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Comparison of Chick Quality, Health, and Inflammation from Two Hatchery Environments

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Abstract

Hatchery contamination can result in ingested or inhaled microbes that may modify colonization of the intestinal and respiratory tract, with potential to influence early growth, inflammation, and overall health. Six experiments were completed to compare chick quality, inflammation, and health between two hatcheries (H1 and H2). On embryonic d0, 45 eggs from the same breeder flock were set at each hatchery. On d0, length, abdominal height, navel and leg abnormalities, and self-righting were measured for 36 chicks/hatchery, yolk sacs were weighed, and crop/cloaca swabs were cultured from 12 chicks/hatchery. On d7, mid-ileum and ceca were cultured from 12 chicks/hatchery. On d0 and d7, body weight (BW) and intestinal weight were measured, lung/air sac swabs and liver were cultured, and liver and air sacs were scored for health. Blood was collected on d0 and d7 for serum alpha-1-acid glycoprotein concentrations in Exp 1 and 2. Data was analyzed using Student's *t*-test or χ^2, significance $p < 0.05$. On d0, BW, length, yolk free BW, and intestinal weight were greater for H2 chicks ($p < 0.05$). Liver bacterial recovery was decreased in H2 on d0 ($p < 0.05$) and there were fewer average leg and righting abnormalities in H2 ($p < 0.05$). Decreased lactase positive Enterobacteriaceae were noted in H2 in crop/cloaca and lung/air sac swabs ($p < 0.05$), and of alpha and beta hemolysis in crop/cloaca swabs, and alpha and gamma hemolysis in lung/air sac swabs ($p < 0.05$) on d0. By d7, only alpha hemolytic bacteria were increased in lung/air sac swabs of H2. Based on factors measured, chicks from H2 showed favorable microbial colonization, starting quality, and improved health on d0. While not sustained through d7, differences in d0 microbial recovery may have shifted microbial development and potentially influenced immune response development. These experiments elucidated the importance of hatchery environment on early chick quality, microbial colonization, overall inflammation, and chick health.

Keywords

Chick Quality, Hatchery Environment, Enterobacteriaceae, Hemolysis

1. Introduction

Hatchery environment and contamination have the potential to expose chicks to pathobionts which may result in altered microbial environments in day of hatch chicks with capacity to influence early growth, microbial development, inflammation, and overall health [1] [2] [3]. Removal of eggs from hens in commercial production schemes has resulted in eggs being exposed to opportunistic pathogens such as *E. coli*, *Salmonella spp.*, and *Campylobacter spp.*, as well as other environmental microbes [4] [5] [6], despite cleaning and disinfecting between hatches, and are therefore likely to be pioneer colonizing bacteria within the gastrointestinal tract at a time when they are critically susceptible to infection. Since the hatchery environment would present the greatest opportunity for early influence on chick quality, understanding the role of hatcheries on chick health and overall quality may improve poultry production.

Hatcheries play a vital role in the broiler production scheme, as they have continued to provide chicks to producers, who expect a quality product. Poultry producers rely on high chick quality, with expectations of exceptional growth rate, high breast meat yield, low feed conversion, and disease resistance for continued production of a profitable, high quality, low cost meat source [7] [8]. While hatcheries have continued to target high quality chick production, parameters with which to measure chick quality are not well defined or are subjective [9] [10]. Physiological metrics studied as measures of chick quality include d0 or d1 body weight (**BW**), d7 BW, d0 chick length, chick length to BW ratio, abdominal height, navel abnormalities, leg abnormalities, yolk free BW (**YFBW**, body weight without the residual yolk), and intestinal weight [10]-[15], while health metrics such as spleen weight and liver bacterial translocation have also been used [16] [17]. Combined metrics for measuring chick quality can be used to evaluate role of hatchery environment on chick quality and health through the first week of life.

Measures of microbial infection status include spleen weight and bacterial translocation to the liver, which may hint toward lymphocyte activation and leukocyte migration to the spleen, and mark increased intestinal permeability, respectively [18] [19]. Another marker indicative of inflammation and potential microbial infection would include alpha-1-acid glycoprotein (**A1GP**), a major acute phase protein produced mainly by the liver in chickens [20]. Some of the bacteria responsible for these changes can include Enterobacteriaceae and hemolytic bacteria [21]. Enterobacteriaceae are generally recognized as opportunistic pathogens that include lactose fermenting coliforms, and may normally inhabit the intestine of chickens [21] [22]. On the other hand, hemolytic microbes are considered more detrimental due to their ability to utilize red blood cells

as a nutrient source [23]. Therefore, monitoring both Enterobacteriaceae status and hemolytic activity may be an important consideration when assessing chick health and early chick quality.

The objective of these experiments was to determine whether hatchery environment influenced early microbial colonization, generalized inflammation, and overall chick health and quality. When compared to a local commercial hatchery, a research facility hatchery was hypothesized to provide a cleaner hatchery environment, expected to result in improved BW, growth, decreased bacterial recovery of the liver and lower incidence of hemolytic activity of intestinal and respiratory tissues, decreased serum A1GP concentrations, and improved scores related to chick health.

2. Materials and Methods

2.1. Animals and Housing

A total of six experiments were completed at the Poultry Center of the Ohio Agricultural Research and Development Center, Wooster, Ohio under approved animal care protocols (2016A00000038 and 2021A00000010) from The Ohio State University Institutional Animal Care and Use Committee. In all experiments, Ross 708 broiler eggs were obtained from a local commercial hatchery (**H1**), and half were set at H1 while the other half were set in a hatcher cabinet at the Turkey Center of the Ohio Agricultural Research and Development Center, Wooster, Ohio (**H2**). On d0, chicks were obtained from the local commercial hatchery, and all chicks were neck tagged, and randomly placed in floor pens with fresh pine shavings. Nutritionally complete feed and water were provided *ad libitum*, and ambient temperature and lighting were maintained at age-appropriate levels [24].

2.2. Experimental Design

For each of the six experiments, a total of 90 eggs were set on embryonic d0, with 45 eggs set at H1 and 45 eggs set at H2. Eggs at H2 were candled on embryonic d8 to determine fertility, with infertile eggs discarded, and were moved to a hatcher cabinet on embryonic d18. On d0, all hatched chicks were obtained from H1 and H2, and chicks from H2 were moved to carboard chick transport boxes, then driven for 75 minutes to mimic transportation time and stress that chicks in H1 experienced. A total of 36 chicks each were randomly selected from H1 and H2 and neck-tagged, with 12 birds per pen and three replicate pens per hatchery, while any remaining chicks were euthanized. On d0, all chicks were weighed and subjected to an observational health assessment that included chick length, abdominal height, navel abnormalities, leg abnormalities, and chick righting described in Table 1. In addition, four chicks per pen were killed and sampled which included assessment of air sac and liver, aseptic crop/cloaca swab and lung/air sac swab for presence of Enterobacteriaceae on MacConkey agar and hemolytic colonies on blood agar, aseptic collection of liver for aerobic bacterial recovery on tryptic soy agar (**TSA**), yolk sac weight to calculate YFBW, in-

testinal weight, and spleen weight, described in Table 1. On d7, feed was removed 6h prior to sampling to minimized contents within the intestinal tract. All chicks were weighed, then four chicks per pen were killed and sampled which included assessment of air sac and liver, aseptic lung/air sac swab for identification of Enterobacteriaceae on MacConkey agar and hemolytic colonies on blood agar, aseptic collection of liver for aerobic bacterial recovery on TSA, aseptic collection of mid-ileum and ceca for Enterobacteriaceae recovery on MacConkey agar and identification of hemolytic colonies on blood agar, intestinal weight, and spleen weight, described in Table 1. Blood was collected both on d0 and d7 for A1GP analysis only in experiments 1 and 2. All birds were euthanized via CO_2.

Table 1. Descriptions for observational health assessments and measurements. On d0, a total of 36 chicks from each hatchery, the commercial hatchery or research hatchery, were subjected to an observational health assessment that included body weight, chick length, abdominal height, chick righting, navel abnormalities, and leg abnormalities. In addition, a total of 12 chicks from each hatchery were sampled for additional qualitative and quantitative measures, including liver score, air sac score, yolk sac weight, yolk free body weight, intestinal weight, and spleen weight.

Length (cm)	Lay chick on ventral side and measure chick from tip of beak to the tip of the nail on the third toe of the outstretched right leg.
Abdominal height (cm)	Lay chick on dorsal side and measure vertically to the highest point of the abdomen.
Righting (0/1)	Place chick on dorsal side and hold until the chick stops struggling, then time whether chick can right itself in less than 3 seconds. If the chick is successful, score as 0. If chick is unsuccessful in righting itself in less than 3 seconds, score as 1.
Navel abnormalities	
Black button/ bruised (0/1)	If navel does not appear to have a black button or to be bruised, score as 0. If navel appears to have a black button or to be bruised, score as 1.
"String" navel (0/1)	If navel does not appear to have a "string" navel, score as 0. If navel appears to have a "string" navel, score as 1.
Open and unhealed (0/1)	If navel appears closed and healed, score as 0. If navel appears open or unhealed, score as 1.
Infected (0/1)	If navel does not appear infected, score as 0. If navel appears infected, red, or inflamed, score as 1.
Leg abnormalities	
Red hocks (0/1)	If hocks appear yellow/yellow-orange, score as 0. If hocks appear red and/or inflamed, score as 1.
Dehydrated legs (0/1)	If legs appear smooth and feel soft, score as 0. If legs appear scaly and feel hard/rough, score as 1.
Spread legs (0/1)	If the chick is able to stand upright on a smooth surface (stainless steel surface of the scale), score as 0. If the chick's legs spread and chick is unable to stand upright on a smooth surface, score as 1.
Liver (0/1)	If liver is yellow to reddish brown in color, score as 0. If liver is abnormally colored, spotted, pale, or enlarged, score as 1.
Air sac (0/1)	If air sac is clear and transparent, score as 0. If air sac is cloudy or translucent, score as 1.
Yolk sac weight (g)	Remove the yolk sac and stalk, weigh, and record yolk sac weight in grams. Use yolk sac weight to calculate yolk free body weight.
Yolk free body weight (g)	To calculate yolk free body weight, subtract yolk sac weight from chick body weight on d0.
Intestinal weight (g)	Aseptically remove intestine from duodenum to cloaca, weigh, and record intestinal section weight in grams.
Spleen weight (g)	Remove the spleen, weigh, and record spleen weight in grams.

2.3. Bacterial Translocation and Recovery

To measure translocation of enteric bacteria to the liver, as well as Enterobacteriaceae recovery in the mid-ileum and ceca, livers, mid-ileum and ceca were collected aseptically into sterile bags, homogenized, and diluted 1:4 (w:v) with sterile 0.9% saline. Ten-fold serial dilutions were made in sterile 96-well plates. Livers were plated on TSA (Merck KGaA, EMD Millipore Cooperation, Billerica, MA, USA), and mid-ileum and ceca were plated on MacConkey agar (Becton, Dickinson and Co., Difco, Sparks, MD, USA), for total aerobic bacterial translocation to the liver or total Enterobacteriaceae recovery in the mid-ileum and ceca. All plates were incubated at 37˚C for 20 - 24 h to assess bacterial recovery reported as Log_{10} CFU/g of tissue.

2.4. Identification of Lactase Positive and Negative Enterobacteriaceae, and Hemolytic Bacteria

To measure differences in the presence of lactase positive and lactase negative Enterobacteriaceae in addition to the hemolytic activity of bacteria in the intestines, including alpha, beta, and gamma hemolysis, aseptic swabs of the crop/cloaca, and lung/air sac were placed in 15 mL tubes containing 1mL of sterile 0.9% saline, and mid-ileum, and ceca were aseptically collected into sterile bags, homogenized, and diluted 1:4 (w:v) with sterile 0.9% saline. Both the lung/air sac and crop/cloaca swabs were each streaked for isolation onto MacConkey agar and blood agar (Becton, Dickinson and Co., Franklin Lakes, NJ, USA), while mid-ileum and ceca were only streaked onto blood agar. All plates were incubated at 37˚C for 18 - 24 h, and colonies were identified as either lactase positive (pink colonies), or lactase negative (colorless colonies) Enterobacteriaceae on MacConkey agar, or as having alpha hemolysis (partial zone of hemolysis with a green-ish hue), beta hemolysis (complete zone of hemolysis), or gamma hemolysis (no hemolysis) on blood agar.

2.5. Serum A1GP Analysis

Blood was collected from the jugular vein (d0) or femoral vein (d7) after euthanasia, allowed to clot at room temperature for approximately 3 h, centrifuged at 2000× g for 15 min for serum separation and collection, then stored at −20˚C. Serum was diluted and A1GP serum concentrations were evaluated according to manufacturer instructions of the A1GP ELISA Kit (AGP-5, Life Diagnostics, Inc., West Chester, PA, USA).

2.6. Statistical Analysis

Hatcheries were considered the experimental unit, experiment number was considered the block, and BW, BWG, chick length, abdominal height, yolk sac weight, YFBW, intestinal weight, spleen weight, and bacterial recovery were subject to Analysis of Variance [25], with data expressed as mean ± standard error. Significant differences among means were determined using Student's *t* test

at $p < 0.05$. Air sac and liver assessment, navel and leg abnormalities, chick righting, presence of lactase positive and lactase negative Enterobacteriaceae, and presence of alpha, beta, and gamma hemolytic colonies were analyzed using Chi-Squared Analysis (SAS 9.4, SAS Inc., 2016) at $p < 0.05$.

3. Results

With respect to d0 quantitative health assessment metrics, differences were noted in BW, chick length, yolk sac weight, yolk free body weight, intestinal weight, and d0 liver bacterial recovery ($p < 0.05$, Table 2), while there were no differences in BWG, abdominal height, spleen weight, and A1GP ($p > 0.05$, Table 2). On d7, no differences were measured for BW, intestinal weight, spleen weight, A1GP, or bacterial recovery of the liver, mid-ileum, or ceca ($p > 0.05$, Table 2). On d0, BW was found to be greater in H2 chicks, at 37.53 ± 0.28 g, compared to 36.61 ± 0.29 g for H1 chicks ($p = 0.018$, Table 2). There was no difference in BW on d7, or BWG from d0-7 ($p > 0.05$, Table 2). Chicks from H2 not only tended to be heavier on d0, but also had increased length compared to H1, at 18.75 ± 0.05 cm versus 18.17 ± 0.07 cm, respectively ($p < 0.001$, Table 2). Yolk sac weight was reduced in H2, at 2.80 ± 0.12 g compared to 3.68 ± 0.15 g in H1, which aligned with increased yolk free body weight for H2, 35.08 ± 0.47 g versus 32.52 ± 0.45 g for H1 ($p = 0.007$ and $p = 0.011$, respectively, Table 2). Intestinal weight was also increased in H2, weighing 1.98 ± 0.03 g contrasted with 1.75 ± 0.04 g for H1 ($p = 0.011$, Table 2). Liver bacterial recovery was reduced by approximately 90% in H2, at 0.83 ± 0.13 Log_{10} CFU/g versus 1.85 ± 0.15 Log_{10} CFU/g in H1 ($p = 0.006$, Table 2).

Table 2. Body weight, body weight gain, yolk sac weight, yolk free body weight, chick length, abdominal height, intestinal weight, spleen weight, serum alpha-1-acid glycoprotein, Log_{10} CFU/g aerobic bacterial recovery of the liver on tryptic soy agar, and Log_{10} CFU/g Enterobacteriaceae recovery of mid-ileum and ceca. On d0, a total of 36 chicks from each hatchery, the commercial hatchery (H1) or research hatchery (H2), were subjected to an observational health assessment that included body weight, chick length, abdominal height, chick righting, navel abnormalities, and leg abnormalities. In addition, a total of 12 chicks from each hatchery were sampled on d0 for yolk sac weight and yolk free body weight, and on d0 and d7, intestinal weight and spleen weight were measured, and blood was collected for serum alpha-1-acid glycoprotein (experiments 1 and 2). Liver was aseptically collected on d0 and d7, diluted in 0.9% sterile saline, and plated on tryptic soy agar to quantify total aerobic bacterial translocation to the liver. On d7, mid-ileum and ceca were additionally collected aseptically, diluted in 0.9% sterile saline, and plated on MacConkey agar to quantify aerobic Enterobacteriaceae. All data presented as mean ± standard error.

	Body weight (g) and body weight gain (g)		
	Body weight (g)		Body weight gain (g)
	d0	d7	d0 - 7
H1[1]	36.61 ± 0.29[b]	126.26 ± 2.69	88.76 ± 2.57
H2	37.53 ± 0.28[a]	129.00 ± 2.86	91.65 ± 2.70
SEM	0.46	1.37	1.44
p-Value	0.015	0.514	0.486

Continued

D0 yolk sac weight (g) and yolk free body weight (g)				
	Yolk sac (g)	YFBW[2] (g)		
H1	3.68 ± 0.15[a]	32.52 ± 0.45[b]		
H2	2.80 ± 0.12[b]	35.08 ± 0.47[a]		
SEM	0.44	1.28		
p-Value	<0.001	<0.001		
D0 chick length and abdominal height (cm)				
	Length (cm)	Abdominal height (cm)		
H1	18.17 ± 0.07[b]	2.66 ± 0.01		
H2	18.75 ± 0.05[a]	2.66 ± 0.02		
SEM	0.29	0.00		
p-Value	<0.001	0.793		
Intestinal weight (g) and spleen weight (g)				
	Intestinal weight (g)		Spleen weight (g)	
	d0	d7	d0	d7
H1	1.75 ± 0.04[b]	16.06 ± 0.39	0.0709 ± 0.0275	0.1586 ± 0.0268
H2	1.98 ± 0.03[a]	15.63 ± 0.41	0.0417 ± 0.0164	0.1060 ± 0.0153
SEM	0.12	0.22	0.0146	0.0263
p-Value	<0.001	0.567	0.411	0.234
Alpha-1-acid glycoprotein (μg/mL)				
	d0	d7		
H1	159.85 ± 20.94	178.92 ± 64.13		
H2	158.6 ± 24.98	314.56 ± 43.33		
SEM	0.62	67.82		
p-Value	0.926	0.316		
Log_{10} CFU/g aerobic recovery of liver on tryptic soy agar and Log_{10} CFU/g Enterobacteriaceae recovery of mid-ileum and ceca on MacConkey agar				
	Liver (TSA)[3]		Mid-Ileum (MacConkey)	Ceca (MacConkey)
	d0	d7	d7	d7
H1	1.85 ± 0.15[a]	2.45 ± 0.12	4.28 ± 0.23	8.51 ± 0.08
H2	0.83 ± 0.13[b]	2.43 ± 0.14	4.11 ± 0.24	8.40 ± 0.06
SEM	0.51	0.01	0.09	0.06
p-Value	<0.001	0.890	0.624	0.264

[a,b]Mean values with different superscript letters within a column and within a block indicate a significant difference ($p < 0.05$). [1]Commercial hatchery = H1, Research hatchery = H2; [2]Yolk free body weight = yolk free body weight; [3]Tryptic soy agar = TSA.

With regard to d0 qualitative health assessment metrics, differences were noted in red hocks, average leg score, chick righting, presence of lactase positive Enterobacteriaceae in both crop/cloaca swabs, as well as lung/air sac swabs, al-

pha and beta hemolysis in crop/cloaca swabs, and increased alpha and gamma hemolysis in lung/air sac swabs ($p < 0.05$, Table 3 and Table 4). There were no differences in navel abnormalities, average navel score, dehydrated or spread legs, liver score, air sac score, presence of lactase negative Enterobacteriaceae in both crop/cloaca swabs and lung/air sac swabs, gamma hemolysis in crop/cloaca swabs, and beta hemolysis in lung/air sac swabs ($p > 0.05$, Table 3 and Table 4). On d0, H1 chicks were more likely to have red hocks, 10% compared to 3%, and had a greater average leg score at 0.10 ± 0.02 versus H2 at 0.03 ± 0.01 ($p = 0.002$ and $p = 0.007$, respectively, Table 3). In addition, H1 chicks had more difficulty righting themselves on d0, with a failure rate of 13% compared to 5% in H2 ($p = 0.005$, Table 3). Chicks from H1 had higher rates of lactase positive Enterobacteriaceae in crop/cloaca swabs, 83% versus 29%, and lung/air sac swabs, 74% versus 10% ($p < 0.001$, Table 4). With respect to hemolytic activity on d0, H1 had greater alpha hemolysis, 71% versus 32%, and beta hemolysis, 22% versus 7%, in crop/cloaca swabs ($p = 0.008$ and $p < 0.001$, respectively, Table 4). Further, lung/air sac swabs on d0 resulted in increased alpha hemolysis in H1, 26% versus 8%, as well as increased gamma hemolysis in H1, 82% versus 25% ($p = 0.003$ and $p < 0.001$, respectively, Table 4). For d7 qualitative metrics, no differences were found in lactase positive and lactase negative Enterobacteriaceae, or in hemolytic activity of lung/air sac swabs, mid-ileum, and ceca ($p > 0.05$, Table 4), except for alpha hemolysis of lung/air sac swabs ($p < 0.05$, Table 4). Specifically, H1 had decreased alpha hemolytic activity in lung/air sac swabs at 52% versus 66% ($p = 0.048$, Table 4).

4. Discussion

These experiments were conducted to reveal the importance of hatchery environment on measures of chick quality, inflammation, and early microbial colonization. Many of the measures used to assess early chick quality were not able to be interpreted independently, as a combination of various metrics was required to interpret results more confidently. For example, d0 or d1 chick weight has been cited as a poor predictor of chick quality, with greater correlation to egg weight as opposed to later growth performance noted across several studies [10] [13] [26] [27]. While egg weights were not measured during these experiments, eggs from the same flock collected at approximately the same time were used to minimize effects of flock, age, or storage time, and standard hatchery conditions were used. Therefore, greater d0 BW observed in H2 compared to H1 was suggestive of hatchery effect, and not necessarily differences in the eggs. As a measure of chick quality, d0 BW has been suggested as a predictor of d42 growth performance in Ross, though the correlation was significant, but low, at $r = 0.25$ and $r = 0.33$ for 53 week and 39 week-old flocks [13]. On the other hand, d7 BW has been noted as a better predictor of growth performance than hatch weight to minimize egg related differences, and can be used to better assess chick quality, though results have been mixed [10] [13] [26]. While there were no differences in d7 BW in these experiments, H2 remained slightly elevated in relation to H1

on both d0 and d7 (Table 2). It would be difficult to declare this trend in greater BW would have been maintained had the chicks been extended through a normal production timeline, however, previous studies would suggest that this slight advantage in growth rate may have resulted in improved BW through d40, though not always statistically greater [10] [13]. In addition to BW, chick length measurements have resulted in some low to moderate correlations with later growth performance [12] [13] [14]. These experiments indicated that chick length was greater in chicks from H2 (Table 2), with improved chick length aligning with greater d0 BW measured in H2, which suggested that these parameters aligned with a difference in hatchery environment and chick quality.

Table 3. Qualitative measurements of navel abnormalities, leg abnormalities, chick righting, liver scores, and air sac scores. On d0, a total of 36 chicks from each hatchery, the commercial hatchery (H1) or research hatchery (H2), were subjected to an observational health assessment that included body weight, chick length, abdominal height, chick righting, navel abnormalities, and leg abnormalities. In addition, a total of 12 chicks from each hatchery were sampled on d0 and d7 to score liver and air sac for abnormalities. All data presented as total positive/total sampled (percentage positive).

	Navel abnormalities				
	Black button/bruised	String navel	Open and unhealed	Infected	Average navel score
H1[1]	44/205 (21%)	29/205 (14%)	20/205 (10%)	6/205 (3%)	0.48 ± 0.04
H2	37/214 (17%)	28/214 (13%)	19/214 (9%)	1/214 (0%)	0.40 ± 0.03
SEM	2%	1%	0%	1%	0.04
p-Value	0.281	0.800	0.826	0.089	0.095
	Leg abnormalities				
	Red hocks	Dehydrated legs	Spread legs	Average leg score	
H1	21/205 (10%)[a]	1/205 (0%)	0/205 (0%)	0.10 ± 0.02[a]	
H2	6/214 (3%)[b]	1/214 (0%)	0/214 (0%)	0.03 ± 0.01[b]	
SEM	4%	0%	0%	4%	
p-Value	0.002	1.000	1.000	0.007	
	Righting				
H1	26/205 (13%)[a]				
H2	10/214 (5%)[b]				
SEM	4%				
p-Value	0.0053				
	Liver and air sac scores				
	Liver		Air sac		
	d0	d7	d0	d7	
H1	0/72 (0%)	8/71 (11%)	1/72 (1%)	0/71 (0%)	
H2	0/72 (0%)	7/71 (10%)	0/72 (0%)	5/71 (7%)	
SEM	0%	1%	1%	4%	
p-Value	1.000	0.782	0.979	0.976	

[a,b]Mean values with different superscript letters within a column and within a block indicate a significant difference ($p < 0.05$). [1]Commercial hatchery = H1, Research hatchery = H2.

Table 4. Qualitative measurements of Enterobacteriaceae lactose fermentation on MacConkey agar and blood hemolysis activity on blood agar. On d0, a total of 12 chicks from each hatchery, the commercial hatchery (H1) or research hatchery (H2), were sampled and swabs were taken of the crop/cloaca and lung/air sac. On d7, 12 chicks from each hatchery were sampled and swabs were taken of lung/air sac, mid-ileum, and ceca. All samples were streaked onto MacConkey agar, except mid-ileum and ceca, to determine differences in lactose fermenting activity (lactase positive or lactase negative) of Enterobacteriaceae. All samples were also streaked onto blood agar to observe beta hemolysis (complete zone of hemolysis), alpha hemolysis (partial zone of hemolysis with a green-ish hue), and/or gamma hemolysis (no hemolysis). All data presented as total positive/total sampled (percentage positive).

	MacConkey		Blood agar		
	Lactase +	Lactase −	Beta hemolysis	Alpha hemolysis	Gamma hemolysis
			d0 Crop/cloaca swab		
H1[1]	60/72 (83%)[a]	4/72 (6%)	16/72 (22%)[a]	51/72 (71%)[a]	43/72 (60%)
H2	21/72 (29%)[b]	1/72 (1%)	5/72 (7%)[b]	23/72 (32%)[b]	45/72 (63%)
SEM	27%	2%	8%	19%	1%
p-Value	<0.001	0.194	0.008	<0.001	0.695
			d0 Lung/air sac swab		
H1	53/72 (74%)[a]	3/72 (4%)	3/72 (4%)	19/72 (26%)[a]	59/72 (82%)[a]
H2	7/72 (10%)[b]	0/72 (0%)	0/72 (0%)	6/72 (8%)[b]	18/72 (25%)[b]
SEM	32%	2%	2%	9%	28%
p-Value	<0.001	0.977	0.977	0.003	<0.001
			d7 Lung/air sac swab		
H1	24/72 (34%)	2/71 (3%)	3/71 (4%)	37/71 (52%)[b]	44/71 (62%)
H2	18/71 (25%)	5/71 (7%)	0/71 (0%)	47/71 (66%)[a]	42/71 (59%)
SEM	4%	2%	2%	7%	1%
p-Value	0.471	0.258	0.977	0.048	0.664
			d7 Mid-ileum		
H1	64/70 (93%)	19/71 (27%)	12/71 (17%)	62/71 (87%)	38/71 (54%)
H2	60/67 (90%)	26/67 (39%)	10/71 (14%)	66/71 (93%)	42/71 (59%)
SEM	2%	6%	1%	3%	3%
p-Value	0.751	0.052	0.658	0.158	0.175
			d7 Ceca		
H1	71/72 (100%)	34/71 (48%)	21/71 (30%)	71/71 (100%)	13/71 (18%)
H2	71/71 (100%)	31/71 (44%)	28/71 (39%)	71/71 (100%)	15/71 (21%)
SEM	0%	2%	5%	0%	1%
p-Value	0.979	0.567	0.192	1.000	0.297

[a,b]Mean values with different superscript letters within a column and within a block indicate a significant difference ($p < 0.05$). [1]Commercial hatchery = H1, research hatchery = H2.

With regard to physical conformation, both the navel area and legs have been regarded as important factors to assess chick quality [7]. Unhealed navels have been well established as a potential pathogen entry port [28], with poor navel

conditions associated with poor quality chicks and negative production impact [29]. The navel scores in these experiments evaluated black button or bruised navel, string navel, open and unhealed navel, and infected navel. None of these measures resulted in differences based on hatchery at either an individual level, or when the scores were averaged. However, H1 was trending toward elevated average navel scores at 0.48 ± 0.04, versus 0.40 ± 0.03 in H2 ($p = 0.095$, **Table 3**). These navel deformities are generally considered an indicator of poor chick quality [7]. While differences in the average navel scores were not significant, this trend of a higher average navel score followed alongside lower BW and chick length in H1 chicks. Other characteristics, such as poor leg conformation and red hocks, have been deemed undesirable, and may indicate inflammation, damage, or weakness [7] [11]. Specifically, Tona and coauthors (2003) evaluated toe confirmation, articulation of the knees for inflammation and/or redness, and the ability of chicks to remain upright to assess chick quality in relation to legs [11]. Leg abnormalities measured in these experiments included red hocks, dehydrated legs, and spread or splayed legs. Red hocks were the only individual metric that resulted in differences, with greater incidence in H1 chicks. It has been noted that red hocks may be related to hatchery temperatures, thus providing a misleading comparison of hatcheries [7]. However, following hatch, chicks from each hatchery were driven for approximately 75 minutes prior to physiological assessment, which should have limited the effects of hatchery temperatures due to the extended period they were removed from the hatchery prior to assessment. Therefore, these results suggest H1 chicks were likely more sedentary, spending more time down with pressure on their hocks, resulting in hock redness. This idea was supported by results of chick righting, as chicks in H1 were more likely to fail the chick righting exercise, failing to return to a standing position from being on their back within three seconds. Alertness and activity are an important aspect of chick quality [7] that was captured by the chick righting exercise. Studies have noted that poorly formed navels and red hocks were not always related to deficient performance, and suggested that these measurements may not indicate early chick quality, especially when comparing hatcheries [10] [15]. Issues associated with leg abnormalities, such as femoral head necrosis, gait score, and hock burn all became worse with age, but were not indicative of differences between hatchery conditions [10]. In all, navel, leg, and alertness assessment may provide a method for assessing chick quality at time of hatch, but the parameters may not result in lasting influence on birds. These measurements should still be considered, however, in the interest of analyzing the entirety of early chick health.

Studies have shown the yolk sac to be a factor in initiating growth and early BW gain in chicks [30] [31] [32]. The yolk sac is considered the main nutrient source for chicks prior to introduction of exogenous feed and its size can be affected by how much chicks utilize the sac for nutrition. The yolk sac can comprise anywhere from 10% to 25% of chick weight at hatch, pointing to its im-

portance as a nutrient reservoir for the first few days post-hatch [33] [34] [35] [36]. Height and consistency of the abdomen of newly hatched chicks has been used as a quality measure [11]. Furthermore, abdominal palpations have been proposed as a proxy for residual yolk sac weight, since it has been shown to estimate yolk reserves fairly accurately [12]. In these experiments, abdominal height was measured by laying a chick on its back and measuring the height of the abdomen at its highest point to provide a quantitative metric for abdominal distention, and a potential estimation of yolk sac retention. However, no differences were found in abdominal height between H1 and H2 chicks, indicating abdominal height as a poor estimation for residual yolk sac size (**Table 2**). Yolk sac utilization can also be used as an indicator of growth and development in the form of YFBW since YFBW represents how much of the egg has been converted to live chick [37]. While this metric can be influenced by parent flock age, access to feed, and hatchery conditions, it has been utilized as an indicator of chick quality [10] [14] [15]. Attempts were made to control for these potential influences as previously described. Not only does YFBW provide a "real" chick weight, but it has also been cited to indicate yolk sac utilization and stimulation of GI development [14] [27]. Both yolk sac weight and YFBW were measured in these experiments, and resulted in both lower yolk sac weight and greater YFBW for H2 chicks, suggestive of greater yolk sac utilization and a potential jump start for intestinal development. This was supported by the significantly greater d0 intestinal weight for H2 chicks, where post hatch development of the GI tract occurs rapidly in the first few days of life. The first 48h post hatch has demonstrated a rapid increase in the number of crypts per villus in addition to invagination in duodenal, and upper and lower ileal segments [38]. Further, intestinal weight can be used to approximate development of the intestinal tract, as increased intestinal weight was found to indicate greater number of enterocytes and villus height [39]. Changes and rapid development of the GI tract would support the idea of intestinal weight as an indicator of chick quality. Therefore, internal measures, such as YFBW and intestinal weight can provide valuable and reliable measures to assess chick quality.

Physical measures of assessing chick quality can provide valuable information regarding health status of chicks, but including measures associated with inflammatory and microbial status may enhance the meaning of chick quality and ability to extrapolate long-term health. Recognized as an immunopoietic secondary lymphoid organ, the spleen has been shown to increase size, as measured by weight, in response to bacterial antigen exposure [19]. Lymphocyte activation that occurs within the spleen may account for some of the change in weight, as well as leukocyte migration to the spleen [19] [40]. While spleen weights were not different at either d0 or d7, spleen weight was always numerically greater in H1, and was approximately 70% larger in H1 on d0, and approximately 30% larger in H1 on d7 (**Table 2**). This may have indicated greater immune activity in the spleens of H1 chicks, though not enough to result in significant differenc-

es. This was somewhat supported by liver bacterial recovery. Liver bacterial translocation has been identified as a marker for increased gastrointestinal permeability [18] [41], and can be used in addition to spleen weight to denote chick health. Due to the direct connection between the GI tract and the liver via the portal vein, increased transport of bacteria may signify disrupted intestinal health [42]. On d0, liver bacterial translocation for H1 was nearly 10-fold greater than in H2, indicative of greater intestinal permeability and bacterial translocation to the liver (**Table 2**). While this difference did not carry through d7, the suggested difference in bacterial translocation at d0 helps support the idea that hatchery environment may influence early intestinal development and susceptibility to bacterial translocation.

Enterobacteriaceae are considered normal inhabitants of poultry environment and/or poultry intestinal tracts, and include *Escherichia*, *Klebsiella*, *Citrobacter*, *Enterobacter*, *Salmonella*, and *Proteus*, among others [21] [43]. Many of these genera can be considered opportunistic pathogens due to their capacity to produce endotoxins, as *E. coli*, the most commonly identified Enterobacteriaceae in chickens, and *Salmonella enterica* serovars have been linked to poultry diseases and foodborne illnesses in humans [22] [44] [45] [46]. Furthermore, Enterobacteriaceae classified as coliforms include lactose fermenters *Escherichia*, *Klebsiella*, *Enterobacter*, and *Citrobacter* [21]. In these experiments, lactose fermenting Enterobacteriaceae were more prevalent in H1 chicks on d0 for both crop/cloaca as well as lung/air sac samples, which indicates the possibility of greater exposure to coliforms within the hatchery environment (**Table 4**). While this did not carry through to d7, the early presence of detectable differences in lactose fermenting Enterobacteriaceae would indicate differences in early microbial colonization. This trend was further observed for hemolytic activity of both crop/cloaca, and lung/air sac swabs. Blood hemolysis has been identified as a virulence factor, as premature destruction of red blood cells that are then used as a nutrient source by these bacteria signify a route of systemic infection that may be detrimental to hosts [23] [47]. Further, hemolytic activity has been suggested to be linked to biofilm formation, as [48] found a correlation of 92.5% between the two when testing *Staphylococcus xylosus* isolates from poultry bioaerosol. Both of these factors have been associated with persistence and pathogenesis of bacteria, which are important to consider when assessing chick quality. In the present experiments, both beta and alpha hemolysis were found more often in d0 crop/cloaca swabs for H1 chicks, as well as increased incidence of alpha and gamma hemolysis in lung/air sac swabs, suggestive of greater incidence of virulent bacteria present within the intestinal and respiratory tract of those chicks (**Table 4**). This supports the idea that H1 chicks were exposed to different microbes within their hatchery environment, and these pioneer colonizing bacteria may have influenced early microbial colonization, with ripple effects touching immune development and later growth. A study completed by [49] evaluated dust and airborne components, including pathogen associated molecular pat-

terns, on immune responses and BWG, and found that these dust components resulted in decreased BWG, as well as improved humoral immune responsiveness as measured by antibody titers. While the hemolytic activity observed on d0 did not carry over through d7, as only lung/air sac had greater alpha hemolytic activity in H2 on d7, the early microbial exposure had the potential to influence overall microbial colonization and immune activity. Several studies have shown the influence of early pioneer colonizers which may result in shifts in microbial populations with downstream consequences on immune development as well [3] [50] [51] [52]. For instance, day of hatch exposure to *Salmonella* resulted in changes to the immune profile that showed early pro-inflammatory state 2 days after inoculation that shifted to anti-inflammatory 4 days after inoculation, indicative of tolerance, before transitioning to a non-inflammatory response characterized by an increase of IL-10 and TNF-α and decrease of IL-6 and IL-1β [52] [53]. This state of tolerance may not be specific to *Salmonella*, as indicated by *Citrobacter* exposure in which proteomic analysis suggested *in ovo* inoculation resulted in changes to proteomic profiles, particularly with regard to capacity for inflammatory response [54]. Pathway analysis in this work reported an increase of inflammation, but a decrease in inflammatory response, as well as diminished movement of leukocytes and granulocytes, indicating an inability of the innate immune system to act on the apparent GI inflammation [3]. These changes at 10d were in contrast to increased stress response, immune cell trafficking, and cell-to-cell signaling indicated at day of hatch in the same treatment group [50]. Early shifts observed in microbial colonization would need to be further studied for their influence on growth, overall health, and immune development, but considered in context of other studies, hatchery environment appeared to influence early microbial development, with potential influences on microbial environment and immune stimulation.

While only two experiments were tested for differences in A1GP and no differences were found, patterns of A1GP seemed to allude to subtle differences in innate immune response. As a major acute phase protein synthesized and released by the liver as part of the acute phase response in poultry, A1GP can be triggered by stress, burns, infection, and other chronic inflammatory conditions [55] [56]. Several studies in poultry have evaluated changes in A1GP associated with various bacterial diseases and inflammatory conditions, with normal serum concentrations generally in the range of 150 - 400 μg/mL [20] [57] [58] [59] [60]. Both d0 and d7 A1GP levels fell within the normal range and resulted in no differences, but the near doubling of A1GP in H2 chicks, as opposed to the 12% increase in H1 between d0 and d7 signified a potential alteration in immune responsiveness (**Table 2**). This may allude back to differences in early microbial exposure, and how hatchery environments may have primed different immune responses in each set of chicks, as described previously in the study by [49].

Overall, the measurements used to assess chick quality on d0 revealed the importance of including a variety of measures, both qualitative and quantitative,

for a holistic perspective of chick health. The measures that proved most useful on d0 were BW, chick length, yolk sac weight, YFBW, intestinal weight, liver bacterial recovery, average navel score, average leg score, lactose fermenting Enterobacteriaceae, and hemolytic activity of both crop/cloaca, and lung/air sac. These measures suggested that hatchery environment played a role in chick quality, as well as early microbial colonization, while a change in inflammatory responsiveness was not conclusive. Further studies would need to be completed to assess influences of chick quality on growth, overall health, disease susceptibility, microbial shifts, and changes to immune responsiveness throughout the production timeline of broilers.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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