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Acoustic features and morphological parameters of the domestic chickens

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ABSTRACT Acoustic characteristics reflect male quality and play a role in female mate choice. Thus, the frequency of vocalizations and temporal characteristics are often related to body size within and across species. However, it is less clear whether acoustic features can reveal information about individual quality in the domestic chicken (*Gallus gallus domesticus*) populations. Here, we investigated the relationship between morphological parameters and acoustic features in male and female free-ranged domestic chickens in Liuzhi, Guizhou, southwest China, and further examined whether acoustic characteristics correlate with internal organs, including the heart, liver, testis, and spleen in male chickens, and whether the cackling call of females indicates body size and mass. We found that both male and female chickens differ significantly in their morphological parameters; however, based on acoustic parameters, they only differ in high frequency. Morphological parameters displayed no relationship with the frequency and duration of calls in both male and female chickens. Furthermore, none of the frequency or temporal parameters of the calls we studied were related to the internal body parameters of males.

Key words: acoustic feature, cackle, domestic chicken, internal body parameter, male quality

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INTRODUCTION

Sound signals play a crucial role in animal communication. As one of the most important aspects of animal communication, sound signals serve numerous functions, including the recognition of individuals and the selection of potential mates (Matyjasiak 2005; Puswal et al., 2022). Acoustic features may provide information regarding the social status, emotional state and quality of vocal individuals during sexual encounters (Otter et al., 1997; Briefer et al., 2015). In addition, birds' acoustic signals can also be used as biomarker of health condition. For example, by analyzing acoustic signals, researchers can develop non-invasive and early detection methods for diseases such as infections and other poultry illnesses (Mahdavian et al., 2021; Tao et al., 2022; Ginovart-Panisello et al., 2024).

Physical differences among signalers can affect vocal characteristics (Mason and Burns 2015). For example, body size is a particularly well-known correlate of several acoustic features in fishes (Balebail and Sisneros, 2022), birds (Marcolin et al., 2022), frogs (Wang et al., 2012) and primates (Fitch, 1997). Body mass in Himalayan leaf-nosed bats *Hipposideros armiger* is linked negatively with minimum frequency and positively with syllable duration (Sun et al., 2021). A recent study on pasture-raised red junglefowls *Gallus gallus* also found that vocalizations of red junglefowls' are indicators of body size and individual health (Hao et al., 2022).

Furthermore, acoustic signals may also provide information about passerines' body size and condition. A study comparing the vocalizations of three Neotropical passerines found that both inter and intraspecific song frequencies were significantly correlated with body sizes (García et al., 2014). In green-winged saltator (*Saltator similis*), acoustic variables such as slope of the first syllable, peak frequency, high frequency of the last syllable, and calling rate act as an honest signal of the body condition of the caller (Lyra et al., 2022). While the relationship between body condition and acoustic features is well-studied, few studies have examined it in different domestic chicken populations (Leonard and Horn, 1995; Asmara et al., 2020a).

Moreover, variation in body size affects the size of the syrinx, which alters the frequency range that an organism can produce (Ryan and Brenowitz, 1985). As the bill is part of the vocal tract, its shape and size also influence sound production and sexual selection (Christensen et al., 2006). For example, Palacios and Tubaro (2000) found that woodcreepers (*Dendrocolaptinae*) with larger bills produce low-frequency sounds (Palacios and

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Tubaro, 2000), while the opposite was found in corvid species (Laiolo and Rolando, 2003). Likewise, Medina−García et al. (2015) found a positive correlation between bill length and fundamental frequency in Neotropical parrots; however, no relationship was found between call features and bill morphology in black-bellied seed-crackers, *Pyrenestes ostrinus* (Slabbekoorn and Smith, 2000). Thus, the effect of bill morphology on song varies greatly among species. Other body parameters, like testis size and eye size, also correlate with call features and body size. Greig et al. (2013) found that species with larger testes mass produced songs with lower frequencies and shorter, faster-repeating notes (Greig et al., 2013). Likewise, studies have demonstrated relationship between eye size, predation risk, and song timing in birds (Thomas et al., 2002; Møller and Erritzøe, 2010); however, the relationship between eye size and the acoustic features of calls is less understood.

In addition, the duration of calls and syllables is also a good indicator of quality and body size in many species, as larger individuals often have larger lungs, which results in longer vocalizations (Fitch and Hauser, 2003; Ey et al., 2007). The duration of loud two-syllable calls within baboon fights is a good indicator of the level of male competitiveness, as shown by the loud "wahoo" calls of high-ranking males (Fischer et al., 2004). A number of studies have demonstrated an association between temporal call features and body size in avian species, but the results are mixed. For example, Zhao et al. (2018) found a positive relationship between body size and call duration in bats (Zhao et al., 2018). An opposite pattern was found in domestic roosters, *Gallus gallus domesticus*, and Pelung chickens (Leonard and Horn, 1995; Asmara et al., 2020a), while no relationship was found between temporal call features and body weight in red junglefowls *Gallus gallus* (Hao et al., 2022).

Domestic chickens derived from red jungle fowls are one of the world's most widely distributed domestic species (Lawal and Hanotte, 2021). They are an excellent source of animal protein and also play a significant role in sociocultural development (Lawler, 2020; Sykes, 2012). Aside from this, the cock's crow call is among the most familiar of all avian vocalizations. Male chickens produce loud vocalizations that usually consist of 4 notes (Miller, 1978). They produce these calls to advertise territory and assert dominance (Joos and Collias, 1953; Leonard and Horn, 1995). In particular call duration, rate and frequency are important factors in determining the quality of a chicken because body weight and other morphological parameters influence call features (Asmara et al., 2020b). Thus, a lot of information about chickens' health can be gained through their sounds (Manteuffel et al., 2004). Likewise, hens typically give cackles after laying eggs (Konishi, 1963). A cackle is a loud, repetitive call consisting of a series of brief elements followed by a final, more extended syllable (McBride et al., 1969). Cackle calls are thought to be used to attract males to escort the hen back to the flock (McBride et al., 1969) or these calls may be followed by copulation (Austin et al., 2021). However, an experimental study conducted in Sweden on domestic fowls found no significant difference in male fowl responses to control and cackle calls (Pizzari and Birkhead, 2001). Further research is needed to understand the exact function of cackle calls.

In this study, we performed a comprehensive analysis of the vocalizations of domestic chickens, male and female. We selected these species because their calls are simple, clear, and easily recognizable on the spectrograms of recordings. This makes them ideal species for testing body conditions and acoustic features. The aim of the study was to address the following questions: 1) Are acoustic features correlate with the body quality and morphology of domestic cocks and hens? 2) Does the size of the heart, liver, and testis affect the vocal features in cocks?

MATERIALS AND METHODS

Study Area and Species

This study was performed on domestic chickens in Liuzhi, Guizhou, southwest China (26°10′N−26°14′ N, 105°13′E−105°24′ E). The total area is about 1800 ha with an average elevation of 1442 m. a. s. l. The climate of the study area is subtropical monsoon, with an average annual temperature of 13.9°C (summer 21°C) and total precipitation of 1,515.5 mm (Liu et al., 2023).

Data Collection

All the data on body parameters and sound recordings were collected from June to August 2022. A total of 18 males and 18 females were selected from the local area for the experiment. After that, we brought the males into the farm house (Figure S1 a), and each male was placed at least two meters apart. The recordings were made using a Sony PCM-A10 (Tokyo, Japan) recorder at a sampling rate of 44.1 kHz on clear, sunny days between 6 am and 2 pm. As part of the recording experiment, the recorder was placed 1.5 meters from the chickens, and every time a cock called, SMP audio tagged it. These tags were later used to identify cocks' calls for acoustic analysis. All recordings were stored as WAV files in secure digital cards. The hens' calls were recorded at different locations where they laid eggs (Figure S1 b).

A total of 25 variables were measured, 13 morphological parameters and 12 internal body parameters. The morphological parameters were divided into the following categories: body weight, body length, wing length, tail length, tarsus length, eye size, bill size, and head size.

Morphological Measurements

The measurements were taken by 2 operators, one holding the bird and the other measuring. Chickens were measured on their right-hand sides for all organ pairs (Francesch et al., 2011). Body weight was measured using a Pesola spring scale in grams. Body length, wing length, and tail length were measured in centimeters with a ruler. Body length was measured from the tip of the beak to the end of the tail (Francesch et al., 2011). The length of the tail was measured from the tip to where it appears from the skin. Later, we converted the value from centimeters to millimeters by multiplying the length by 10. Tarsus length was measured from shinbone to the other end (in mm) (Francesch et al., 2011). The bill length was measured from tip of the beak to insertion into the skull, width (taken across the center of the culmen), and depth (taken through the center of the beak vertically) in mm (Christensen et al., 2006; Favaro et al., 2017). Head length was measured from the occipital bone to the point where the beak enters into the skull, head width at eyes level and head height vertically with caliper (±0.01 mm) (Francesch et al., 2011). Then to calculate the bill and head volume (mm^3) we multiplied the width in millimeters by the length in millimeters by the height in millimeters. Next, to get the data on eye morphology we measured the axial length and width of the eye with a caliper (±0.01 mm). From this, we calculated the eye volume using the following formula:

Eye volume (cm^3) $= 2 \times 1.33\ \pi\ \times a^2\ (cm^2)\ \times b\ (cm)$ where a is the largest and b the smallest radius of the eyes (Garamszegi et al., 2002; Liu et al., 2023). All the morphological measurements were made at the end of the experiment, when each cock finished calling.

Internal Body Organ Measurements

To measure the internal organs, chickens were killed by cutting just below the jaw line into the jugular vein with a sharp knife (Kumar et al., 2023). As a result, the chicken's blood drained and it died immediately. In order to drain all the blood, we left the chicken for a few minutes. We then removed chicken feathers and dissected them to measure their internal organs. Following the dissection process, the heart, testis, spleen, and liver were placed on a piece of white cardboard, and their length and width were measured using a caliper (±0.01 mm). Once the data was recorded, the area of each organ was calculated by multiplying its length and width. The mass of heart, liver, spleen and testis was weighed in grams using a digital weighing scale and the relative weight of organs was calculated by dividing the total body weight of each sampled chicken (Rowe and Pruett-Jones, 2011; Jia et al., 2022).

Acoustic Analysis

The recordings were visualized in Raven Pro 1.5 using Hann windows, 512 FFTs, 50% overlap, and 100% frame size (Bioacoustics Research Program 2014). Calls of male chickens have a simple structure with 4 notes (Figure 1a), whereas female cackle call consists of several brief elements followed by a longer syllable at the end (McBride et al., 1969). In our study, we considered one cackling sound as "buck-buck-buck-badaaack," "buck-buck-buck-buck-badaaack" and "buck-buck-buck-buck-badaaack-buck" (Figure 1b). For each chicken, we selected three good-quality calls with no background noise from the recorded spectrograms. The features of calls were selected by using the time and frequency cursors displayed on the window of Raven software. To study the structure of songs, we noted the minimum, maximum, and peak frequency of calls. Because the lowest call frequency was almost zero for all chickens, we have excluded it from further analysis. To examine the

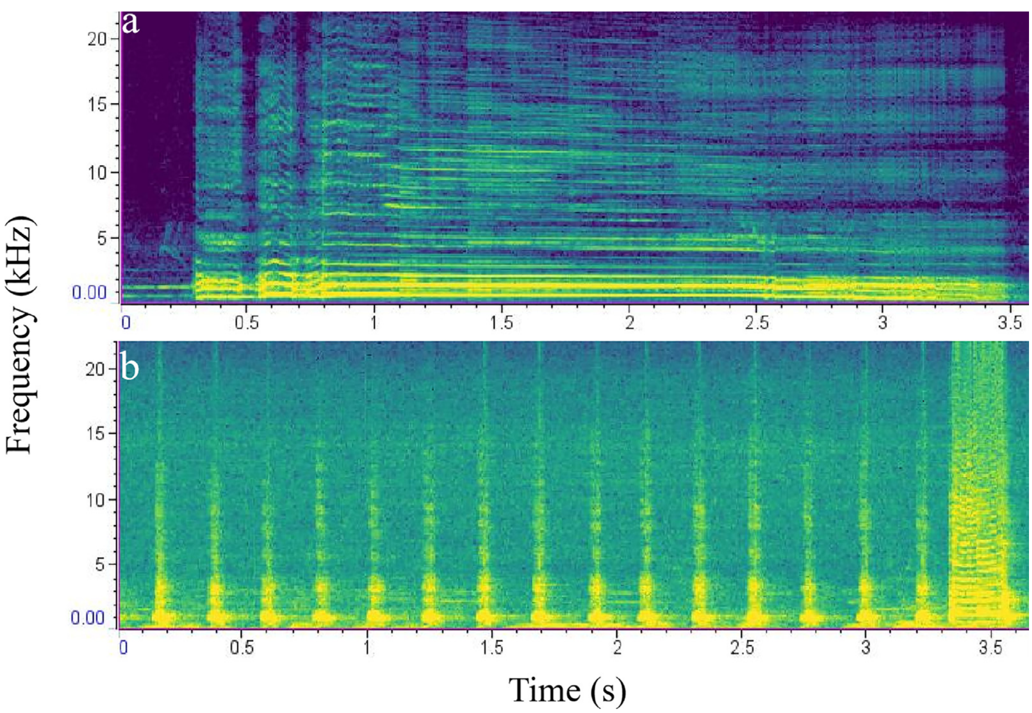

Figure 1. Spectrograms of the calls of domestic chickens. X-axis represent time in seconds while Y-axis shows frequency in hertz. (a) the male chicken call and (b) cackling call of the female chicken.

temporal parameters of calls we calculated the duration of each call in seconds by subtracting start time of the call from the end time of the call. The mean values of duration and frequency characteristics of calls were used for statistical analysis.

Statistical Analysis

All the data were analyzed by using R.V. 4. 2. 2 (R Development Core Team, 2018). Prior to analyses, the data normality was inspected by the residual histogram and normal Q-Q plots. The log 10-transformation was applied to some of the response variables to improve normality and then we applied multivariate and one-way ANOVA tests to examine the morphology and vocalizations of male and female domestic chickens. In the next step, we checked multicollinearity among acoustic variables using the VIF function in the car package (Fox and Weisberg, 2019). The results detected variance inflation factors (**VIF**) less than 1.04 for males and less than 2.04 for females in all multicollinearity models. Since, there was no collinearity we used the original acoustic variables for further analysis. In order to test whether the acoustic features of domestic chicken males and females could affect the morphological and internal body parameters, we used call duration, high and peak frequency as predictor variables and body, heart, liver, testis and spleen mass, body, wing, tail and tarsus length, and beak, eye, head, liver, heart, spleen and testis size as well as the relative weight of organs as response variables in multiple linear regression models.

RESULTS

Male and female domestic chickens differ significantly in several morphological parameters (Manova: F = 62.86, $df = 8, 27$, $P > 0.0001$, N = 18; Table 1). There are significant differences in frequency characteristics (Manova: F = 4.76, $df = 3, 32$, $P > 0.007$, N = 18); however, this variation is noticeable only at high frequencies (see Table 1). There was no significant difference in the duration of chicken calls between males and females (Table 1).

Table 1. Analysis of variance presented as a one-way ANOVA of the acoustic features of male and female domestic chickens (significant results are presented in bold).

	F	*P*
Acoustic features		
High frequency	8.47	**0.006***
Peak frequency	1.82	0.18
Call duration	0.61	0.44
Morphological parameters		
Body weight	0.03	0.84
Body length	14.61	**0.0005***
Wing length	21.61	**0.00005***
Tarsus length	23.26	**0.00002***
Tail length	10.1	**0.003***
Eye size	2.65	0.11
Head size	15.45	**0.0003***
Bill size	121.8	**0.0002***

The mean duration of chicken calls was 2.79 s in males and 2.56 s in females, respectively.

Acoustic Features and Morphological Parameters of the Male and Female Chickens

Based on multiple regression models, there were no significant correlations between acoustic parameters and body weight and length ($F_{3,14} = 1.07$, adjusted $R^2 = 0.01$, $P = 0.39$; $F_{3,14} = 0.91$, adjusted $R^2 = -0.01$, $P = 0.45$), respectively, in male chickens (Figure 2). A similar pattern was observed in female chickens (Body weight: $F_{3,14} = 1.47$, adjusted $R^2 = 0.07$, $P = 0.29$; Body length: $F_{3,14} = 0.79$, adjusted $R^2 = -0.03$, $P = 0.51$: Figure 3). The results indicated that acoustic parameters were not related to (wing length: $F_{3,14} = 0.72$, adjusted $R^2 = -0.05$, $P = 0.55$; tail length: $F_{3,14} = 1.75$, adjusted $R^2 = 0.11$, $P = 0.20$; and tarsus length: $F_{3,14} = 0.80$, adjusted $R^2 = -0.03$, $P = 0.50$) in both male (wing length: $F_{3,14} = 0.77$, adjusted $R^2 = -0.04$, $P = 0.52$; tail length: $F_{3,14} = 1.03$, adjusted $R^2 = 0.005$, $P = 0.40$; and tarsus length: $F_{3,14} = 0.67$, adjusted $R^2 = -0.06$, $P = 0.58$;) and female chickens, respectively (Figures 2 and 3). Likewise, no significant association was found between acoustic parameters and beak size ($F_{3,14} = 1.79$, adjusted $R^2 = 0.12$, $P = 0.19$; $F_{3,14} = 0.23$, adjusted $R^2 = -0.15$, $P = 0.87$), eye size ($F_{3,14} = 1.04$, adjusted $R^2 = -0.007$, $P = 0.40$; $F_{3,14} = 1.008$, adjusted $R^2 = 0.001$, $P = 0.41$) and head size ($F_{3,14} = 1.05$, adjusted $R^2 = -0.009$, $P = 0.39$; $F_{3,14} = 0.60$, adjusted $R^2 = -0.07$, $P = 0.62$) of male and female chickens, respectively (Figures 2 and 3; Table 2 and Table 3).

Acoustic Features and Internal Parameters of Male Chickens

Multiple linear regression analysis indicated that there was no significant relationship between call features and testis size and mass ($F_{3,14} = 0.79$, adjusted $R^2 = -0.03$, $P = 0.51$; $F_{3,14} = 0.27$, adjusted $R^2 = -0.14$, $P = 0.84$), heart size and mass ($F_{3,14} = 1.86$, adjusted $R^2 = 0.13$, $P = 0.18$; $F_{3,14} = 1.29$, adjusted $R^2 = 0.05$, $P = 0.31$), liver size and mass ($F_{3,14} = 0.28$, adjusted $R^2 = -0.14$, $P = 0.83$; $F_{3,14} = 1.01$, adjusted $R^2 = 0.002$, $P = 0.41$), spleen size and mass ($F_{3,14} = 0.26$, adjusted $R^2 = -0.14$, $P = 0.85$; $F_{3,14} = 1.01$, adjusted $R^2 = 0.002$, $P = 0.41$), respectively (Table 4). These results suggest that there is no association between call features and the size and mass of the organs studied. The relative weight of organs was also non-significant for all the acoustic features of male and female chickens (Figure S2−S5 and Table S1).

DISCUSSION

We investigated the morphological correlates of call features in domestic chickens, both males and females. We discovered that male and female chickens have substantial morphological differences. Based on the acoustic

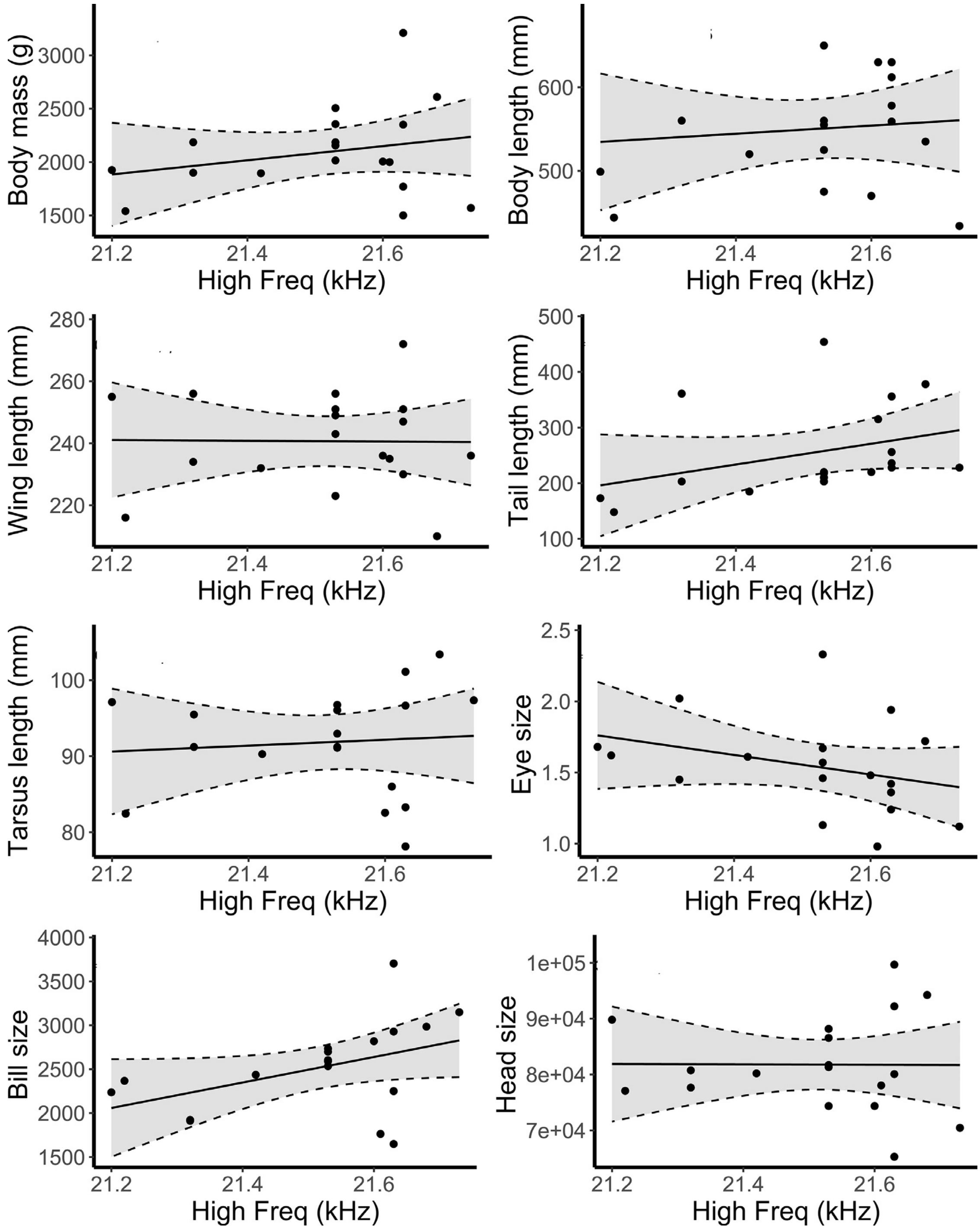

Figure 2. Relationship between morphological parameters and the high frequency of calls in male chickens. The X-axis indicates the frequency in Hz, and the Y-axis represents the body parameters of male chickens. The line indicates the linear regression between two variables, and the shaded grey area represents the 95% confidence interval.

characteristics, they only differ in high frequencies of the call. Furthermore, the calls of male chickens were not related to any of the morphological parameters, nor were internal body parameters affected by them. For females, this finding was also non-significant.

Animals use acoustic signals to attract mates and defend territories (Puswal et al., 2021; Mei et al., 2022). Nevertheless, mating does not occur randomly, and certain behavior patterns play an important role in sexual selection, including the frequency of calls, their duration, and the complexity of songs (Hedrick, 1986). Earlier studies suggested that these characteristics change with body size, age and weight (Ey et al., 2007; Taylor and Reby, 2010). In red deer (Cervus elaphus), large males produced low-pitched roars with a high roaring rate, and females were more attracted to these low-pitched roars, which had a better reproductive success (Reby and McComb, 2003; Reby et al., 2010). Hao et al. (2022) found a positive relationship between frequency parameters and body size in *Gallus* adults, where frequency was relatively higher in larger individuals. In our study, we did not find any significant relationship between call

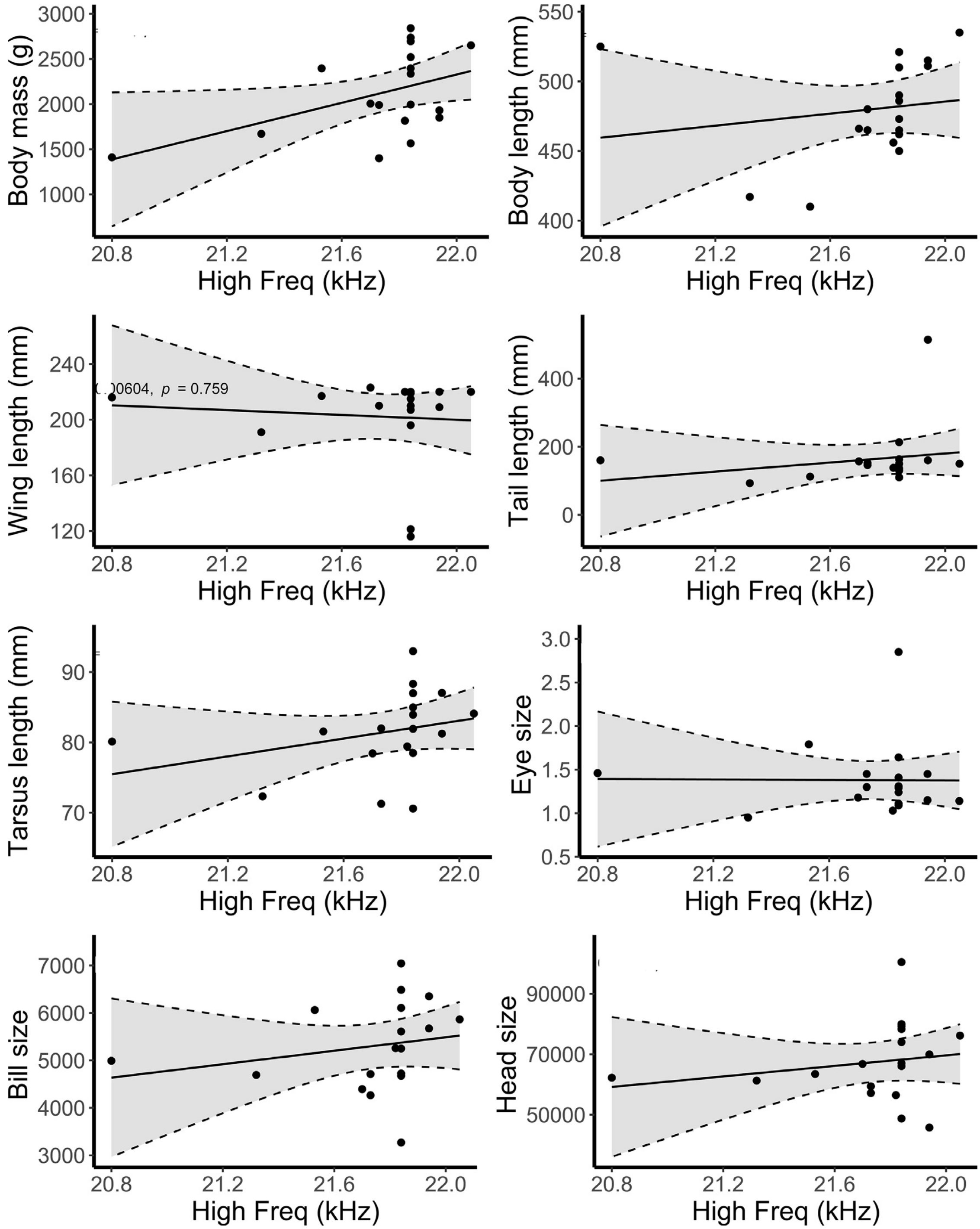

Figure 3. Relationship between morphological parameters and the high frequency of calls in female chickens. The X-axis represents the frequency in Hz, and the Y-axis shows the body parameters of female chickens. The line indicates the linear regression between two variables, and the shaded grey area represents the 95% confidence interval.

features and body weight, size, wing length, tail length and tarsus length in male and female chickens. There could be differences in the results of studies due to differences in the timing of recordings. For example, if recordings are made during different times of the day or under different weather conditions, this could affect how the signals propagate and detected (Henwood and Fabrick, 1979; Larom et al., 1997). The difference in habitat can also significantly impact acoustic signals (Slabbekoorn et al., 2002). For example, Morton (1975) found that edge bird sounds have a high variance in the frequency-emphasized component, while grassland bird sounds have a positive correlation between increasing frequency and increasing attenuation (Morton, 1975). Furthermore, in prairie warblers, element rate, number of elements and frequency of songs were correlated both with the female mate choice and male quality (Byers et al., 2015; 2016). It is possible that chickens do not recognize individuals based on sounds, and that call features have no effect on mating success (Hao et al., 2022).

Given the size of the vocal organ, the most accurate prediction relating frequency to body size involves the

Table 2. Multiple regression of acoustic features with morphological parameters of male domestic chickens (significant results are presented in bold).

Response	Predictor	Estimate	SE	*t*	*P*
Body weight	Intercept	−10270	13990		
	Peak frequency	0.26	0.27	0.93	0.36
	High frequency	0.58	0.64	0.91	0.37
	Duration	−203.9	214.8	−0.94	0.35
Body length	Intercept	−120.25	2341.30		
	Peak frequency	−0.04	−0.04	−1.04	0.31
	High frequency	0.03	0.10	0.36	0.71
	Duration	−50.01	35.94	−1.39	0.18
Wing length	Intercept	349.74	537.20		
	Peak frequency	0.0007	0.01	0.67	0.51
	High frequency	−0.004	0.024	−0.16	0.87
	Duration	−9.51	8.24	−1.15	0.26
Tarsus length	Intercept	0.423	238.2		
	Peak frequency	0.007	0.004	1.51	0.15
	High frequency	0.003	0.011	0.34	0.73
	Duration	0.86	3.657	0.23	0.81
Tail length	Intercept	−3408	2600		
	Peak frequency	−0.072	0.051	−1.39	0.18
	High frequency	0.179	0.12	1.49	0.15
	Duration	−47.77	39.91	−1.19	0.25
Eye size	Intercept	17.92	11.19		
	Peak frequency	−0.00009	0.0002	−0.40	0.69
	High frequency	−0.01	0.02	−0.75	0.46
	Duration	−9.20	7.34	−1.25	0.23
Head size	Intercept	123019.07	289764.1		
	Peak frequency	7.48	5.76	1.29	0.21
	High frequency	−1.70	13.40	−0.12	0.90
	Duration	−4175.2	4448.8	−0.93	0.36
Bill size	Intercept	−28120	16330		
	Peak frequency	0.40	0.325	1.26	0.22
	High frequency	1.409	7.55	1.86	0.08
	Duration	−26.91	250.7	−0.10	0.91

lowest frequency (Bradbury and Vehrencamp, 1998). However, in our study there was no obvious difference in minimum frequency of chickens so we have excluded it from analysis. This explanation also supports the findings of an earlier study which found a positive relationship between body size and frequency parameters. Furthermore, small variation in chicken sizes may explain the nonsignificant results. Because differences in size among conspecifics are generally small, and so are the predicted differences in frequency (Cardoso et al., 2008; Patel et al., 2010). For instance, a correlation between frequency and size is found only when males and females are analyzed together, but not when each sex is examined separately, where size variation is lower (Ey et al., 2007). As it turned out in our case, there was a significant difference in the high frequency of calls between males and females (Table 1). Additionally, in birds body weight could also influence parents' foraging behavior, and heavier individuals appear to be better at feeding their chicks properly (Saraux et al., 2011). This might be the reason that in birds mate choice is influenced by the factors that are linked with body weight and size (Byers et al., 2016; Wang et al., 2019). However, this may not be true for domestic chickens.

Previous studies examined the relationship between bill size and acoustic features and indicated that song is a reliable indicator of bill morphology and vocal performance predicts male pairing success (Christensen et al.,

Table 3. Multiple regression of acoustic features with internal body organs of male domestic chickens (significant results are presented in bold).

Response	Predictor	Estimate	SE	*t*	*P*
Testis size	Intercept	5368.49	23790.1		
	Peak frequency	−0.16	0.47	−0.35	0.73
	High frequency	−0.10	1.10	−0.09	0.92
	Duration	−564.24	365.25	−1.54	0.14
Testis mass	Intercept	19.2	176		
	Peak frequency	0.002	0.003	0.59	0.55
	High frequency	−0.0001	0.008	−0.01	0.98
	Duration	−1.48	2.7	−0.54	0.59
Heart size	Intercept	4679.4	11577.2		
	Peak frequency	0.27	0.23	1.19	0.25
	High frequency	−0.012	0.53	−0.22	0.82
	Duration	−314.8	177.7	−1.77	0.09
Heart mass	Intercept	14.82	140.4		
	Peak frequency	0.003	0.002	1.16	0.26
	High frequency	0.0001	0.006	0.02	0.97
	Duration	−2.86	2.15	−1.33	0.2
Liver size	Intercept	1631.8	46744.8		
	Peak frequency	−0.50	0.93	−0.54	0.59
	High frequency	0.42	2.16	0.19	0.84
	Duration	−575.1	717.6	−0.80	0.43
Liver mass	Intercept	−415.6	404.8		
	Peak frequency	0.005	0.008	0.69	0.49
	High frequency	0.02	0.01	1.16	0.26
	Duration	−5.22	6.21	−0.84	0.41
Spleen size	Intercept	−4598.8	10422.5		
	Peak frequency	0.12	0.20	0.59	0.56
	High frequency	0.23	0.48	0.47	0.64
	Duration	95.66	160.02	0.59	0.55
Spleen mass	Intercept	−104	124.3		
	Peak frequency	0.0002	0.002	0.08	0.93
	High frequency	0.01	0.05	0.86	0.4
	Duration	1.24	1.90	0.60	0.52

Table 4. Multiple regression of acoustic features with morphological parameters of female domestic chickens (significant results are presented in bold).

Response	Predictor	Estimate	SE	*t*	*P*
Body weight	Intercept	−14,660	12630		
	Peak frequency	−0.25	1.15	−0.22	0.82
	High frequency	0.78	0.55	1.43	0.17
	Duration	−34.85	111.2	−0.31	0.75
Body length	Intercept	−159.07	1024.28		
	Peak frequency	−0.003	0.09	−0.35	0.72
	High frequency	0.03	0.04	0.72	0.48
	Duration	−9.42	9.02	−1.04	0.31
Wing length	Intercept	−272.66	915.09		
	Peak frequency	0.03	0.08	0.42	0.67
	High frequency	0.02	0.03	0.53	0.59
	Duration	−11.84	8.06	−1.47	0.16
Tarsus length	Intercept	−26.21	173.12		
	Peak frequency	−0.007	0.01	−0.5	0.62
	High frequency	0.005	0.007	0.71	0.48
	Duration	−0.44	1.52	−0.29	0.77
Tail length	Intercept	1,125.09	2595.4		
	Peak frequency	−0.18	0.23	−0.77	0.45
	High frequency	−0.03	0.11	−0.34	0.73
	Duration	34.91	22.86	1.52	0.14
Eye size	Intercept	626.20	522.9		
	Peak frequency	−0.07	0.04	−1.65	0.12
	High frequency	−0.01	0.02	−0.86	0.40
	Duration	0.06	4.60	0.01	0.98
Head size	Intercept	−158266.7	379723.5		
	Peak frequency	−11.53	34.87	−0.33	0.74
	High frequency	11.27	16.56	0.68	0.50
	Duration	−2756.08	3344.61	−0.82	0.42
Bill size	Intercept	−12130	28570		
	Peak frequency	0.10	2.62	0.04	0.96
	High frequency	0.80	1.24	0.64	0.53
	Duration	−37.05	251.7	−0.14	0.88

2006; Gémard et al., 2019). Conversely, in our study, we did not find any relationship between bill size and call features in domestic chickens. A possible explanation for this contrasting finding is that bill morphology may be more important in filter feeding birds such as blue petrels (*Halobaena caerulea*), where it impacts feeding efficiency (Gémard et al., 2021; Klages and Cooper, 1992). Domestic chickens are omnivorous, and their diet is more varied than blue petrels. Thus, they may not rely on bill morphology for feeding efficiency as much as petrels do. Nonetheless, some other studies also did not find a significant relationship between bill measurements and song frequencies (García and Tubaro, 2018), suggesting that small variations in bill sizes could also influence results. Furthermore, selective pressure for enhanced transmission in the habitat where acoustic signals are produced and received may also influence their structure (Morton, 1975). For example, Derryberry (2009) found that vegetation density and bill size explained significant variation in the song structure of white-crowned sparrows (*Zonotrichia leuco phrys*) (Derryberry, 2009).

It is well known that birds have larger eyes compared to their body sizes (Brooke et al., 1999). The relative size of an organ may reflect its functional significance, adaptations to the environment, or constraints to adaptation caused by genetic correlations with other characters or recent changes in the environment (Harvey and Krebs, 1990; Møller et al., 1998). For example, body reserves enable individuals to survive during food shortages, while vision is important to detect predators and forage (Reinhardt, 2002; Aubret and Bonnet, 2005). Therefore, the 2 physiological variables influence foraging decisions suggesting that sight is critical to the survival of animals. In our study, we found that eye size did not show a significant relationship with any of the studied call features. A possible explanation is that eye size may be more important for species living in dense habitats, but not for chickens living in open habitats, as a recent study indicated that avian eye size is primarily influenced by light availability, food need, and cognitive ability (Liu et al., 2023). Lastly, internal structures such as the testis have also been reported to be good predictors of bird song and male quality (Greig et al., 2013). The parameters we selected, however, did not appear to be related to call parameters, therefore, it is unclear whether they are indicators of chicken body size.

To sum up, acoustic signals may be more critical for species that live in dense habitats where visual acuity is low, so they rely on them for mating success. For example, monarcha flycatchers use acoustic signals for recognition in dense habitats whereas they use other visual signals in open habitats (Uy and Safran, 2013). In addition, the non-significant results could also be due to the small sample size. Furthermore, the habitats in which birds communicate also affect the frequency and structure of song, imposing different sources of attenuation and degradation depending on their specific characteristics. There is a need for further comparative analysis with larger sample size from different habitats to find out whether acoustic signals serve as indicators in domestic chickens and whether or not they influence sexual selection.

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Ethical Approval: No research permit was required for this study. The experiments comply with the current laws of China. All procedures were approved by the Animal Research Ethics Committee of Hainan Provincial Education Centre for Ecology and Environment, Hainan Normal University (permit no. HNECEE-2020-005).

Data Accessibility: Data used for this study was provided as supplementary material (Figure S1-S5, Table S1 and Data Table S2).

Author Contributions: WL designed the study, SMP carried out field experiments, performed the analyses and wrote the draft manuscript, and WL edited the manuscript. All authors approved the final submission.

DISCLOSURES

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2024.103758.

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