

Growth and humoral immune effects of dietary Original XPC in layer pullets challenged with *Mycoplasma gallisepticum*^{a,b,c}

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ABSTRACT Effects of dietary Original XPC (XPC) in commercial layer pullets challenged with the virulent, low passage R strain of *Mycoplasma gallisepticum* (R_{low} MG) were investigated. Hy-Line W-36 pullets sourced from MG-clean breeders were fed a basal diet with or without (CON) XPC (1.25 kg/metric ton) from hatch until 12 wk of age (woa). At 8 and 10 woa, half of the birds in each dietary treatment were challenged with R_{low} MG. Body weight was recorded at 3, 8, and 12 woa, and ovary, ceca, and bursa weights were recorded at 3 and 12 woa. Blood samples were taken immediately before the initial R_{low} MG challenge at 8 woa and again at 12 woa to test for IgM and IgG antibody production against MG. All birds were evaluated for MG lesion scores at 12 woa. Regardless of challenge, inclusion of XPC in the diet did not significantly alter BW at 3 or 8 woa or relative organ weights at 3 or 12 woa. However, at 12 woa, BW of XPC-fed birds, regardless of challenge

was significantly ($P = 0.0038$) heavier than CON by 25.7 g. All birds tested negative for MG antibodies before the 8 woa challenge. Respective percentage serum plate agglutination and ELISA positive birds at 12 woa were 0 and 0% (CON, nonchallenged), 1.4 and 0% (XPC, nonchallenged), 100 and 47.2% (CON, challenged), and 100 and 50.0% (XPC, challenged). Diet did not significantly affect ELISA titers, but they were significantly ($P < 0.0001$) increased due to challenge. Furthermore, lesion scores were significantly higher for R_{low} MG-challenged birds ($P = 0.0012$), and dietary treatment with XPC in challenged birds numerically reduced MG lesion scores from 0.278 to 0.194. In conclusion, although dietary XPC did not significantly alter the humoral immune response, antibody titer levels, or severity of MG lesions in layer pullets that were or were not challenged with R_{low} MG, it led to an increase in their rate of growth through 12 woa.

Key words: growth, immunity, layer pullets, *Mycoplasma gallisepticum*, Original XPC

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INTRODUCTION

Well-recognized as a successful and persistent respiratory pathogen through its ability to evade the immune response of its host, *Mycoplasma gallisepticum*

(MG), has caused significant economic loss to the poultry industry. This is particularly true for commercial egg-laying hens subjected to stressful and highly concentrated housing conditions (Bradbury, 2005). Financial losses in the table egg industry are predominately because of reductions in egg production and egg quality in layer flocks infected by natural or field-strains of MG (Peebles and Branton, 2012). The R strain of MG has been shown to significantly increase the incidence of air sac lesions and the severity of airsacculitis in chickens (Rodriguez and Kleven, 1980). Subsequently, the R strain of MG has been commonly used as a pathogenic strain for studies involving MG challenges (Ley, 2003).

The use of commercial prebiotic compounds has been shown to modulate the immune system, reduce pathogen colonization in the gut, and promote intestinal

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development and growth performance in chickens (El-Husseiny et al., 2008; Roto, 2016; Roto et al., 2017; Peebles, 2020). A reduction in the use of antibiotics as a feed additive has further prompted the investigation and use of dietary probiotics, prebiotics, and microbe-based feed additives (Dizaji and Pirmohammadi, 2009; Suarez Martinez et al., 2018; Peebles, 2020). The effects of a diversity of microbe fermentation products on layer performance have been investigated in various studies (Harms and Miles, 1988; Grimes et al., 1997; Fujiwara et al., 2008). Grimes et al. (1997) observed an improvement in the feed conversion of laying hens when a fermentation product was included in their diets.

Original XPC (XPC; Diamond V Corp., Cedar Rapids, IA) is a fermentation product derived from *Saccharomyces cerevisiae* and has been shown to positively affect commercial layer egg yolk yield and composition. More specifically, Suarez Martinez et al. (2018) have recently demonstrated that use of this product significantly increased the yolk weight and the percentages of yolk yield and yolk solids of Hy-Line W-36 laying hens. Because it was hypothesized that XPC also has the potential to improve the immune response and well-being of birds against an MG challenge, the objective of the current study was to initially evaluate Hy-Line W-36 layer pullets through 12 wk of age (woa) when subjected to a virulent MG challenge for the effects of dietary XPC on the humoral immune response, weight gain of the birds, and effects on the weight of the ovary, ceca, and bursa.

MATERIALS AND METHODS

Treatments

Beginning on the day of hatch, Hy-Line W-36 pullets were continuously fed 1 of 2 dietary treatments for the duration of the study (12 woa). The dietary treatments were a basal control diet (CON) or a basal control diet containing XPC at 1.25 kg/metric ton (MT).

For the first 2 wk, the chicks were brooded under standard brooding conditions in floor pens with fresh pine shavings consisting of 4 rooms each containing 4 pens within a room. Forty-eight female chicks (sex-determined via feather sexing) were placed in each of the 16 total pens. Each pen measured 2 m² for a stocking density of 0.04 m² per bird. Mean BW of the birds in each replicate pen was equated within a 10% range to avoid an initial bias in BW between dietary treatments. Within each room, 2 pens of birds were fed the CON diet, and 2 pens of birds were fed the XPC diet. Thus, for 0 to 14 D of age, there were 8 pens per each dietary treatment, blocked by 4 rooms.

At 14 D of age, birds were then allocated into groups of 18 based on dietary treatment and moved to 32 isolation chambers each of which measured 0.92 m² (n = 576 birds; Branton and Simmons, 1992). The isolation chambers consisted of 16 chambers per each of 2 rooms. Feed treatments were alternately placed

within each room for a total of 8 replicate chambers per each feed treatment in each room, with room serving as a block. The additional treatment of MG challenge or no MG challenge (administered at 8 woa) was assigned. The 18 birds per each isolation chamber were selected from each of the 8 floor pens within a treatment for nearly equal pen representation within each isolation chamber. Each bird placed in each isolation chamber was individually weighed and wing banded. Thus, from 2 to 8 woa, there was a dietary treatment (CON or XPC) with a total of 16 replicates per each treatment across 2 rooms. After the MG challenge at 8 woa, the treatment combination of dietary treatment (CON or XPC) and challenge status (challenged with MG or sham challenged) was considered with a total of 8 replicates for each treatment combination across 2 rooms.

The birds were challenged twice with the virulent, low passage (13 in vitro passages) R strain of MG (R_{low} MG) that was generously provided by Dr. Steven Geary (University of Connecticut). The first challenge was at 8 woa (eye drop), and the second challenge was at 10 woa (tracheal gavage). For the 8 woa challenge, the R_{low} MG inoculum was plated immediately before challenge and observed to contain 1.5×10^8 CFU/mL. A 20 μ L volume of inoculum culture containing 3.0×10^6 CFU of R_{low} MG was applied to the same 1 eye of all challenged birds using a 200 μ L pipette. For the 10 woa challenge, the same inoculum that was used at 8 woa was frozen and thawed, with less than a 10% loss of viable bacteria, and a 200 μ L volume of that inoculum culture, containing 3.0×10^7 CFU of R_{low} MG, was discharged into the trachea using a 1,000 μ L pipette. For challenge titer determinations, R_{low} MG colony counts were performed on Frey's plate medium (Frey et al., 1968) supplemented with 35 mL of yeast extract solution per liter (#18180-059, Invitrogen/Gibco, Carlsbad, CA) following incubation at 37°C. The presence of viable bacteria in the inocula cultures was confirmed by color indicator (phenol red) change (18 h). The study was terminated at 12 woa. Bird husbandry, handling, and sampling were approved by a USDA-ARS Animal Care and Use Committee (Mississippi State, MS).

Body and Organ Weights

The BW of 7 birds in each of 16 replicate units per dietary treatment at 3 woa, of 11 birds in each of 16 replicate units per dietary treatment at 8 woa, and of 9 birds in each of 8 replicate units per dietary-challenge treatment combination group at 12 woa were recorded. At 3 woa, the select organ (ovary, bursa, and ceca) weights of 4 birds in each of 16 replicate units in each dietary treatment group were recorded, and at 12 woa, the select organ weights of 4 birds in each of 8 replicate pens in each dietary-challenge treatment combination group were recorded. Relative organ weights were expressed as percentages of total BW.

Bursal Follicle Evaluation

At 12 woa, the bursae of 2 birds per pen were collected, weighed, and placed into 10% buffered formalin for histological evaluation. A 5 mm portion of each bursa was prepared by sagittal plane dissection and placed into tissue embedding cassettes and were subsequently embedded in paraffin. The 5 μm sections of the embedded samples were prepared and stained with hematoxylin and eosin. For morphometric analysis of bursal follicles, the bursal follicles were imaged under a 4 x objective with an Olympus model BX60 microscope (Olympus Optical Co., Tokyo, Japan). The images were used to determine the total area of 5 follicles for each bursal section. Based on the criteria of [Muniz et al. \(2006\)](#), a region of a section where follicles were cut midline was selected for evaluation with Infinity Analyze software (Lumenera Corporation, Ottawa; Canada). On the images, mean total area (μm^2) of the 5 individual follicles of each bursal section was determined and recorded.

MG DNA Evaluation and Humoral Antibody Production

At 8 woa, choanal cleft swab and blood (2 mL) samples ([Elliott et al., 2018](#)) were collected from 3 birds belonging to each of 16 replicate units per dietary treatment immediately before the challenge to confirm that all birds had not been exposed to MG before the challenge. Furthermore, at 12 woa, 9 birds belonging to each of 8 replicate units per dietary-challenge treatment combination group were also similarly swabbed and bled for postchallenge testing. Choanal cleft swab samples were obtained using prewetted sterile rayon tipped applicators in PBS (Puritan Sterile Rayon Tipped Applicators REF 25-800 R 50, Puritan Medical Products Co. LLC, Guilford, ME), and the swab sample was then transferred into 110 μL of PBS. Tests for the presence of MG DNA on all swab samples collected at 8 and 12 woa were accomplished by using Real Time PCR. Swab samples were stored at 4°C until DNA isolation, and following isolation, DNA samples were frozen until PCR was performed. The procedures employed for DNA extraction were as specified by [Elliott et al. \(2017\)](#). The primers and probe utilized for the real-time PCR reaction were designed by [Callison et al. \(2006\)](#) to amplify a 139 bp fragment of the MGA0319 gene within the MG genome. Each swab sample was run in duplicate utilizing a 7,500 Fast Real Time PCR System (Applied Biosystems, Foster City, CA). Each PCR reaction contained a total 25 μL reaction volume with 12.5 μL Taqman Universal Master Mix (Applied Biosystems), 0.5 μmol of both primers, 0.1 μmol of the probe, 2.5 μL of the template sample, and sterile water. The cycling parameters were 50°C for 2 min, 95°C for 15 min, and optics on for 40 cycles of 94°C for 15 s and 60°C for 60 s. For the 8 wk prechallenge swab samples and the nonchallenged bird 12 wk swab samples, individual bird swab samples were pooled for each isolation

unit. Pooling was accomplished by combining 2.5 μL from each individual bird sample into a common corresponding tube for their respective unit (3 samples per unit were pooled for the 8 wk samples and 9 samples per unit were pooled for the 12 wk nonchallenged unit samples). Any pooled samples that resulted in findings of MG DNA were rerun for each individual bird sample in the contaminated unit. All MG-challenged birds at 12 wk were tested on an individual bird basis. Dilutions of extracted DNA from a laboratory cultured R_{low} MG sample (unknown quantity) were utilized for the standard curve with an arbitrarily assigned quantity for the 10^{-2} , 10^{-4} , and 10^{-6} dilutions of the stock. Relative quantitative evaluations of the samples were performed for the purpose of evaluating if the dietary treatment reduced the relative quantity of bacteria.

At 8 and 12 woa, MG-specific circulating humoral antibody production in the birds was tested for confirmation of a humoral immune response and to determine antibody class switching. Extracted serum samples collected at 8 and 12 woa were used for the subsequent determination of MG-specific serum IgM antibody (serum plate agglutination [SPA]) production and serum IgG antibody (ELISA) titers. The SPA test results using MG antigen (Charles River Laboratories International, Wilmington, MA) were based on a 0 to 3 scale as designated by [Evans et al. \(2007\)](#), where a score of 1 or higher is considered as a positive test. The ELISA tests were performed by the Mississippi State Chemical Laboratory using the IDEXX MG antibody test kit (IDEXX Laboratories, Westbrook, ME).

Scoring of Lung Conjunctivitis and Airsacculitis

At 12 woa, the lungs and air sacs (cranial thoracic, caudal thoracic, and abdominal) were grossly examined by a single experienced observer for MG lesions in 9 birds in each of 8 replicate units per dietary-challenge treatment combination group. The combined lesion incidences for lung conjunctivitis and airsacculitis were graded following the scoring system (0–3) described by [Nunoya et al. \(1987\)](#): 0 = no significant changes; 1 = cloudy appearance or several yellowish foci; 2 = cloudy thickening and/or more than several yellowish foci; and 3 = diffuse yellowish thickening with caseous exudates.

Statistical Analysis

All procedures of data analysis used were of SAS software (Version 9.4, [SAS Institute, 2012](#)). A mixed model ANOVA employing PROC MIXED was used to analyze the data. A one-way ANOVA was used to analyze the main effect of diet at 3 and 8 woa, and the effect of diet within the challenged treatment group for the quantity of MG DNA at 12 woa, and a 2-way ANOVA was used to analyze the main and interactive effects of diet and challenge at 12 woa. Fixed effects in the model were diet and challenge treatment. Random effects

included room, isolation unit within room and diet \times challenge \times isolation unit within room interaction. Analysis of challenge effect for SPA and ELISA tests and of diet effect for SPA tests were not possible because of a lack of data variation with convergence criteria of estimation procedure not being met. Least-squares means were separated by least significant difference (Steel and Torrie, 1980). Differences in least-squares means were considered significant at $P \leq 0.05$.

RESULTS

There were no significant effects of dietary treatment on the BW of birds at 3 ($P = 0.8513$) and 8 ($P = 0.4923$) woa. The mean BW of birds at 3 woa in the CON and XPC dietary treatment groups were 174.2 and 173.7 g, respectively (pooled SEM = 1.27 g). The mean BW of birds at 8 woa in the CON and XPC dietary treatment groups were 648.6 and 653.0 g, respectively (pooled SEM = 3.00 g). There was also no significant ($P = 0.7116$) main effect because of challenge and no significant ($P = 0.3407$) diet \times challenge interaction for BW at 12 woa. However, there was a significant ($P = 0.0038$) main effect because of diet on the BW of the birds at 12 woa (Table 1). The BW of the birds at 12 woa that were fed XPC diets was significantly heavier than that of birds fed CON diets (Table 1).

There was no significant effect because of dietary treatment on the relative weights of the ovary ($P = 0.6361$), bursa ($P = 0.7807$), and ceca ($P = 0.4555$) at 3 woa. Mean relative weights of the ovary, bursa, and ceca in the CON and XPC dietary treatment groups at 3 woa were respectively, 0.033 and 0.034% (pooled SEM = 0.001%), 0.509 and 0.505% (pooled SEM = 0.008%), and 0.980 and 1.010% (pooled SEM = 0.020%). There was no significant main effect

due to diet, and no significant diet \times challenge interaction for relative ovary, bursa, or ceca weight at 12 woa (Table 1). Furthermore, there were no significant main effects due to diet ($P = 0.7437$) or challenge ($P = 0.2482$) treatment, and no significant ($P = 0.6036$) diet \times challenge interaction on the mean total area of individual follicles in the bursal sections of the birds at 12 woa. However, there was a significant main effect due to challenge treatment on relative ovary ($P = 0.0385$), bursa ($P = 0.0014$), and ceca ($P = 0.0046$) weight at 12 woa. Challenging the birds with R_{low} MG at 8 and 10 woa significantly increased relative ovary and ceca weights, but decreased relative bursa weight at 12 woa (Table 1). These results indicate that although the R_{low} MG challenge affected the relative weights of those organs at 12 woa, and their relative weights were not influenced by diet, regardless of challenge treatment.

Real-time PCR results showed that there was no presence of MG DNA in any of the swabbed birds at 8 woa. At 12 woa, out of the 16 nonchallenged isolation units, 12 of the units were negative for MG DNA detection. Out of all 36 birds among the other 4 nonchallenged isolation units, 9 tested positive for MG DNA detection. This approximated 50, 63, 22, and 89%, respectively, of the birds within each of the 4 units. In 2 of the units, there were 3 samples and 1 sample, respectively, that were lost because of evaporation of the samples during storage. In the challenged birds at 12 woa ($n = 144$), there was a total of 5 samples that were lost because of sample storage evaporation. Two challenged bird samples (1 in a control diet unit and 1 in an XPC diet unit) tested negative for MG DNA, and the remaining 137 samples tested positive for MG DNA. Statistical analysis of the quantity of DNA within the challenged birds showed no difference between the 2 dietary treatments ($P = 0.5545$). The quantity of DNA in the

Table 1. Effects of diet and challenge treatments on layer pullet BW and relative select organ (ovary, bursa, and ceca) weights at 12 wk of age.

Treatments	BW ¹ (g)	Ovary ² (%)	Bursa ² (%)	Ceca ² (%)
Diet				
Control	1,000.3 ^b \pm 7.15	0.046 \pm 0.0021	0.394 \pm 0.0106	0.499 \pm 0.0220
XPC	1,026.0 ^a \pm 7.10	0.046 \pm 0.0021	0.397 \pm 0.0106	0.535 \pm 0.0220
Main effect P -value	0.0038	0.8274	0.8489	0.1204
Challenge				
Nonchallenged	1,014.6 \pm 7.10	0.045 ^b \pm 0.0021	0.420 ^a \pm 0.0106	0.483 ^b \pm 0.0220
R _{low} MG-challenged	1,011.6 \pm 7.15	0.048 ^a \pm 0.0021	0.371 ^b \pm 0.0105	0.551 ^a \pm 0.0220
Main effect P -value	0.7116	0.0385	0.0014	0.0046
Diet-challenge				
Control and Nonchallenged	997.8 \pm 9.11	0.045 \pm 0.0023	0.424 \pm 0.0150	0.480 \pm 0.0270
Control and R _{low} MG-challenged	1,002.7 \pm 9.26	0.047 \pm 0.0023	0.364 \pm 0.0148	0.519 \pm 0.0270
XPC and Nonchallenged	1,031.4 \pm 9.11	0.044 \pm 0.0023	0.415 \pm 0.0150	0.486 \pm 0.0270
XPC and R _{low} MG-challenged	1,020.5 \pm 9.11	0.048 \pm 0.0023	0.378 \pm 0.0148	0.583 \pm 0.0270
Interaction P -value	0.3407	0.3858	0.4562	0.2031

^{a,b}Means in a column within type of variable and main effect or interaction treatment with no common superscript differ significantly ($P \leq 0.05$).

Bold indicates $P < 0.05$.

Abbreviations: R_{low} MG, low passage R strain of *Mycoplasma gallisepticum*; XPC, original XPC.

¹Nine birds in each of 16 replicate units were used to calculate means in the diet and challenge treatment groups, and 9 birds in each of 8 replicate units were used to calculate means in the diet-challenge treatment combination groups.

²Four birds in each of 16 replicate units were used to calculate means in the diet and challenge treatment groups, and 4 birds in each of 8 replicate units were used to calculate means in the diet-challenge treatment combination groups.

challenged birds that tested positive averaged 96% greater than that in the nonchallenged birds that tested MG positive at 12 woa.

Serum IgM and IgG antibody results, as determined by respective SPA and ELISA tests, showed that all birds across the CON and XPC dietary treatment groups were negative for MG antibody production before the first 8 woa R_{low} MG challenge. Similarly, across dietary treatment, all nonchallenged birds but 1 (143/144 = 99.3%) at 12 woa were SPA negative for IgM antibody production against MG. The nonchallenged bird that tested SPA positive tested negative for MG DNA detection via PCR. In addition, all (100%) of the birds across dietary treatment that did not receive an R_{low} MG challenge also tested ELISA negative for IgG antibody production against MG at 12 woa. However, after an R_{low} MG challenge, all challenged birds mounted a humoral immune response. All (100%) challenged birds across dietary treatment at 12 woa were SPA positive for IgM antibody production against MG. Furthermore, approximately 48% of the birds across dietary treatment in the challenged group tested ELISA positive for IgG antibody production at 12 woa. Within the challenged group of birds, there was no significant ($P = 0.6417$) effect because of diet on the percentage of ELISA positive birds. Within the challenged group of birds, $47.2 \pm 4.14\%$ of the birds in the CON dietary treatment tested positive, and $50.0 \pm 4.14\%$ of the birds in the XPC dietary treatment tested positive.

There was no significant main effect because of diet and no significant diet \times challenge interaction for ELISA IgG titers at 12 woa. However, there was a significant ($P < 0.0001$) main effect because of challenge on IgG titers at 12 woa (Table 2). ELISA IgG antibody titers were observed to increase in response to the R_{low}

MG challenge (Table 2). These results showed that both IgM and IgG antibody titers at 12 woa were significantly increased by the R_{low} MG challenge at 8 and 10 woa. Nevertheless, the inclusion of XPC had no apparent effect on the above results. Dietary XPC did not significantly influence the number of birds that produced antibodies and did not influence their levels in those birds that did produce antibodies.

There was no significant main effect because of diet and no significant diet \times challenge interaction, for scores of the combined incidences of lung conjunctivitis and airsacculitis at 12 woa because of MG. However, dietary treatment numerically reduced MG lesion scores in R_{low} MG-challenged birds from 0.28 to 0.19 (Table 2). There was a significant ($P = 0.0012$) main effect because of challenge with MG lesion scores at 12 woa being higher for R_{low} MG-challenged birds. Although not statistically significant, supplementation of the basal diet with XPC led to a numerical reduction in the lung and air sac MG lesion scores of the birds (Table 2).

DISCUSSION

The current study evaluates for benefits to feeding layer pullets XPC in the face of an MG challenge. Other studies have been conducted on the effects of XPC on the cecal microbiota (McIntyre et al., 2014; Guyard-Nicodème et al., 2016; Rubinelli et al., 2016; Park et al., 2017; Roto et al., 2017), fecal bacteria shedding (Feye et al., 2016), organ weight (Osweiler et al., 2010), and performance (El-Husseiny et al., 2008; Osweiler et al., 2010; Feye et al., 2016; Roto et al., 2017) of broilers. Furthermore, its effects on the performance of broiler breeder hens (Kidd et al., 2013) and on the intestinal health (Lensing et al., 2012), production performance (Lensing et al., 2012; Suarez Martinez et al., 2018), and egg component yield and composition (Suarez Martinez et al., 2018) in laying hens have been investigated. In the current study, we focused on our attention on the effect of the XPC diet on the immune response to MG, its effect on the ovary, ceca, and bursa, and the weight gain of the pullets to 12 woa.

BW and Relative Organ Weight

In an in vitro study by Rubinelli et al. (2016), in which the effects of feed containing XPC on the *Salmonella typhimurium* population in an anaerobic mixed culture containing cecal microbiota were evaluated, it was found that dietary XPC reduced *S. typhimurium* in the cecal microbiota. Based on the results of the study, it was suggested that XPC may decrease the proportions of *Salmonella* in the ceca of broilers and layers. Moreover, Feye et al. (2016) observed that 1.25 kg/MT of dietary XPC fed between 21 and 49 D posthatch inhibited *Salmonella* fecal shedding, postliminary virulence, and antibiotic resistance in Cobb broilers. These effects were likewise associated with a heavier BW and increased BW gain.

Table 2. Effects of diet and challenge treatments on ELISA IgG titer and MG lesion score (combined incidences of lung conjunctivitis and airsacculitis) in layer pullet at 12 wk of age.

Treatments	IgG titer ¹	Lesion score ¹
Diet		
Control	1,367.8 \pm 161.52	0.139 \pm 0.0463
XPC	1,366.1 \pm 159.79	0.097 \pm 0.0459
Main effect <i>P</i> -value	0.9939	0.5289
Challenge		
Nonchallenged	72.7 ^b \pm 159.79	0.000 ^b \pm 0.0459
R_{low} MG-challenged	2,661.2 ^a \pm 161.52	0.236 ^a \pm 0.0463
Main effect <i>P</i> -value	0.0001	0.0012
Diet-challenge		
Control and Nonchallenged	79.0 \pm 225.98	0.000 \pm 0.0650
Control and R_{low} MG-challenged	2,656.6 \pm 230.84	0.278 \pm 0.0659
XPC and Nonchallenged	66.3 \pm 225.98	0.00 \pm 0.0650
XPC and R_{low} MG-challenged	2,665.8 \pm 225.98	0.194 \pm 0.0650
Interaction <i>P</i> -value	0.9616	0.5289

^{a,b}Means in a column within type of variable and main effect or interaction treatment with no common superscript differ significantly ($P \leq 0.05$).

Bold indicates $P < 0.05$.

Abbreviations: R_{low} MG, low passage R strain of *Mycoplasma gallisepticum*; XPC, original XPC.

¹Nine birds in each of 16 replicate units were used to calculate means in the diet and challenge treatment groups, and 9 birds in each of 8 replicate units were used to calculate means in the diet-challenge treatment combination groups.

Oswailer et al. (2010) showed that dietary XPC at either 0.0625 or 0.125% concentrations did not increase the 28 D BW gain or affect the day 28 relative liver weights of male Ross 308 broilers fed diets containing 2,280 ng/g of aflatoxin B₁ and 1,123 ng/g of aflatoxin G₁. However, El-Husseiny et al. (2008) found that 0.075 and 0.100% dietary XPC did significantly increase the 35 D BW gain and immune response of mixed sex Ross 308 broilers when compared with controls fed no feed additive.

Like the results of El-Husseiny et al. (2008), but in contrast to those of Oswailer et al. (2010), dietary XPC significantly increased the BW of the pullets at 12 woa in the present study. Liver weight was not examined in the pullets in this study, but in resemblance to the findings of Oswailer et al. (2010), the relative organ (ovary, bursa, and ceca) weights that were determined were not affected by the use of dietary XPC in the R_{low} MG-challenged birds. Nevertheless, unlike El-Husseiny et al. (2008), dietary XPC did not affect the immune response of the pullets belonging to both the unchallenged and challenged treatment groups. The differing immune responses observed in the 2 studies may be attributed to differences in bird type and the immune variables examined and testing techniques employed.

Humoral Immune Response

Lensing et al. (2012) observed that an *Eimeria maxima* challenge in Brown Nick laying hens resulted in a reduction in their production performance, as indicated by significant decreases in their egg weight, rate of lay, feed intake, and feed efficiency. In that report, in which the effects of dietary supplementation with XPC_{LS} (a *S. cerevisiae* fermentation product similar to Original XPC) was tested, it was also shown that although XPC_{LS} had no major effects on the production performance or humoral immunity of the hens infected by *E. maxima*, it reduced the intestinal damage caused by the *E. maxima* infection. It was surmised that the XPC_{LS} treatment may have caused the coccidiosis infection to be milder and gut integrity to be less impaired in the birds. Furthermore, in a later study by Roto et al. (2017), it was suggested that 1.25 g/kg of dietary XPC could be effectively used concurrently with a live coccidiosis vaccine to increase the feed conversion and growth rate of Ross 708 male broilers.

The current MG DNA and humoral antibody results showed that birds that were challenged with R_{low} MG were infected by the organism and elicited an ensuing immune response to the infection. Furthermore, the R_{low} MG significantly reduced the relative bursal weights of the birds. The detection of MG DNA in 25% of the nonchallenged isolation units at 12 woa indicates some contamination in those units. These inadvertently contaminated birds, however, had DNA quantities far lower than the challenged birds, and they had not yet mounted an antibody response by the end of the study. However, the results further showed

that the inclusion of XPC in the diets of the pullets had no significant influence on those results. Tracheal washing could be utilized to detect any dietary treatment differences in the localization of antibodies in the trachea for protection of the trachea epithelial cells; however, this was not conducted in the current study (Avakian and Ley, 1993). Nevertheless, similar to the results reported by Lensing et al. (2012), in which XPC_{LS} reduced the intestinal damage caused by an *E. maxima* infection, it was observed in the current investigation that dietary supplementation with XPC numerically reduced MG lesion scores in the lungs and air sacs of the layer pullets. The gut microbiota profile of the birds in the present study were not evaluated. However, the combined results from those of the current study and of Lensing et al. (2012) suggest that XPC may promote tissue integrity and provide associated protection against tissue invasion by pathogens.

Future Research Areas

Supplementation of the diets of Cobb 500 broiler breeder hens with XPC at a rate of 0.68 kg/ton has proved to reduce contamination incidence in the eggs laid at 32 woa (Kidd et al., 2013). The hatchability of fertile eggs laid at 32 and 39 woa was likewise increased by incorporating XPC in the diets of the hens. Effects of supplemental XPC in the breeder hen diets on their subsequent progeny were further observed, in that feed conversion and breast meat yield of the broilers hatched at 39 wk of breeder hen age were improved. Results of the investigation by Suarez Martinez et al. (2018) have shown that supplementing the standard layer rations of Hy-Line W-36 laying hens with 1.25 kg/MT of XPC did not affect hen-day egg production or egg mass between 19 and 53 woa. However, supplementing the diets with XPC increased feed conversion ratio, yolk weight, percentage of yolk yield, percentage of yolk solids, and percentage of albumen nitrogen but decreased percentage of yolk nitrogen. The performance results of broiler breeder and layer hens reported by Kidd et al. (2013) and Suarez Martinez et al. (2018), respectively, would support the contention that further studies should be conducted to investigate the possible effects of dietary XPC on the physiology and performance of commercial laying hens having been subjected to a prelay R_{low} MG challenge.

In conclusion, dietary XPC did not alter the BW or relative select organ (ovary, bursa, and ceca) weights of the layer pullets at 3 woa in the current study. Conversely, the R_{low} MG challenge at 8 and 10 woa decreased the relative bursal weight and increased the relative ovarian and cecal weights of the pullets at 12 woa. Furthermore, all challenged birds mounted a humoral immune response to the R_{low} MG challenge. Nevertheless, although dietary XPC did not significantly alter the relative select organ weights, the morphometry of the bursal follicles, or the humoral immune response and antibody titer levels of the challenged layer pullets,

it numerically reduced their air sac MG lesion scores and led to an increase in their rate of growth through 12 woa.

The current study evaluated pullets up to 12 woa. Further study should evaluate the effects of feeding XPC to layer chickens during egg production. The best egg production scenario is to have MG-clean layer hens: field-strain MG infections as well as vaccinating birds against MG diminish egg production (Carpenter et al., 1981). The goal of future research should be to determine the potential benefits of supplemental dietary XPC on the quality and production of eggs laid by commercial layers throughout a complete lay cycle after a prelay R_{low} MG challenge. Furthermore, antibody production alone provides limited information concerning immunocompetence. Therefore, the influence of XPC on antibody activity and the responses of other immune factors subsequent to a field-strain MG infection should likewise be examined in future research.

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REFERENCES

- Avakian, A. P., and D. H. Ley. 1993. Protective immune response to *Mycoplasma gallisepticum* demonstrated in respiratory-tract washings from *M. gallisepticum*-infected chickens. *Avian Dis.* 37:697–705.
- Bradbury, J. M. 2005. Poultry mycoplasmas: sophisticated pathogens in simple guise. *Br. Poult. Sci.* 46:125–136.
- 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.
- Callison, S. A., S. M. Riblet, S. Sun, N. Ikuta, D. Hilt, V. Leiting, S. H. Kleven, D. L. Suarez, and M. García. 2006. Development and validation of a real-time Taqman polymerase chain reaction assay for the detection of *Mycoplasma gallisepticum* in naturally infected birds. *Avian Dis.* 50:537–544.
- 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.
- Dizaji, S. B., and R. Pirmohammadi. 2009. Effect of *Saccharomyces cerevisiae* and Bioplus 2B on performance of laying hens. *Int. J. Agric. Biol.* 11:495–497.
- El-Husseiny, O. M., A. G. Abdallah, and K. O. Abdel-Latif. 2008. The influence of biological feed additives on broiler performance. *Int. J. Poult. Sci.* 7:862–871.
- Elliott, K. E. C., S. L. Branton, J. D. Evans, P. D. Gerard, and E. D. Peebles. 2017. Layer chicken embryo survival to hatch when administered an *in ovo* vaccination of strain F *Mycoplasma gallisepticum* and locations of bacteria prevalence in the newly hatched chick. *Poult. Sci.* 96:3879–3884.
- Elliott, K. E. C., S. L. Branton, J. D. Evans, and E. D. Peebles. 2018. Early post-hatch survival and humoral immune response of layer chickens when *in ovo* vaccinated with strain F *Mycoplasma gallisepticum*. *Poult. Sci.* 97:3860–3869.
- Evans, J. D., S. A. Leigh, S. L. Branton, D. S., and Collier. 2007. Effects of increased dosages of the *Mycoplasma gallisepticum* vaccine MYCOVAC-L® in layer chickens subsequently challenged with virulent *M. gallisepticum*: egg production and serologic response. *Avian Dis.* 51:912–917.
- Feye, K. M., K. L. Anderson, M. F. Scott, D. R. McIntyre, and S. A. Carlson. 2016. Inhibition of the virulence, antibiotic resistance, and fecal shedding of multiple antibiotic-resistant *Salmonella Typhimurium* in broilers fed Original XPC™. *Poult. Sci.* 95:2902–2910.
- Frey, M. C., R. P. Hanson, and D. P. Anderson. 1968. A medium for the isolation of avian *Mycoplasma*. *Am. J. Vet. Res.* 29:2163–2171.
- Fujiwara, K., Y. Miyaguchi, A. Toyoda, Y. Nakamura, M. Yamazaki, K. Nakashima, and H. Abe. 2008. Effect of fermented soybean natto supplement on egg production and qualities. *Asian Australas. J. Anim. Sci.* 21:1610–1615.
- Grimes, J. L., D. V. Maurice, S. F. Lightsey, and J. G. Lopez. 1997. The effect of dietary fermacto on layer hen performance. *J. Appl. Poult. Res.* 6:399–403.
- Guyard-Nicodème, A. Keita, S. Quesne, M. Amelot, T. Poezevara, B. Le Berre, J. Sánchez, P. Vasseur, Á. Martín, and M. Chemaly. 2016. Efficacy of feed additives against *Campylobacter* in live broilers during the entire rearing period. *Poult. Sci.* 95:298–305.
- Harns, R. H., and R. D. Miles. 1988. Influence of fermacto on the performance of laying hens when fed diets with different levels of methionine. *Poult. Sci.* 67:842–844.
- Kidd, M. T., L. Araujo, C. Araujo, C. D. McDaniel, and D. McIntyre. 2013. A study assessing hen and progeny performance through dam diet fortification with a *Saccharomyces cerevisiae* fermentation product. *J. Appl. Poult. Res.* 22:872–877.
- Lensing, M., J. D. van der Klis, I. Yoon, and D. T. Moore. 2012. Efficacy of *Saccharomyces cerevisiae* fermentation product on intestinal health and productivity of coccidian-challenged laying hens. *Poult. Sci.* 91:1590–1597.
- Ley, D. H. 2003. *Mycoplasmosis-Mycoplasma gallisepticum* infection. Pages 722–744 in *Diseases of Poultry*. Y. M. Saif, H. J. Barnes, J. R. Glisson, A. M. Fadly, L. R. McDougald and D. E. Swayne eds, 11th ed. Iowa State Press, Ames.
- McIntyre, D. R., C. L. Hofacre, and G. F. Mathis. 2014. Feeding XPC can reduce *Campylobacter* in broilers. *Poult. Sci.* 93(E-Suppl. 1):90–91.
- Muniz, E. C., V. B. Fascina, P. P. Pires, A. S. Carrijo, and E. B. Guimaraes. 2006. Histomorphology of bursa of Fabricius: effects of stock densities on commercial broilers. *Braz. J. Poult. Sci.* 8:217–220.
- Nunoya, T., M. Tajima, T. Yagihashi, and S. Sannai. 1987. Evaluation of respiratory lesions in chickens induced by *Mycoplasma gallisepticum*. *Jpn. J. Vet. Sci.* 49:621–629.
- Osweller, G. D., S. Jagannatha, D. W. Trampel, P. M. Imerman, S. M. Ensley, I. Yoon, and D. T. Moore. 2010. Evaluation of XPC and prototypes on aflatoxin-challenged broilers. *Poult. Sci.* 89:1887–1893.
- Park, S. H., S. Roto, H. Pavlidis, D. McIntyre, K. Striplin, L. Brammer, and S. C. Ricke. 2017. Effects of feeding Original XPC™ to broilers with a live coccidiosis vaccine under industrial conditions: Part 2. Cecal microbiota analysis. *Poult. Sci.* 96:2400–2411.
- Peebles, E. D. 2020. *In ovo* development of the chicken gut microbiome and impact on later gut function. Pages 95–119 in *Understanding and Improving Gut Function in Poultry*. S. C. Ricke ed. Burleigh Dodds Science Publishing, Sawston, Cambridge, UK.
- Peebles, E. D., and S. L. Branton. 2012. *Mycoplasma gallisepticum* in the commercial egg-laying hen: a historical perspective considering the effects of pathogen strain, age of the bird at inoculation, and diet on performance and physiology. *J. Appl. Poult. Res.* 21:897–914.
- Rodriguez, R., and S. H. Kleven. 1980. Pathogenicity of two strains of *Mycoplasma gallisepticum* in broilers. *Avian Dis.* 24:800–807.
- Roto, S. M. 2016. A molecular approach to understanding the effects of Original-XPC on the modulation of the cecal microbiota and the survival of *Salmonella* in the poultry host. Theses and Dissertations. <http://scholarworks.uark.edu/etd/1480>.
- Roto, S. M., S. H. Park, S. I. Lee, P. Kaldhone, H. O. Pavlidis, S. B. Frankenbach, D. R. McIntyre, K. Striplin, L. Brammer, and S. C. Ricke. 2017. Effects of feeding Original XPC™ to broilers with a live coccidiosis-vaccine under industry conditions: Part 1. Growth performance and *Salmonella* inhibition. *Poult. Sci.* 96:1831–1837.

- Rubinelli, P., S. Roto, S. A. Kim, S. H. Park, H. O. Pavlidis, D. McIntyre, and S. C. Ricke. 2016. Reduction of *Salmonella* Typhimurium by fermentation metabolites of Diamond V Original XPC in an *in vitro* anaerobic mixed chicken cecal culture. *Front. Vet. Sci.* 3:1–7.
- SAS Institute 2012. SAS Proprietary Software 9.4. SAS Institute Inc., Cary, NC, USA.
- Steel, R. G. D., and J. H. Torrie. 1980. Principles and Procedures of Statistics. A Biometrical Approach, 2nd ed. McGraw-Hill, New York, NY.
- Suarez Martinez, J. C., R. L. Blount, J. Park, D. R. McIntyre, H. O. Pavlidis, and J. B. Carey. 2018. Effects of feeding original XPC™ to laying hens on egg production, component yield and composition. *J. Appl. Poult. Res.* 27:603–608.