr., and M. S. Hofstad. 1964. Characterization of avian Mycoplasma. Avian Dis. 8:481–512.

Yoder, H. W., Jr., S. R. Hopkins, and B. W. Mitchell. 1984. *Mycoplasma gallisepticum* oil-emulsion bacterins for protection against airsacculitis in broilers. Avian Dis. 28:224–234.

Zander, D. V. 1984. Principles of disease prevention. Pages 1–37 in Diseases of Poultry. 8th ed. M. S. Hofstad, H. J. Barnes, B. W. Calnek, W. M. Reid, and H. W. Yoder, Jr., ed. Iowa State University Press, Ames, IA.