Effects of F-Strain *Mycoplasma gallisepticum* Inoculation at Twelve Weeks of Age on the Blood Characteristics of Commercial Egg Laying Hens[1,2]

M. R. Burnham,* E. D. Peebles,*[,3] S. L. Branton,‡ M. S. Jones,* and P. D. Gerard†

*Department of Poultry Science and †Experimental Statistics Unit, Mississippi State University, Mississippi State, Mississippi 39762; and ‡South Central Poultry Research Laboratory, Agricultural Research Service, United States Department of Agriculture, Mississippi State, Mississippi 39762

ABSTRACT In two trials, the effects of an F-strain *Mycoplasma gallisepticum* (FMG) inoculation at 12 wk of age on the blood characteristics of commercial Single Combed White Leghorn laying hens were investigated throughout lay. Variables measured in both trials were whole blood hematocrit, plasma protein (PP), and serum cholesterol, triglycerides (ST), and calcium. In both trials, hematocrit at 20 wk of age was significantly increased in birds inoculated with FMG. In trial 1, ST and PP were significantly increased at 22 wk of age by FMG, while ST and PP were significantly decreased in FMG-inoculated birds at wk 54 and 52, respectively. When combined with the establishment of an FMG infection, the initial weeks of egg production become particularly stressful to the bird. Increases in these independent blood parameters between 8 and 10 wk postchallenge are suggestive of compensatory responses in these birds to an FMG challenge. Postpeak decreases in both ST (54 wk) and PP (52 wk) in FMG-infected birds may be the result of a more chronic effect of FMG on lipid and protein synthesis in the liver. These data are the first to suggest that alterations in egg production in response to FMG-infection in commercial layers, as noted in a previous report, may be associated with changes in hematocrit. However, because ST and PP were not affected by FMG in both trials, the responses of these blood parameters to FMG-infection may be inconsistent among flocks.

(*Key words*: blood, hematology, layer, lipid, *Mycoplasma gallisepticum*)

2003 Poultry Science 82:1397–1402

INTRODUCTION

The differential leukocyte counts of birds after exposure to *Mycoplasma* species have been described (Kerr and Olson, 1970; Branton et al., 1997), but further characterization of the blood from birds infected with *Mycoplasma gallisepticum* (MG) is lacking in the literature. Estrogen release and the onset of egg production in laying hens drastically increases liver metabolism (Lorentz et al., 1938; Hillyard et al., 1956) and its production of neutral lipids (Heald and Badman, 1963), serum triglycerides (ST) and phospholipids (Dashti et al., 1983). Eventually, these components are destined for yolk lipid deposition (Nimpf and Schneider, 1991; Walzem et al., 1999), but colonization of the liver by F-strain MG (FMG) (Sahu and Olson, 1976) may disrupt yolk lipid synthesis and subsequently reduce egg production.

The objective of the current study was to determine possible changes in blood characteristics associated with those in performance of FMG-inoculated hens. Blood characteristics included whole blood hematocrit (HCT), plasma protein (PP), serum cholesterol (SCH), ST, and serum calcium (SCA). Determinations of the effects of FMG on blood components in association with various production characteristics may provide vital information as to the physiological mechanisms behind previously observed alterations in the performance of FMG-infected hens.

MATERIALS AND METHODS

Pullet Housing and Management

In each of two trials, 1,000 day-old pullets of a single genetic strain were obtained from a commercial source that was monitored and certified free of MG and *Mycoplasma synoviae* (MS) (National Poultry Improvement Plan and Auxiliary Provisions, 1995). Chickens were vacci-

Received for publication October 18, 2002.

Accepted for publication April 2, 2003.

[1]This is Journal Article No. J-10168 from the Mississippi Agricultural and Forestry Experiment Station supported by MIS-321010.

[2]Use of trade names in this publication does not imply endorsement by Mississippi Agricultural and Forestry Experiment Station of these products, nor similar ones not mentioned.

[3]To whom correspondence should be addressed: dpeebles@poultry.msstate.edu.

Abbreviation Key: FMG = F-strain *Mycoplasma gallisepticum*; HCT = hematocrit; HI = hemagglutination-inhibition; MG = *Mycoplasma gallisepticum*; MS = *Mycoplasma synoviae*; PP = plasma protein; SCA = serum calcium; SCH = serum cholesterol; SPA = serum plate agglutination; ST = serum triglycerides.

nated 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, ten randomly selected pullets were bled from the left cutanea ulnea wing vein and tested for antibodies to MG and MS using both serum plate agglutination (SPA) and 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. An aliquot of the positive samples 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 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).

Pullets were maintained on clean dry litter in a 5.5- × 6.1-m floor pen from 1 d until 12 wk of age. Flock density at placement was 0.034 m^2/bird. A daily artificial lighting schedule followed a 13L:11D 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 have been reported by Burnham et al. (2002a). All diets were formulated to meet or exceed NRC (1994) specifications. No medication was administered during the course of either trial.

At 12 wk of age, 11 pullets were randomly selected and placed in each of eight (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). All birds were beak trimmed upon placement in the isolation units at 12 wk. In each trial, half of the total number of isolation units contained FMG-free control birds, whereas the other half contained FMG-inoculated birds. Four replicate units per treatment were in trial 1, and eight replicate units per treatment were in trial 2. Beginning at 18 wk of age, the artificial lighting schedule was increased 15 min/d until a 16 h 15 min L:7 h 45 min D daily cycle was achieved. Chickens were maintained on that schedule through the remainder of each trial.

Ingredient percentages and dietary analyses of the basal developer, prelay, and layer diets used in both trials have been reported by Burnham et al. (2002a). 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, quadruplicate feed samples per lot of mixed feed were taken at 26 and 54 wk of age and 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. Beginning at wk 20, 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 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 was tested for the presence of *Mycoplasma* species as previously described for pullets.

Data Collection

In each trial, hens were bled following an overnight fast. Blood was harvested at the same time of day at 16, 20, 22, 24, 28, 30, 32, 36, 40, 44, 48, 52, and 54 wk of age in trial 1 and at 16, 20, 24, 28, 34, 40, 46, 52, and 58 wk of age in trial 2 from four hens per isolation unit. Hematocrit, expressed as percentage blood packed cell (primarily red blood cell) volume, was determined through use of capillary tubes that were centrifuged in a micro-HCT centrifuge and were then read with a microcapillary reader. Serum cholesterol and ST expressed in milligrams per deciliter and PP expressed in grams per deciliter 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[5] as described by Latour et al. (1996b). Similarly, SCA concentrations expressed in milligrams per deciliter were determined by placing 10 μL of serum on a test slide which was analyzed on a Kodak Ektachem DTSC module analyzer[5], according to procedures of Tietz (1986). Control analyses were performed to assure that each sample was in the appropriate test range for accurate analysis.

[4]University of Georgia, Athens, GA.
[5]Eastman Kodak Co., Rochester, NY.

TABLE 1. Whole blood hematocrit in F-strain *Mycoplasma gallisepticum* (FMG)-free and FMG-inoculated Single Combed White Leghorn laying hens at various ages between 16 and 58 wk of age in trials 1 and 2

	Trial 1[1] (%)		Trial 2[1] (%)	
Age (wk)	FMG-free[2]	FMG-inoculated[2]	FMG-free[2]	FMG-inoculated[2]
16	30.9	30.2	29.5	29.1
20	30.1[b]	31.7[a]	26.9[b]	28.1[a]
22	27.6	27.2	ND	ND
24	27.0	26.2	28.2	27.2
28	26.7	25.7	27.8	27.6
30	26.3	25.0	ND	ND
32	25.2	25.7	ND	ND
34	ND[3]	ND	25.2	25.3
36	25.3	26.6	ND	ND
40	26.9	26.5	27.9	27.2
44	26.1	25.7	ND	ND
46	ND	ND	27.2	26.1
48	25.4	25.6	ND	ND
52	24.9	24.7	27.6	28.0
54	23.9	23.7	ND	ND
58	ND	ND	28.3	29.4

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

[1]Based on pooled estimate of variance SEM = 0.51 in trial 1 and 0.48 in trial 2.

[2]n = 16 samples for the calculation of means within treatment and week.

[3]Not determined.

Statistical Analysis

A completely randomized experimental design was utilized. All parameters were subjected to a repeated measures analysis in which 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 software (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, agar plate fluorescent antibody 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.

In each trial, there were significant ($P \leq 0.05$) age by FMG treatment interactions for HCT (Table 1). In comparison to FMG-free birds, inoculation with FMG resulted in an increase in the percentage of packed blood cells at 20 wk in both trials. In trial 1, there were significant ($P \leq 0.05$) age by FMG treatment interactions for both ST (Table 2) and PP (Table 3). Results from trial 1 indicated significant increases in ST and PP at 22 wk of age, followed by a significant decrease in ST at 54 wk and a significant decrease in PP at 52 wk in FMG-inoculated birds. There were no significant FMG treatment main effects or age by FMG treatment interactions for ST or PP in trial 2 or for SCH or SCA in either trial.

In trial 2, significant ($P \leq 0.0001$) main effects due to layer age were observed for ST and PP. Also, in both trials, significant ($P \leq 0.0001$) main effects due to layer age were observed for SCH and SCA. In the interest of

TABLE 2. Serum triglycerides in F-strain *Mycoplasma gallisepticum* (FMG)-free and FMG-inoculated Single Combed White Leghorn laying hens at various ages between 16 and 54 wk of age in trial 1[1,2]

Age (wk)	FMG-free (mg/dL)	FMG-inoculated (mg/dL)
16	167	131
20	2,597	1,781
22	2,857[b]	4,239[a]
24	3,274	3,518
28	3,219	3,214
30	3,362	3,029
32	3,558	2,813
36	3,127	2,802
40	3,803	3,386
44	3,551	4,089
48	3,321	3,364
52	3,393	3,013
54	4,028[a]	2,758[b]

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

[1]Based on pooled estimate of variance SEM = 364.3.

[2]n = 16 samples for the calculation of means within treatment and week.

TABLE 3. Plasma protein in F-strain *Mycoplasma gallisepticum* (FMG)-free and FMG-inoculated Single Combed White Leghorn laying hens at various ages between 16 and 54 wk of age in trial 1[1,2]

Age (wk)	FMG-free (g/dL)	FMG-inoculated (g/dL)
16	4.5	4.4
20	5.5	5.6
22	4.9[b]	5.9[a]
24	5.7	5.4
28	5.6	5.4
30	5.6	5.5
32	5.7	5.4
36	5.1	4.8
40	6.2	5.7
44	5.6	5.8
48	6.1	5.7
52	5.8[a]	5.0[b]
54	5.6	5.0

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

[1]Based on pooled estimate of variance SEM = 0.22.

[2]n = 16 samples for the calculation of means within treatment and week.

brevity, tabular data of ST and PP at each age in trial 2, and SCH and SCA at each age in both trials are not presented. In trial 2, ST and PP, respectively, were 35.8 mg/dL and 4.54 g/dL at 16 wk (initiation of study), 2,044 mg/dL and 4.09 g/dL at 28 wk, and 1,895 mg/dL and 4.96 g/dL at 58 wk (trial termination). In trial 1, SCH and SCA, respectively, were 116 and 11.2 mg/dL at 16 wk (initiation of study), 172 and 39.0 mg/dL at 28 wk, and 161 and 30.8 mg/dL at 54 wk (trial termination). In trial 2, SCH and SCA, respectively, were 114 and 10.7 mg/dL at 16 wk, 139 and 20.7 mg/dL at 28 wk, and 104 and 20.0 mg/dL at 58 wk. Levels of ST, PP, SCH, and SCA across treatment were most notably higher at 28 and 54 wk in trial 1 when compared with 28 and 58 wk, respectively, in trial 2. Higher blood levels of these particular parameters in trial 1 were associated with higher levels of egg production. As reported by Burnham et al. (2002a) for these same flocks, egg production at wk 28 and 54 in trial 1 was 95 and 63%, respectively, whereas at wk 28 and 58 in trial 2, egg production was 82 and 58%, respectively.

DISCUSSION

At the beginning and end of both trials, SPA tests from swabs and sera, and HI sera tests along with the agar plate fluorescent antibody tests, verified systemic infections in FMG-inoculated birds. Conversely, sham-inoculated birds remained FMG-free throughout each trial. There can be marked interactions between mycoplasmas, respiratory viruses, and bacteria (Saif et al., 1970; Jordan, 1972; Springer et al., 1974; Kleven, 1998). Manifestations of MG usually occur in the respiratory system and lesions become extensive when complicated by other bacteria. Environmental factors, such as dust and ammonia, along with intensive rearing or stress, crowding, cold weather, live virus vaccination, or natural virus infection may also be important in lesion incidence and severity (Jordan 1972; Springer et al., 1974). However, when there are no secondary infections to mycoplasmas, infection is often subclinical or mild (Kerr and Olson, 1967). Nevertheless, in this study in which birds were housed in isolation units and were free of secondary infectious agents and other environmental stressors, responses to FMG were manifested in the blood. Furthermore, as indicated in a previous report (Burnham et al., 2002a), these same birds also exhibited a subsequent delay in onset of lay and a decrease in total egg production in response to FMG inoculation.

By 8 wk after challenge (20 wk), FMG-inoculated birds in both trials exhibited an increase in HCT, which would suggest a compensatory polycythemic response to the insult imposed on their respiratory system by the FMG. Conversely, HCT levels returned to those of controls after this time, which would indicate that the birds adjusted through other physiological means to the FMG. Serum triglycerides are normally elevated in hens during lay. Likewise, when a stressor such as FMG is introduced, ST may become elevated in response to the infection. An elevation in ST is known to be a common response to the presence of infectious disease agents (Guyton and Hall, 1996). Furthermore, elevated circulating corticosterone concentrations (a condition associated with stress) after adrenocorticotropin administration, has been reported to increase plasma triglyceride concentrations in chickens (Davison et al., 1985; Latour et al., 1996a). In FMG-inoculated birds in trial 1, the concentrations of ST and PP were elevated during prepeak egg production (22 wk), but during postpeak lay, ST (54 wk) and PP (52 wk) levels were depressed. It is known that the establishment of systemic FMG infections in birds takes approximately 6 wk (Soeripto et al., 1989; Nunoya et al., 1995). Increases in HCT, ST, and PP between 20 and 22 wk of age or between 8 and 10 wk postinoculation suggest that the initial weeks of egg production are stressful to the bird, particularly when combined with the establishment of a full systemic FMG infection. Increases in these independent blood parameters may be initial indicators of a bird's compensatory response to an FMG challenge. Postpeak decreases in both ST (54 wk) and PP (52 wk) in FMG-infected birds would further suggest a more chronic inhibition on lipid and protein synthesis in the liver by FMG. Nevertheless, the absence of corresponding changes in ST and PP in response to FMG at these various ages in trial 2 suggests that the effects of FMG on ST and PP may differ among flocks. Changes in ST and PP in response to FMG-infection in different commercial layer flocks may, therefore, be unpredictable.

Burnham et al. (2002a) reported that initiation of lay was delayed and that both weekly egg production after 42 wk and average weekly egg production throughout a complete cycle were reduced in layer hens inoculated with FMG at 12 wk of age. Reductions in numbers of mature ovarian follicles, ovarian follicle size, and magnal, isthmal, and vaginal portions of the oviduct in hens previously inoculated with FMG at 12 wk of age were reported by Burnham et al. (2002b). Furthermore, increased incidences of fatty liver hemorrhagic syndrome were re-

ported in those same birds (Burnham et al., 2002b). Although SCA is known to increase in response to estrogen stimulation (Urist et al., 1958; Tsang and Grunder, 1983), which occurs with the onset of egg production, SCA or SCH did not change significantly in either trial due to FMG-inoculation in the current study.

These data suggest that decreases in ST and PP may be associated with ovarian follicular regression, reproductive tissue atrophy, and the onset of fatty liver hemorrhagic syndrome in FMG-infected hens during the egg laying cycle. Burnham et al. (2003) reported decreases in total yolk lipid and yolk cholesterol between 22 and 28 wk of age in birds inoculated with FMG. In that same study, decreases in yolk myristic, palmitoleic, and oleic acid percentages, and increases in yolk linoleic, stearic, and arachidonic acid percentages were reported in FMG-inoculated birds. Decreased concentrations of lipids in the blood may be directly responsible for reductions in total yolk lipid, yolk cholesterol, and yolk fatty acid deposition in FMG-inoculated hens. Supplemental dietary fat may help provide the lipids necessary for the maintenance of egg yolk formation and subsequent egg production in infected birds. Peebles et al. (2003) suggested that 1.5% supplemental dietary poultry fat may alleviate reductions in early egg production due to FMG in commercial laying hens. Decreased PP may also contribute to drops in egg production, as protein alterations may, likewise, affect albumen deposition in the magnum.

ACKNOWLEDGMENTS

This work was funded by a grant from the USDA. The authors appreciate the expert technical assistance of Sharon Whitmarsh (Mississippi State University, Mississippi State, Mississippi), Jerry Drott, and Dana Chamblee (USDA, SCPRL, Starkville, Mississippi), and secretarial assistance of Denise Richardson (Mississippi State University, Mississippi State, Mississippi). Also, a sincere debt of gratitude is extended to all personnel at the Mississippi State University Poultry Science Department and USDA.

REFERENCES

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

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.

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

Branton, S. L., J. D. May, B. D. Lott, and W. R. Maslin. 1997. Various blood parameters in commercial hens acutely and chronically infected with *Mycoplasma gallisepticum* and *Mycoplasma synoviae*. Avian Dis. 41:540–547.

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. 2002a. 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, M. S. Jones, P. D. Gerard, and W. R. Maslin. 2002b. Effects of F-strain *Mycoplasma gallisepticum* inoculation at twelve weeks of age on digestive and reproductive organ characteristics of commercial egg laying hens. Poult. Sci. 81:1884–1891.

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

Dashti, N, J. L. Kelley, R. H. Thayer, and J. A. Ontko. 1983. Concurrent inductions of avian hepatic lipogenesis, plasma lipids, and plasma apo lipoprotein B by estrogen. J. Lipid Res. 24:368–380.

Davison, T. F., B. M. Freeman, and J. Rea. 1985. Effects of continuous treatment with synthetic $ACTH^{1-24}$ or corticosterone on immature *Gallus domesticus*. Gen. Comp. Endocrinol. 59:416–423.

Freund, R. J., and W. J. Wilson. 1997. Statistical Methods. Academic Press, San Diego, CA 92101.

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.

Guyton, A. C., and J. E. Hall. 1996. Textbook of Medical Physiology. 9th ed. W. B. Saunders Co. Philadelphia, PA.

Heald, P. J., and H. G. Banman. 1963. Lipid metabolism and the laying hen 1. Plasma-free fatty acids and the onset of laying in the domestic fowl. Biochim. Biophys. Acta 70:381–388.

Hillyard, L. A., C. Entenman, and I. L. Chaikoff. 1956. Concentration and composition of serum lipoproteins of cholesterol-fed and stilbestrol-injected birds. J. Biol. Chem. 223:359–368.

Jordan, F. T. 1972. The epidemiology of disease of multiple aetiology: the avian respiratory disease complex. Vet. Rec. 90:556–562.

Kerr, K. M., and N. O. Olson. 1967. Pathology in chickens experimentally inoculated or contact-infected with *Mycoplasma gallisepticum*. Avian Dis. 11:559–578.

Kerr, K. M., and N. O. Olson. 1970. Pathology of chickens inoculated experimentally or contact infected with *Mycoplasma synoviae*. Avian Dis. 14:291–320.

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

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

Latour, M. A., S. A. Laiche, J. R. Thompson, A. L. Pond, and E. D. Peebles. 1996a. Continuous infusion of adrenocorticotropin elevates circulating lipoprotein cholesterol and corticosterone concentrations in chickens. Poultry Sci. 75:1428–1432.

Latour, M. A., E. D. Peebles, C. R. Boyle, S. M. Doyle, and J. D. Brake. 1996b. Effects of breeder hen age and dietary fat on embryonic and neonatal broiler serum lipids and glucose. Poult. Sci. 75:695–701.

Lorentz, F. W., C. Entenman, and I. L. Chaikoff. 1938. The influence of age, sex, and ovarian activity on the blood lipids of the domestic fowl. J. Biol. Chem. 117:619–663.

National Poultry Improvement Plan and Auxiliary Provisions. 1995. Published in Chapter 9 Code of Federal Regulations (9CFR)USDA-APHIS-VS, 1498 Klondike Road, Suite 200, Conyers, GA 30094.

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

Nimpf, J., and W. J. Schneider. 1991. Receptor mediated lipoprotein transport in laying hens. J. Nutr. 121:1471–1474.

Nunoya, T., T. Yagihashi, M. Tajima, and Y. Nagsawa. 1995. Occurrence of keratoconjunctivitis apparently caused by *Mycoplasma gallisepticum* in layer chickens. Vet. Pathol. 32:11–18.

Peebles, E. D., S. L. Branton, M. R. Burnham, and P. D. Gerard. 2003. Influences of supplemental dietary poultry fat and F-strain *Mycoplasma gallisepticum* infection on the early perfor-

mance of commercial egg laying hens. Poultry Sci. 82:596–602.
Petersen, R. G. 1985. Design and Analysis of Experiments. Marcel Dekker, Inc., New York, NY 10016.
Sahu, S. P., and N. O. Olson. 1976. Use of the agar-gel precipitin test to evaluate broiler breeder and commercial layer flocks for *Mycoplasma gallisepticum* infection. Avian Dis. 20:563–573.
Saif, Y. M., P. D. Moorhead, and E. H. Bohl. 1970. *Mycoplasma meleagridis* and *Escherichia coli* infections in germfree and specific-pathogen-free turkey poults: production of complicated airsacculitis. Am. J. Vet. Res. 31:1637–1643.
SAS Institute. 1996. SAS/STAT Software: Changes and Enhancements Through Release 6.11. Version 8. SAS Institute, Inc., Cary, NC.
Soeripto, K., G. Whithear, G. S. Cottew, and K. E. Harrigan. 1989. Virulence and transmissibility of *Mycoplasma gallisepticum*. Aust. Vet. J. 66:65–72.
Springer, W. T., C. Luskus, and S. S. Pourciau. 1974. Infectious bronchitis and mixed infections of *Mycoplasma synoviae* and *Escherichia coli* in gnotobiotic chickens. Synergistic role in the airsacculitis syndrome. Infect. Immun. 10:578–589.
Steel, R. G. D., and J. H. Torrie. 1980. Principles and Procedures of Statistics. A Biometrical Approach. 2nd ed., McGraw-Hill, New York, NY.
Tietz, N. W. 1986. Fundamentals of clinical chemistry. Page 1,816 in Clinical Chemistry. WB Saunders Co., Philadelphia.
Tsang, C. P. W., and A. A. Grunder. 1983. Effect of active immunization against estradiol on egg shell quality. Poultry Sci. 62:1297–1304.
Urist, M. R., O. A. Schjeide, and F. C. McLean. 1958. The partition and binding of calcium in the serum of the laying hen and of the estrogenized rooster. Endocrinology 63:570–585.
Walzem, R. L., R. J. Hansen, D. L. Williams, and R. L. Hamilton. 1999. Estrogen induction of VLDLy assembly in egg-laying hens. J. Nutr. 129:467S–472S.
Yoder, H. W., Jr. 1975. Pages 109–116 in Mycoplasmosis: Isolation and Identification of Avian Pathogens. American Association of Avian Pathologists, College Station, TX.