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 (RlowMG) 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 RlowMG. Blood samples were taken immediately before the initial RlowMG 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 RlowMG-challenged group than the unchallenged group of birds regardless of dietary treatment. The RlowMG 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 RlowMG 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 RlowMG 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

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 airsacculitis and the incidence of airsac 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).
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 (pCO₂), corticosterone, osmolality, and bicarbonate (HCO₃⁻) levels, while reducing their BW as well as their blood pH, oxygen partial pressure (pO₂), and sodium (Na⁺) and chloride (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 (R₀MG) 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 Simmonds, 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 R₀MG 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 R₀MG 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 R₀MG inoculum was conducted at 8 woa (eye drop) and at 10 woa (tracheal gavage). For the 8 woa challenge, the R₀MG inoculum was plated immediately before challenge and observed to contain 1.5 × 10⁸ CFU/mL. A 20 µL volume of inoculum culture containing 3.0 × 10⁶ CFU of R₀MG 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 × 10⁷ CFU of R₀MG, 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, R₀MG colony counts were performed on Frey’s plate medium (Frey et al., 1968) supplemented with 35 mL of yeast extract solution per liter (#18150-059; Invitrogen/Gibco, Carlsbad, CA) after incubation at 37°C. The presence of viable bacteria in the inoculum
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, pCO₂, pO₂, arterial oxygen saturation or oxyhemoglobin concentration (SaO₂), oxygen concentration dissolved or carried in the blood (sO₂), calcium (Ca²⁺), potassium (K⁺), Na⁺, Cl⁻, pH, anion gap, HCO₃⁻, 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 SaO₂ 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 ≤ 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_gowMG 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_gowMG-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 sO₂ concentration (concentration of oxygen dissolved or carried in the blood) at 12 woa (Table 7). Blood sO₂ levels were significantly lower in the R_gowMG-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 sO₂ levels. There was a significant main effect due to R_gowMG challenge treatment on 12 of the 17 blood variables. The R_gowMG challenge significantly affected blood pCO₂, pO₂, SaO₂ (arterial oxygen saturation, or oxyhemoglobin concentration), and sO₂ (Table 7); Ca²⁺, Na⁺, and Cl⁻ (Table 8); pH, anion gap, and osmolality (Table 9); and glucose and corticosterone levels (Table 10). More specifically, the R_gowMG challenge increased blood pCO₂, Ca²⁺, Na⁺, anion gap, osmolality, glucose, and corticosterone levels but decreased blood pO₂, SaO₂, sO₂, Cl⁻, and pH levels.
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 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) |
|----------|------------|----------|-------------|
| Diet2    |            |          |             |
| 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 |
| Challenge3 |          |          |             |
| 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-Challenge4 |          |          |             |
| 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.
1Challenge treatment designations were preassigned, as main and interaction effect means involving challenge were before challenge.
2Main effect means based on 4 birds in each of 16 units in each dietary treatment group across challenge treatment (64 total birds).
3Main effect means based on 4 birds in each of 16 units in each challenge treatment group across dietary treatment (64 total birds).
4Interaction effect means based on 4 birds in each of 8 replicate units in each diet-challenge treatment combination group (32 total birds).

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) | $SaO_2$ (%) | $sO_2$ (%) |
|----------|----------------|---------------|-------------|------------|
| Diet2    |                |               |             |            |
| 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 |
| Challenge3 |            |               |             |            |
| 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 | 0.0067 | 0.9186 | 0.3305 | 0.3632 |
| Diet-Challenge4 |            |               |             |            |
| 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.
1Challenge treatment designations were preassigned, as main and interaction effect means involving challenge were before challenge.
2Main effect means based on 4 birds in each of 16 units in each dietary treatment group across challenge treatment (64 total birds).
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4Interaction 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 RlowMG infection have not been previously reported. However, on investigating the pathomechanism of MG in chickens, Vogl et al. (2008) detected the presence of RlowMG on the inside and on the surface of their erythrocytes 8 h after a RlowMG infection was initiated. The ability of RlowMG 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

| Variable                      | Ca²⁺ (mEq/L) | K⁺ (mEq/L) | Na⁺ (mEq/L) | Cl⁻ (mEq/L) |
|-------------------------------|--------------|------------|-------------|-------------|
| Diet                          |              |            |             |             |
| 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      |
| Challenge¹                    |              |            |             |             |
| Unchallenged                  | 3.01         | 4.58       | 148         | 109         |
| RlowMG 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      |

Abbreviation: woa, wk of age.

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

The blood profiles of commercial egg-laying hens in response to a RlowMG infection have not been previously reported. However, on investigating the pathomechanism of MG in chickens, Vogl et al. (2008) detected the presence of RlowMG on the inside and on the surface of their erythrocytes 8 h after a RlowMG infection was initiated. The ability of RlowMG 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

| Variable                      | pH | Anion gap (mEq/L) | HCO₃⁻ (mm Hg) | Osmolality (mmol/kg) |
|-------------------------------|----|------------------|--------------|---------------------|
| Diet                          |    |                  |              |                     |
| 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              |
| Challenge³                    |    |                  |              |                     |
| Unchallenged                  | 7.34a | 19.8             | 23.9         | 309                 |
| RlowMG challenged             | 7.32b | 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              |

Abbreviation: woa, wk of age.

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

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

³Main effect means based on 4 birds in each of 16 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

| Variable                                      | Glucose (mg/dL) | Corticosterone (pg/mL) |
|-----------------------------------------------|-----------------|------------------------|
| Diet                                          |                 |                        |
| Control                                       | 242             | 4,799                  |
| XPC                                           | 244             | 5,192                  |
| Pooled SEM                                    | 1.9             | 710                    |
| Main effect P-value                           | 0.4813          | 0.6984                 |
| Challenge                                     |                 |                        |
| 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                                |                 |                        |
| Control and unchallenged                      | 239             | 4,308                  |
| Control and R_{low}MG challenged              | 244             | 5,289                  |
| XPC                                           | 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                 |

Abbreviation: woa, wk of age.

| Variable                                      | Hct (% PCV) | Hb (g/dL) | McHc (g/dL) |
|-----------------------------------------------|-------------|-----------|-------------|
| Diet                                          |             |           |             |
| 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                                     |             |           |             |
| 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                                |             |           |             |
| 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.

| Variable                                      |                  |           |             |
|-----------------------------------------------|------------------|-----------|-------------|
| Diet                                          |                  |           |             |
| Control                                       |                  |           |             |
| XPC                                           |                  |           |             |
| Pooled SEM                                    |                  |           |             |
| Main effect P-value                           |                  |           |             |

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.

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.
10-wk-old sham inoculation or a 22-wk-old S6MG inoculation, a 10-wk-old S6MG inoculation increased serum Ca\textsuperscript{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\textsubscript{low}MG challenge in the present study increased blood Ca\textsuperscript{2+} concentrations of S6MG elevated the blood Hct of the birds at 20 woa.

### Table 7. Mean blood pCO\textsubscript{2}, pO\textsubscript{2}, SaO\textsubscript{2} (arterial oxygen saturation, or oxyhemoglobin concentration), and sO\textsubscript{2} (oxygen concentration dissolved or carried in the blood) values at 12 woa in birds after a R\textsubscript{low} Mycoplasma gallisepticum (R\textsubscript{low}MG) challenge at 8 and 10 woa and that belonged to basal (control) and XPC-supplemented (XPC) dietary treatment groups.

| Variable                  | pCO\textsubscript{2} (mm Hg) | pO\textsubscript{2} (mm Hg) | SaO\textsubscript{2} (%) | sO\textsubscript{2} (%) |
|---------------------------|-------------------------------|----------------------------|--------------------------|-------------------------|
| Diet\textsuperscript{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\textsuperscript{2} |                               |                            |                          |                         |
| Unchallenged              | 47.8\textsuperscript{a}      | 75.6\textsuperscript{a}    | 86.0\textsuperscript{a}  | 56.4\textsuperscript{a} |
| R\textsubscript{low}MG challenged | 50.6\textsuperscript{a}    | 69.9\textsuperscript{b}    | 84.6\textsuperscript{b}  | 50.8\textsuperscript{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\textsuperscript{3} |                               |                            |                          |                         |
| Control and unchallenged  | 47.6                          | 76.3                       | 86.2                     | 57.6\textsuperscript{a} |
| Control and R\textsubscript{low}MG challenged | 51.7                          | 69.8                       | 84.3                     | 49.0\textsuperscript{b} |
| XPC and unchallenged      | 48.0                          | 75.0                       | 85.7                     | 55.2\textsuperscript{a} |
| XPC and R\textsubscript{low}MG challenged | 49.5                          | 69.9                       | 84.9                     | 52.0\textsuperscript{b} |
| Pooled SEM                | 0.8                           | 1.1                        | 0.4                      | 1.1                     |
| Interaction P-value       | 0.1145                        | 0.5489                     | 0.1113                   | 0.0356                  |

\textsuperscript{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.

\textsuperscript{1}Main effect means based on 4 birds in each of 16 units in each dietary treatment group across challenge treatment (64 total birds).

\textsuperscript{2}Main effect means based on 4 birds in each of 16 units in each challenge treatment group across dietary treatment (64 total birds).

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

| Variable                  | Ca\textsuperscript{2+} (mEq/L) | K\textsuperscript{+} (mEq/L) | Na\textsuperscript{+} (mEq/L) | Cl\textsuperscript{-} (mEq/L) |
|---------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| Diet\textsuperscript{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\textsuperscript{2} |                               |                               |                               |                               |
| Unchallenged              | 2.94\textsuperscript{a}      | 4.46                          | 148\textsuperscript{b}        | 113\textsuperscript{a}       |
| R\textsubscript{low}MG challenged | 3.00\textsuperscript{a}    | 4.44                          | 150\textsuperscript{a}        | 111\textsuperscript{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\textsuperscript{3} |                               |                               |                               |                               |
| Control and unchallenged  | 2.93                          | 4.45                          | 148                           | 113                           |
| Control and R\textsubscript{low}MG challenged | 3.02                          | 4.51                          | 150                           | 111                           |
| XPC and unchallenged      | 2.95                          | 4.47                          | 148                           | 112                           |
| XPC and R\textsubscript{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                        |

\textsuperscript{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.

\textsuperscript{1}Main effect means based on 4 birds in each of 16 units in each dietary treatment group across challenge treatment (64 total birds).

\textsuperscript{2}Main effect means based on 4 birds in each of 16 units in each challenge treatment group across dietary treatment (64 total birds).

\textsuperscript{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 RlowMG) 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

| 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).

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**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.

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**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 RlowMG-challenged birds would occur. In contrast to the results reported by Peebles et al. (2006), the RlowMG 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\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{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), RlowMG (present study), and TS-11MG (Peebles et al., 2009) increased serum Ca\textsuperscript{2+} concentration, but neither FMG (Burnham et al., 2003) nor 6/85MG (Peebles et al., 2008) alone had any subsequent effect on serum Ca\textsuperscript{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\textsuperscript{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 RlowMG (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\textsuperscript{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\textsubscript{2}, anion gap, osmolality, and Na\textsuperscript{+}, glucose, and corticosterone concentrations of the blood were also currently observed in response to the RlowMG challenge. These elevations were likewise associated with decreases in blood pO\textsubscript{2}, Cl\textsuperscript{-}, SaO\textsubscript{2}, and sO\textsubscript{2} concentrations; and pH. The layer pullets challenged with RlowMG experienced a stress response, as indicated by the increase in their plasma corticosterone concentrations. A decrease in pH and increase in pCO\textsubscript{2}, in association with a lack of change in HCO\textsubscript{3}\textsuperscript{-} 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\textsubscript{2} and CO\textsubscript{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\textsubscript{2} to be increased and of CO\textsubscript{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 RlowMG at 30 woa. The changes in pO\textsubscript{2} and pCO\textsubscript{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\textsuperscript{2+}, the other blood variables currently examined were not observed by Peebles et al. (2006). Nevertheless, the significant effects of RlowMG 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 lowMG. Similar to the results reported by Lensing et al. (2012) in which XPC in the diets of the layer pullets led to an increase in various physiological blood values in commercial laying hens throughout a complete lay cycle subsequent to an R lowMG challenge during the pullet period, 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 lowMG undergo a stress response associated with changes in various physiological blood variables. A decrease in pH and increase in pCO2, in association with a lack of change in HCO3−, also indicates that the stress response caused by the R lowMG 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 lowMG challenge during the pullet period.

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