Influence of supplemental dietary poultry fat on the digestive and reproductive organ characteristics of commercial layers inoculated before or at the onset of lay with F-strain *Mycoplasma gallisepticum* \(^{(1,2)}\)

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ABSTRACT Effects of F-strain *Mycoplasma gallisepticum* (FMG) inoculation and 1.5% supplemental dietary poultry fat (PF) on the digestive and reproductive organ characteristics of commercial layers at 58 wk of age were investigated. Sham and FMG inoculations were administered at 12 (before lay) and 22 (early in lay) wk of age, and dietary treatments (basal control diets and basal control diets with PF) were initiated at 20 wk of age. Supplemental PF increased BW and decreased isthmal length relative to total oviduct length in hens. Various oviduct segments were also affected by the type and age of inoculation, and these effects were further influenced by the use of PF. In comparison to their time-specific sham-inoculated controls, infundibulum weight relative to BW was increased when birds were inoculated with FMG at 22 wk, whereas isthmus weight relative to total oviduct weight was increased by FMG inoculation at 12 wk of age. However, PF affected infundibulum length relative to total oviduct length only in sham-inoculated birds, and PF increased magnum weight relative to total oviduct weight only in birds inoculated at 22 wk of age (sham or FMG). Furthermore, PF decreased isthmus weight relative to total oviduct weight only in birds that were sham-inoculated (12 or 22 wk). In conclusion, the inoculation of FMG at 12 or 22 wk may increase the relative contributions of the isthmus and infundibulum, respectively, to the total mass of the oviduct. In addition, PF may decrease the relative length of the isthmus and increase the relative weight of the magnum in the oviducts of birds that have been inoculated at 22 wk of age (sham or FMG). Previous studies have shown 1.5% supplemental dietary PF to influence feed consumption throughout lay and performance early in lay in hens that were inoculated with FMG at 12 wk of age. However, the current results suggest that these influences are associated with gross changes in the oviduct but not the digestive tract of layers.

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

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INTRODUCTION To prevent outbreaks of virulent *Mycoplasma gallisepticum* infections and to control associated egg production (EP) losses in commercial layer flocks, vaccination programs have been implemented, which include the use of the F-strain of *M. gallisepticum* (FMG). Producers (Jack Self, Cal-Maine Foods Inc., Jackson, MS; personal communication) and researchers (Branton et al., 1997) have observed that FMG may not adversely affect subsequent EP when used as a prelay vaccine. In a companion study using the same birds and treatments as in this study, Park et al. (2009a) more recently reported that the inoculation of commercial layers with FMG at 22 wk of age led to a significant increase in total EP between 22 and 58 wk of age. However, the inoculation of FMG at either 12 or 22 wk also caused an associated increase in feed consumption during the early and late stages of lay and an increase in egg weight throughout lay.

In subsequent companion reports, Park et al. (2009b) showed that total lipid and fatty acid contents of the yolk early and late in lay were differentially affected by the inoculation of FMG at either 12 or 22 wk of age, and Peebles et al. (2009) reported that serum triglyceride and calcium levels at peak EP and late in lay responded differently to the inoculation of FMG at 12 or 22 wk.
of age. The inoculation of commercial layers with FMG at 12 wk of age has also been shown to result in an increased incidence of fatty liver hemorrhagic syndrome, ovarian follicular regression, and decreased isthmal and vaginal proportions of the reproductive tract (Burnham et al., 2002a). It was concluded in that report that alterations in the performance and egg characteristics of layers inoculated with FMG at 12 wk of age are related to mutual functional disturbances in the liver, ovary, and oviduct, without concomitant intestinal changes. However, the effects of an FMG inoculation at 22 wk of age on the digestive and reproductive organ characteristics of the birds were not investigated. Park et al. (2009b) has suggested that 1.5% supplemental dietary poultry fat (PF) was not effective in modulating the effects of a wk 12 FMG inoculation on the egg yolk characteristics of hens between 24 and 58 wk of age. However, Peebles et al. (2003) earlier demonstrated that the effects of a wk 12 FMG inoculation on EP and feed consumption through 26 wk of age in commercial egg-laying hens can be modified by 1.5% supplemental dietary PF. Park et al. (2009a) also reported that 1.5% supplemental PF reduced feed consumption across a 20 to 55-wk laying cycle in layers that had likewise experienced a wk 12 inoculation (sham or FMG) and interacted with the age (12 or 22 wk) and type (sham or FMG) of inoculation to influence eggshell quality. Peebles et al. (2009) further noted that the use of 1.5% supplemental dietary PF may interact with the age (12 or 22 wk) at which an inoculation (sham or FMG) is given to influence serum cholesterol and total plasma protein concentrations in layers. Nevertheless, no information has been published concerning the possible effects of these treatment regimens on the digestive and reproductive organ characteristics of layers. Therefore, the objective of this study was to examine the possible effects of 1.5% supplemental PF fed beginning at 20 wk of age and FMG inoculations administered at 12 or 22 wk of age on the digestive and reproductive organ characteristics of commercial egg-laying hens.

MATERIALS AND METHODS

Bird Management

In all 3 trials, 1-d-old Hy-Line W-36 Single Comb White Leghorn pullets were obtained from a commercial hatchery certified free of M. gallisepticum and Mycoplasma synoviae (USDA-APHIS-VS, 2003). Until 12 wk of age, birds were raised, fed, vaccinated, and tested for the presence of M. gallisepticum and M. synoviae as described by Peebles et al. (2003). At 12 and 22 wk of age in each trial, 120 sham-inoculated (control) and 120 FMG-inoculated (treated) birds were wing-banded and randomly assigned to individual cages in 1 of 2 enclosed and isolated ends of a caged layer facility according to inoculation treatment. Sham and FMG inoculations at 12 and 22 wk and Mycoplasma detection during lay were as described by Peebles et al. (2003) and Park et al. (2009a).

Treatments

Two isocaloric and isonitrogenous treatment layer diets were randomly provided to birds within each end of the layer house at 20 wk of age, with both dietary treatments assigned to birds belonging to each inoculation type (sham- or FMG-inoculated) and inoculation age (12 or 22 wk) treatment combination. One diet served as a basal control diet (BC; contained 0.5% PF), and the other was the basal diet supplemented with 1.5% PF (BCPF; 2.0% total PF). There were 3 replicate groups (10 birds per replicate group) for each inoculation type, inoculation age, and dietary treatment combination. Feed and water were provided for ad libitum consumption, and birds in each side of the house were watered, fed, and ventilated separately. Descriptions of the diets fed to the layers are provided by Peebles et al. (2003) and Park et al. (2009a). All pullet and layer diets were formulated to meet or exceed NRC (1994) specifications, and all trials were conducted under an approved USDA animal care and use protocol.

Data Collection

At the end of each trial (wk 58), 2 birds from each replicate unit were weighed, killed by cervical dislocation, their organs removed, and the following parameters determined: liver weight, liver moisture and lipid contents, ovary weight, mature ovarian follicle number (those that are ≥12 mm in diameter), total weights and lengths of the oviduct and small intestine, and segmental weights and lengths of the oviduct (infundibulum, magnum, isthmus, uterus, and vagina) and small intestine (duodenum, jejunum, and ileum). Liver and ovary weights and total oviduct and small intestine weights were calculated as percentages of BW. Furthermore, oviduct and small intestine segment weights were calculated as percentages of BW and total organ weight, and oviduct and small intestine segment lengths were calculated as percentages of total organ length. The number of mature follicles in an ovary was assigned a category from 0 to 6, in which 0 indicated the absence of mature follicles and 6 was the number of maximum follicles recorded. The percentage of birds in each unit possessing 0, 1, 2, 3, 4, 5, or 6 follicles was calculated. Livers were examined for incidence of fatty liver hemorrhagic syndrome (FLHS) by the same individual observer for all birds in each trial. Two categories were used for classification of FLHS incidence: normal and those exhibiting FLHS to any degree. Birds with normal livers or those exhibiting FLHS were calculated as percentages of the total number of birds in each unit.
Statistical Analysis

A completely randomized experimental design, with trial as a block, was used. The data of all 3 trials were pooled and analyzed together. All data were subjected to a mixed model ANOVA. The fixed effects were type of inoculation, age of inoculation, and diet, factorially arranged, along with their interactions, and the random effects were trial, replicate within trial, trial × type of inoculation, type of inoculation × replicate within trial, and age of inoculation × diet × type of inoculation × replicate within trial. Individual sample data within each replicate unit 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 using the MIXED procedure of SAS software (SAS Institute, 2003).

RESULTS

There were no significant treatment effects of any kind for liver, ovary, oviduct, magnum, isthmus, uterus, vagina, small intestine, duodenum, jejunum, or ileum weights as percentages of BW; infundibulum, uterus, and vagina weights as percentages of oviduct weight; magnum, uterus, and vagina lengths as percentages of oviduct length; duodenum, jejunum, and ileum weights and lengths as percentages of small intestine weight and length, respectively; liver moisture and lipid concentrations; mature ovarian follicle number; and incidence of FLHS. Descriptions of the effects for only those parameters in which significance was found are described below.

There was a significant main effect due to diet for BW (\( P \leq 0.05 \)) and isthmus length as a percentage of total oviduct length (ISLPOL; \( P \leq 0.04 \)) on wk 58. In comparison to the birds fed the BC diet, BW was higher and ISLPOL was lower in birds fed the BCPF diets, with those inoculated at 12 wk of age and fed BCPF diets but that were sham-inoculated on wk 22, those inoculated with FMG at 22 wk or FMG-inoculated at 12 wk in comparison to those that were concurrently sham-inoculated, with those that were sham- or FMG-inoculated at 12 wk being intermediate. Conversely, ISWPOW was significantly greater in birds that were sham-inoculated at 22 wk or FMG-inoculated at 12 wk in comparison to birds that were sham-inoculated at 12 wk of age, with birds inoculated with FMG at 22 wk of age being intermediate.

A significant (\( P \leq 0.03 \)) type of inoculation × age of inoculation × dietary treatment interaction was found for infundibulum length as a percentage of total oviduct length (INLبول; Table 2). The INLبول of birds that were sham-inoculated at 12 wk of age and fed BCPF diets was significantly greater than that of birds similarly inoculated but fed BC diets, those that were also fed BCPF diets but that were sham-inoculated on wk 22, those inoculated with FMG at 12 wk and fed BC diets, and those inoculated with FMG at 22 wk and fed either diet. Also, the INLبول of birds sham-inoculated at 22 wk of age and fed BC diets was greater than those similarly inoculated but provided 1.5% supplemental dietary PF and those fed BC diets and sham- or FMG-inoculated at 12 wk of age.

A significant (\( P \leq 0.04 \)) age of inoculation × dietary treatment interaction was found for magnum weight as a percentage of oviduct weight (Table 3), whereas a significant (\( P \leq 0.01 \)) type of inoculation × dietary treatment interaction was found for ISWPOW (Table 4). Compared with birds that were inoculated (sham or FMG) on wk 22 and that were fed BC diets, magnum weight as a percentage of oviduct weight was greater in birds that were similarly inoculated on wk 22 but fed BCPF diets, with those inoculated at 12 wk of age and

| Table 1. Infundibulum weight as a percentage of BW (INWPBW) and isthmus weight as a percentage of total oviduct weight (ISWPOW) in birds that were sham- or F-strain Mycoplasma gallisepticum (FMG)-inoculated at 12 or 22 wk of age across dietary treatment1,2 |
|-----------------|-----------------|-----------------|-----------------|
| Item            | Sham            | FMG             |                 |
|                 | 12 wk           | 22 wk           | 12 wk           | 22 wk           | Pooled SEM |
| INWPBW          | 0.129<sup>a</sup>| 0.106<sup>b</sup>| 0.118<sup>b</sup>| 0.140<sup>a</sup>| 0.0119      |
| ISWPOW          | 8.73<sup>b</sup>| 10.69<sup>a</sup>| 10.30<sup>a</sup>| 9.72<sup>b</sup>| 0.413       |

<sup>a</sup>Means among treatments within a row (parameter) with no common superscript differ (\( P \leq 0.05 \)).
<sup>b</sup>Means are representative of significant type of inoculation × age of inoculation interactions for INWPBW (\( P \leq 0.04 \)) and ISWPOW (\( P \leq 0.005 \)).
<sup>n</sup>n = 18 and 12 replicate units used for the calculation of treatment means for INWPBW and ISWPOW, respectively.
Table 2. Infundibulum length as a percentage of total oviduct length in birds that were sham- or F-strain Mycoplasma gallisepticum (FMG)-inoculated at 12 or 22 wk of age and fed basal control diets (BC) or basal control diets supplemented with 1.5% poultry fat (BCPF)\textsuperscript{1,2,3}

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<thead>
<tr>
<th>Group</th>
<th>Sham</th>
<th>FMG</th>
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<tr>
<td></td>
<td>12 wk</td>
<td>22 wk</td>
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<tr>
<td>BC</td>
<td>14.4\textsuperscript{a}</td>
<td>16.7\textsuperscript{ab}</td>
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<tr>
<td>BCPF</td>
<td>17.4\textsuperscript{a}</td>
<td>14.4\textsuperscript{c}</td>
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\textsuperscript{1}\textsuperscript{2}\textsuperscript{3}Means among treatments with no common superscript differ (\(P \leq 0.05\)).

\textsuperscript{1}Means are representative of a significant (\(P \leq 0.03\)) type of inoculation \(\times\) age of inoculation \(\times\) dietary treatment interaction for infundibulum length as a percentage of total oviduct length.

\textsuperscript{2}n = 9 replicate units used for the calculation of treatment means.

\textsuperscript{3}Standard error of the mean based on pooled estimate of variance = 1.48.

Table 3. Magnum weight as a percentage of total oviduct weight in birds that were inoculated at 12 or 22 wk of age and fed basal control diets (BC) or basal control diets supplemented with 1.5% poultry fat (BCPF) across type of inoculation\textsuperscript{1,2,3}

<table>
<thead>
<tr>
<th>Group</th>
<th>Magnum wt (% of total oviduct wt)</th>
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<tr>
<td></td>
<td>12 wk</td>
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<tr>
<td>BC</td>
<td>14.4\textsuperscript{a}</td>
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<tr>
<td>BCPF</td>
<td>17.4\textsuperscript{a}</td>
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\textsuperscript{1}\textsuperscript{2}\textsuperscript{3}Means among treatments with no common superscript differ (\(P \leq 0.05\)).

\textsuperscript{1}Means are representative of a significant (\(P \leq 0.04\)) age of inoculation \(\times\) dietary treatment interaction for magnum weight as a percentage of total oviduct weight.

\textsuperscript{2}n = 12 replicate units used for the calculation of treatment means.

\textsuperscript{3}Standard error of the mean based on pooled estimate of variance = 2.13.

DISCUSSION

Mycoplasma gallisepticum has the ability to invade cells (Winner et al., 2000). Sahu and Olson (1976) have cultured \(M.\) gallisepticum from the livers of infected chickens, and Domermuth and Gross (1962) have further provided evidence that \(M.\) gallisepticum alone is capable of producing salpingitis (inflammation of the oviduct) in Single Comb White Leghorn chickens. Ball et al. (1969) have specifically noted that microorganisms causing infertility in turkeys have an apparent selectivity for the infundibulum and isthmus of the oviduct. These researchers also observed that in comparison to \(M.\) gallisepticum-free turkeys, mild inflammation associated with an increased incidence of lymphoid loci and plasma-cell infiltration occurred in the oviducts of turkeys infected with \(M.\) meleagridis. These findings suggest that \(M.\) gallisepticum may be capable of interfering with liver lipid metabolism and oviduct function. A delay in onset of lay by 1 wk and a decrease in total EP (Burnham et al., 2002b), as well as significant alterations in liver, ovarian, and oviduct characteristics (Burnham et al., 2002a), have been reported to occur in commercial layers in response to the inoculation of FMG at 12 wk of age. Colonization of the liver and oviduct by FMG may, therefore, be a possible means by which EP in FMG-infected hens can be altered.

Burnham et al. (2002a) more precisely documented that FMG inoculation at 12 wk of age resulted in an increased incidence of FLHS, follicular regression, and decreased isthmal and vaginal proportions of the reproductive tract, without any concomitant effects on the intestine. Similarly, in the current report, the inoculation of FMG on wk 12 affected the isthmal segment of the oviduct and there were no effects of 12 or 22 wk FMG inoculations on the intestinal characteristics examined. However, unlike the results of Burnham et al. (2002a), these inoculation treatments had no associated effects on the liver or ovary, and the relative proportion of the oviduct occupied by the isthmus was increased in response to a wk 12 FMG inoculation. In addition, the infundibulum rather than the vagina was influenced by treatment, with an FMG inoculation on wk 22 increasing relative infundibulum weight. The increased relative weights of the infundibulum and isthmus of the birds in this study are suggestive of the organism’s selective invasion of these oviductal segments with subsequent inflammation and cell infiltration, as
noted in turkeys infected with *M. meleagridis* by Ball et al. (1969).

Related reports have described the effects of other *M. gallisepticum* strains on the reproductive tract characteristics of layers (Nunoya et al., 1997; Peebles et al., 2006; Vance et al., 2009; Viscione et al., 2009). Peebles et al. (2006) found that isthmus weight as a percentage of total oviduct weight was increased at 60 wk of age in birds that had been inoculated with S6-strain *M. gallisepticum* at 45 wk. Nunoya et al. (1997) also reported that S6-strain *M. gallisepticum* infections consequentially caused salpingitis and decreased EP in layers. Vance et al. (2009) observed that only vaginal length as a percentage of total oviduct length was greater in hens that had been vaccinated with ts-11-strain *M. gallisepticum* at 10 wk of age, whereas Viscione et al. (2009) found that a 6/85-strain *M. gallisepticum* vaccine administered at 10 wk of age exerted no effect on the oviduct. The varying results among strains may be due to differences in their virulence. The S6-strain of *M. gallisepticum* is recognized as being one of the more virulent strains of *M. gallisepticum* in the field (Levisohn et al., 1986). Conversely, FMG has been described as mildly pathogenic, and 6/85- and ts-11-strains are apathogenic, exhibit minimal virulence, and are considered to be safer than FMG (Levisohn and Kleven, 1981; Kleven et al., 1990).

It has been reported that 1.5% supplemental dietary PF interacts with the age (12 or 22 wk) and type (sham or FMG) of inoculation to influence eggshell quality (Park et al., 2009a), may alleviate reductions in early EP (18 to 26 wk of age) due to a wk 12 FMG inoculation (Peebles et al., 2003), and reduces feed consumption between 20 and 55 wk of age in layers having been sham- or FMG-inoculated on wk 12 (Park et al., 2009a). The use of 1.5% supplemental dietary PF in this study increased BW and had additional variable influences on relative isthmus length across all treatments, relative infundibulum length in 12 and 22 wk sham-inoculated birds, relative magnum weight in birds that received sham or FMG inoculations on wk 22, and relative isthmus weight in birds that were sham-inoculated regardless of inoculation age. These effects occurred without any observed associated influences on their liver, ovary, or digestive tract. Supplemental PF influenced the relative proportions of the oviduct occupied by the infundibulum, magnum, and isthmus, with its effects on these components interacting with the type and age of inoculation. Therefore, consideration should be given to the possible influence that supplemental PF may exert on the oviduct and subsequent eggshell quality of layers with further attention to the fact that its influence may be modified by the concurrent inoculation regimen imposed. Nevertheless, despite these responses in the oviduct to 1.5% supplemental dietary PF, earlier research reports indicate that its use does not augment EP or eggshell quality throughout lay in hens that have been subjected to inoculation (sham or FMG) at 12 or 22 wk of age (Park et al., 2009a), that it is not effective in modulating the effects of an FMG inoculation at 12 wk of age on hens’ egg yolk characteristics (Park et al., 2009b), and that it has no influence on the effects that an FMG challenge has on their blood characteristics (Peebles et al., 2009).

**REFERENCES**


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