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The Effects of Sound Stimulation During Incubation on Developmental Stability, Performance Traits, and Fear Responses in Japanese Quail

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Abstract

The aim of this study was to determine the effects of different sound stimulant applied to embryos during the incubation on performance characteristics, fear responses, and developmental stability of Japanese quails. For this purpose, a quiet environment, species-specific relaxing sounds, and species-specific disturbing sounds were applied to quail eggs placed in three homologous incubators in different rooms. A constant sound pressure (65 decibels) was used throughout the incubation, with stimulation for two minutes out of every twenty minutes. There were no differences between the experimental groups in terms of hatch weight and weekly body weights. Similarly, sound stimulation had no positive or negative effects on parameters of the Gompertz growth curve and slaughter-carcass traits. The mean values of tonic immobility duration of quails in both the disturbance and comfort group (72.15 and 71.35 sec, respectively) were higher than that of the control group (51.14 sec, P <0.05). Moreover, when the developmental stability of the bilateral features was examined, fewer fluctuating asymmetry cases were detected in the disturbance and comfort groups. As a result, it is possible to say that the sound models applied to the embryos during incubation did not affect the performance characteristics, but had negative effects on developmental stability and fear responses.

Introduction

Domestic fowls are capable of vocal learning and are one of the few groups of animals known to use acoustic communication for territorial possession, mate selection, offspring recognition, alarm signaling, and individual recognition (Rubel and Fritzsch, 2002; Alladi et al., 2002). Most birds produce infrasonic sounds that humans cannot hear, and they are sensitive to sound pressure up to 110 decibels with an ability to hear sounds in the range of 60-11950 Hz at 60 decibels (Brandes, 2008; Tefera, 2012). It is wellknown that the auditory system of poultry develops in the early stages of the incubation period (Rubel and Fritzsch, 2002). The auditory system ontogeny of domestic chickens begins approximately between 10-12 days of incubation, and hatched chicks have auditory abilities similar to adults (Alladi et al., 2002). Compared to humans, chickens are sensitive to lower sound frequencies, and therefore low-frequency communication (usually below 800 Hz) between hen and chick is important. It has been determined that the interaction between the hen and the embryo occurs via vocal communication a few days before hatching (Edgar *et al.*, 2015).

In nature, the hatching window of a group of chicks under a broody hen is quite short. Broody hen applies sound, movement, and thermal stimuli to minimize the differences in the development and incubation time of embryos (Reed and Clark, 2011). In industrial hatching, all environmental conditions are kept constant during incubation, and all maternal effects are excluded. Therefore, the hatching window can last up to 48 hours. This spread in hatching negatively affects the first access to feed and water and the growth characteristics of day-old chicks (Narinç and Aydemir, 2021). For this reason, most of the studies on sound stimulation during the incubation period have been designed to provide hatching synchronization. Recently, many studies have been carried out on bioacoustics in incubation,

sound responses during embryonic development, and indicators and algorithms related to the biorhythm and timings of the hatching process (Veterany *et al.*, 2005; Bamelis *et al.*, 2005; Exadaktylos *et al.*, 2011; Tong *et al.*, 2015).

Few studies have investigated the effects of sound stimulation during embryonic development on yield, behavior, and welfare characteristics in the postincubation period (Tong et al., 2015). The embryo's vocal communication causes physiological and behavioral changes. For example, the determination of physiological parameters related to vocal communication, such as blood values and hormones, responds to vocalization and its importance for the well-being of the bird (Manteuffel et al., 2004). It has been determined that acute noise during incubation increases pessimistic judgment in mature chickens. In enhanced morphological studies, biochemical changes were detected in the chick hippocampus as a result of sound stimulation during hatching using 65-decibel species-specific sounds and sitar music. Also, some spatial orientations and environmental learning were facilitated 12 hours after hatching (Chaudhury et al., 2006; 2009). Tong et al. (2015) investigated the growth performance of stimulated and unstimulated broiler embryos during incubation. They found that the growth performance of stimulated embryos was comparable to that of the control group. According to the findings of researchers, the species-specific sound stimulation had no effect on the hatching window, chick quality (Tona score), blood parameters (pH, partial pressure of carbon dioxide and oxygen, total carbon dioxide,base excess, sodium, potassium, ionised calcium, glucose, lactate, haemoglobin haematocrit,), or plasma corticosterone levels. Kesar (2013) exposed domestic chicken eggs to chronic excessive acoustic stimulation at 110 dB sound pressure (between 30 and 3000 Hz sound frequency) from the 10th day of incubation until hatching. The researchers found a decrease in neuronal nuclear diameter in the MNH region (Medio-rostral neostriatum/hyperstriatum ventral region; a higher auditory association area in chick forebrain), an increase in neuron density, and a decrease in brain weight in the experimental group (Kesar, 2013). It has been argued that fetal chronic exposure to noise may have a disruptive effect on growth and development. The aim of this study was to determine the effects of different sound stimuli applied to embryos during incubation on developmental stability, performance traits, and fear responses of Japanese quails.

Materials and Methods

The study was carried out at Akdeniz University (Antalya-Turkey) and with the decision of the Animal Experiments Local Ethics Committee of Akdeniz

University, dated 28.04.2020 and numbered 128. A total of 390 fertile eggs were obtained from a commercial breeder flock and placed randomly in three identical automatic incubators (130 eggs per each). For the first 14 days, hatching quail eggs were exposed to 37.5 °C and 55% relative humidity, and for the final three days, they were exposed to 37.2 °C and 68% relative humidity. The incubators were in three different soundproof rooms and no sound was applied to the control group. A remote access sound system was installed on the other two machines and constant sound pressure of 65 decibels was provided. In the second incubator, the species-specific sounds made by adult quails in their comfort state were played (comfort trial group). In the third incubator, species-specific sounds made by quails in situations of fear, fighting, and stress were palyed (disturbance trial group). These sounds were played continuously for 2 minutes out of every twenty minutes from the beginning to the end of the embryonic period in the 2nd and 3rd incubators.

The wing numbers were attached to 300 one-day-old quail chicks in the experimental groups after hatching. Before being transported to rearing cages (150 cm²/quail), the chicks were housed for three weeks in five-floor, heated, battery-type brooder cages (82.56 cm²/quail). The quails were fed a diet containing 24% CP and 2800 kcal/kg ME for the first three weeks. From the third week to the end of the trial, a diet containing 21% CP and 2950 kcal/kg ME was applied (NRC, 1994). Feed and water were given *ad libitum* in the study. A 23-hour monochromatic yellow lighting program was applied throughout the study (Narinç *et al.*, 2015).

Weekly body weight data of each quail were used to estimate the growth curves of quails and estimation of parameters were performed individually. The Gompertz function, one of the three-parameter sigmoidal models, was used for the analysis of the growth curve (Alkan *et al.*, 2012).

$$\mathbf{y}_t = \boldsymbol{\beta}_0 e^{\left(-\beta_1 e^{-\beta_2 t}\right)} \left(\mathbf{I}\right)$$

where y_t is the weight at age t, β_0 is the asymptotic (mature) weight parameter, β_1 is the scaling parameter (constant of integration) and β_2 is the instantaneous growth rate (per day) parameter (Narinç et al., 2017). The inflection point of the Gompertz equation, which is an S-shaped model, is realized in the ratio of the value of e of the asymptotic weight. Thus, the coordinates of the inflection point of the Gompertz growth model, the age and weight at the point of inflection (IPA and IPW, respectively), are obtained as follows:

$$IPW = \beta_0/e \text{ (II)}$$

 $IPA = ln(\beta_1)/\beta_2 \text{ (III)}$

On the 21st and 42nd days of the study, tonic immobility durations were measured to determine the fear responses of 20 quails (male and female equally) selected by chance from each group. For the

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application of the tonic immobility test, the expert operator laid the bird on its back on a special device with its head hanging down. The operator placed one hand on the chicken's chest gently and without pressure, and waited for 10 seconds for the bird to immobilize. At the end of 10 seconds, the operator took his hand slowly and measured the immobility time of the bird with a stopwatch. In the measurement of the inactivity time, the maximum value was determined as 300 seconds (Campo and Davila, 2002).

Four bilateral characteristics (face, wing, shank diameter, and shank length) were measured in millimeters by calipers to determine developmental stability in Japanese quail at 56 days of age. The recorded morphological traits were right and left lengths and diameters of the shank (metatarsus), right and left lengths of the wing (radius), and face lengths. For a bilateral characteristic, the difference is determined by measuring the right and left sides. This practice is conducted at the group level; so the bilateral symmetry type is not measured individually. The one sample T-test is utilized to determine whether or not the mean of the group's differences is different from zero. Depending on the sample size, the Shapiro-Wilk or Kolmogorov-Smirnov tests evaluate whether or not the distribution of the group's differences is normal. Based on the results, the group's symmetry type is determined. The symmetry type is characterized as fluctuation asymmetry when the distribution is normal, and the mean is equal to zero. When the distribution is normal and the mean is found to be different from zero, the symmetry type is directional asymmetry. If the distribution of the differences is not normal, the symmetry type is antisymmetry (Campo et al., 2008; Narinç et al., 2016).

Standard slaughtering procedures were applied when the quails were eight weeks old. Feed was withdrawn for eight hours to allow the birds' digestive systems to become empty. Birds were bled from the neck via the jugular vein in an experimental processing plant. The slaughtered birds were kept in 55 °C water for about two minutes to soften the feather bottoms. The feathers were removed using a machine. The viscera and abdominal fat were removed, and rapid cooling was performed. After 24 hours, the carcasses were divided into parts. Cold carcass, breast, leg, and wing yields were calculated according to body weight (Narinç *et al.*, 2013).

Statistical analysis

The nonlinear regression analysis for Gompertz growth curves of birds was performed using NLIN procedure of SAS 9.3 statistics software. The descriptive statistics, Shapiro Wilk normality tests, and hypothesis tests of the traits were obtained using the UNIVARIATE procedure of SAS 9.3 statistics

software. Data of weekly body weights, growth curve parameters and relative asymmetry were analyzed via SAS 9.3 software by analysis of variance comparing treatment groups. When significant, the means were compared by Duncan's multiplerange test.

Results

The average values of body weights of female and male quails at hatch, 42 and 56 days of age, and the results of variance analysis are given in Table 1. As can be seen from Table 1, exposure to different sounds did not affect the mean values of hatching weights of Japanese quails. This situation is similar to the body weights of quails at 42 and 56 days of age, and there is no statistical difference between the experimental groups (P > 0.05 at both ages). In the study, statistical differences were determined between the sexes in terms of mean values of body weight at 42 and 56 days of age. While female quails had higher body weights than males at 42 and 56 days of age (P < 0.05) bring weight data here), the treatment×sex interaction effect was not significant in terms of body weight for all ages (P > 0.05).

The results of the Gompertz growth curve analysis using weekly live weight data of female and male quails in the experimental groups are given in Table 2. The coefficients of determination in all nonlinear regression analyses were found to be between 0.994 and 0.999 (not included in any table). In this case, it was determined that the Gompertz growth curve model was quite sufficient to explain the quail data. The mean values of the mature (asymptotic) weight parameter (β_0) of the Gompertz growth model were estimated as 235.11, 241.35, and 237.82 g for control, disturbance, and comfort groups, respectively (Table 2). The mean β_0 value of the control group was not statistically different from those in the disturbance and comfort groups (P > 0.05). At the same time, the mean value of β_0 parameter of female quails was higher than that of males (P < 0.05). There was no significant difference between the experimental groups (P > 0.05) but between the sexes for the β_1 parameter (P < 0.05), which is defined as the integration constant or maturation rate. The highest mean value (3.97) for the β_1 parameter was found in males in the disturbance group, while the lowest mean value (3.56) was determined in females in the control group. Similar to the other two parameters, there was no statistical difference between the experimental groups in terms of the instantaneous growth rate parameter (β₂) of the Gompertz growth model (P > 0.05). There was no statistical difference between the experimental groups in terms of both inflection point age and inflection point weight averages (P > 0.05). While the inflection point of the sigmoidal curve occurred earlier in males, the females had a higher mean weight at the inflection point (P < 0.05).

Table 1. Live body weight (at hatch, 42 and 56 day) of Japanese quils exposed to sound stimulation during the incubation period

| | | | Live body weight | |
|-----------------|--------|-----------|------------------|----------|
| | | Hatch day | d 42 | d 56 |
| Main effects | | | | |
| Sound treatment | S | | | |
| Control | | 8.71 | 203.63 | 217.18 |
| Disturbance | | 8.87 | 206.77 | 221.31 |
| Comfort | | 8.74 | 207.53 | 220.21 |
| Sex | | | | |
| Female | | 8.76 b | 221.27 a | 238.36 a |
| Male | | 8.78 a | 190.67 в | 200.77 b |
| Interactions | | | | |
| C1 | Female | 8.61 | 217.42 | 233.16 |
| Control | Male | 8.81 | 189.84 | 201.19 |
| D: | Female | 8.97 | 219.31 | 240.10 |
| Disturbance | Male | 8.76 | 194.22 | 202.52 |
| Comfort | Female | 8.72 | 227.10 | 241.83 |
| | Male | 8.76 | 187.96 | 198.59 |
| SEM | | 0.06 | 1.26 | 1.29 |
| P-Value | | | | |
| Treatment | | 0.507 | 0.379 | 0.401 |
| Sex | | 0.893 | 0.000 | 0.000 |
| Treatment × Sex | | 0.336 | 0.067 | 0.172 |

The means with different letters in the same column for the subgroups of main effect or interaction are statistically different (P < 0.05).

Table 2. The mean values of Gompertz growth curve parameters and results of variance analyses from Japanese quils exposed to sound stimulation during the incubation period____

| • | | β0 | β1 | β2 | IPT | IPW |
|------------------------|--------|----------|----------|----------|--------------------|----------|
| Main effects | | | • | • | | |
| Sound treatments | | | | | | |
| Control | | 235.11 | 3.64 | 0.079 | 16.59 | 86.49 |
| Disturbance | | 241.35 | 3.76 | 0.078 | 17.43 | 88.79 |
| Comfort | | 237.82 | 3.68 | 0.078 | 16.89 | 87.49 |
| Sex | | | | | | |
| Female | | 266.54 a | 3.57 b | 0.070 b | 18.32 a | 98.05 a |
| Male | | 209.65 b | 3.82 a | 0.087 a | 15.61 ^b | 77.12 b |
| Interactions | | | | | | |
| Control | Female | 262.18 | 3.55 | 0.073 b | 17.45 | 96.45 |
| Collifor | Male | 208.04 | 3.74 | 0.085 a | 15.73 | 76.53 |
| D:-4 | Female | 269.28 | 3.56 | 0.067 b | 19.10 | 99.06 |
| Disturbance | Male | 213.43 | 3.97 | 0.089 a | 15.76 | 78.52 |
| Comfort | Female | 268.16 | 3.62 | 0.071 b | 18.42 | 98.65 |
| Collifort | Male | 207.48 | 3.74 | 0.086 a | 15.35 | 76.33 |
| SEM | | 1.75 | 0.03 | 0.001 | 0.15 | 0.64 |
| P-Value | | | | | | |
| Treatment | | 0.369 | 0.166 | 0.657 | 0.083 | 0.369 |
| Sex | | < 0.000 | < 0.0001 | < 0.0001 | < 0.0001 | < 0.0001 |
| $Treatment \times Sex$ | | 0.708 | 0.061 | 0.015 | 0.059 | 0.708 |

 β_0 : asymptotic weight parameter, β_1 : integration constant parameter, β_1 : instantaneous growth rate parameter, IPT: Point of inflection time, IPW: Point of inflection weight. The means with different letters in the same column are statistically different (P < 0.05).

The mean values of cold carcass weight, cold carcass yield, and percentages of breast, leg, and wing (% body weight) of quails slaughtered at the age of six weeks in the control, disturbance, and comfort experimental groups, as well as variance analysis results are presented in Table 3. Exposure to different sounds during the incubation period had no effect on

carcass weight, carcass yield, and carcass piece ratios in Japanese quails (P>0.05 in all). While the mean value of carcass weight of female quails was higher than that of males, both the carcass yield and the percentages of all carcass parts except the wing of males were higher (P<0.05). Cold carcass yields of quails varied between 65.72 and 73.72%.

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Table 3. The mean values of the carcass characteristics (%) of Japanese quails exposed to sound stimulation

during the incubation period

| · | | Cold Carcass | Cold Carcass | Breast | Leg | Wing |
|------------------------|--------|--------------|--------------|--------|-------|-------|
| | | (g) | (%) | (%) | (%) | (%) |
| Main effects | | | | | | |
| Sound treatments | | | | | | |
| Control | | 161.34 | 70.61 | 27.41 | 15.88 | 5.32 |
| Disturbance | | 162.14 | 67.93 | 27.10 | 15.27 | 4.98 |
| Comfort | | 157.51 | 69.72 | 26.93 | 15.44 | 5.45 |
| Sex | | | | | | |
| Female | | 164.58 | 66.96 | 26.29 | 15.10 | 5.11 |
| Male | | 156.08 | 71.88 | 28.01 | 15.96 | 5.39 |
| Interactions | | | | | | |
| Ct1 | Female | 162.07 | 68.69 | 26.63 | 15.60 | 5.41 |
| Control | Male | 160.62 | 72.53 | 28.19 | 16.16 | 5.23 |
| D: | Female | 166.47 | 66.47 | 26.13 | 15.23 | 4.99 |
| Disturbance | Male | 157.81 | 69.39 | 28.07 | 15.31 | 4.98 |
| Cft | Female | 165.21 | 65.72 | 26.10 | 14.47 | 4.95 |
| Comfort | Male | 149.81 | 73.72 | 27.75 | 16.40 | 5.96 |
| SEM | | 2.04 | 0.45 | 0.25 | 0.17 | 0.09 |
| P-Value | | | | | | |
| Treatment | | 0.602 | 0.061 | 0.729 | 0.344 | 0.095 |
| Sex | | 0.042 | 0.000 | 0.001 | 0.016 | 0.134 |
| Treatment \times Sex | | 0.389 | 0.053 | 0.949 | 0.072 | 0.019 |

The means with different letters in the same column for the subgroups of main effect or interaction are statistically different (P < 0.05).

The mean values of tonic immobility durations and statistical analysis results of Japanese quails in the experimental groups are presented in Table 4. There is a statistical difference between the experimental groups in terms of tonic immobility duration. While the mean value of tonic immobility

of the quails in the control group was 51.14 seconds, the mean values (72.15 and 71.35 seconds, respectively) of those in the disturbance and comfort groups were higher (P < 0.05). There was no statistical difference between tonic immobility durations of female and male quails (P > 0.05).

Table 4. The mean values of the tonic immobility duration of Japanese quails exposed to sound stimulation during the incubation period

| | | Tonic Immobility Duration (sec) |
|------------------|--------|---------------------------------|
| Main effects | | · |
| Sound treatments | | |
| Control | | 51.14 ^b |
| Disturbance | | 72.15 ^a |
| Comfort | | 71.35 ^a |
| Sex | | |
| Female | | 63.36 |
| Male | | 66.40 |
| Interactions | | |
| G 1 | Female | 45.71 |
| Control | Male | 56.58 |
| D' . 1 | Female | 73.22 |
| Disturbance | Male | 71.09 |
| C f | Female | 71.15 |
| Comfort | Male | 71.54 |
| SEM | | 3.79 |
| P-Value | | |
| Treatment | | 0.033 |
| Sex | | 0.689 |
| Treatment × Sex | | 0.755 |

The means with different letters in the same column for the subgroups of main effect or interaction are statistically different (P < 0.05).

Table 5 shows the symmetry status of the quails in the experimental groups, in which the developmental

stability and the presence of stress were measured in some bilateral features. Fluctuating asymmetry was

determined for the face and shank length and shank diameter of the quails in the control group, and antisymmetry was determined only for the wing length. Fluctuating asymmetry was determined for the two bilateral features (length of face and shank) of the quails in the disturbance experimental group, while anti-symmetry was observed for the other two traits. Fluctuating asymmetry was determined for only one bilateral characteristic (length of the face) of the quails in the comfort experimental group, while antisymmetry and directional asymmetry were observed in the other three traits.

Table 5. The mean values of the asymmetry status in bilateral traits of Japanese quails exposed to sound stimulation during the incubation period

| Treatment | Criterion | Face Length | Wing Length | Shank Length | Shank Diameter |
|-------------|---------------------|-------------|---------------|-----------------|-------------------|
| Control | Shapiro Wilk | 0.694 | 0.008 | 0.940 | 0.634 |
| | One Sample T | 0.097 | 0.742 | 0.455 | 0.357 |
| | Type of Symmetry | Fluctuating | Anti-Symmetry | Fluctuating | Fluctuating |
| | Type of Symmetry | Asymmetry | Anu-Symmeny | Asymmetry | Asymmetry |
| Disturbance | Shapiro Wilk | 0.962 | 0.013 | 0.201 | 0.027 |
| | One Sample T | 0.058 | 0.007 | 0.137 | 0.043 |
| | Trung of Crimmotory | Fluctuating | Anti-Symmetry | Fluctuating | Anti-Symmetry |
| | Type of Symmetry | Asymmetry | Anu-Symmeny | Asymmetry | Anu-symmeny |
| Comfort | Shapiro Wilk | 0.304 | 0.670 | 0.001 | 0.037 |
| | One Sample T | 0.084 | 0.000 | 0.005 | 0.002 |
| | Type of Symmetry | Fluctuating | Directional | Anti Crimmoturi | Anti Crimonosturi |
| | Type of Symmetry | Asymmetry | Asymmetry | Anti-Symmetry | Anti-Symmetry |

Discussion

Most studies on sound stimulation during incubation have been done to reduce the hatching window (Veterany et al., 2005; Exadaktylos et al., 2011). The main purpose of these studies was to synchronize access to food and water and to reduce economic losses. Numerous studies have been conducted on the neurophysiological effects of sound stimulation in incubation (Chaudhury et al., 2009; Kesar, 2013; Sanyal et al., 2013a, 2013b). However, most of these studies are on the follow-up of biological events and not much work has been done to understand the practical implications for the poultry industry (Tong et al., 2015; Veterany et al., 1999; Donofre et al., 2020). Tong et al. (2015) reported that prenatal auditory stimulation did not affect body and organ weight, chick quality, and embryonic growth during incubation. Likewise, Donofre et al (2020) reported that there was no difference in length and weight of embryos exposed to both species-specific sounds and noise at two different decibels. In this study, there was no difference between the experimental groups in terms of body weight values measured at hatch, 42, and 56 days of age, which is consistent with the results reported by Tong et al. (2015) and Donofre et al. (2020). It was determined that the gender effect influenced the mean values of body weight. There is a reverse dimorphism between males and females in Japanese quail compared to other poultry species. In many studies, it has been reported that weekly live weight values and feed consumption characteristics of female quails are higher than males (Narinç and Aksoy, 2012).

There were no statistical differences between the Gompertz growth curve parameters of Japanese quails exposed to different sounds during the

incubation period and those in the control group. According to our knowledge, there is no study on the effect of sound stimulation on growth curve parameters of poultry embryos . Alkan and Birgül (2016) applied high temperature for epigenetic adaptation during early and late embryogenesis periods in broiler chickens. Researchers reported that exposure to heat caused an increase in the β_0 and β_1 parameters, and inflection point weight of the Gompertz growth model. As it is known, high temperature in incubation reduces the incubation period, as a result, chicks hatch before they are fully developed, and compensatory growth can occur in the post-hatch period. However, the effect of exposure to sound on embryo development is not fully known yet. Although there are findings that this application reduces the time of onset of internal pipping and the incubation period (Veterany et al., 1999; Tong et al., 2015), no difference has been reported in terms of chick weights or growth.

In studies in which the growth of Japanese quails was analyzed with the Gompertz model, the mature weight parameter (β_0) was found in the range of 222.10-265.78 g (Akbaş and Oğuz, 1998; Fırat et al., 2016; Narinç and Genç, 2021). In our study, β_0 parameter averages (235.11-241.35 g) obtained from all three experimental groups were found to be compatible with mature weight parameter values reported in the literature. In the current study, the integration coefficient parameter (β_1) of the Gompertz growth curve model for quail growth samples was estimated in the range of 3.64-3.76. The results obtained were found to be compatible with the estimated values (3.40-3.89) by many researchers (Akbaş and Yaylak, 2000; Alkan et al., 2009). In all experimental groups in the study, the average values Şahin et al., 2022 165

of the inflection point age of the Gompertz model were between 16.59 and 17.43 days, and the mean values of body weight at the inflection point were between 86.49 g and 88.79 g. According to the results of many studies in which the growth samples of Japanese quails were analyzed with the Gompertz function, it was reported that the values obtained for the inflection point age of the curve were between 14.76-19.48 days of age, and the weight of the growth curve inflection point was between 76.22-98.45 g (Kaplan and Gürcan, 2018; Narinç *et al.*, 2014; Raji *et al.*, 2014). The values of inflection point age and weight determined for the Japanese quails included in this study were found to be consistent with the averages reported in these studies.

The effect of sound stimulation during the incubation period on carcass weight, carcass yield, and carcass part ratios of Japanese quails was insignificant. In accordance with the results of this study, many researchers (Yalçın et al., 1995; Bonos et al., 2010; Narinç et al., 2014) reported that the carcass yields of Japanese quail were between 68.33-73.00%. Having obtained similar results, Walita et al. (2017) reported that the mean values of breast and leg were 29.1% and 15.9%, respectively. Lotfi et al. (2011) found similar averages to the mean values determined in this study, and the yield of breast and leg were found to be 24.1%, and 14.5%, respectively. In the current study, significantly higher tonic immobility durations were observed in soundstimulated embryos. Surprisingly, these high levels of fear were detected in both the comfort group and the disturbance group. Therefore, stimulating the birds with comfort sounds at the same sound pressure and frequency may have negatively affected the birds' fear responses. Veterany et al. (1999) pointed out that prenatal sound can cause stress in birds. These stimuli can also cause an increase in the level of fear in poultry. To our knowledge, there is no study in the literature examining the effects of sound stimulation in the prenatal period on the fear responses of birds. The mean value of tonic immobility duration (51.14 sec) of quails in the control group was in agreement with those reported by many researchers (Sarica and Özdemir, 2018; Sabuncuoğlu et al., 2018; Flores et

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al., 2019) which range from 38.40 to 64.19 sec. In the current study, the tonic immobility duration of birds were lower than those published by Satterlee *et al.* (1993), Mignon-Grasteau, and Minvielle (2003), Minvielle *et al.*, (2005) who reported tonic immobility of 183.1-201.8 s in Japanese quail. The duration of tonic immobility can vary widely from bird to bird and flock to flock as it is affected by both numerous environmental and genetic factors (Narinç and Genç., 2021).

When the types of asymmetries detected in terms of bilateral symmetrical traits were evaluated, fluctuating asymmetries were observed for three of the four traits in the control group (Table 5). Antisymmetry was detected only for the shank length feature in the control group. Whereas, fluctuating asymmetries were detected for fewer bilateral features in the disturbance and comfort trial groups (two features in the distraction group and only one feature in the comfort group). Tong et al. (2015) reported that the timing of sound application and the type of sound during incubation are important. Researchers have claimed that inappropriate sound stimulation can negatively affect development and create stress. The results of the developmental stability of bilateral traits in the current study support the claim of Tong et al. (2015).

Conclusion

As a result, both sound stimuli (disturbing and relaxing) applied in this study did not affect the live weight, growth curve, and slaughter carcass characteristics of the birds. However, it was determined that the sound stimulation model from the beginning to the end of the incubation (2 minutes of every 20 minutes and constant sound pressure of 65 decibels) prolonged the tonic immobility duration and negatively affected the developmental stability of Japanese quails.

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