

Early infection with *Histomonas meleagridis* has limited effects on broiler breeder hens' growth and egg production and quality

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ABSTRACT A study was conducted to determine differences between *Histomonas meleagridis*-infected and control pullets based on disease signs, hen growth, and egg production and quality. Ross 708SF females were weighed and then placed in pens on the day of hatch (92 chicks/pen). At 25 D, 4 pens were infected with *H. meleagridis* in the cloaca, whereas 4 pens were control. At 5, 10, and 20 D after inoculation, 5 birds per pen (2 birds per pen at 20 D) were subjectively scored for blackhead disease. Birds were feed restricted based on BW and/or egg production. Individual BW were collected at 3, 5, 13, 15, 20, and 64 wk. Egg production was recorded at 24–63 wk. Egg quality was measured at 30, 34, 39, 42, and 56 wk and included shell and vitelline membrane strength, shell thickness, egg weight, and Haugh units. Hatchability was measured at 27, 37, and 60 wk and fertility at 27 and 37 wk. Treatment effects were

determined by JMP Pro 14 using GLM with means separated using the Student *t* test ($P \leq 0.05$). Cecal lesions were apparent on 5, 10, and 20 D and liver lesions on 10 and 20 D for the infected birds. The control had no histomoniasis lesions. Flock uniformity differed on wk 13 and 20 ($P = 0.04$; 0.04). Infected birds weighed less at 64 wk ($P = 0.002$). The onset of lay was not delayed. Infected birds produced more eggs during 1 period ($P = 0.02$). The infected birds produced heavier eggs at 30 wk ($P = 0.04$), eggs with a stronger and thicker shell at 42 wk ($P = 0.05$, 0.03), and eggs with a stronger vitelline membrane at 56 wk ($P = 0.049$). Hatchability and fertility did not differ ($P > 0.05$). *H. meleagridis* was observed in the infected birds' cecal samples at trial termination. This study indicates early infection with *H. meleagridis* has limited effects on pullet egg production and quality.

Key words: *Histomonas meleagridis*, blackhead disease, broiler breeder, egg production

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INTRODUCTION

Histomonas meleagridis is a protozoal parasite that causes histomoniasis or blackhead disease in gallinaceous birds. Histomoniasis is considered a reemerging disease owing to the number of outbreaks in intensive chicken and turkey facilities increasing over the past few decades as available treatments decrease (Liebhart et al., 2017). Chickens mount a more effective immune response against *H. meleagridis* compared with the turkey (Powell et al., 2009). Generally, infected chickens suffer from morbidity, specifically lesions in the ceca, but have reduced liver involvement and mortality than turkeys

(Liebhart et al., 2011). Negative effects on chicken production performance can include poor flock uniformity, delayed onset of lay, and a decrease in the quality and quantity of eggs produced leading to economic losses (Gerth et al., 1985; Hu and McDougald 2002; Esquenet et al., 2003; Graff et al., 2011; Dolka et al., 2015). Conversely, some *H. meleagridis* outbreaks in layer and breeder facilities go unnoticed because of absence of clinical signs or gross pathologic lesions (McDougald, 1998, 2005; Hu et al., 2006; Sulejmanovic et al., 2013).

Economic losses after a histomoniasis outbreak in commercial chicken facilities are attributed to *H. meleagridis* but do not account for losses because of coinfections or timing of the *H. meleagridis* introduction (Desowitz, 1951; Gerth et al., 1985; Esquenet et al., 2003). Coinfection of *H. meleagridis* with other pathogens has led to an increase in layer mortality and a decrease in performance (Gerth et al., 1985; Esquenet et al., 2003). Liebhart et al. (2013) found that experimentally inoculating layers with only *H. meleagridis* at

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the peak of lay caused a decrease in egg production, whereas conversely, Sigmon et al. (2019) found that inoculating layers during rearing did not alter their egg production.

Broiler breeders are more likely to cycle *Heterakis gallinarum*, a cecal nematode known for being a vector of *H. meleagridis*, than layers owing to floor rearing used in commercial breeder facilities (Waters et al., 1994). This indicates an increased potential for the broiler breeder to be exposed to *H. meleagridis*. Case reports on the interaction between broiler breeder flocks and *H. meleagridis* are documented in the context of a natural infection without other environmental and disease factors being controlled (Waters et al., 1994; Dolka et al., 2015). Experimental inoculation has indicated that broiler breeder pullets suffer from inflammation and lesions in the ceca, but these studies have only focused on acute disease signs and not production (Hu and McDougald, 2002). The long-term effects of only *H. meleagridis* on broiler breeder pullets' performance are yet to be investigated. This study aimed to determine 1) if an inoculation with a virulent strain of *H. meleagridis* would cause morbidity of commercially raised broiler breeder pullets and 2) if histomoniasis during rearing causes alterations to breeder hen egg production and quality.

MATERIALS AND METHODS

Experimental Animals

Seven hundred thirty-six Ross 708SF female pullets were obtained from a commercial hatchery (Pageland, South Carolina) on day of hatch and individually tagged. The pullets were randomly assigned to 4 pens per treatment (infected or control). Each pen housed 92 pullets. Breeders were vaccinated to control coccidiosis (*Eimeria acervulina*, *Eimeria brunetti*, *Eimeria maxima*, *Eimeria necatrix*, and *Eimeria tenella*) and Marek's disease at the hatchery. At 2, 5, 12, and 18 wk of age, breeders received vaccinations for Newcastle disease and infectious bronchitis. At 14 wk of age, they received a fowl pox vaccine. Pullets were raised in a blackout rearing facility from 0 to 21 wk of age; then, they were transferred to a curtain-sided laying facility from 21 to 64 wk of age. Cockerels were reared in separate pens and were not infected directly with *H. meleagridis* during rearing. Eighteen males were housed in each pen during grow out, and at 21 wk of age, 8 males per pen were selected for mating and transferred to the laying house. Birds were monitored twice daily and fed based on the feeding program provided in the following section. Onset of lay and the number of eggs produced were recorded daily for the duration of study.

Housing

Pullet pens during the first 20 wk of age of rearing measured 3.33 m × 4.65 m and had fresh shavings applied before bird placement. In the rearing facility,

birds were given 23 h of light for the first 2 wk of age, followed by 8 h of light until moving to the laying house. At 21 wk of age, 64 pullets averaging the mean BW of the pen, within 1 SD of the mean, from each pen were moved to a curtain-sided layer house with each pen measuring 4.65 m × 3.73 m containing 0.51 m high slats, used litter and 8 egg boxes. Birds maintained their original treatment status. In the laying facility, birds were given 14 h of light until 22 wk of age, 15 h of light through 24 wk of age, 15.5 h of light for 25–27 wk of age, and 16 h of light until trial termination.

Feeding Program

Diets were formulated to meet or exceed the Aviagen Female Parent Stock Nutrient Specifications and the NRC (1994) requirements (Tables 1 and 2). Pullets were provided feed ad libitum until 2 wk of age and then were placed on a modified "skip-a-day" feeding program from 2 to 22 wk of age. Feed was adjusted for mortality as needed. After 22 wk of age, birds were fed based on hen population and egg production on a per pen basis.

Inoculation and Detection of *H. meleagridis*

A culture of *H. meleagridis* was collected from a broiler breeder outbreak in Buford, Georgia, and then preserved in liquid nitrogen. The isolates were removed from storage and grown at 42°C in modified Dwyer's medium consisting of 0.8% (wt/vol) rice powder and 5% horse serum in Medium 199 with Hank's balanced salt solution (Hauck et al., 2010). The number of *H. meleagridis* cells was determined using a hemocytometer. At 25 D, pullets in the infected treatment (4 pens) were inoculated in the cloaca with an average of 100,000 *H. meleagridis* cells per pullet. At 5 and 10 D after inoculation, 5 pullets per pen per sampling day were euthanized and subjectively scored for histomoniasis based on cecal and liver lesions. On 20 D, 2 pullets per pen were euthanized and subjectively scored. At 64 wk of age, 10 hens per pen were euthanized, cecal samples collected, and placed in media; then, the cultures were incubated at 42°C for 48 h. After incubation, cultures were examined under light microscopy for the presence of *H. meleagridis*.

Data Collection

Morbidity was assessed by observing disease signs after inoculation; BW and flock uniformity were assessed at various points through rearing and at trial termination. Pen weights were collected at placement to ensure initial BW uniformity. At 3, 5, 13, 16, 20, and 63 wk of age, individual BW of the breeder pullets were documented and analyzed for average BW and uniformity.

Monitoring of egg production occurred twice daily from onset of lay until trial termination. To determine egg quality characteristics, 24 eggs per treatment representing a sample population were measured for shell and vitelline membrane strength, shell thickness, egg weight,

Table 1. Ingredient composition throughout the rearing and laying periods.

Age fed (wk)	0 to 3	3 to 5	5 to 19	19 to 5% production	5% production to 35 wk	35 to 50	50 to trial termination
	Starter		Grower	Layer			
Ingredients	1	2		Prelay	1	2	3
	(%)						
Corn	57.47	49.75	42.58	50.02	65.28	66.35	67.17
Wheat middlings	11.21	30.96	44.98	30.2	6.5	6.24	4.73
Soybean meal, 46%	27.12	15.6	8.98	15.71	19.12	17.8	17.97
Poultry fat	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Limestone fine	1.35	1.57	1.56	2.3	7.06	7.65	8.2
DCP, 18.5%	1.06	0.65	0.38	0.19	0.48	0.39	0.37
Salt (NaCl)	0.26	0.2	0.18	0.2	0.31	0.28	0.28
Sodium bicarbonate	0.29	0.24	0.31	0.3	0.14	0.19	0.19
L-Lysine-HCl, 78.8%	0.09	-	-	0	0	0	0
DL-Methionine, 99%	0.2	0.08	0.08	0.16	0.17	0.15	0.15
L-Threonine, 98%	0.02	-	-	0.02	0.03	0.02	0.02
Mineral premix ¹	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Vitamin premix ²	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Selenium premix	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Phytase ³	0.015	0.015	0.015	0.015	0.015	0.015	0.015
Filler	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Choline chloride, 60%	0.07	0.1	0.09	0.04	0.06	0.06	0.06
Total	100	100	100	100	100	100	100

¹Trace minerals provided per kg of premix: manganese (Mn SO₄), 60 g; zinc (ZnSO₄), 60 g; iron (FeSO₄), 40 g; copper (CuSO₄), 5 g; iodine (Ca(IO₃)₂), 1.25 g.

²Vitamins provided per kg of premix: vitamin A, 13,227,513 IU; vitamin D3, 3,968,253 IU; vitamin E, 66,137 IU; vitamin B12, 39.6 mg; riboflavin, 13,227 mg; niacin, 110,229 mg; d-pantothenic acid, 22,045 mg; menadione, 3,968 mg; folic acid, 2,204 mg; vitamin B6, 7,936 mg; thiamine, 3,968 mg; biotin, 253.5 mg.

³Quantum Blue 5G, 80 g/ton to supply 1,000 FYT (AB Vista) delivering 0.11% of nonphytate phosphorus P, and 0.10% of calcium.

and Haugh units at 30, 34, 39, 42, and 56 wk of age. Egg weight and Haugh units (Haugh, 1937) were measured using the TSS QCD system (Technical Services and Supplies, Dunnington, York, the UK). Shell strength and vitelline membrane strength determinations were conducted using a Texture Analyzer (Texture Technologies, Scarsdale, NY). Shell thickness was recorded with an iGAGING Absolute Origin SpeedMic Micrometer (San Clemente, CA), and the average of 2 shell thickness measurements was presented. To determine hatchability and fertility, eggs were collected from each pen and then stored at 15°C for 1 wk before placement in the incubator. One hundred eighty eggs were chosen at random from each pen and incubated under standard conditions. Twenty-one days later, hatchability and fertility were documented based on treatment. Hatchability was determined at 27, 37, and 63 wk of age, whereas fertility was analyzed on wk 27 and 37.

Statistical Analysis

Feed allocation and egg production were recorded daily and analyzed based on 28-D periods (10 total) during the laying period. The BW, flock uniformity, and egg production and quality for the 2 treatment groups were analyzed in JMP Pro 14 via GLM. Differences between treatments were found using Student *t* test with significance considered if $P \leq 0.05$.

Animal Care and Use

This experiment was conducted in agreement with the Institutional Animal Care and Use Committee at North

Carolina State University, where husbandry practices and euthanasia were followed keeping animal welfare and well-being in mind.

RESULTS

Gross Pathology and Microscopy

Inflammation of the cecal tissue and surrounding mesentery with visually obvious thickening of the cecal wall and changes in the luminal contents were observed in 80% of the infected pullets 5 D after inoculation, 90% at 10 D, and in all infected pullets at 20 D (Table 3). Very few white, pinpoint-shaped liver lesions characteristic with *H. meleagridis* were found sporadically in the infected pullets at 10 D and in all infected pullets at 20 D after inoculation. None of the control pullets had lesions associated with *H. meleagridis*. Lesions associated with coccidia were apparent in the upper intestinal tract but not the ceca for both treatments during the sampling day. At 64 wk of age, *H. meleagridis* was observed via light microscopy in 18 of the 40 cultures from the infected hens sampled and 0 of the 40 control hens. The protozoan *Blastocystis* was apparent in cultures for both treatments.

BW and Flock Uniformity

Individual BW only differed between treatments at trial termination ($P = 0.002$), where the infected pullets weighed significantly less (Figure 1). Flock uniformity differed between treatments at 13 and 20 wk of age, where the CV was higher for the infected treatment,

Table 2. Nutrient composition throughout the rearing and laying periods.

Age fed (wk)	0 to 3		3 to 5	5 to 19	19 to 5% production	5% production to 35 wk		35 to 50	50 to study termination
	Starter		Grower	Prelay	Layer				
	1	2C			1	2	3		
Nutrient									
ME, kcal/kg	2,767	2,653	2,600	2,700	2,800	2,800	2,800	2,800	2,800
CP, %	19.43	16.26	14.58	16.00	15.00	14.40	14.40	14.30	14.30
Calcium, %	0.85	0.85	0.80	1.05	2.85	3.05	3.05	3.25	3.25
Total P, %	0.79	0.84	0.88	0.73	0.58	0.56	0.56	0.54	0.54
NPP, %	0.50	0.45	0.42	0.35	0.35	0.33	0.33	0.32	0.32
Total Lys, %	1.08	0.78	0.64	0.76	0.74	0.70	0.70	0.70	0.70
Total Trp, %	0.25	0.22	0.20	0.21	0.18	0.17	0.17	0.17	0.17
Total Thr, %	0.74	0.59	0.52	0.60	0.60	0.57	0.57	0.56	0.56
Total Val, %	0.93	0.78	0.68	0.75	0.71	0.68	0.68	0.68	0.68
Total Arg, %	1.30	1.08	0.95	1.05	0.97	0.92	0.92	0.92	0.92
Total SAA, %	0.82	0.63	0.59	0.69	0.67	0.64	0.64	0.63	0.63
Dig Lys, %	0.96	0.66	0.52	0.64	0.65	0.62	0.62	0.62	0.62
Dig Met, %	0.47	0.31	0.28	0.38	0.39	0.36	0.36	0.36	0.36
Dig Cys, %	0.27	0.24	0.22	0.23	0.22	0.21	0.21	0.21	0.21
Dig SAA, %	0.74	0.54	0.50	0.61	0.60	0.57	0.57	0.57	0.57
Dig Thr, %	0.63	0.48	0.41	0.49	0.51	0.48	0.48	0.48	0.48
Dig Trp, %	0.22	0.19	0.17	0.18	0.15	0.15	0.15	0.14	0.14
Dig Iso, %	0.73	0.55	0.45	0.54	0.55	0.53	0.53	0.53	0.53
Dig Leu, %	1.48	1.18	0.99	1.16	1.22	1.18	1.18	1.18	1.18
Dig Val, %	0.81	0.66	0.56	0.64	0.62	0.60	0.60	0.60	0.60
Dig Arg, %	1.20	0.96	0.83	0.93	0.89	0.84	0.84	0.84	0.84
Sodium, %	0.20	0.16	0.17	0.18	0.18	0.18	0.18	0.18	0.18
Potassium, %	0.81	0.63	0.50	0.60	0.62	0.60	0.60	0.60	0.60
Chloride, %	0.22	0.18	0.18	0.18	0.24	0.22	0.22	0.22	0.22
DEB, mEq/100 g	240	182	150	180	170	170	170	170	170

indicating greater ($P = 0.04$) variation in the pullet size (Table 4).

DISCUSSION

Egg Production and Quality

More eggs ($P = 0.03$) were produced in the infected treatment at 48 to 51 wk of age (Figure 2). Egg quality was affected at various sampling times, where at 30 wk of age, the infected pullets produced heavier eggs ($P = 0.04$); at 42 wk of age, there was a stronger ($P = 0.05$) and thicker shell ($P = 0.03$); and at 56 wk of age, there was a stronger ($P = 0.049$) vitelline membrane (Table 5). Hatchability and fertility did not differ between treatments for the time periods analyzed (Table 6).

Host-Parasite Interaction

Infection with *H. meleagridis* has varying consequences dependent on the gallinaceous bird host (Lund and Chute, 1972). Chickens have milder clinical signs than the turkey, where there is pathologic lesions and inflammation in the ceca with minimal, if any, damage to the liver (Lund, 1967; Powell et al., 2009; Mitra et al., 2018). Cecal lesions and an inflammatory response have been found as early as 3 D after inoculation and can be seen up to 3 wk after (Hauck and Hafez, 2013). At 6 to 8 D after infection, the damaged cecal mucosa recovers, and within a mo, the breeders can gain immunity while remaining positive for the parasite (Dolka et al., 2015).

Table 3. Identification of *Histomonas meleagridis* using gross pathology and microscopy.

D after inoculation	Treatment	N	Average BW (kg)	<i>H. meleagridis</i> positive ¹ (%)	Ceca score	Liver score
5	Control	80	0.62	0	0	0
	Infected	80	0.59	80	1.5	0
10	Control	80	0.68	0	0	0
	Infected	80	0.65	90	3	0.2
20	Control	40	0.84	0	0	0
	Infected	40	0.77	100	1	1
420*	Control	40	4.29	0	0	0
	Infected	40	4.17	45	0	0

n = number of birds sampled for that treatment during that time period.

An asterisk (*) beside days post inoculation indicates statistical differences found between treatments for BW at a specific time period ($P \leq 0.05$).

¹The 2 methodologies used to determine if samples were positive for *Histomonas meleagridis*. On 5, 10, and 20 D after inoculation, birds were subjectively scored, and any bird with *H. meleagridis* disease signs was considered positive. On 420 D after inoculation, samples were considered positive if *H. meleagridis* cells were observed under light microscopy in culture.

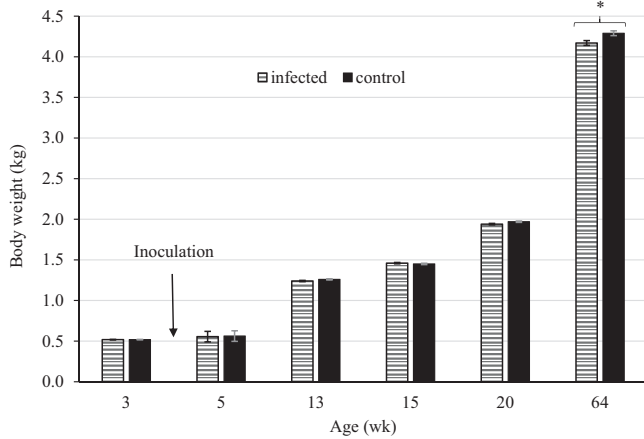


Figure 1. Average BW. The average pullet BW for each treatment for various time points during rearing as well as trial termination presented as the mean BW \pm SEM. An asterisk (*) above the bars indicate statistical differences found between treatments at a specific time period ($P \leq 0.05$).

Chickens have been shown most vulnerable to *H. meleagridis* at around 4 wk of age, therefore the pullets in this experiment were inoculated at 25 D (Desowitz, 1951). Five D after the inoculation, 80% of the infected treatment pullets sampled had inflammation of the cecal tissue (Table 3). Cecal cores were found in some of the infected pullets at 10 D, whereas visually apparent recovery of the ceca via decrease inflammation and core formation was seen at 20 D. Liver damage was minimal and sporadically identified at 10 D, whereas all infected pullets had 1 to 22 necrotic pinpoint lesions per liver at 20 D. No mortality due to the *H. meleagridis* inoculation was recorded. The pathogenesis of *H. meleagridis* in the breeders mimics the time points observed by Zahoor (2011) who experimentally inoculated various chicken species.

Concurrent infections of *H. meleagridis* with an inoculation of *E. tenella* (10^4 oocysts per bird) in broilers at 10 or 14 D of age has been shown to increase pathologic lesions in the liver and cause growth stunting (McDougald and Hu, 2001). In this study, the coccidia vaccine given on the day of hatch contained *E. tenella*,

Table 4. The flock uniformity presented as the CV for multiple time periods during rearing and at trial termination.

Age (wk)	Treatment	CV (%)	<i>P</i> -value
3	Infected	12.6	0.26
	Control	11.4	
5	Infected	12.8	0.11
	Control	11.1	
13	Infected	12.3 ^b	0.04
	Control	10.6 ^a	
15	Infected	13.9	0.15
	Control	12.6	
20	Infected	14.1 ^b	0.04
	Control	11.9 ^a	
64	Infected	10.9	0.16
	Control	9.8	

Values are means of 4 pens (replicates) per treatment.

^{a,b}Means in a column not sharing a common superscript are significantly different ($P < 0.05$) by Student *t* test.

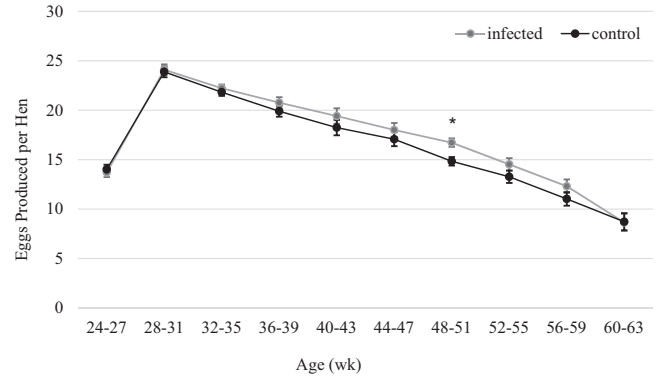


Figure 2. Average egg production. The average eggs produced per hen per period within each treatment group \pm SEM. An asterisk (*) above a time period indicates statistical differences found between treatments ($P \leq 0.05$).

but no macroscopic lesions in the ceca were visually apparent in the control birds and damage from *E. tenella* could not be observed in the infected treatment owing to histomoniasis. In addition, no difference in weight was found at the 5, 10, and 20 D after inoculation necropsies (data not shown). Differences seen in the present study compared with that by McDougald and Hu (2001) could be explained by the timing and dosage of *Eimeria* administered. Similar to the findings by Welter (1960), our findings suggest that proper timing and dosage of vaccines do not exacerbate histomoniasis in breeders. Coinfection disease trials are necessary to conclude if specific pathogens worsen histomoniasis in chickens.

It is hypothesized that *H. meleagridis* inhabits the cecal lumen, becoming part of the microflora for the duration of the chicken's life (Lund, 1967). At termination, *H. meleagridis* was isolated and grown in culture from the infected birds. The recovery of *H. meleagridis* in almost half of the cecal samples collected from the infected birds supports this claim and indicates that the broiler breeder chicken is an ideal host of this protozoan (McDougald, 2005). It should be noted that *Blastocystis*, a protozoan commonly found in the bird's intestines, was apparent in the cultures for both treatments (Grabensteiner and Hess, 2006). Generally, *Blastocystis* is an enteric endosymbiont with limited clinical relevance, but this statement has been debated (Stensvold et al., 2009). It has also been documented to grow well in the media used for *H. meleagridis* (McDougald, 2005). From the present study, it is unknown when the breeders became contaminated with this protozoan. However, the ceca were not inflamed for either treatment during trial termination nor were there signs of gastrointestinal distress owing to the protozoan presence. Therefore, the presence of *Blastocystis* most likely did not alter the outcomes from this experiment.

Flock Growth and Uniformity

Infection with *H. meleagridis* has been reported to cause morbidity, altering BW and flock uniformity in

Table 5. Effect of treatment on physical qualities of the eggs produced throughout lay.

Age of hens (wk)		Shell strength (g force)	Average shell thickness(mm)	Egg weight (g)	Haugh units	Vitelline membrane strength (g force)
30	Infected	4,608.8	0.34	58.78	92.5	2.26
	Control	4,553	0.338	56.67	94.1	2.36
	SEM	180	0.005	0.69	0.83	0.1
	<i>P-value</i>	0.83	0.81	0.04	0.17	0.45
34	Infected	3,800	0.335	60.23	87.2	1.97
	Control	4,177.9	0.339	59.43	84.5	2
	SEM	142	0.005	0.83	1.17	0.09
	<i>P-value</i>	0.07	0.65	0.5	0.11	0.81
39	Infected	4,297.8	0.341	61.33	87.3	2.06
	Control	3,954.8	0.339	60.62	87.6	2.04
	SEM	154	0.005	0.74	1.63	0.09
	<i>P-value</i>	0.12	0.8	0.5	0.92	0.9
42	Infected	4,223.7	0.352	63 ± 0.90	83.5	2.29
	Control	3,842.6	0.334	63 ± 0.92	84.1	2.03
	SEM	135	0.005		1.4	0.01
	<i>P-value</i>	0.05	0.03	0.99	0.74	0.06
56	Infected	3,987.2	0.349	70	83.5 ± 1.4	2.69
	Control	4,265	0.349	70	80.1 ± 1.2	2.34
	SEM	162	0.006	0.93		0.21
	<i>P-value</i>	0.23	0.99	0.57	0.81	0.049

A significant difference between treatment groups was considered if $P \leq 0.05$.

chickens (Lund 1967; Gerth et al., 1985; McDougald and Hu, 2001; Esquenet et al., 2003). We hypothesized that histomoniasis leads to growth variation because of the inflammation and granuloma development in the ceca. This organ is used for immune processes and water and electrolyte absorption, so when its functionality is altered by pathogens, bird growth can suffer (Clench and Mathias, 1995; Stephens and Hampson, 2001; Svihus, 2014). Differences in BW were observed only at trial termination, where the infected treatment weighed significantly less than the control (Figure 1). Although BW did not differ during rearing, the infected treatment had significantly poorer uniformity (Table 4). Disease states during rearing that cause poor flock uniformity often correlate with lasting effects on the uniformity of the flock (Abbas et al., 2010). This was not seen in the present study, potentially owing to the selection process in the transfer of pullets to the laying facility. Greater variation in flock uniformity has been documented with layers suffering from *H. meleagridis* (Gerth et al., 1985; Esquenet et al., 2003). However, these studies are field reports with birds suffering from concurrent infections unlike the current controlled research experiment. Disease signs only during rearing and not production could be the reason why there are different growth outcomes between previous *H. meleagridis* publications and the present study; however, further investigations are necessary.

Egg Production and Quality

To date, no studies have analyzed the effects of *H. meleagridis* on the egg production and quality of broiler breeder pullets. Poor uniformity is associated with variation in the hen sexual maturity, where underweight pullets have a delayed onset of egg production and altered egg quality traits (Abbas et al., 2010). The

inoculation during rearing in this study correlated with variation in treatment BW uniformity, but there was no delay in the onset of lay. During one period, the infected produced more eggs than the control (Figure 2). This could be because of the lighter BW of the infected treatment, where heavier hens can have lower egg production (Abbas et al., 2010). The increase in the number of eggs produced per treatment in the present study differ from that reported in the studies by Gerth (1985) and Liebhart (2013) most likely because of the timing of the infection, where the previous publications had birds suffering from blackhead disease during lay, whereas the present study was during rearing. Similar to the study by Sigmon (2019), inoculating pullets during rearing did not have a negative effect on egg production. Favorable physical qualities of the eggs were also periodically identified with the infected treatment throughout this experiment (Table 5). To the authors' knowledge, this is the first study to analyze breeder pullet hatchability and fertility changes because of *H. meleagridis*, and no effects ($P > 0.05$) were seen (Table 6). Long-term benefits of early acute stressors in poultry have correlated with better overall health

Table 6. Hatchability and fertility of each treatment group and different time periods during lay.

Wk	Treatment	Hatched eggs (%)	Infertile eggs (%)
27	Infected	89.65	0.84
	Control	91.93	0.7
	<i>P-value</i>	0.19	0.72
37	Infected	85.05	2.51
	Control	83.61	2.5
	<i>P-value</i>	0.84	0.99
63	Infected	68.40	-
	Control	83.61	-
	<i>P-value</i>	0.39	-

Significance was considered if $P \leq 0.05$.

and performance of the bird (Zulkifli and Siegel, 1995; Smit et al., 1998). In the present study, the *H. meleagridis* stressor only during rearing and not production supports this association. Inoculation of only this parasite during lay is necessary to determine there is an effect on older broiler breeders' egg production and quality.

Based on the recovery of *H. meleagridis* over a year after inoculation, it can be inferred that the broiler breeder is an ideal host for this protozoan and supports the claim that chickens become carriers for life. Breeder pullets have pathologic lesions the first few wk of infection, but mortality was not observed. Negative effects on egg production and quality were not observed when pullets were infected at 4 wk of age suggesting that inoculation at this time could offer protection against blackhead outbreaks during the laying period.

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