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Effect of species-specific sound stimulation on the development and hatching of broiler chicks

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Abstract

1. Previous research has reported that chicken embryos develop a functionary auditory system during incubation and that prenatal sound may play an important role in embryo development and alter the hatch time. In this study the effects of prenatal auditory stimulation on hatch process, hatch performance, the development of embryo and blood parameters were investigated.
2. Four batches of Ross 308 broiler breeder eggs were incubated either in control or sound-stimulated groups. Sound-stimulated embryos were exposed to a discontinuous sound of species-specific calls by means of a speaker at 72dB for 16 hours a day: maternal calls from day 10 to day 19 of incubation time and embryo/chick calls from day 19 until hatching. The species-specific sound was excluded in the control group.
3. The onset of hatch (IP) was delayed (P=0.05) in the sound-stimulated group compared to controls. This was also supported by comparison of the exact hatching time of individual focal chicks within the two groups. However, the sound-stimulated embryos had a lower hatchability than the control group, mainly due to significant increased numbers of late deaths (P<0.01). The embryos exhibited a similar growth pattern between the sound-stimulated group and the control group. Although sound exposure decreased body weight at day 16, no consistent effect of sound on body weight at incubation stage was observed. Species-specific sound stimulation also had no impact on chick quality, blood values and plasma corticosterone concentrations during hatch.

Introduction

In nature, a clutch of eggs incubated under the mother hen hatches within a short 'hatch window' (HW), which is defined as the time between the early-hatching and late-hatching chicks. During artificial incubation, maternally-derived components of incubation control and sound communication are excluded. In the industrial hatchery setting, the HW can be as long as 48 h due to differences in genetics and handling (i.e. storage conditions of the eggs before incubation) between batches of eggs. As the spread of hatch increases and thus the HW, the time of first access to feed and water also increases. This delay in access to feed for day-old chicks ultimately impairs post hatch growth (Decuypere et al., 2001, Gonzales et al., 2003, Willemsen et al., 2010).

Several studies report that the auditory development in birds is precocious during incubation (Friauf and Lohmann, 1999, Konishi, 1973, Rubel and Fritzsch, 2002). In domestic chickens, the ontogeny of hearing is thought to begin as early as day 10 of incubation (Alladi et al., 2002). Chicken embryos have been reported to respond to external sound below 90 dB from late day 16 (Jones et al., 2006). Early studies indicated that the specific interactions between hen and embryo take place the day before hatching, by means of vocal communication (Tuculescu and Griswold, 1983, Gottlieb, 1965). Perception of vocalised communication by the embryo may result in physiological and/or behavioural changes. The determination of physiological parameters in relation to vocalised communication, for instance blood values and hormones, can lead to a deeper understanding of how the embryo responds to vocalisation and the significance of this with respect to the well-being of the animal (Manteuffel et al., 2004).

In some avian species, parents exert considerable control (vocalisation, movement and thermal signalling), which minimises developmental and hatching time differences in a clutch

(Reed and Clark, 2011). Research has shown that hen's vocalisations delay internal pipping (IP) when the chick penetrates the air cell membrane of the egg with its beak until most embryos reach the hatching phase well-developed and begin hatching in sync (Greenlees, 1993). In addition to maternal vocalisation, embryo vocalisation plays a role in the synchronisation of hatching (Veterany et al., 1999; Vergne and Mathevon, 2008). Avian embryos produce the first sounds at IP and this true vocalisation via the syrinx gradually develops into a species specific sound (Rumpf and Tzschentke, 2010). In addition, embryos begin to regularly produce clicking sounds at external pipping (EP) due to the egg tooth tapping against the eggshell. Clicks are accompanied by the development of breathing and respiration movements and are not a real vocalisation (Tong et al., 2013). Earlier studies (Vince, 1966; White, 1984) have demonstrated that accelerated hatch during artificial incubation is only in response to clicking sound produced by the embryos and not maternal calls.

The aim of this study was to achieve a delayed and narrowed hatch window through manipulation of maternal and embryo sounds during incubation. We wanted to reveal the underline mechanism via embryonic parameters and hatch related hormone. Hatch window, hatching time of individual chicks, hatchability, body and organ weights, blood values and plasma corticosterone (CORT) concentrations were compared between the sound-stimulated and control groups.

MATERIAL AND METHODS

Incubation and sound protocols

Four experiments were conducted and each experiment consisted of two incubators. In total, 4 batches of fertilised Ross 308 eggs (n = 600 each batch) were obtained from a local supplier (Henry Stewart & Co. Ltd, Lincolnshire, UK). Eggs were incubated in the small custom-built "BioStreamer" incubators (Petersime NV, Zulte, Belgium) under standard incubation conditions with an eggshell temperature of 37.8°C and a relative humidity around 60%.

A background sound of 70 dB, which emanates from the motor and fan, was present in all groups and could not be eliminated. In the control incubator, there was no additional species-specific sound stimulation. In the sound-stimulated incubator, embryos were exposed to pre-recorded files based on natural incubation sounds which were recorded from 9 Ross broody hens at 53 weeks of age and their incubated eggs (Greenlees, 1993). Any effect of the individual incubator was negated by swapping the incubator used between control group and sound group in the four experimental repeats. The sound stimulations were given in two phases. The maternal calls, which are of low-frequency range (500-1000 Hz), were delivered from day 10 to internal pipping (IP) followed by embryo/chick calls, which are of high frequency (2000 – 4500 Hz), from IP until hatch. The one hour maternal sound file was a composition of several call types (cluck sound, beak-clapping and alarm sounds) with 65% silence. The one hour embryo/chick sound file consisted of distress and pleasure calls with 11% silence (Collias, 1987, Wood-Gush, 1971). The auditory stimulation was given at 72dB and over a continuous period of 16 hours per day through a built-in speaker connected to the PC with VLC media player installed.

Monitoring of hatching process

Animal experiments were performed with ethical approval from the Royal Veterinary College Animal Ethics Committee.

The onset of hatch (IP) and the end of hatch were detected and recorded by the incubator controller (Petersime BIO-IRIS^{TM}) which indicates the start and the end of hatching process. The HW of entire batch is defined as the duration between IP and Hatch for each incubation.

In total, 40 focal eggs of each group in four experiments were randomly selected and individually labeled. The focal eggs were placed at fixed location on the tray and after transfer they were placed separately in a specially constructed area (8 x 8 x 8 cm metallic mesh grid) of the top basket. The hatching time of individual focal eggs was determined using an analogue colour video camera (VDC 413, Inter M, Korea) which was attached to the ceiling of the incubator. Additional light (intensity 80 lux) was provided from day 18 of incubation time to ensure a clear view of the baskets. The video image was recorded every 5 minutes for 5 seconds at a frame rate of 1 frame per second (fps) using Milestone surveillance software (NW Systems Group Limited, Scotland). The labeling of hatching time was based on seeing the chick just emerge from the egg and recorded as the incubation time (hours). Twenty out of 40 focal eggs in each group were successfully hatched and hatching times of these focal chicks were determined.

Hatch performance

All eggs were candled at day 18 and those with evidence of a living embryo were transferred from the turning trays to hatching baskets. Both machines were stopped after 512h (21 days and 8 hours) of incubation. Hatchability (the percentage of fertile eggs that hatch), early death (ED) from day 0 to day 7, middle death (MD) from day 8 to day 15 and late death (LD) from day 16 to day 21 were determined at the end of incubation based on breakout results. All

hatched chicks were scored for quality using a standard method (Tona et al., 2003). This method assesses chick quality based on several physical conditions (activity, feather, eye, leg, comb, navel area and remaining yolk) and chicks with full score (100%) were considered as first class chicks.

Embryo and blood parameters

Samples of five eggs or chicks selected randomly from each group were collected at eight incubation stages: day 10, day 12, day 14, day 16, day 18, day 19 (IP), day 20 (external pipping; EP) and day 21. Embryos or chicks were killed and their organs (heart, liver and stomach) were dissected and weighed. Arterialised blood of embryos at d 18, IP, EP and chicks at d 21 was collected from allantoic veins or the left ventricle, respectively. Blood was collected into heparin-coated syringes and 200 µl whole blood was immediately analysed using epoc Portable Blood Gas Electrolyte and Critical Care Analyser (Woodley Equipment Company Ltd, UK) for the blood values including pH, partial pressure of carbon dioxide (pCO2; mmHg), partial pressure of oxygen (pO2; mmHg), bicarbonate (HCO3-; mmol/l), total carbon dioxide (TCO2; mmol/l), base excess (BE; mmol/l), sodium (Na; mmol/l), potassium (K; mmol/l), ionised calcium (iCa; mmol/l), glucose (Glu; mmol/l), lactate (lac; mmol/l), haematocrit (Hct; %) and haemoglobin (Hb; g/dl). The remaining blood was centrifuged at 3000 rpm for 10 min. The plasma was decanted into 1.5 ml tubes and frozen at −20°C for CORT analysis. Plasma CORT was measured using a commercially available double antibody RIA-kit (IDS Ltd, Boldon, England) (Tona et al., 2007)

Statistical analysis

Data were analysed using SPSS (PASW statistics 20) and expressed as mean ± SE of the mean (SEM). Hatch window, hatchability, mortality, chick score and chick weight at the end of incubation were obtained from 4 experiment repeats. Hatching time, embryo and blood parameters were measured from individual chicks from different incubation stages. A linear

mixed model, taking into account sound treatment, incubator and incubation stage as fixed effects, and batch as a random effect, was used to determine the statistical significance between the control and the sound-stimulated groups. When the means of the linear mixed model were statistically different, the means were compared using the least significant difference (LSD) test. Significance was based on $P \leq 0.05$.

Results

Effect on hatch performance

No effect of incubator was observed on hatch performance. The IP and HW detected in groups are presented as the hours of incubation time in the Table. IP occurred in the sound-stimulated group approximately 4 h later than that of the control group ($P = 0.05$). However, there was no statistical difference in HW between the control group and the sound-stimulated group ($P = 0.5$).

Table. Mean (±SE) time of onset of internal pipping (IP) and length of hatch window (HW) in additional natural sound exposed embryos compared to controls

Table 1.

Group	IP[a]	HW (h)
Control	465.3±1.5	27.0±2.0
Sound-stimulated	469.5±1.0	25.5±1.3
P-value	0.05	0.5

[a] *hours of incubation time*

The individual hatching time of 20 focal chicks of each group is shown in Figure 1. The sound-stimulated focal chicks started hatching later than the control focal chicks. However, the average hatching time of the sound-stimulated focal chicks (483.9 ± 1.3 h) was not statistically significantly different from that of the control focal chicks (485.8 ± 1.5 h).

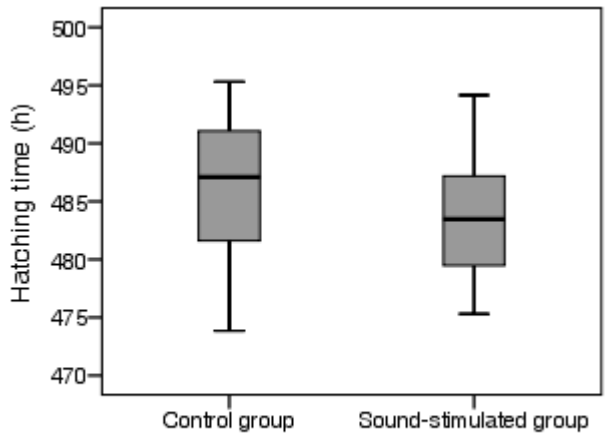

Figure 1. *Boxplot of hatching time of focal chicks in the control group and the sound stimulated group (n=20 chicks of each group).*

The mean values of hatchability, early death, middle death and late death of the control group and the sound-stimulated groups of four repeats are shown in Figure 2. No significant differences in hatchability were detected between groups and incubators. However, significantly higher late death was found in the sound-stimulated group than the control group ($P < 0.01$). No differences in chick quality and chick weight were found between the control and sound-stimulated groups.

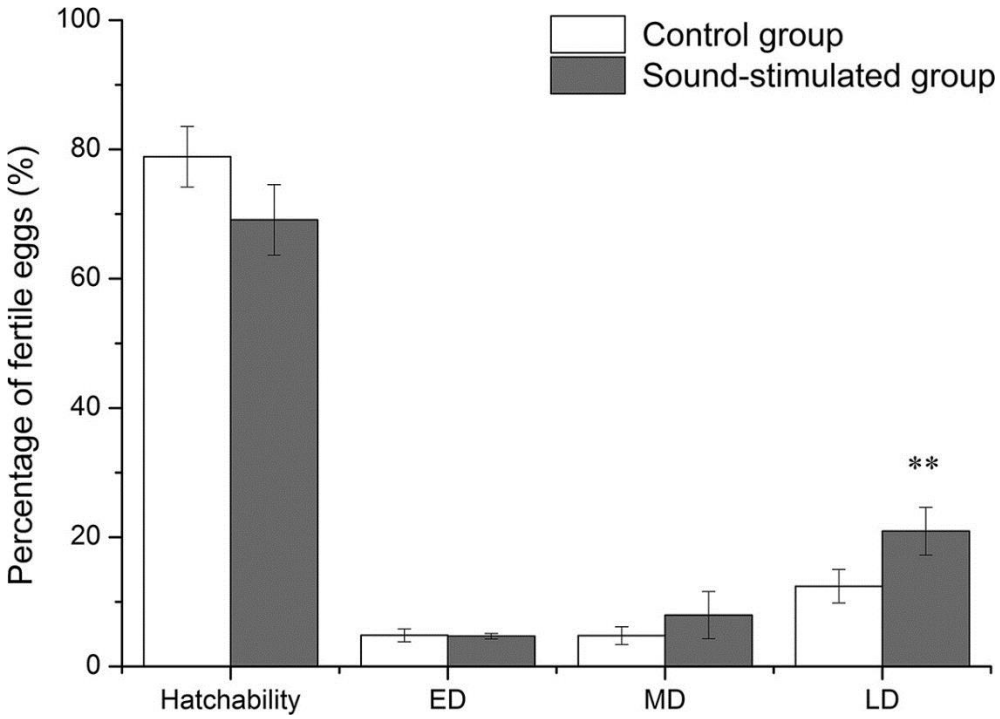

Figure 2. *Mean of hatchability and mortality in the control group and the sound-stimulated group over 4 experiments (n = 1200 eggs at setting of each group, fertility was about 85%–95%). ED: early death, d 0–7; MD: middle death, d 8–15; LD: late death, d 16–21; **values were significantly different between the control and sound-stimulated groups at P < 0.01.*

Effect on embryonic development

The average egg weight at setting in the control group and the sound-stimulated group was 59.7 ± 0.5 g and 59.3 ± 0.5 g, respectively. Because results of the relative body and organ weights were very similar to those of absolute body and organ weights, only the findings of absolute organ weights were compared between different treatments. Except for incubation stage ($P < 0.01$), sound treatment and incubator had no significant effect on body weight and organ weights. In both groups, embryonic body weight increased steadily up to d 19 before plateauing.

Effects on blood values and corticosterone levels

No significant differences in blood values were found between the different sound treatments and incubators (data not shown). Figure 3 shows the general profile of the plasma CORT levels during hatch. There were no significant differences in CORT between control and sound-stimulated groups at the specific time points measured (d 18, d 19, d 20 and d 21). However, within the control group and within the sound group, there were significant differences between the individual time points, and the pattern of these differences was not identical between the groups. In both groups, plasma CORT levels increased significantly ($P < 0.01$) from d 18 (5 ng/ml) and reached a peak at IP (10.5 ng/ml) before dropping to 8.6 ng/ml when chicks emerged from the eggshell at d 20. However, CORT showed slight differences in newly hatched chicks on d 21, being 12.7 ng/ml in control chicks and 10.3 ng/ml in the sound-stimulated chicks; however, this was not significantly different.

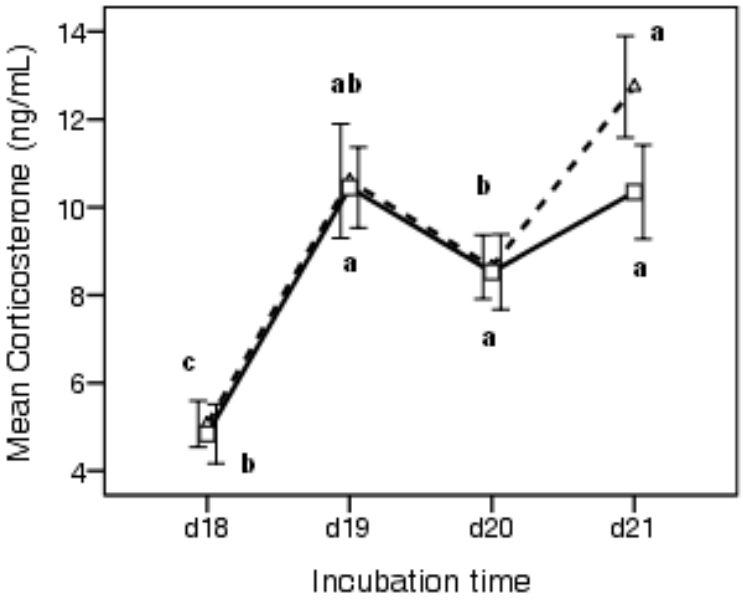

Figure 3. *Effect of prenatal auditory stimulation on plasma corticosterone (CORT) levels during hatch. Each value represents the mean ± SEM (n = 11 to 15). Mean values sharing a superscript letter (a–c) are not statistically different at P < 0.01.*

Discussion

Balaban et al. (2012) found that chick embryos show selective sensitivity to maternal vocalisations before the forebrain becomes active and maternal vocalisation causes the entire brain to become active as an integrated system earlier than expected.

There has been little documentation on how incubating hen sound can affect the hatching of domestic chickens. Greenlees (1993) reported that acoustic enrichment during incubation may be responsible for the initial delay in hatch, but the functional incubating hen calls were unclear. The delayed pipping observed in the sound-stimulated embryos in this study could be due to the exposure of maternal calls before pipping. Hen vocalisations may serve as an evolutionary parenting mechanism to prevent some eggs from needing additional incubation time while hatched chicks were ready to explore. In this study, the sound was switched to embryo/chick vocalisation calls around 469.5 h of incubation. The intent was to synchronise the hatching process when the whole group started pipping. From this stage, embryos were expected to be well developed and able to adjust pipping behaviour due to vocalisation cues. Whereas a shorter HW was achieved via manipulation of real vocalisation, there was no synchronising effect of the embryonic/chick calls. The individual hatching time of focal chicks was not accelerated in the present study, although there are some other embryo sound communications that have been reported to affect hatching synchronisation. Veterany et al. (2005) demonstrated that the onset and time intervals of synthetic pipping sounds have different influences on chicken hatching but without affecting hatchability. It was concluded that the stimulation by a synthetic sound beginning after 433 h of incubation or with a time interval of 176 min resulted in an earlier pipping and shorter hatch window. Furthermore, to achieve acceleration of hatch, the rate and amplitude of artificial click stimulation are also very important (Vince et al., 1976; Ockleford and Vince, 1985) Schwagmeyer et al. (1991)

pointed out that the synchronisation effect due to chick clicking sounds depends on physical contact between siblings in the egg.

A dramatic increase in the plasma CORT concentrations from day 18 to IP was observed in both control group and sound-stimulation group. This confirms the findings of Kalliecharan et al. (1974, 1976) and Scott et al. (1981) that CORT concentrations reach a peak at IP. The CORT profile during hatching did not show any significant difference between treatments, indicating that this sound exposure did not influence plasma corticosterone levels. After hatching, CORT levels increased in both control and sound-stimulated groups. In the control group, the CORT levels of chicks at take-off increased significantly from the external pipping embryos. Furthermore, the plasma CORT levels in control chicks was also higher than sound-stimulated chicks but were not statistically significant which was likely due to limited sample size. The higher CORT levels of control chicks might be due to the early hatch and longer holding period in the incubator.

In this study, prenatal auditory stimulation did not physically improve chick quality and embryonic growth in terms of body weight and organ weight during incubation. However, significantly higher late death was observed in the sound-stimulated embryos.

The causes of increased mortality in sound-stimulated group were unclear. An increased mortality occurred in duck eggs which were incubated under the artificial sound stimulation (Veterany et al., 1999). Stress, caused by prenatal sound, could be a factor influencing mortality. However, there is no evidence that stress was related to this increased rate of embryo death. The timing of presentation of prenatal auditory stimulation and type of sound are important. If the sounds were not in a proper sequence, rate, frequency and duration compared to the natural timing and patterning, it might have a negative impact and cause prenatal stress, which has the potential to impair embryo development and animal well-being. Considering this negative impact on hatchability, further study is needed to identify as to

what degree and via which mechanisms, prenatal auditory stimulation affects hatchability and hatching behaviour.

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