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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

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# An F-Strain *Mycoplasma gallisepticum* Inoculation at Twelve Weeks of Age Does Not Modify the Effects of Fasting During Lay on the Blood Characteristics of Commercial Egg Laying Hens

E.D. Peebles[1], M.R. Burnham[1], S.L. Branton[2] and P.D. Gerard[3]
[1]Department of Poultry Science, Mississippi State University, Mississippi State, Mississippi 39762, USA
[2]Poultry Research Unit, Agricultural Research Service, USDA, Mississippi State, Mississippi 39762, USA
[3]Department of Mathematical Sciences, Clemson University, Clemson, SC 29634, USA

**Abstract:** The interactive effects of an F-strain *Mycoplasma gallisepticum* (FMG) inoculation at 12 wk of age with those of a 24 h fast during lay on the BW, mortality, egg production and blood characteristics of commercial egg laying hens at 34 and 46 wk of age were investigated. Blood parameters measured were whole blood hematocrit; plasma protein concentration (PP); serum total cholesterol, triglycerides and calcium concentrations; and percentage serum cholesterol recovered in very low, low and high density lipoprotein fractions. There were no interactive effects involving FMG inoculation and fasting (inoculation x fasting or age x inoculation x fasting) on any of the parameters investigated. These results suggest that a pre-lay (12 wk of age) FMG inoculation does not influence the effects of a 24 h fast during lay (34 and 46 wk of age) on the performance and blood characteristics of commercial layers.

**Key words:** Blood, fasting, layer, lipid, *Mycoplasma gallisepticum*)

## INTRODUCTION

The colonization of F-strain *Mycoplasma gallisepticum* (FMG) in the liver of laying hens (Sahu and Olson, 1976) may disrupt the generation of hepatic lipids (Heald and Badman, 1963; Dashti *et al*., 1983) destined for yolk deposition (Burley and Vadehra, 1989; Nimpf and Schneider, 1991; Schneider, 1996; Walzem *et al*., 1994, 1999) in association with estrogen release and the initiation of Egg Production (EP). In subsequent research, the onset of lay (Burnham *et al*., 2002) and the egg (Burnham *et al*., 2002), yolk (Burnham *et al*., 2003b) and blood (Burnham *et al*., 2003a) characteristics of commercial layers have been further shown to change in response to an FMG infection.

Burnham *et al*. (2002) reported that layer BW, mortality and EP between 12 and 58 wk of age, including wk 34 and 46, were not affected by an FMG inoculation at 12 wk of age, however, it delayed the onset of lay by approximately 1 wk. Furthermore, in another trial, total EP between 18 and 54 wk of age, exclusive of wk 34 and 46, was significantly reduced in laying hens in response to the inoculation of FMG at 12 wk of age (Burnham *et al*., 2002). Burnham *et al*. (2003a) noted no effects of a wk 12 FMG inoculation on layer blood parameters on wk 34 and 46 including whole blood hematocrit (HCT), total plasma protein (PP) and total serum cholesterol (SCH), triglycerides (ST) and calcium (SCA) concentrations and Burnham *et al*. (2003c) showed that the inoculation of FMG at 12 wk of age did not significantly affect the concentration of SCH within each of the circulation populations of very low (VLDL), low (LDL) and high (HDL) density lipoprotein particles at 34 or 46 wk of age in commercial egg-laying hens. Nevertheless, Burnham *et al*. (2003a) showed in 2 trials that HCT at 20 wk of age was increased and in 1 of the 2 trials that PP and ST underwent variable significant changes on wk 22, 52 and 54 due to an FMG inoculation at 12 wk of age.

In a separate study by Peebles *et al*. (2004), it was found that across wk 34 and 46 wk of age a 24 h fast did not affect mortality or EP but it significantly increased HCT and decreased BW, PP, total SCH, ST and SCA in the egg-laying hen. Furthermore, in response to fasting, the percentage of SCH recovered from LDL was increased whereas the percentage of SCH recovered from HDL was decreased on wk 34. Conversely, on wk 46, the percentage of SCH recovered from VLDL was decreased whereas the percentage of SCH recovered from HDL was increased due to fasting.

It was hypothesized after review of the above reports that a pre-lay FMG inoculation may modify the effects of a 24 h fast during lay on the performance and associated blood characteristics of laying hens and vice-versa. Because no previous studies have explored the possible interactive effects of a pre-lay FMG inoculation and a 24 h fast during lay on the performance and blood characteristics of commercial egg laying hens, it was the objective of the current study to determine possible changes in the performance and blood characteristics of hens that were inoculated with FMG at 12 wk of age before being subjected to a 24 h fast at 34 and 46 wk of age. In addition to BW, mortality and EP, the blood characteristics investigated in this report included HCT,

**Corresponding Author:** E.D. Peebles, Department of Poultry Science, Mississippi State University, Mississippi State, MS 39762, USA

PP, SCH, ST, SCA and concentrations of SCH distributed among VLDL, LDL and HDL particles.

## MATERIALS AND METHODS

**Pullet housing and management:** One thousand 1 d-old Single Combed White Leghorn pullets of a single genetic strain were obtained from a commercial source that was monitored and certified free of MG and *Mycoplasma synoviae* (National Poultry Improvement Plan and Auxiliary Provisions, 1995) and the subsequent trial was conducted under an approved USDA animal care and use protocol. Chickens were vaccinated at 10 d of age for infectious bursal disease and at 12 d and again at 4 wk of age, chickens were vaccinated for Newcastle Disease and infectious bronchitis. The above vaccines were delivered via the drinking water. At 5 wk of age, blood and choanal cleft swab tests used for detection of the presence of *Mycoplasma* species and the specific presence of FMG were performed on 30 randomly selected pullets according to the procedures described by Burnham *et al.* (2003a). Pullets were maintained in a floor pen from 1 d until 12 wk of age. Feed and water were provided for ad libitum consumption. Bird housing conditions and ingredient percentages and dietary analyses of the basal starter and grower diets used have been reported by Burnham *et al.* (2002).

At 12 wk of age, 11 pullets were randomly selected and placed in each of 16 (total of 176 pullets) negative pressure fiberglass biological isolation units (BIU; 1.16 $m^2$). The BIU were housed in a previously described poultry disease isolation facility (Branton and Simmons, 1992). Half of the total number of BIU contained FMG-free control birds, whereas, the other half contained FMG-inoculated birds. Furthermore, half of the BIU containing FMG-free control birds were fasted as were half of the BIU containing FMG-inoculated birds. The 4 treatment groups were sham-inoculated, non-fasted; sham-inoculated, fasted; FMG-inoculated, non-fasted; and FMG-inoculated, fasted. There were 4 replicate BIU per treatment group. Beginning at 18 wk of age, the artificial lighting schedule was increased 15 min/d until a 16 h 15 min light:7 h 45 min dark daily cycle was achieved. Chickens were maintained on that schedule through the remainder of the trial. 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 the trial. Feed adjustments, ingredient percentages and dietary analyses of the basal developer, pre-lay and layer diets used have been reported by Burnham *et al.* (2002). All diets were formulated to meet or exceed National Research Council (1994) specifications. No medication was administered during the course of the trial.

**FMG inoculation, mycoplasma detection and fasting period:** 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 (University of Georgia, Athens, GA). Inoculum titers were $1.0 \times 10^5$ cfu/mL. 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 (Frey *et al.*, 1968) broth medium. At 20 and 58 wk, 1 randomly selected hen from each of 4 FMG-free control and FMG-treated BIU was bled and swabbed. Each of these samples was tested for the presence of *Mycoplasma* species as previously described for pullets. On wk 34 and 46 of age which began on Monday and ended on Sunday, feed was removed for 24 h (1230 h on Wednesday to 1230 h on Thursday) from the BIU of hens belonging to the fasted treatment, whereas hens in BIU that belonged to the nonfasted treatment had ad libitum access to feed over that same 24 h period.

**Data collection:** Body weight was determined and then blood was harvested from 4 hens in each of the 16 BIU at the same time of day (1230 h on Thursday) on wk 34 and 46. Percentage mortality and hen day EP on wk 34 and 46 of age were subsequently determined on a BIU basis for birds in all BIU over a 4 d period (Thursday-Sunday) after blood collection. Hematocrit was expressed as percentage blood packed cell (primarily red blood cell) volume, PP was expressed in g/dL and SCH, ST and SCA were expressed in mg/dL. Analyses of PP, SCH, ST and SCA were performed according to the procedures of Burnham *et al.* (2003a). Size exclusion chromatography as described by German *et al.* (1996) was used to determine the percentage of SCH contained in VLDL, LDL and HDL particle classes. A detailed description of the size exclusion chromatography system and the sample sizes used are provided by Peebles *et al.* (2004).

**Statistical analysis:** A split plot treatment arrangement in a completely randomized experimental design was utilized. The whole plot treatment structure was a factorial arrangement of inoculation and fasting and the sub plot factor was the age. The fixed effects tested were age, inoculation, age x inoculation, fasting, age x fasting, inoculation x fasting and age x inoculation x fasting; however, only significant interactive effects involving inoculation and fasting treatments (inoculation x fasting or age x inoculation x fasting) were reported. Random effect was the replication nested within inoculation and fasting combination. 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 9.2 (2008). Statements of statistical significance were based on $P \leq 0.05$ unless otherwise stated.

## RESULTS AND DISCUSSION

All initial test results obtained from 5-wk-old pullets were negative for MG and *Mycoplasma synoviae*. Control serum samples obtained at 20 and 58 wk of age were negative for MG, while the same tests were positive for MG in the FMG-inoculated hens. Similarly, culture results for swabs obtained at 20 and 58 wk of age were negative for Mycoplasma species growth for 4 out of 4 FMG-free hens tested, while growth was evident for 4 out of 4 FMG-inoculated hens tested.

Unlike Burnham *et al.* (2002) and Peebles *et al.* (2004), in which EP on wk 34 and 46 was determined for the entire 7 d period including the 3 d before the 24 h fasting period, EP in the current study was determined only during the 4 d period following the fast. Therefore, it is noted for the 4 d period that although there was no significant main effect due to the inoculation of FMG, there was a significant ($P \leq 0.05$) main effect due to fasting for EP. This is in contrast to the lack of a significant fasting effect on EP at wk 34 and 46 that was previously reported by Peebles *et al.* (2004). In the current study, EP was higher in non-fasted than in fasted birds. Mean EP in non-fasted and fasted birds was 85.3±3.72 and 73.3±4.11 %, respectively.

There were no significant interactive effects involving inoculation and fasting treatments for mortality, BW, or EP in this study. Burnham *et al.* (2002) and Peebles *et al.* (2004), respectively reported no significant effects of a pre-lay inoculation of FMG or a 24 h fast during lay on bird mortality which would support the currently reported lack of an interaction between these 2 factors for mortality. However, despite the fact that BW was shown by Peebles *et al.* (2004) to be depressed due to fasting and that there was an observed depressing effect on EP due to fasting in the current study, the wk 12 FMG inoculation had no influence on these effects. An interactive effect of FMG and fasting on BW was not expected, considering the report by Burnham *et al.* (2002) showing no effect of a pre-lay FMG inoculation on BW; but, because Burnham *et al.* (2002) found that total EP was reduced in 1 trial due to the inoculation of FMG at 12 wk of age, it was expected that FMG would have exacerbated the reducing effect of fasting on EP. However, the pre-lay FMG inoculation provided no additional influence.

The earlier reported effects of fasting on HCT, PP, SCH, ST, SCA and SCH recovered from VLDL, LDL and HDL by Peebles *et al.* (2004), were also shown in the current study to not be influenced by a pre-lay FMG inoculation. Peebles *et al.* (2004) reported that a 24 h fast on wk 34 and 46 of layer age resulted in a significant increase in HCT and decreases in PP and SCA and Chamblee and Morgan (1982) earlier found that a 12 h fast resulted in a decrease in the BW and an increase in the HCT of broilers. A decrease in SCA would be expected to occur in association with the observed decreases in BW and EP. It was proposed by Peebles *et al.* (2004) that a decrease in PP in the fasted hens was in part due to a decrease in the diameter of VLDL particles belonging to the 90th population percentile and was based on earlier research having shown that plasma lipoproteins undergo dramatic changes during regression of the ovary and oviduct in association with molts caused by prolonged fasting (Barron *et al.*, 1999).

Conversely, it was proposed by Burnham *et al.* (2003a) that the observed increase in HCT 8 wk after a pre-lay FMG inoculation was a polycythemic response to the colonization of FMG in the bird's respiratory system and Burnham *et al.* (2003a) found that PP concentrations in layers were significantly elevated 10 wk later in response to the inoculation of FMG at 12 wk of age. Knowing that approximately 6 wk are necessary to establish systemic FMG infections in birds (Yoder, 1986; McMartin *et al.*, 1987; Soeripto *et al.*, 1989; Nunoya *et al.*, 1995), Burnham *et al.* (2003a) indicated that an increase in PP 10 wk post-inoculation was suggestive of the bird's compensatory response to an FMG infection. In consideration of the finding by Burnham *et al.* (2003a) that SCA was not affected by an FMG inoculation at 12 wk of age, the observed lack of influence of FMG on the effect that fasting had on SCA was expected. However, the inability of FMG to exacerbate the elevation of HCT in response to fasting was unexpected. Nonetheless, the lack of a counteracting influence by FMG on a reduction in PP in response to fasting indicates that the potential stimulatory effect of an FMG infection on PP was unable to supersede the reducing effect caused by fasting.

Peebles *et al.* (2004) concluded that a 24 h fast during lay was able to significantly decrease the serum lipids of egg-laying hens. Contrariwise, an increase in ST is known to occur as a reaction to the presence of infectious disease organisms (Guyton and Hall, 1996) and elevated circulating corticosterone concentrations in response to a stressor can elevate ST concentrations in chickens (Davison *et al.*, 1985; Latour *et al.*, 1996). Nevertheless, an FMG inoculation at 12 wk of age failed to influence the effects of fasting on ST and the other blood lipid parameters examined in the current study. In conclusion, the current results suggest that in addition to its lack of influence on the effects of fasting on layer performance, the inoculation of FMG at 12 wk of age also did not have a subsequent associated influence on the HCT, PP, SCA and blood lipids of commercial layers.

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