MANAGEMENT AND PRODUCTION

Effects of dietary Original XPC on selected blood variables in layer pullets challenged with *Mycoplasma gallisepticum*[1,2,3]

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ABSTRACT Effects of dietary Original XPC (**XPC**) on 17 selected blood variables 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 *M. gallisepticum*–clean layer breeders were fed a basal diet with XPC (1.25 kg/metric ton) or without 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. Blood samples were taken immediately before the initial R_{low}MG challenge at 8 woa and again at 12 woa (4 wk after challenge). At 8 woa, blood pH was lower and glucose concentration was higher in the pre-assigned challenge treatment groups. At 12 woa, the concentration of oxygen dissolved in the blood was significantly lower in the R_{low}MG-challenged group than the unchallenged group of birds regardless of dietary treatment. The R_{low}MG challenge significantly increased blood carbon dioxide partial pressure, calcium, sodium, anion gap, osmolality, glucose, and corticosterone levels but significantly decreased blood oxygen partial pressure, oxyhemoglobin concentration, concentration of oxygen dissolved in the blood, chloride, and pH levels. Because blood pH and glucose concentration at 8 woa were examined before challenge, their baseline values were biased with respect to challenge treatment before treatment was applied. However, the lack of a significant main effect due to diet at 8 woa for blood pH and glucose concentration, along with the other 15 blood variables, indicate that the baseline data with respect to dietary treatment were unbiased, allowing for real dietary effects to be accurately assessed. In conclusion, layer pullets challenged with R_{low}MG undergo a stress response associated with changes in various physiological blood variables, and a decrease in pH and increase in carbon dioxide partial pressure, in association with a lack of change in bicarbonate, indicates that the stress response caused by the R_{low}MG challenge was associated with respiratory acidosis. Nevertheless, feeding XPC did not influence the effects of challenge treatment on these post-challenge physiological blood values.

Key words: egg production, immunity, layer pullet, *Mycoplasma gallisepticum*, Original XPC

2020 Poultry Science 99:4373–4383
https://doi.org/10.1016/j.psj.2020.06.005

INTRODUCTION

In layer hen flocks subjected to intensive and stressful housing conditions, *Mycoplasma gallisepticum* (**MG**) has caused significant economic losses in the table egg industry (Mohammed et al., 1987). These financial losses are largely a result of reductions in the quality and production of eggs laid by commercial layer flocks infected by field strains of MG (Peebles and Branton, 2012). Known for its relatively higher level of pathogenicity, as evidenced by its ability to significantly increase the severity of air-sacculitis and the incidence of air sac lesions in chickens (Rodriguez and Kleven, 1980), the R strain of MG has been largely used as a pathogenic strain in MG challenge trials (Ley, 2003).

Received February 6, 2020.
Accepted June 3, 2020.
[1]This publication is a contribution of the Mississippi Agricultural and Forestry Experiment Station.
[2]This material is based upon work that is supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, Hatch project under accession number 329260.
[3]Use of trade names in this publication does not imply endorsement by Mississippi Agricultural and Forestry Experiment Station of these products, nor similar ones not mentioned.
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Alterations in various blood variables have been commonly used as indices for evaluating the health of chickens and the influences of different pathologic agents (Heath, 1970; Kral and Suchy, 2000; Olanrewaju et al., 2017). Furthermore, changes in diverse blood variables, including those related to electrolyte levels, blood gas partial pressures, and acid–base balance, have been associated with physiological stress responses in broilers (Sandercock et al., 2001; Olanrewaju et al., 2006, 2007). Physiological stress is known to be associated with modifications in the regulation and function of the hypothalamic-pituitary-adrenal axis (Smith and Vale, 2006). Olanrewaju et al. (2007) has more specifically shown that the infusion of broilers with adrenocorticotropic hormone resulted in increases in their blood hematocrit (**Hct**), hemoglobin (**Hb**), carbon dioxide partial pressure ($\mathbf{pCO_2}$), corticosterone, osmolality, and bicarbonate ($\mathbf{HCO_3^-}$) levels, while reducing their BW as well as their blood pH, oxygen partial pressure ($\mathbf{pO_2}$), and sodium ($\mathbf{Na^+}$) and chloride ($\mathbf{Cl^-}$) levels. It has also been reported that the inoculation of layers with the F strain of MG (**FMG**, Olanrewaju et al., 2009) and TS-11 strain of MG (**TS-11MG**; Olanrewaju et al., 2011) caused an increase in their arterial oxygen partial pressures.

Original XPC (**XPC**; Diamond V Corp., Cedar Rapids, IA), a fermentation product derived from *Saccharomyces cerevisiae*, has been shown to enhance immune activation (Chou et al., 2017) and has been reported to reduce gut pathogen colonization and to improve intestinal development and growth in chickens (El-Husseiny et al., 2008; Roto, 2016; Roto et al., 2017). Furthermore, XPC was more recently shown to increase the yolk weight and percentages of yolk yield and yolk solids of eggs laid by Hy-Line W-36 layers (Suarez Martinez et al., 2018). In a companion article by Elliott et al. (2020), it was also reported that dietary XPC increased the growth rate of Hy-Line W-36 layer pullets through 12 wk of age (**woa**) and numerically reduced the air sac MG lesion scores at 12 woa of pullets that had been previously challenged with the virulent, low-passage (13 in vitro passages) R strain of MG ($\mathbf{R_{low}MG}$) at 8 and 10 woa. To the knowledge of the authors, no previous research has been conducted concerning the effects of a virulent strain of MG, as well as the influence of dietary XPC, on the blood panel variables, including the electrolyte levels, blood gas partial pressures, and acid–base balance, of commercial layers. Therefore, the objective of the present study was to determine the effects of dietary XPC on the comprehensive profile of various physiologically relevant blood variables in Hy-Line W-36 layer pullets through 12 woa, after being subjected to a virulent MG challenge.

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 (Elliott et al., 2020). In each of 4 rooms in an environmentally controlled facility, there were 2 replicate floor pens belonging to each of the 2 dietary treatments (4 pens per room; 16 total pens). In each pen, 48 female chicks were placed on fresh litter and were maintained under standard brooding conditions. 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. The birds were raised in the floor pens from 0 to 3 woa, at which time they were then moved to disease challenge biological isolation units. Birds were randomly preassigned to 4 replicate biological isolation units belonging to each preassigned dietary challenge combination treatment group in each of 2 replicate rooms (blocks) in an environmentally controlled poultry disease isolation facility (Branton and Simmons, 1992). Initially, 18 birds were placed in each biological isolation unit.

Birds in half of the isolation pens in each dietary treatment group were unchallenged, and the other half were challenged. Unchallenged birds were sham challenged with media that lacked challenge bacteria. Challenged birds were inoculated with $R_{low}MG$ that was generously provided by Dr. Steven Geary (University of Connecticut). Frey's broth base (Frey et al., 1968) was used as the medium for the sham challenge, and the $R_{low}MG$ was diluted in Frey's broth base for the actual challenge. The challenge dosages and their application methods were those used in the companion article by Elliott et al. (2020). Challenge with the $R_{low}MG$ inoculum was conducted at 8 woa (eye drop) and 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 μL volume of inoculum culture containing 3.0×10^6 CFU of $R_{low}MG$ was applied to the eye using a 200-μL pipette. For the 10 woa challenge, the same inoculum that was used at 8 woa was frozen and thawed and held on ice at approximately 4°C until being administered, with less than a 10% loss of viable bacteria. A 200 μL volume of that inoculum culture, containing 3.0×10^7 CFU of $R_{low}MG$, was discharged into the trachea using a 1,000-μL pipette.

In the article by Elliott et al. (2020) in which these same treatments were used, it was reported that the combined incidences of lung conjunctivitis and airsacculitis at 12 woa were significantly increased by the RMG challenge treatment. However, these incidences were not observed at 10 woa in response to the eye drop challenge, which led to the secondary tracheal gavage challenge at 10 woa. This secondary challenge led to the challenge treatment effect at 12 woa. 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) after incubation at 37°C. The presence of viable bacteria in the inoculum

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-Agricultural Research Service Animal Care and Use Committee (Mississippi State, MS).

Blood Collection and Chemical Analyses

In total, 17 blood variables were examined at 8 and 12 woa and included Hct, Hb, mean corpuscular Hb concentration, pCO_2, pO_2, arterial oxygen saturation or oxyhemoglobin concentration ($\mathbf{SaO_2}$), oxygen concentration dissolved or carried in the blood ($\mathbf{sO_2}$), calcium ($\mathbf{Ca^{2+}}$), potassium ($\mathbf{K^+}$), Na^+, Cl^-, pH, anion gap, HCO_3^-, osmolality, glucose, and corticosterone. At 8 woa, blood samples were taken from birds before challenge treatment. Blood samples were collected from the wing brachial vein between 800 and 1,000 h on the day of sampling from 4 randomly selected birds from each replicate biological isolation unit. Different birds were sampled at 8 and 12 woa. All blood was collected within 45 s after the birds were caught for bleeding (Olanrewaju et al., 2019). As described by Olanrewaju et al. (2016), a 3 mL volume of blood was drawn into heparinized (50 IU/mL) Monovette syringes and was directly and immediately transferred to a blood gas analyzer (ABL80 CO-OX Flex; Radiometer America, Westlake, OH) for analysis. Calculation of mean corpuscular Hb concentration and SaO_2 values and the plasma collection and subsequent measurement of plasma corticosterone concentrations were performed in accordance with the procedures of Olanrewaju et al. (2016).

Statistical Analysis

All procedures of data analysis used were of SAS software (version 9.4, SAS Institute, 2012). A mixed-model ANOVA using PROC MIXED was used to analyze the data. A 2-way ANOVA was used to analyze the main and interactive effects of diet and challenge at 8 and 12 woa. Fixed effects in the model were diet and challenge treatment. Random effects included room, isolation unit within room, and diet × challenge × isolation unit within room interaction. Analysis of challenge effect for serum plate agglutination and ELISA tests and of diet effect for serum plate agglutination tests was not possible owing to 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

In the previous companion trial by Elliott et al. (2020) in which the same birds were tested, it was shown that all birds in the control and XPC dietary treatment groups tested negative for MG-specific serum IgM and IgG antibody titers before the first 8 woa challenge. At 12 woa, all but 1 unchallenged bird tested IgM negative, whereas all unchallenged birds tested IgG negative. However, at 12 woa, all challenged birds tested positive for IgM antibody production, and 48% of the challenged birds tested IgG positive. There was no significant effect of XPC dietary treatment on the aforementioned results. Elliott et al. (2020) also observed that scores for the combined incidences of lung conjunctivitis and airsacculitis at 12 woa due to R_{low}MG challenge were significantly increased but that dietary XPC led to a numerical reduction in their incidence.

For 15 of the 17 blood variables examined at 8 woa (Tables 1–5), there were no significant main or interactive effects involving diet or challenge treatment. The lack of an effect owing to challenge treatment for these 15 variables was expected, as these blood variables were examined before challenge treatment. However, there was a significant main effect because of challenge treatment for blood pH (Table 4) and glucose concentration (Table 5) at 8 woa. Blood pH was lower, whereas glucose concentration was higher, in the R_{low}MG-challenged treatment group compared with the unchallenged treatment group. These results were unexpected and are difficult to explain, as the blood samples were collected before challenge treatment. However, they do indicate that the baseline values of these 2 variables were biased or weighted with respect to challenge treatment before treatment was applied. Nevertheless, there was no significant main or interactive effect due to diet for blood pH (Table 4) or glucose concentration (Table 5), as well as the other 15 blood variables, at 8 woa. The baseline data with respect to dietary treatment, therefore, were unbiased or unweighted so that real effects due to dietary treatment could be more accurately assessed.

For 16 of the 17 blood variables examined at 12 woa, there were no significant main or interactive effects because of dietary treatment (Tables 6–10). However, there was a significant ($P = 0.0356$) diet × challenge treatment interaction for sO_2 concentration (concentration of oxygen dissolved or carried in the blood) at 12 woa (Table 7). Blood sO_2 levels were significantly lower in the R_{low}MG-challenged group than in the unchallenged group of birds regardless of dietary treatment. This suggests that despite a significant challenge × dietary treatment interaction, XPC supplementation did not have a significant influence on the effects of challenge treatment on blood sO_2 levels. There was a significant main effect due to R_{low}MG challenge treatment on 12 of the 17 blood variables. The R_{low}MG challenge significantly affected blood pCO_2, pO_2, SaO_2 (arterial oxygen saturation, or oxyhemoglobin concentration), and sO_2 (Table 7); Ca^{2+}, Na^+, and Cl^- (Table 8); pH, anion gap, and osmolality (Table 9); and glucose and corticosterone levels (Table 10). More specifically, the R_{low}MG challenge increased blood pCO_2, Ca^{2+}, Na^+, anion gap, osmolality, glucose, and corticosterone levels but decreased blood pO_2, SaO_2, sO_2, Cl^-, and pH levels.

Table 1. Mean blood hematocrit (Hct), hemoglobin (Hb), and mean corpuscular hemoglobin concentration (McHc) values at 8 woa in layer pullets before a R_{low} *Mycoplasma gallisepticum* (R_{low}MG) challenge at 8 and 10 woa and that belonged to basal (control) and XPC-supplemented (XPC) dietary treatment groups.[1]

Variable	Hct (% PCV)	Hb (g/dL)	McHc (g/dL)
Diet[2]			
Control	29.2	9.44	32.3
XPC	29.3	9.48	32.3
Pooled SEM	0.25	0.08	0.01
Main effect *P*-value	0.7079	0.7079	0.6987
Challenge[3]			
Unchallenged	29.3	9.47	32.3
R_{low}MG challenged	29.3	9.45	32.3
Pooled SEM	0.25	0.08	0.01
Main effect *P*-value	0.9146	0.9146	0.8565
Diet-Challenge[4]			
Control and unchallenged	29.3	9.46	32.3
Control and R_{low}MG challenged	29.2	9.42	32.3
XPC and unchallenged	29.3	9.48	32.3
XPC and R_{low}MG challenged	29.4	9.49	32.3
Pooled SEM	0.35	0.12	0.01
Interaction *P*-value	0.8512	0.8512	0.8363

Abbreviation: woa, wk of age.
[1]Challenge treatment designations were preassigned, as main and interaction effect means involving challenge were before challenge.
[2]Main effect means based on 4 birds in each of 16 units in each dietary treatment group across challenge treatment (64 total birds).
[3]Main effect means based on 4 birds in each of 16 units in each challenge treatment group across dietary treatment (64 total birds).
[4]Interaction effect means based on 4 birds in each of 8 replicate units in each diet-challenge treatment combination group (32 total birds).

DISCUSSION

Elliott et al. (2020) reported the humoral antibody results for the same birds that were used in this study. Those results confirmed that only the pullets that were challenged with R_{low}MG showed positive antibody responses to the organism and that it subsequently evoked an immune response in those birds. In addition, the relative bursal weights of the pullets were significantly reduced by the R_{low}MG challenge.

Table 2. Mean blood pCO_2, pO_2, SaO_2 (arterial oxygen saturation, or oxyhemoglobin concentration), and sO_2 (oxygen concentration dissolved or carried in the blood) values at 8 woa in layer pullets before a R_{low} *Mycoplasma gallisepticum* (R_{low}MG) challenge at 8 and 10 woa and that belonged to basal (control) and XPC-supplemented (XPC) dietary treatment groups.[1]

Variable	pCO_2 (mm Hg)	pO_2 (mm Hg)	SaO_2 (%)	sO_2 (%)
Diet[2]				
Control	49.7	68.3	82.2	47.0
XPC	48.9	69.1	82.6	48.5
Pooled SEM	0.5	0.9	0.4	1.1
Main effect *P*-value	0.2765	0.4764	0.4589	0.3352
Challenge[3]				
Unchallenged	48.6	68.8	82.7	48.5
R_{low}MG challenged	50.0	68.6	82.2	47.0
Pooled SEM	0.5	0.9	0.4	1.1
Main effect *P*-value	0.0667	0.9186	0.3305	0.3632
Diet-Challenge[4]				
Control and unchallenged	48.7	68.4	82.6	48.0
Control and R_{low}MG challenged	50.6	68.1	81.8	45.9
XPC and unchallenged	48.4	69.1	82.8	48.9
XPC and R_{low}MG challenged	49.3	69.1	82.5	48.1
Pooled SEM	0.7	1.2	0.5	1.6
Interaction *P*-value	0.5024	0.9186	0.6785	0.6675

Abbreviation: woa, wk of age.
[1]Challenge treatment designations were preassigned, as main and interaction effect means involving challenge were before challenge.
[2]Main effect means based on 4 birds in each of 16 units in each dietary treatment group across challenge treatment (64 total birds).
[3]Main effect means based on 4 birds in each of 16 units in each challenge treatment group across dietary treatment (64 total birds).
[4]Interaction effect means based on 4 birds in each of 8 replicate units in each diet-challenge treatment combination group (32 total birds).

Table 3. Mean blood calcium (Ca^{2+}), potassium, (K^+), sodium (Na^+), and chloride (Cl^-) values at 8 woa in layer pullets before a R_{low} *Mycoplasma gallisepticum* (R_{low}MG) challenge at 8 and 10 woa and that belonged to basal (control) and XPC-supplemented (XPC) dietary treatment groups.[1]

Variable	Ca^{2+} (mEq/L)	K^+ (mEq/L)	Na^+ (mEq/L)	Cl^- (mEq/L)
Diet[2]				
Control	3.01	4.63	148	109
XPC	3.02	4.58	148	109
Pooled SEM	0.02	0.05	0.4	0.2
Main effect *P*-value	0.2565	0.4798	0.3403	0.1410
Challenge[3]				
Unchallenged	3.01	4.58	148	109
R_{low}MG challenged	3.02	4.64	148	109
Pooled SEM	0.02	0.05	0.4	0.2
Main effect *P*-value	0.6183	0.3478	0.5759	0.9637
Diet-Challenge[4]				
Control and unchallenged	3.00	4.57	148	109
Control and R_{low}MG challenged	3.01	4.69	148	109
XPC and unchallenged	3.02	4.58	148	109
XPC and R_{low}MG challenged	3.02	4.58	148	109
Pooled SEM	0.02	0.07	0.5	0.3
Interaction *P*-value	0.4865	0.3478	0.8101	0.8914

Abbreviation: woa, wk of age.
[1]Challenge treatment designations were preassigned, as main and interaction effect means involving challenge were before challenge.
[2]Main effect means based on 4 birds in each of 16 units in each dietary treatment group across challenge treatment (64 total birds).
[3]Main effect means based on 4 birds in each of 16 units in each challenge treatment group across dietary treatment (64 total birds).
[4]Interaction effect means based on 4 birds in each of 8 replicate units in each diet-challenge treatment combination group (32 total birds).

The blood profiles of commercial egg-laying hens in response to a R_{low}MG infection have not been previously reported. However, on investigating the pathomechanism of MG in chickens, Vogl et al. (2008) detected the presence of R_{low}MG on the inside and on the surface of their erythrocytes 8 h after a R_{low}MG infection was initiated. The ability of R_{low}MG to reside intracellularly is indicative of its invasiveness and its capability to survive and multiply systemically in the chicken. The S6-strain of MG (**S6MG**) is also recognized as one of the more

Table 4. Mean blood pH, anion gap, bicarbonate (HCO_3^-), and osmolality values at 8 woa in layer pullets before a R_{low} *Mycoplasma gallisepticum* (R_{low}MG) challenge at 8 and 10 woa and that belonged to basal (control) and XPC-supplemented (XPC) dietary treatment groups.[1]

Variable	pH	Anion gap (mEq/L)	HCO_3^- (mm Hg)	Osmolality (mmol/kg)
Diet[2]				
Control	7.33	20.1	23.8	309
XPC	7.33	20.3	23.5	310
Pooled SEM	0.007	0.6	0.3	0.8
Main effect *P*-value	0.9834	0.7089	0.2947	0.2887
Challenge[3]				
Unchallenged	7.34[a]	19.8	23.9	309
R_{low}MG challenged	7.32[b]	20.6	23.4	310
Pooled SEM	0.007	0.6	0.3	0.8
Main effect *P*-value	0.0301	0.0747	0.1614	0.3045
Diet-Challenge[4]				
Control and unchallenged	7.34	19.8	24.0	309
Control and R_{low}MG challenged	7.32	20.4	23.6	309
XPC and unchallenged	7.33	19.9	23.7	309
XPC and R_{low}MG challenged	7.32	20.7	23.2	311
Pooled SEM	0.009	0.7	0.4	1.0
Interaction *P*-value	0.7869	0.8628	0.7923	0.7124

[a,b]Means in a column within type of variable and main effect or interaction treatment with no common superscript differ significantly ($P \leq 0.05$).
Abbreviation: woa, wk of age.
[1]Challenge treatment designations were preassigned, as main and interaction effect means involving challenge were before challenge.
[2]Main effect means based on 4 birds in each of 16 units in each dietary treatment group across challenge treatment (64 total birds).
[3]Main effect means based on 4 birds in each of 16 units in each challenge treatment group across dietary treatment (64 total birds).
[4]Interaction effect means based on 4 birds in each of 8 replicate units in each diet-challenge treatment combination group (32 total birds).

Table 5. Mean blood glucose and corticosterone concentrations at 8 woa in layer pullets before a R_{low} *Mycoplasma gallisepticum* (R_{low}MG) challenge at 8 and 10 woa and that belonged to basal (control) and XPC-supplemented (XPC) dietary treatment groups.[1]

Variable	Glucose (mg/dL)	Corticosterone (pg/mL)
Diet[2]		
Control	242	4,799
XPC	244	5,192
Pooled SEM	1.9	710
Main effect *P*-value	0.4813	0.6984
Challenge[3]		
Unchallenged	239[b]	4,571
R_{low}MG challenged	246[a]	5,420
Pooled SEM	1.9	710
Main effect *P*-value	0.0129	0.4051
Diet-Challenge[4]		
Control and unchallenged	239	4,308
Control and R_{low}MG challenged	244	5,289
XPC and unchallenged	239	4,833
XPC and R_{low}MG challenged	248	5,551
Pooled SEM	2.7	1,005
Interaction *P*-value	0.4742	0.8970

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

Abbreviation: woa, wk of age.

[1]Challenge treatment designations were preassigned, as main and interaction effect means involving challenge were before challenge.

[2]Main effect means based on 4 birds in each of 16 units in each dietary treatment group across challenge treatment (64 total birds).

[3]Main effect means based on 4 birds in each of 16 units in each challenge treatment group across dietary treatment (64 total birds).

[4]Interaction effect means based on 4 birds in each of 8 replicate units in each diet-challenge treatment combination group (32 total birds).

virulent MG strains (Levisohn et al., 1986), and its effects on the blood profiles of egg-laying hens have been investigated. The inoculation of commercial layers with this virulent field strain of MG has been reported to have varied effects on several of their blood variables, including those measured in the present study. Peebles et al. (2006) showed that in comparison with sham-inoculated controls, 10 or 22 woa inoculations of S6MG caused elevations in their serum Ca^{2+} levels across 24, 32, and 43 woa. Furthermore, in comparison with a

Table 6. Mean blood hematocrit (Hct), hemoglobin (Hb), and mean corpuscular hemoglobin concentration (McHc) values at 12 woa in birds after a R_{low} *Mycoplasma gallisepticum* (R_{low}MG) challenge at 8 and 10 woa and that belonged to basal (control) and XPC-supplemented (XPC) dietary treatment groups.

Variable	Hct (% PCV)	Hb (g/dL)	McHc (g/dL)
Diet[1]			
Control	26.7	8.60	32.2
XPC	26.7	8.61	32.2
Pooled SEM	0.2	0.08	0.01
Main effect *P*-value	0.9342	0.9342	0.9744
Challenge[2]			
Unchallenged	26.7	8.62	32.2
R_{low}MG challenged	26.7	8.60	32.2
Pooled SEM	0.2	0.08	0.01
Main effect *P*-value	0.9123	0.9123	0.9404
Diet-Challenge[3]			
Control and unchallenged	26.8	8.62	32.2
Control and R_{low}MG challenged	26.7	8.59	32.2
XPC and unchallenged	26.7	8.61	32.2
XPC and R_{low}MG challenged	26.8	8.62	32.2
Pooled SEM	0.3	0.11	0.01
Interaction *P*-value	0.8473	0.8473	0.8226

Abbreviation: woa, wk of age.

[1]Main effect means based on 4 birds in each of 16 units in each dietary treatment group across challenge treatment (64 total birds).

[2]Main effect means based on 4 birds in each of 16 units in each challenge treatment group across dietary treatment (64 total birds).

[3]Interaction effect means based on 4 birds in each of 8 replicate units in each diet-challenge treatment combination group (32 total birds).

Table 7. Mean blood pCO_2, pO_2, SaO_2 (arterial oxygen saturation, or oxyhemoglobin concentration), and sO_2 (oxygen concentration dissolved or carried in the blood) values at 12 woa in birds after a R_{low} *Mycoplasma gallisepticum* (R_{low}MG) challenge at 8 and 10 woa and that belonged to basal (control) and XPC-supplemented (XPC) dietary treatment groups.

Variable	pCO_2 (mm Hg)	pO_2 (mm Hg)	SaO_2 (%)	sO_2 (%)
Diet[1]				
Control	49.6	73.0	85.3	53.6
XPC	48.8	72.4	85.3	53.6
Pooled SEM	0.6	0.8	0.3	0.8
Main effect *P*-value	0.2771	0.6043	0.8904	0.9759
Challenge[2]				
Unchallenged	47.8[b]	75.6[a]	86.0[a]	56.4[a]
R_{low}MG challenged	50.6[a]	69.9[b]	84.6[b]	50.8[b]
Pooled SEM	0.6	0.8	0.3	0.8
Main effect *P*-value	0.0006	0.0001	0.0010	0.0001
Diet-Challenge[3]				
Control and unchallenged	47.6	76.3	86.2	57.6[a]
Control and R_{low}MG challenged	51.7	69.8	84.3	49.6[b]
XPC and unchallenged	48.0	75.0	85.7	55.2[a]
XPC and R_{low}MG challenged	49.5	69.9	84.9	52.0[b]
Pooled SEM	0.8	1.1	0.4	1.1
Interaction *P*-value	0.1145	0.5489	0.1113	0.0356

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

Abbreviation: woa, wk of age.

[1]Main effect means based on 4 birds in each of 16 units in each dietary treatment group across challenge treatment (64 total birds).

[2]Main effect means based on 4 birds in each of 16 units in each challenge treatment group across dietary treatment (64 total birds).

[3]Interaction effect means based on 4 birds in each of 8 replicate units in each diet-challenge treatment combination group (32 total birds).

10-wk-old sham inoculation or a 22-wk-old S6MG inoculation, a 10-wk-old S6MG inoculation increased serum Ca^{2+} concentrations across 47 and 58 woa. Peebles et al. (2006) likewise reported that in comparison with sham-inoculated controls, that a 10-wk-old prelay inoculation of S6MG elevated the blood Hct of the birds at 20 woa.

Similar to the results reported by Peebles et al. (2006) in which an S6MG challenge was imposed, the R_{low}MG challenge in the present study increased blood Ca^{2+}

Table 8. Mean blood calcium (Ca^{2+}), potassium, (K^+), sodium (Na^+), and chloride (Cl^-) values at 12 woa in birds after a R_{low} *Mycoplasma gallisepticum* (R_{low}MG) challenge at 8 and 10 woa and that belonged to basal (control) and XPC-supplemented (XPC) dietary treatment groups.

Variable	Ca^{2+} (mEq/L)	K^+ (mEq/L)	Na^+ (mEq/L)	Cl^- (mEq/L)
Diet[1]				
Control	2.97	4.48	149	112
XPC	2.97	4.42	149	112
Pooled SEM	0.04	0.11	0.9	0.3
Main effect *P*-value	0.6615	0.2970	0.2521	0.7768
Challenge[2]				
Unchallenged	2.94[b]	4.46	148[b]	113[a]
R_{low}MG challenged	3.00[a]	4.44	150[a]	111[b]
Pooled SEM	0.04	0.11	0.9	0.3
Main effect *P*-value	0.0011	0.7023	0.0020	0.0001
Diet-Challenge[3]				
Control and unchallenged	2.93	4.45	148	113
Control and R_{low}MG challenged	3.02	4.51	150	111
XPC and unchallenged	2.95	4.47	148	112
XPC and R_{low}MG challenged	2.98	4.37	149	111
Pooled SEM	0.05	0.12	0.9	0.4
Interaction *P*-value	0.1371	0.1951	0.3276	0.3549

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

Abbreviation: woa, wk of age.

[1]Main effect means based on 4 birds in each of 16 units in each dietary treatment group across challenge treatment (64 total birds).

[2]Main effect means based on 4 birds in each of 16 units in each challenge treatment group across dietary treatment (64 total birds).

[3]Interaction effect means based on 4 birds in each of 8 replicate units in each diet-challenge treatment combination group (32 total birds).

Table 9. Mean blood pH, anion gap, bicarbonate (HCO_3^-), and osmolality values at 12 woa in birds after a R_{low} *Mycoplasma gallisepticum* (R_{low}MG) challenge at 8 and 10 woa and that belonged to basal (control) and XPC-supplemented (XPC) dietary treatment groups.

Variable	pH	Anion gap (mEq/L)	HCO_3^-(mm Hg)	Osmolality (mmol/kg)
Diet[1]				
Control	7.31	18.8	23.0	312
XPC	7.32	18.5	23.0	311
Pooled SEM	0.01	1.5	0.5	2.0
Main effect *P*-value	0.4445	0.5826	0.9793	0.2231
Challenge[2]				
Unchallenged	7.33[a]	17.0[b]	23.1	310[b]
R_{low}MG challenged	7.30[b]	20.3[a]	22.9	314[a]
Pooled SEM	0.01	1.5	0.5	2.0
Main effect *P*-value	0.0035	0.0001	0.3666	0.0001
Diet-Challenge[3]				
Control and unchallenged	7.33	16.6	23.3	310
Control and R_{low}MG challenged	7.30	21.0	22.8	314
XPC and unchallenged	7.33	17.3	23.0	310
XPC and R_{low}MG challenged	7.31	19.6	23.0	313
Pooled SEM	0.01	1.6	0.6	2.0
Interaction *P*-value	0.1102	0.1454	0.5175	0.4052

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

Abbreviation: woa, wk of age.

[1]Main effect means based on 4 birds in each of 16 units in each dietary treatment group across challenge treatment (64 total birds).

[2]Main effect means based on 4 birds in each of 16 units in each challenge treatment group across dietary treatment (64 total birds).

[3]Interaction effect means based on 4 birds in each of 8 replicate units in each diet-challenge treatment combination group (32 total birds).

levels at 12 woa. A physiological mechanism by which a virulent MG (S6MG or R_{low}MG) infection could increase blood Ca^{2+} concentrations has not been proposed. However, the general cellular invasiveness of the organism may play a role in this effect. Furthermore, Branton et al. (1997) have shown that an acute FMG infection leads to a high degree of lymphoid loci within the bone marrow of layer pullets between 6 and 10 woa. Changes in the cellular constitution of the bone marrow of these birds would likely have a subsequent effect on their serum Ca^{2+} levels. It would be expected that a similar or even more extensive change in the cellular profile of

Table 10. Mean blood glucose and corticosterone concentration values at 12 woa in birds after a R_{low} *Mycoplasma gallisepticum* (R_{low}MG) challenge at 8 and 10 woa and that belonged to basal (control) and XPC-supplemented (XPC) dietary treatment groups.

Variable	Glucose (mg/dL)	Corticosterone (pg/mL)
Diet[1]		
Control	250	9,255
XPC	248	10,819
Pooled SEM	3.4	2,472
Main effect *P*-value	0.4217	0.6553
Challenge[2]		
Unchallenged	237[b]	5,354[b]
R_{low}MG challenged	261[a]	14,720[a]
Pooled SEM	3.3	2,472
Main effect *P*-value	0.0001	0.0084
Diet-Challenge[3]		
Control and unchallenged	239	7,220
Control and R_{low}MG challenged	262	11,290
XPC and unchallenged	235	3,488
XPC and R_{low}MG challenged	260	18,150
Pooled SEM	4.1	3,496
Interaction *P*-value	0.7853	0.1323

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

Abbreviation: woa, wk of age.

[1]Main effect means based on 4 birds in each of 16 units in each dietary treatment group across challenge treatment (64 total birds).

[2]Main effect means based on 4 birds in each of 16 units in each challenge treatment group across dietary treatment (64 total birds).

[3]Interaction effect means based on 4 birds in each of 8 replicate units in each diet-challenge treatment combination group (32 total birds).

the bone marrow of the R_{low}MG-challenged birds would occur. In contrast to the results reported by Peebles et al. (2006), the R_{low}MG challenge in the present study did not affect the Hct of the pullets. The adhesin, pMGA, on the surfaces of MG cells, allows for their attachment to chicken erythrocytes (Markham et al., 1992). Markham et al. (1992) have reported differences in the molecular weight of pMGA structures in the S6 and R strains of MG. These differences may play a role in the abilities of the S6 and R strains to cause hemagglutination in chickens, leading to their differential effects on Hct.

The effects of vaccine strains of MG on the blood characteristics of layers have also been explored in various studies (Burnham et al., 2003; Peebles et al., 2008, 2009; Olanrewaju et al., 2009, 2011). These effects have been determined using either low virulence (FMG) (Burnham et al., 2003; Olanrewaju et al., 2009) or avirulent (TS-11MG and 6/85 strain [**6/85MG**]) (Peebles et al., 2008, 2009; Olanrewaju et al., 2011) vaccine strains of MG. In 2 trials conducted by Burnham et al. (2003), it was shown that although serum Ca^{2+} concentrations were not affected, Hct was increased at 20 woa in layers that had been eye drop inoculated with FMG at 12 woa. It was suggested that the increases in Hct 8 wk after challenge may have been a compensatory physiological response to the FMG challenge. Peebles et al. (2009) observed that serum Ca^{2+} concentrations in layers at 22 woa were significantly increased in response to a TS-11MG vaccination at 10 woa. Further significant elevations in Ca^{2+} levels at 22 woa were observed when the 10 woa TS-11MG vaccination was overlaid by an FMG inoculation at 22 woa. However, this response was not accompanied by any changes in Hct during lay. Conversely, using a live 6/85MG vaccine at 10 woa, Peebles et al. (2008) observed no effects on serum Ca^{2+} or Hct during lay. However, in comparison with sham-inoculated controls and those inoculated with 6/85MG at 10 woa and FMG at 22 woa, serum Ca^{2+} concentrations at 47 woa were greater in birds that were administered 6/85MG at 10 woa in conjunction with an FMG overlay at 45 woa.

When administered alone in the pullet period, S6MG (Peebles et al., 2006), R_{low}MG (present study), and TS-11MG (Peebles et al., 2009) increased serum Ca^{2+} concentration, but neither FMG (Burnham et al., 2003) nor 6/85MG (Peebles et al., 2008) alone had any subsequent effect on serum Ca^{2+} levels. Yet, the administration of 6/85MG in the pullet period in combination with an FMG inoculation overlay at 45 woa resulted in an increase in serum Ca^{2+} concentration (Peebles et al., 2008). In addition, when inoculated in the pullet period, S6MG (Peebles et al., 2006) and FMG (Burnham et al., 2003) afterward increased Hct, whereas R_{low}MG (present study), TS-11MG (Peebles et al., 2009), and 6/85MG (Peebles et al., 2008) had no effect. Possible differences in the physiological conditions of the birds at the time of inoculation in these separate studies may have led to the divergent effects of these individual strains of MG on circulating Ca^{2+} concentrations and Hct. Nevertheless, the duration of the postinoculation period and the virulence level of the MG do not appear to be factors controlling the results for these 2 blood variables.

Elevations in the pCO_2, anion gap, osmolality, and Na^+, glucose, and corticosterone concentrations of the blood were also currently observed in response to the R_{low}MG challenge. These elevations were likewise associated with decreases in blood pO_2; Cl^-, SaO_2, and sO_2 concentrations; and pH. The layer pullets challenged with R_{low}MG experienced a stress response, as indicated by the increase in their plasma corticosterone concentrations. A decrease in pH and increase in pCO_2, in association with a lack of change in HCO_3^- in those birds, is also indicative of respiratory acidosis. Olanrewaju et al. (2009) reported that the eye drop inoculation of layer pullets with FMG caused the blood partial pressures of O_2 and CO_2 to be increased and decreased, respectively, at 9 woa. In a subsequent study by Olanrewaju et al. (2011), it was also noted that when compared with MG-clean controls and those inoculated at 10 woa with a killed vaccine (bacterin) followed at 18 woa by live TS-11MG, the inoculation of layers with a live TS-11MG vaccine alone at 10 woa caused the partial pressures of O_2 to be increased and of CO_2 to be decreased in their blood at 56 woa. It was further noted that all but the control birds received a subsequent eye drop inoculation of R_{low}MG at 30 woa. The changes in pO_2 and pCO_2, subsequent to the MG challenges imposed by Olanrewaju et al. (2009, 2011), were opposite to those noted in the present study. However, the responses reported in the previous and present studies are indicative of the ability of these various strains of MG to cause alterations in the blood gases of layers. Contrasts in the changes of these blood gases may be due to differences in the MG strains tested and the timing of their inoculation.

Because blood lipid profiles were not examined in this study, they could not be compared with those of Peebles et al. (2006). Moreover, other than Hct and Ca^{2+}, the other blood variables currently examined were not observed by Peebles et al. (2006). Nevertheless, the significant effects of R_{low}MG on 12 of the 17 blood variables in this study attest to its high level of virulence, its systemic colonization, and its considerable influence on the blood profile and subsequent physiological condition of the birds. Elliott et al. (2020) showed that these same birds experienced significantly increased IgG titers, incidences of airsacculitis and lung conjunctivitis, and percentage ovary and cecal weights in association with decreased percentage bursal weights.

Suarez Martinez et al. (2018) have shown that although supplementing the standard layer rations of Hy-Line W-36 laying hens with 1.25 kg/metric ton of XPC increased their feed conversion ratio, yolk weight, percentage of yolk yield, percentage of yolk solids, and percentage of albumen nitrogen, it decreased their yolk nitrogen percentages and did not affect their hen-day egg production or egg mass between 19 and 53 woa. Elliott et al. (2020) showed that although the inclusion

of XPC in the diets of the layer pullets led to an increase in their rate of growth through 12 woa, it had no significant influence on their humoral response or antibody titer levels whether or not they were challenged with R_{low}MG. Similar to the results reported by Lensing et al. (2012) in which XPC_{LS} reduced the intestinal damage caused by an *Eimeria maxima* infection, Elliott et al. (2020) observed 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 that study were not evaluated. However, the combined results from those of Elliott et al. (2020) and of Lensing et al. (2012) suggest that XPC may promote tissue integrity and provide associated protection against tissue invasion by pathogens. Moreover, this tissue protection may not be limited to enteric pathogens but may also include pathogens, such as MG, that can invade the respiratory system.

In conclusion, these results suggest that layer pullets challenged with R_{low}MG undergo a stress response associated with changes in various physiological blood variables. A decrease in pH and increase in pCO_2, in association with a lack of change in HCO_3^-, also indicates that the stress response caused by the R_{low}MG challenge was associated with respiratory acidosis. However, in this study, feeding XPC did not influence the effects of challenge treatment on these postchallenge physiological blood values. Nevertheless, because field-strain MG infections have engendered losses in commercial table egg production, future research should focus on the potential benefits of long-term supplemental dietary XPC usage on the production and quality of eggs laid by commercial laying hens throughout a complete lay cycle subsequent to an R_{low}MG challenge during the pullet period.

ACKNOWLEDGMENTS

This work was funded by grant number DVRP147526 from Diamond V.

Conflict of Interest Statement: There are no conflicts of interest for this work.

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