ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

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International Journal of Poultry Science 14 (5): 293-299, 2015
ISSN 1682-8356
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Timing of Light Exposure During Incubation to Improve Hatchability, Chick Quality and Post-Hatch Well-Being in Broiler Chickens: 21 or 18 Days

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Abstract: Providing light during incubation has been shown to hatchability and post hatch development; however, the optimal timing of this light is still not known. To determine if there is an effect of exposing embryos to light for the first 18 d of incubation or the entire 21 d of incubation, we incubated broiler chicken eggs (N = 3096) for either 18 d (18DL), 21 d (21DL) or not at all (DARK). Embryo mortality, chick weight, proportion of cull or dirty chicks, or those with leg or other abnormalities was not affected by incubation conditions (p>0.05). The DARK broilers had a lower hatch rate, a lower proportion of unhealed navels and no defect chicks than both the 18DL and the 21DL (p<0.05) broilers. There was no effect of incubation condition (p>0.05) on 45 d weight gain or 45 d feed conversion. The DARK broilers vocalized more during isolation and had longer latency to right during tonic immobility than both the 18DL and the 21DL (p<0.05) broilers. The DARK broilers had higher composite asymmetry scores and corticosterone concentrations than both the 18DL and the 21DL (p<0.05) broilers. There were no differences observed between the lighting treatments in any measure (p>0.05). Hatchability, chick quality, fear response and stress susceptibility were all improved with lighted incubation irrespective if the lighting occurred for 18 or 21 days. Providing light during the first 18 d of incubation can improve production and bird welfare.

Key words: Broiler, incubation, light, hatchability

INTRODUCTION

The concept of implementing lighting during incubation and hatching has been a subject of study for many years, but only recently has new technology become available to make it feasible for use in commercial hatcheries. The conventional procedure utilized in the commercial poultry industry is to incubate fertilized eggs in complete darkness, with the eggs only being intermittently exposed to light when the incubator is opened. Overall hatchability has been shown to be increased in poultry with the addition of light (Cooper, 1972; Shafey and Al-Mohsen, 2002; Shafey, 2004; Archer and Mench, 2014b; Huth and Archer, 2015), though it seems to vary depending on factors like type of light used or strain of birds. There are some reports of depressed hatchability and increased embryo mortality when light is introduced, which may be attributed to excess heat produced by incandescent bulbs (Tamimie and Fox, 1967; Erwin *et al*., 1971). Rate of growth is also affected, with embryos usually showing an accelerated growth rate when exposed to light (Siegel *et al*., 1969; Lauber, 1975; Fairchild and Christensen, 2000; Shafey and Al-Mohsen, 2002; Shafey, 2004; Veterany *et al*., 2004). Differences in post-hatch growth as a result of lighted incubation have also been seen in previous studies though results have been inconsistent with some reporting differences in growth and weight (Ozkan *et al*., 2012b; Zhang *et al*., 2012) and others reporting no changes in performance (Archer *et al*., 2009). Providing light exposure during incubation has been shown to reduce stress and fear levels in broilers post-hatch (Archer and Mench, 2013; Archer and Mench, 2014b). It has been noted that photoperiodic lighting during incubation resulted in birds that adapted more easily to novel environments than their dark incubated counterparts, which may result in better post-hatching development (Ozkan *et al*., 2012a; Ozkan *et al*., 2012b). This may be attributed to visual lateralization (Johnston and Rogers, 1999), entrainment of circadian rhythms (Hill *et al*., 2004), or changes in hormone levels (Ozkan *et al*., 2012b).

The earliest measurement of an embryo's ability to sense light is at 2 days of incubation, where light exposure stimulates mitosis in neural crest mesoderm (Cooper *et al*., 2011). This accelerates the closing of the neural tube, which in turn differentiates into the precursor of the central nervous system (Isakson *et al*., 1970) and is consistent with observations that high intensity light stimulates embryonic cell proliferation (Cooper *et al*., 2011). The eye and more specifically the retina, is the most obvious light sensing organ to consider as it is the primary light sensing organ in an adult bird (Prescott *et al*., 2003). It is made up of many photoreceptors, divided into 2 main subgroups-rods and cones-which sense light and relay it to the brain (Witkovsky, 1963). However, light sensing opsins (photoreceptor molecules) were not detected in an embryonic chick until 14 days of development, with development completing on day 18 (Bruhn and Cepko, 1996). The pineal gland, which forms at day 3 of

incubation in chickens (Cooper *et al*., 2011), is another light sensitive organ possessed by chicks. Aige-Gil and Murillo-Ferrol (1992) directly exposed an embryo's pineal gland to light, which found a significant increase in the number and size of pineal intracytoplasmic lipid droplets in lit versus unlit embryos after 18 days of exposure. Eighteen days is also the time when pineal circadian clocks develop (Cooper *et al*., 2011). Research has shown that circadian rhythms can be entrained in a prehatch chick by exposing the embryo to light on a 12 h light/12 h dark schedule (Hill *et al*., 2004). Hill *et al*. (2004) found that embryonic light exposure from day 13 to 18 resulted in circadian rhythms being present in post hatch tonic immobility tests and body temperature measurements. This held true if the embryo was only exposed to light for 12 h on a single day between days 13 to 18. The results of this study also showed that short bouts of light during the usual lighted period can entrain a rhythm, but it is not as strong as a full 12 h cycle. When light is sensed by the embryonic pineal gland, it triggers the synthesis of melatonin which affects growth rate and development. Furthermore, light exposure during avian embryonic development can cause changes in brain physiological development (Visual Asymmetry). Visual asymmetry in the visual pathways has been shown to develop as a result of light stimulation prior to hatching on or around day 18 of incubation (Rogers and Krebs, 1996). This is due to the embryo being oriented in the egg such that the left eye is covered and thus only the right eye becomes light stimulated (Rogers and Krebs, 1996). It has been noted that each eye system has unique attributes, with the right eye system using conspicuous clues to assign stimuli to categories and the left eye system taking into account all properties of stimuli including position in space (Andrew, 1988). Though recently it has been demonstrated that brain lateralization can occur before the development of functional visual systems (Chiandetti *et al*., 2013). Lastly, it has been found that light can penetrate to the cellular levels early in embryogenesis and act on cAMP to regulate cell metabolism, which subsequently leads to DNA synthesis. Thus, light may be able to influence gene expression at a very early stage and accelerate the growth process (Cooper *et al*., 2011). The findings of Cooper *et al*. (2011) correlate with the development of lateralization in chicks exposed to light for the first 3 days of incubation vs chicks incubated in the dark or only lit after 18 days of incubation (Chiandetti *et al*., 2013). All of this information illustrates the importance that light can play during the development of a bird.

Lighting during incubation has been shown to impact several aspects of behavior in post-hatch birds, including fear and stress responses (Archer and Mench, 2014a,b). Archer and Mench (2014b) recently showed that broilers incubated in lighted conditions for a minimum of the last 2 weeks of incubation exhibited lower fear response in several fear tests when compared to dark incubated controls. It has been noted that photoperiodic lighting during incubation resulted in birds that adapted more easily to novel environments than their dark incubated counterparts, which may result in better post-hatching development (Ozkan *et al*., 2012a, b). When presented with a stressor, birds that had been incubated in lighted conditions showed a much lower corticosterone response in relation to dark controls (Archer and Mench, 2013). This indicates that the light incubated birds have an overall lower level of stress and may not be impacted to the degree that dark incubated birds are during usual industry handling procedures. It has also been noted that light exposure of eggs during incubation can decrease asymmetry of the birds post hatch, which may indicate that light stimulation can cause a decrease in stress susceptibility later in life (Archer and Mench, 2013).

While it has been shown that light exposure during incubation can increase hatchability and post-hatch behavior and stress susceptibility it is still not known the critical timing of that light exposure. There are several critical time points in the development of the avian embryo that are related to light exposure but the improvement in hatchability, chick quality, fear and stress responses observed in previous studies have not been narrowed down to any of these time points. The objective of this study was to determine if there was a difference in the hatchability, embryo mortality and chick quality of broiler eggs exposed to either 18 or 21 d of light during incubation or no light at all In addition, data was collected to determine if treatments had differential effects on stress, fear and growth as a result of a 45 d grow out. It is hypothesized that eggs incubated under either lighted conditions will result in greater hatchability and lowered stress susceptibility when compared to dark incubated eggs, with possibility that the last three days of light are not needed to see improvements.

MATERIALS AND METHODS

General procedures: Two trials were conducted to investigate the differential effects of providing white LED light for the first 18 days of incubation, the entire incubation, or no illumination during incubation on hatchability, chick quality and post hatch fear, stress and growth of broiler chickens. All methods were approved by the Texas A&M Institutional Animal Care and Use Committee (AUP # 2012-211 and # 2013-0256).

The two trials were conducted using Cobb 500 broiler eggs (N = 3096) from 58 week old breeder flocks. Six GQF 1500 incubators and six GQF 1550 hatchers (GQF Manufacturing, Savannah, GA) were used in each trial and their front windows were blacked out with cardboard to prevent light intrusion into the machines. Two incubators were operated with the traditional dark

method of incubation (0L:24D, DARK), while four others were outfitted with cool white (7500 K) LED strips (Superbrightleds WFLS-X3 Saint Louis, MO; Cool). LED lights were on each level, with 2 strips running the length of the racks. The strips were attached to metal frames, which were in turn attached to the bottom of the rack above them. For the top rack, light strips were held up by a metal frame made to rest on the top rack. The lights were operated by a timer, with a 12L:12D light schedule at 250 lux at egg level as measured using a light meter (Extech 401027, Extech Instruments, Nashua, NH). Two egg trays were set on each rack with each tray holding 43 eggs, for a total of six trays over 3 levels equaling 258 eggs per incubator. The incubators were maintained at standard temperature and humidity levels of 99.5°F and 55% relative humidity. The eggs were incubated for 18 days, at which time they were moved into the hatchers. Two of the hatchers had lights that were outfitted similarly to the incubators, except the metal racks rested on top of each hatch tray instead of being attached to the frame above. Again the lights were kept at a 12L:12D schedule. The other four hatchers had no lights and the eggs were in complete darkness. The eggs were transferred with all room lights off to avoid unneeded light exposure. Each egg was candled with a handheld flashlight and any non-viable eggs were removed and broken out after all eggs were transferred. For each incubator, the number of broken, infertile, early dead, mid dead and late dead eggs were recorded during the breakout. The remaining eggs were incubated in the hatchers for the remaining 3 days of the incubation period. All of the chicks were weighed and counted at hatch. The quality of the live chicks was assessed and they were categorized and counted as either no defect, having an unhealed navel, having leg abnormalities, weak, dirty, having traits a hatchery would cull, or having any other abnormality. The remaining unhatched eggs were broken out and counted as pipped, broken, infertile, early dead, mid dead and late dead.

After hatch analysis, 120 chicks from trial 1 from each treatment were set aside and reared for 45 days. The birds were managed according to the guidelines set forth in the Guide for the Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010). They were housed in pens measuring 1 x 2 meters with 20 birds per pen and placed in a random-block design within the house. They were fed *ad libitum* a standard starter, grower and finisher feed milled at the Texas A and M Poultry Research Center. Water was provided through nipple drinkers. The house was illuminated by incandescent bulbs and dimmed to an average of 20 lux at chick level using a light meter (Extech 401027, Extech Instruments, Nashua, NH) for the first 10 days of growth and then was lowered to 5 lux and set to a 20L:4D light schedule for the entire grow out. All feed was weighed (Ohaus Champ CD-11, Pine Brook, NJ) when added to the feeders and the residual was weighed and subtracted from the total at the end of the 45 day trial to quantify total feed consumed per pen. The chicks were weighed when placed into the pens and at the end of the grow-out. Pen weight and feed conversion ratio was calculated using these numbers.

Fear tests

Isolation: To test the fear response of chicks an isolation test was performed. The isolation tests were performed at 10 days of age by randomly collecting 10 birds from a pen, bringing them to a separate area and placing them in a 133 liter uncovered plastic container. The birds were then individually placed in an unlidded 19-liter bucket. A timer was set for 3 min and the number of vocalizations produced by the bird during this time was counted. Afterward, the bird was placed in a separate holding container. After all 10 birds had been tested, they were returned to their pen and 10 birds from the next pen were collected and tested. More vocalizations were considered to indicate more fearfulness.

Tonic immobility (TI): Tonic Immobility was conducted at 5 weeks of age by again collecting the 10 random birds from a pen, bringing them to a separate area and placing them in a holding container. Methods were modified from previous research by Jones (1986) and Archer and Mench (2014). A 21cm wide by 22 cm high by 30 cm long wooden cradle with the sides sloping out at a 108 degree angle from the base was obtained, covered in a black cloth and placed on a table. Each bird was individually taken and placed on its back in the cradle. The head of the bird was covered with one hand while the breast was held with the other for approximately 15 s to induce tonic immobility, after which time contact was removed and a timer was started. If the bird righted itself in under 15 s, the timer was reset and the above procedure was performed again. If again the bird righted in under 15 s, it was recorded as a time of 0. Otherwise the time of first head movement and time of righting (or attempting to right) was recorded, with a maximum of 10 min. After all 10 birds had been tested they were returned to their pen and the 10 birds from the next pen were collected and tested. Longer times to first head movement and righting were considered to indicate more fearfulness (Jones, 1986).

Stress measures

Physical asymmetry: Physical asymmetry of each bird was measured as per Archer *et al.* (2009) at 45 days immediately after they were euthanized. In brief, using a calibrated Craftsman IP54 Digital Caliper (Sears Holdings, Hoffman Estates, IL), the middle toe length, metatarsal length and metatarsal width were measured for both the right and left legs. The composite asymmetry score was calculated by taking the sum of the absolute

value of left minus right of each trait, then dividing by the total number of traits. Thus the formula for this trial would be $(|L\text{-}R|_{MTL}+|L\text{-}R|_{ML}+|L\text{-}R|_{MW})/3$ = composite asymmetry score.

Corticosterone: At 42 days blood samples were collected from 12 birds per treatment. The area around the wing vein was sanitized with 70% alcohol and in preparation, the inside of a 3 mL syringe was lined with a small amount of heparin. Between 1 to 2 mL of blood were collected from each bird. The blood was injected into a plasma separation gel and lithium heparin vaccutainer (BD 368056, BD, Franklin Lakes, NJ), which was temporarily stored in an ice bath. Once all samples had been taken, the vaccutainers were spun down in a Beckman GS-6R centrifuge (Beckman Coulter, Brea, CA) for 15 min at 4000 RPM to separate the cells from the plasma. The plasma was poured off into 2 mL microcentrifuge tubes and stored at -19°C until further analysis. Plasma corticosterone concentrations were measured using a commercially available ELISA kit (Enzo Life Sciences, ADI-901-097, Farmingdale, NY). The inter and intra-assay %CV were both under 5%.

Statistical analysis: One-way ANOVAs were used to investigate treatment effects on hatchability, embryo mortality, chick quality, composite asymmetry, isolation, tonic immobility, corticosterone, weight gain and feed conversion. The least significant difference test was used to test all planned comparisons. All of the assumptions of ANOVA were tested (Shapiro-Wilk test for normality, Levene's test for homogeneity of variance). No transformations were needed to meet assumptions. All analyses were performed using SAS 9.3 for Windows (SAS Institute Inc.). Significant differences were determined at $p<0.05$.

RESULTS

Hatchability and embryo mortality: There was no effect observed due to incubation conditions on embryo mortality ($p>0.05$, Table 1). There was, however, an effect on the hatchability of fertile eggs ($F_{2,9}$ = 4.02, p = 0.05). The DARK broilers had a lower hatch rate (81.4±0.4%) than both the 18DL (86.1±2.0%, p = 0.03 and the 21DL (85.5±0.8%, p = 0.05) broilers. There were no differences observed between the lighting treatments ($p>0.05$).

Chick quality: There was no effect observed due to incubation conditions on the percent of hatched chicks with leg problems, that were dirty, that were considered cull chicks, or that had other abnormalities ($p>0.05$, Table 1). There was an effect on the percent of hatched chicks with unhealed navels ($F_{2,9}$ = 13.90, p = 0.002). The DARK broilers had a lower percentage of chicks with unhealed navels (50.8±4.0%) than both the 18DL (27.9±3.6%, p = 0.002 and the 21DL (23.8±4.1%, p = 0.001) broilers. There was also an effect on the percent of chicks hatched with no defect due to incubation conditions ($F_{2,9}$ = 13.61, p = 0.002). The DARK broilers had a lower percentage of no defect chicks (46.9±5.2%) than both the 18DL (69.2±3.5%, p = 0.003) and the 21DL (74.0±4.1%, p = 0.001) broilers. The average weight of the chicks did not significantly differ due to incubation conditions with the average chick weight being 47.52±0.49 g. There were no differences observed between the lighting treatments ($p>0.05$).

Growth and feed conversion: There was no effect of incubation condition on 45 d weight gain ($F_{2,15}$ = 1.83, p = 0.19) or 45 d feed conversion ($F_{2,15}$ = 0.02, p = 0.98). The average 45 d weight gain was 2.87±0.06 kg and the average 45 d feed conversion was 1.82±0.04 kg/kg.

Fear response: The number of vocalizations in response to isolation at 10 days of age was affected by incubation conditions ($F_{2,178}$ = 6.24, p = 0.002). The DARK broilers vocalized more (46.15±5.90 vocalizations/3 min) than both the 18DL (25.56±3.37 vocalizations/3 min, p = 0.003) and the 21DL (26.77±3.82 vocalizations/3 min, p = 0.003) broilers. The latency to right during the tonic immobility test was affected by incubation conditions ($F_{2,178}$ = 3.23, p = 0.04). The DARK broilers had longer latencies to right (235.9±28.7 s) than both the 18DL (157.0±23.4 s, p = 0.02) and the 21DL (163.4±20.4 s, p = 0.004) broilers. There were no differences observed between the lighting treatments ($p>0.05$).

Stress response: The composite asymmetry scores were affected by incubation conditions ($F_{2,177}$ = 3.47, p = 0.03). The DARK broilers had higher composite asymmetry scores (2.31±0.24 mm) than both the 18DL (1.83±0.13 mm, p = 0.04) and the 21DL (1.72±0.11 mm, p = 0.01) broilers. Incubation conditions also affected basal plasma corticosterone concentrations ($F_{2,33}$ = 4.85, p = 0.01). The DARK broilers had higher plasma corticosterone concentrations scores (4.02±0.81 ng/ml) that both the 18DL (1.67±0.41 ng/ml, p = 0.02) and the 21DL (1.40±0.67 ng/ml, p = 0.01). There were no differences observed between the lighting treatments ($p>0.05$).

DISCUSSION

The results of this study indicate that the critical time period for providing light to chicken eggs during incubation occurs prior to d 19 of incubation. There were no differences observed between either the 18DL or 21DL treatments in hatchability, chick quality, fear or stress responses; however, both of the lighted treatments showed improvement in all of these categories over the DARK incubated eggs.

Both of the lighting treatments used in this study improve the hatch rate of fertile eggs. These results agree with previous research that also demonstrated that

Table 1: Embryo mortality (%) and chick quality (%) of eggs incubated under 18 days of light (18DL), 21 days of light (21DL) for 12 h a day or no light (dark)

Treat-ment	Early dead	Mid dead	Late dead	Pipped	Un-healed navel	Leg or weak	Dirty feather	Cull	Other	No defect
21DL	6.26	0.60	5.09	2.24	23.76^A	1.06	0.59	0.60	0.00	73.99^A
18DL	5.58	0.62	3.81	3.45	27.86^A	1.34	1.39	0.26	0.00	69.15^A
DARK	7.36	0.71	5.34	3.52	51.24^B	1.50	0.62	0.13	0.12	46.40^B
SEM	1.07	0.30	0.82	0.83	3.83	0.49	0.42	0.20	0.12	3.83

Different letters within column indicate significant differences ($p<0.05$)

hatchability was improved with light exposure during incubation (Huth and Archer, 2015; Shafey and Al-Mohsen, 2002; Shafey, 2004; Veterany, *et al*., 2004). Using LED lights can eliminate the issue of adding heat to the incubators which has been seen to decrease hatchability (Tamimie and Fox, 1967). This increase in hatch rate is not only statistically significant but financially significant to the poultry industry. Improvements of even as little as 1% of hatch could increase revenues substantially. Adding to the increased possible number of chicks this study also observed an increase in chick quality associated with light exposure during incubation. Huth and Archer (2015) also saw this same result not only in broiler chickens but also in layer chickens. The greatest improvement in chick quality was seen in the decrease in unhealed navels when eggs were exposed to light during incubation. This phenomenon can be explained as navel development has been shown to be influenced by light exposure during incubation. This is attributed to the accelerated growth caused by light exposure resulting in improved maturation of the navel over dark incubated eggs (Erwin, *et al*., 1971). While others have seen increased growth in embryos (Siegel *et al*., 1969; Lauber, 1975; Fairchild and Christensen, 2000; Shafey and Al-Mohsen, 2002; Shafey, 2004; Veterany *et al*., 2004) exposed to light during incubation we did not see a difference in embryo weight at hatch. It is possible that this is because previous researchers removed the yolk before weighing the embryos or chicks. We did not do this in this study and it is possible that the yolk weights might differ, which merits future investigation to confirm, but could explain why this study did not see differences in weight of chicks at hatch.

The feed conversion ratio and overall weight gain of the birds was not significantly different between treatments. This is contrary to the results seen by, as has been seen in other light incubation studies (Ozkan *et al*., 2012b; Zhang *et al*., 2012) but consistent with others reporting no changes in performance (Archer *et al*., 2009). This could indicate that differences in post hatch growth rate as a result of lighted incubation may be related to post hatch environmental factors. Possibly, the decreased stress response (discussed below) affects growth more when the environment is more stressful than experienced by the birds in this study.

Both lighting treatments demonstrated a reduced fear response post hatch in all the fear tests used in this study. This agrees with previous research that exposing chicken embryos to light during incubation can reduce fear responses post hatch (Archer and Mench, 2014b; Dharmaretnam and Rogers, 2005). The lighting treatments also resulted in lower stress susceptibility post hatch which again agrees with previous research (Archer *et al*., 2009; Archer and Mench, 2014a; Huth and Archer, 2015). Both of these results demonstrate that providing light during incubation can improve the well-being of poultry post hatch.

Previous research (Archer and Mench, 2014b) concluded in order to reduce fear and stress responses in birds post hatch using lighted incubation that lighting the eggs minimally for the first two weeks of incubation. Archer and Mench (2014b) found that providing light for only the last 7 days of incubation was insufficient to see impartments in fear and stress responses. This does not coincide with the critical time point for visual lateralization (Rogers, 1982) which suggests birds need to be exposed to light on day 18 of incubation. However, more recent research (Chiandetti *et al*., 2013) has demonstrated that light exposure during the first three days of incubation can also result in visual lateralization. By incubating chicken eggs for 18 day with light it is possible to hit both the early and late critical periods demonstrated to exist by both Chiandetti *et al*. (2013) and Rogers (1982) for visual lateralization. This is important as the lateralization of vision in birds is one theorized mechanism for the reduced fear and stress observed in this study and others (Archer *et al*., 2009; Archer and Mench, 2013, 2014b). The physiology of the chicken supports the idea that the critical period (s) fall between 3 and 18 days of incubation. The light sensing opsins of the eye first form on d 14 and complete development on d 18 of incubation (Bruhn and Cepko, 1996). The pineal gland is sensitive to light on d 3 of incubation and the pineal circadian clocks develop on d 18 of incubation (Cooper *et al*., 2011). Hill *et al*. (2004) observed that stimulating embryos for just one day between d 13 and d 18 of incubation set up post hatch rhythms in fear and body temperature. Related to this is the other theorized mechanism for decreased stress and fear response as a result of lighted incubation. Ozkan *et al*. (2012a, b) suggested that melatonin rhythms induced during incubation by periodic lighting could alter the HPA axis and make birds more adaptable to stressors (Ozkan *et al*., 2012a; Ozkan *et al*., 2012b).

Whatever the mechanism it appears that providing light to chicken embryos during incubation can improve hatchability, chick quality and reduce fear and stress responses post hatch in broiler chickens. It also appears that the critical period for improving post hatch fear and stress responses is within the first 18 d of incubation. This fits with previous findings but also is convenient as most commercial hatcheries transfer eggs on d 18 or 19. This means that lighting is only needed in the setters and not the hatchers to obtain the improvements seen in this study. The poultry industry can use lighted incubation as another management technique to improve not only production but animal well-being post-hatch.

ACKNOWLEDGMENTS

Thanks to Jesse Huth and Alyssa Domingue for their help with this study.

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