INTRODUCTION

*Mycoplasma gallisepticum* is an avian pathogen known to infect economically significant avian hosts such as layer and broiler chickens and turkeys, leading to symptoms such as chronic respiratory disease in chickens and sinusitis in turkeys (Ley, 2003; Evans et al., 2005). The outcomes of *M. gallisepticum* infection include decreased egg production, reduced feed efficiency, and downgrading or condemnation of carcasses (Ley, 2003), resulting in significant economic losses for poultry producers (Evans et al., 2005). Several methods are used to control *M. gallisepticum* spread and infection. For meat-type poultry, these include biosecurity and the all-in-all-out management of single-aged rearing facilities (Levisohn and Kleven, 2000). Facilities housing multi-aged flocks, including breeder facilities and layer complexes, must rely on biosecurity and active protection including vaccination where possible (Levisohn and Kleven, 2000).

Three different types of vaccines are available for controlling *M. gallisepticum* infection: bacterin-based (killed) vaccines, live vaccines, and a recombinant viral vaccine (Kleven, 2008). Killed vaccines have been shown to provide limited protection, but the protection is insufficient to control infection at multi-aged flock facilities (Talkington and Kleven, 1985; Levisohn and Kleven, 2000; Feberwee et al., 2006). Live *M. gallisepticum* vaccines include those based on the F-strain (Poulvac Myco F, Fort Dodge Animal Health, Fort Dodge, IA and AviPro MG F, Lohmann Animal Health, Winslow, ME), the 6/85 strain (Mycovac-L, Merck Animal Health, Summit, NJ), and the ts-11 strain (*Mycoplasma gallisepticum* vaccine, Merial Select, Duluth, GA). Each of these vaccines has its benefits and shortcomings.

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Prior vaccination with the 6/85 strain vaccine has been shown to ameliorate the decreased egg production caused by subsequent FMG revaccination (Leigh et al., 2010). The ts-11 and 6/85 strains have been shown to have milder postvaccination effects than the F-strain; however, they also induce lower immunity and are thus less protective than the F-strain vaccines (Abd-el-Motelib and Kleven, 1993; Kleven, 2008).

The recombinant Vectormune FP MG (rFP-MG) vaccine, a genetically modified fowl pox viral vaccine expressing *M. gallisepticum* antigens, is a recent addition to the group of *M. gallisepticum* vaccines. Previous research has demonstrated that the rFP-MG vaccine does not cause gross clinical pathology in vaccinated chickens and also provides protection from subsequent fowlpox virus challenge (Zhang et al., 2010). However, vaccination with rFP-MG vaccine also provided little protection against challenge with a virulent *M. gallisepticum* strain (Ferguson-Noel et al., 2012). The purpose of the present work was to determine the impact of rFP-MG vaccination on egg production and egg quality parameters. Further research was conducted to determine if rFP-MG vaccination before postproduction-peak FMG vaccination (at 45 woa) reduced the previously observed decrease in egg production that has been shown to occur upon postproduction-peak vaccination of *M. gallisepticum*-clean hens with FMG at 45 woa (Branton et al., 1988). This would allow poultry producers to revaccinate their flocks with the more protective FMG vaccine should rFP-MG vaccination prove insufficient at preventing *M. gallisepticum*-associated pathogenesis.

**MATERIALS AND METHODS**

*Housing and Management*

One-day-old Hy-Line W-36 Leghorn pullets obtained from a commercial source were used in each of 2 trials. Pullets were housed on dry pine shavings before the experiment commencing, as previously described (Burnham et al., 2002). At 5 wk of age, 20 pullets were screened by serum plate agglutination (SPA) for antibodies to *M. gallisepticum*. The choanal clefts of the same 20 pullets were also swabbed and cultured for mycoplasma growth (Branton et al., 1984). At 6 wk of age, pullets were randomly assigned to treatment groups and placed in isolation units with 11 pullets per isolation unit (Branton et al., 2002). At onset of lay (approximately 20 wk of age), the number of pullets per box was reduced from 11 to 10.

Pullets were randomly divided into 3 treatment groups with 4 isolation units serving as replicates for each treatment group. The experiment was repeated and the results from both trials were analyzed together for a total of 8 replicates per treatment group. For each trial, chickens were provided ad libitum access to both feed and water as previously described (Branton et al., 2002). Five diets were provided over the course of each trial as follows: 0 to 6 wk, starter; 6 to 12 wk, grower; 12 to 18 wk, developer; 18 wk to onset of lay, prelay; onset of lay to conclusion of experiment, layer. These diets were formulated to meet NRC (1994) recommendations and have been described previously (Burnham et al., 2002). Temperature was maintained at 23°C for the duration of the experiment. Through 18 wk of age, lighting was maintained at 10 h per day. At 18 wk of age, the lighting duration was increased 15 min per week until a duration of 16 h 15 min was achieved as described previously (Branton et al., 2002).

**Vaccination**

Treatment groups used are as follows: 1) control (no vaccine or *M. gallisepticum* exposure), 2) rFP-MG ((Vectormune FP MG at 6 wk, Ceva Biomune, Lenexa, KS), and 3) rFP-MG–FMG (Vectormune FP MG at 6 wk and FMG vaccine at 45 wk). Vectormune FP MG was administered by wing web administration by representatives of Ceva Biomune. All pullets were assessed for vaccination “takes” (swelling or scab formation at the site of vaccination) at 7 d postvaccination by the same individuals. At 45 wk, hens from the rFP-MG–FMG treatment group were removed from their respective isolation units and vaccinated with 0.04 mL of overnight culture of high-passage (99th passage above the unknown passage level) F strain *M. gallisepticum* in Frey’s medium in the left eye, as described previously (Branton et al., 1997). Titers were determined to be 1.4 and 8.3 × 10^8 cfu/mL per 40-µL dose in trials 1 and 2, respectively.

**Data Collection**

Eggs were collected and production data were recorded daily for each isolation unit for the duration of the experiment. Eggs collected on Tuesdays and Wednesdays were tested for eggshell strength and scored for Haugh units. For wk 22 to 43, eggs were only tested on odd weeks (23, 25, 27, 29, 31, 33, 35, 37, 39, 41, and 43). For wk 45 through 52, eggs were tested weekly. Eggshell strength was measured using a stress-strain measuring instrument as described previously (Reece and Lott, 1976). Haugh units were scored using a model EQM egg quality management system (Technical Services and Supplies Limited, York, UK). Egg size classes were determined by egg weights as described previously (Branton et al., 2002).

**Statistical Analysis**

The study was conducted using a completely randomized design with trial as block. The data were analyzed in accordance with the time of FMG vaccination. Egg and eggshell quality data for wk 23 through 43 and wk 45 through 52 were analyzed separately. No trial × treatment effects were observed (*P > 0.05*), so egg production, egg, and eggshell quality data from both
trials were combined for analysis and reporting. The rFP-MG–FMG data were included as a separate treatment group in wk 23 through 43 results even though its treatment is identical to the rFP-MG treatment group during those weeks. Isolation units were considered the experimental treatment group, and all results from within individual isolation units were averaged before analysis. All data were log10 transformed and analyzed using the repeated measures procedure of SAS Analyst (SAS Institute Inc., Cary, NC). A value of $P \leq 0.05$ was considered to be significant.

**RESULTS AND DISCUSSION**

Culture and SPA analysis results for pullets tested before the start of the experiment were shown to be negative for *M. gallisepticum* infection. At the end of the experiment, hens vaccinated with FMG at 45 woa were shown to be *M. gallisepticum* SPA positive consistent with proper vaccination. The rFP-MG vaccine did not produce a positive SPA response, consistent with manufacturer’s literature and previously published results (Zhang et al., 2010).

Vaccination with rFP-MG did not result in significant differences in hen-day egg production compared with the unvaccinated control group (Table 1). Subsequent vaccination of hens with FMG (rFP-MG–FMG) at 45 woa also did not result in decreased egg production, differing from previous results which showed that vaccination of naïve hens with FMG following the onset of lay resulted in a significant decrease in egg production (Branton et al., 1988; Jacobs et al., 2012). Vaccination with rFP-MG also had no effect on Haugh unit scores or eggshell breaking strength compared with unvaccinated hens (Table 1). Subsequent vaccination with FMG also had no effect on these results.

Analysis of egg size results among the differing treatment groups showed no statistical differences for wk 23 to 43 or wk 45 to 52, inclusive (Table 2). As observed in previous publications, trends toward increased extra-large egg production and decreased medium egg production have been observed following postproduction-peak *M. gallisepticum* vaccination (Branton et al., 1999; Branton et al., 2002; Leigh et al., 2010). However, these results were not found to be significant following statistical analysis. Furthermore, a comparison with the FMG treatment group before FMG vaccination suggests that this trend may have existed before FMG vaccination (Table 2).

Results from this work show no difference in egg production, egg, or eggshell quality parameters when comparing the rFP-MG and control groups. This is consistent with previous research that demonstrated no significant effects on chickens following rFP-MG

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**Table 1.** The effect of Vectormune FP MG (rFP-MG; Ceva Biomune, Lenexa, KS) vaccination and subsequent F-strain *Mycoplasma gallisepticum* (rFP-MG–FMG) vaccination at 45 wk of age on hen-day egg production, eggshell strength, and Haugh unit scores

<table>
<thead>
<tr>
<th>Item</th>
<th>Hen-day egg production (%)</th>
<th>Eggshell strength (kg)</th>
<th>Haugh unit scores</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>23 to 43 wk</td>
<td>45 to 52 wk</td>
<td>23 to 43 wk</td>
</tr>
<tr>
<td>Control</td>
<td>88.87</td>
<td>79.97</td>
<td>3.24</td>
</tr>
<tr>
<td>rFP-MG</td>
<td>86.54</td>
<td>79.98</td>
<td>3.23</td>
</tr>
<tr>
<td>rFP-MG–FMG</td>
<td>87.08</td>
<td>78.12</td>
<td>3.19</td>
</tr>
<tr>
<td>SEM1</td>
<td>0.27</td>
<td>0.09</td>
<td>0.0092</td>
</tr>
</tbody>
</table>

1Standard error of the mean based on pooled estimate of variation.

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**Table 2.** The effect of Vectormune FP MG (rFP-MG; Ceva Biomune, Lenexa, KS) vaccination and subsequent F-strain *Mycoplasma gallisepticum* (rFP-MG–FMG) vaccination at 45 wk of age on egg size distribution of eggs collected twice per week on wk 23 to 43 (odd weeks only) and wk 45 to 52, inclusive

<table>
<thead>
<tr>
<th>Item</th>
<th>Jumbo</th>
<th>Extra-large</th>
<th>Large</th>
<th>Medium</th>
<th>Small</th>
<th>Pee wee</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.1</td>
<td>%2</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Wk 23 to 43</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>36</td>
<td>2.4</td>
<td>524</td>
<td>34.98</td>
</tr>
<tr>
<td>rFP-MG</td>
<td>4</td>
<td>0.27</td>
<td>42</td>
<td>2.79</td>
<td>442</td>
<td>29.41</td>
</tr>
<tr>
<td>rFP-MG–FMG</td>
<td>1</td>
<td>0.07</td>
<td>41</td>
<td>2.88</td>
<td>508</td>
<td>35.65</td>
</tr>
<tr>
<td>SEM3</td>
<td>0.0093</td>
<td>0.06</td>
<td>0.23</td>
<td>0.22</td>
<td>0.19</td>
<td>0.015</td>
</tr>
<tr>
<td>Wk 45 to 52</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>134</td>
<td>13.83</td>
<td>633</td>
<td>65.38</td>
</tr>
<tr>
<td>rFP-MG</td>
<td>2</td>
<td>0.2</td>
<td>131</td>
<td>12.82</td>
<td>624</td>
<td>61.06</td>
</tr>
<tr>
<td>rFP-MG–FMG</td>
<td>3</td>
<td>0.32</td>
<td>160</td>
<td>16.86</td>
<td>601</td>
<td>63.33</td>
</tr>
<tr>
<td>SEM3</td>
<td>0.012</td>
<td>0.12</td>
<td>0.18</td>
<td>0.12</td>
<td>0.015</td>
<td>NA4</td>
</tr>
</tbody>
</table>

1Number of eggs laid.
2Percentage of total eggs laid.
3Standard error of the mean based on pooled estimate of variation.
4NA = not applicable.
vaccination (Zhang et al., 2010; Ferguson-Noel et al., 2012). Other research has shown that postproduction-peak vaccination with F MG (45 woa) results in a significant increase in Haugh unit scores compared with vaccination before lay (10 woa; Branton et al., 1988; Branton et al., 1997). Vaccination with rFP-MG before postproduction-peak F MG vaccination ameliorates this effect, as no significant difference was seen for Haugh unit scores. Previous research has also shown that postproduction-peak vaccination of M. gallisepticum clean hens with F MG leads to a reduction in hen-day egg production (Branton et al., 1988). This research indicates that vaccination with rFP-MG ameliorates the negative effect of postpeak-production F MG vaccination on hen-day egg production, whereas rFP-MG does not protect against the pathogenic effects of virulent M. gallisepticum challenge (Ferguson-Noel et al., 2012). The ability to protect from drops in egg production associated with exposure of M. gallisepticum-clean hens to F MG at 45 woa while not protecting against virulent challenge could be due both to the nature of the M. gallisepticum strain used (F MG versus virulent R-low strain) or the route of administration (eye drop versus aerosol). However, it is also possible that gross pathogenic lesions were present in the F MG-vaccinated chickens that had no effect on egg production or egg quality characteristics.

The results of this work suggest that the rFP-MG vaccine Vectormune FP MG has a place in the vaccination regimen against M. gallisepticum infection. Although the vaccine may not be sufficient by itself to protect against the pathological effects of virulent M. gallisepticum challenge (Ferguson-Noel et al., 2012), it is sufficient to protect against the deleterious effects of a more virulent vaccine strain (F MG). Thus, prior vaccination with rFP-MG vaccine provides a mechanism for subsequent M. gallisepticum vaccination without the accompanying decrease in egg production.

REFERENCES


