vocalization

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Vocalization Characters and Forkhead Box P2 (FoxP2) Polymorphism in Indonesian Crowing-Type Chicken (*Gallus gallus domesticus*)

Research Article

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ABSTRACT

Crowing-type chicken is one of the important chicken breeds in Indonesia. This study was conducted to investigate the crowing characters of Pelung chicken and the effect of exon 7 *FoxP2* gene polymorphism in mute chicken. Chicken were obtained from local breeder. Pelung chicken crowing and morphology characterizations were conducted in Cianjur, West Java, Indonesia. Chicken breeding (♀ broiler×♂ Pelung and ♀ Pelung×♂ F_1), DNA extraction and *FoxP2* gene amplification were conducted at the Faculty of Biology, Gadjah Mada University. Results showed that the crowing duration was approximately 8.435 ± 1.647 s, consisting of 1.104 ± 0.210 s first syllable, 5.532 ± 1.274 s second syllable, and 1.858 ± 0.969 s third syllable. Cross-breeding between Pelung and broiler chicken resulted in chicken progeny all of which were of non-crowing-type. Our breeding results indicated that long crowing traits followed recessive autosomal inheritance. Sanger sequencing revealed an identical exon 7 sequence in mute and normal crowing-type chicken. Therefore, crowing is an important character for determining chicken breed purity, and our bioacoustic analysis was applicable in chicken show.

KEY WORDS bioaccoustic, crowing-type chicken, FoxP2, Pelung chicken.

INTRODUCTION

Indonesia is known to have a high diversity of native chicken breeds, including five crowing-type chicken breeds (three pure and two hybrid). The three pure breeds consist of Pelung chicken from Cianjur district, West Java Island; Kokok Balenggek chicken from Solok district, West Sumatra Island; and Gaga chicken from Sidrap district from south Sulawesi Island, and the two hybrid chicken consist of Bekisar derived from the green junglefowl (*Gallus varius*) and the domestic hen and Burgo derived from the red junglefowl (*G. gallus*) and the domestic hen crossbreed (Figure 1).

Based on the variations in the D-loop mtDNA sequence, it has been reported that these breeds could have possibly descended from multiple ancestors (Ulfah *et al*. 2016; Ulfah *et al*. 2017). Among the crowing-type chicken, the Pelung chicken has a very high economic value.

In general, there are 30 sounds in chicken bioacoustics, of which 19 can be interpreted (Tefera, 2012). Chicken sounds can be understood as warning sign, alarm, contact, territorial, laying, nesting, mating, threat, submissive, distress, fear, contentment, indicating food, dust bathing, perching, battle cries, privacy, dominance and time calls (crowing), and short sentences (a combination of two and three syllables/cackle).

Chicken bioacoustics has various applications in poultry such as in predicting the growth rate (Fontana *et al*. 2017). Bioacoustics of the crowing type chicken has been intensively studied in Indonesia (Ulfah *et al.* 2016; Ulfah *et al.* 2017). In this research, the crowing comparisons between pure breed and crossbred chicken using bare ear have revealed visible differences. These results indicate that the crowing sound characteristic can possibly be used as a phenotype marker for determining chicken breed purity. The adult male Pelung chicken produces very long, melodious, repeated and loud crowing sound compared to that produced by kampung and broiler chicken, the so-called crowing trait. Characterization of chicken vocalization, especially crowing, is prominent in popular chicken shows. Standardization of excellent chicken crowing sound would minimize the subjectivity of the jugde during the contest. This could at least help to eliminate the nonstandard Pelung breed during the initial phase of the contest (Ulfah *et al.* 2016). Internal factors that might contribute to the crowing quality include genetics, age, weight, imprinting process, and health status. The external factors include weather condition, nutrition, and environment cleanliness (Ulfah *et al.* 2016).

In molecular biology, Forkhead box P2 (FoxP2) is the most intensively studied gene that is responsible for animal vocalization (Lai *et al*. 2001). The FoxP2 protein is a transcriptional factor for a large number of genes, which is conserved in several taxa. Most of these genes are involved in neuronal cell development and plasticity (Vernes *et al.* 2011). In humans, an arginine-to-histidine substitution (R553H) on the forkhead DNA-binding domain FOXP2 is closely related to dyslexia, the vocalization disorder (Vernes *et al.* 2008; Konopka and Roberts, 2016; Lai *et al.* 2001; Nudel and Newbury, 2013). Another variant, R328X at exon 7, is also associated with KE family's dyspraxia syndromes (Hurst *et al.* 1990). In R328X, cytosine (C) to thimine (T) substitution alter arginine (R) to a premature stop codon (X) at the amino acid sequence number 328 of the FoxP2 protein (MacDermot *et al*. 2005).

In mouse studies, various FoxP2 synthesis manipulation attempts failed at the age of 3 weeks, whereas the heterozygotes exhibited developmental delays (French and Fisher, 2014). These results suggest that FoxP2 protein is involved in not only vocalization. In the bat relative of the mouse, conventional Sanger sequencing elucidated the high variability region of exons 7 and 17 in comparison with other mammals (Li *et al*. 2007). More recent whole-genome sequencing demonstrated higher divergence in exon 3 of FoxP2 in *Myotis davidii* than that in the mammalian consensus sequence (Zhang *et al*. 2013).

In an avian species model of zebra finches, lentiviral shRNA-mediated knock down of *FoxP2* gene resulted in difficulty in developmental and social modulation of song variability (Murugan *et al*. 2013) in both juveniles and adults. Since zebra finches are song-learning species, difficulty or alteration in vocalization can obviously be found. Another study in chicken reported higher expression of corpus striatum FoxP2 mRNA in adult males than in females and juveniles. Comparison of FoxP2 mRNA expression levels among different chicken breeds revealed that the expression patterns in each group (males, females, and juveniles) were not statistically different (Wang *et al*. 2012).

This finding implies the importance of FoxP2 in chicken. In this study, we attempt to describe the pattern of vocalization using bioaccoustic software and the polymorphism of FoxP2 sequence using sanger sequencing in crowing-type chicken in Indonesia.

MATERIALS AND METHODS

Animal material

In this research, 77 adult male pelung chickens were obtained from several local breeders in Cianjur District, West Java, Indonesia. For sanger sequencing, DNA samples were isolated from whole blood collected from three pelung chicken and two broiler chicken. Blood collection was done by professional veteriner.

Phenotyping

A population survey was done in 2018 in Cianjur District, West Java, Indonesia. The quality of crowing voice of adult male chicken was ensured by a trained Pelung chicken fancier, and the crowing voice was recorded using a voice recorder (Sony ICD-UX533F) and then stored in an uncompressed lossless audio (.wav) format. Based on our optimation, the best crowing voice was obtained by recorded 0.5 m away from the chicken cage. For each chicken, crowing voice was recorded five times. The crowing voice was analyzed using the Adobe Audition CS5.5 (Adobe) and PRAAT 5.3.66 (Paul Boersma and David Weenink, Institute of Phonetic Science-University of Amsterdam) software. The voice parameters investigated in this study were crowing duration (second), voice energy (decibel), and voice part (Jenny, 2013; Table 1).

Genotyping

The differences between crowing-type chicken and non-crowing-type chicken were screened using the *FoxP2* gene. In this study, the *FoxP2* gene sequences of three crowing-type chicken, of which one was an abnormal individual (unable to exhibit any vocalization/mute), were compared to those of non-crowing-type chickens (broiler chicken line). The normal crowing-type chickens had a record of winning first in the chicken crowing contest.

Figure 1 Morphological characters of crowing-type chicken in Indonesia: (A) Kokok Balenggek, (B) Pelung, (C) Gaga, and (D) Bekisar chicken

Table 1 Pelung chicken crowing parameters based on syllable

Parameter	Definition
Crowing duration	Entire crowing vocalization
F0 syllable (*pitch*)	Average F0 at first syllable
Min. F0 syllable	F0 (*pitch*)* minimum at first syllable
Max. F0 syllable	F0 (*pitch*)* maximum at first syllable
First syllable duration	Entire first syllable
Element duration	First syllable element duration
Silent interval	Silent duration between element and first syllable
Second syllable duration	Entire second syllable vocalization
Third syllable duration	Entire third syllable vocalization
F0 mean	The average of F0 (*pitch*) at entire crowing vocalization
F0 min	F0 (*pitch*) minimal at entire crowing vocalization
F0 max	F0 (pitch) maximal at entire crowing vocalization
F0 second wave	The average of F0 (*pitch*) at second syllable
F1 mean	The average of *Formant* -1 at entire crowing vocalization
F1 second syllable	The average of *Formant* -1 at second syllable
F2 mean	The average of *Formant* -2 at entire crowing vocalization
F2 second syllable	The average of *Formant* -2 at second syllable
F3 mean	The average of *Formant* -3 at entire crowing vocalization
F3 second syllable	The average of *Formant* -3 at second syllable
The amplitude of element	Amplitude/energy element at first syllable
The amplitude of first syllable	Amplitude/energy at first wave/syllable
The amplitude of second syllable	Amplitude/energy at second wave/syllable
The amplitude of third syllable	Amplitude/energy at third wave/syllable

Blood samples were collected from a vein in the inner part of the chicken femur. Approximately 3 mL of whole blood sample was stored in a vacutainer containing ethylenediaminetetraacetic acid (EDTA) before cooling at −20 ˚C. Genetic analysis was performed at the Laboratory of Genetics and Breeding, Faculty of Biology, Gadjah Mada University. Whole DNA samples were extracted from leukocytes and nucleated red blood cells using commercially available DNA isolation kit (Roche), according to the manufacturer protocol. The DNA quality was assessed using 0.8% agarose gel electrophorsis, while the DNA quantity was measured using a UV-VIS spectrophotometer.

Forward F 5′- CTGGCTTAAGTCCTGCYGARATTC-3′ (Li *et al.* 2007) and reverse R 5′-GCTCATGAGATTACCTGTC-3′ (modified from Webb and Zhang, 2005) primers were used to amplify a 230 bp specific region of exon 7 of the *FoxP2* gene.

The thermal cycler was conditioned as follows: initial denaturation at 95 ˚C for 5 min prior to 30 cycles of denaturation at 95 ˚C for 1 min, annealing at 50 ˚C for 1 min, extension at 72 ˚C for 2 min, and subsequent final extension at 72 ˚C for 2 min. The amplicon was electroporated on 1% flurostained agarose gel and then visualized by a UV transillumintor.

For Sanger sequencing, 50 µL of the PCR product was sent to a commercial DNA sequencing service (1st Base; Singapore). The nucleotide retrieved from the sequencer (ABI 3730XL sequencer) was compared with publicly available sequences at NCBI using BLAST and ClustalX 2.1.

RESULTS AND DISCUSSION

Chicken phenotype

In general, adult chicken male produces a crowing sound to mark its territory and to attract the female, although this is associated with a high risk of being located by predators (Tefera, 2012). In several districts in Indonesia, chicken are specifically bred for their crowing sound. Due to such selective breeding attempts accompanied with a strong tendency to mate among chicken lineages with the best crowing ability for generations, the chicken present today might have acquired their special crowing abilities. Regarding the Pelung chicken, at a glance, the typical adult male produces a long, melodious, repeated, and loud crowing sound compared with the common local chicken type.

The other attribute of Pelung chicken is their heavy weight. Moreover, the Pelung chicken phenotype has been standardized by the Indonesian government (Iskandar and Susanti, 2007), although it is limited in terms of morphological aspects (Table 2).

Several authors have attempted to crossbreed this Pelung chicken breed with commercial broiler chicken line to produce new meat-typed chicken line (Retnoaji *et al.* 2016; Saragih and Daryono, 2016). Their new feed energy was also measured (Perdamaian *et al.* 2017). Unfortunately, according to our research, all hybrid and inbred chickens produced from Pelung and broiler chicken breeds produced extremely different crowing sounds. Based on these findings, we were confident to propose the crowing sound as a biomarker for determining breed purity. The other outcome is the standardization of judging during chicken show. More fair chicken shows will increase the popularity and subsequently maintain the purity of the Pelung chicken breed. Bioacoustic research requires two primary instruments, including an audio recorder and a sound processing software. In this study, the distance from the object and the environment noise level were strictly controlled to minimize bias. Crowing sound was then characterized by two software, Adobe Audition and PRAAT.

Among the Pelung chicken, there were four types of crowing sounds based on the sound loudness, including low (*kukulir*), medium (*kukulur*), loud (*kukudur*), and sometimes their combination (*tetelur*). Loud crowing was the most popular and common. In addition to loudness, sound clearness is also a prominent aspect, as a hoarse (*serak*) sound will earn very low marks from the judge in the chicken contest.

Pelung chicken crowing sounds can be divided into three types based on the sound melody (*balem*, *lunyu*, and standard). *Balem* is the sound produced by nose exhalation and can likely be halted, whereas *lunyu* is the sound produced in combination of nose and mouth exhalation. The standard sound is very loud. In general, Pelung chicken crowing sound consists of three primary sections, front syllable, middle syllable, and end syllable. The typical Pelung chicken crowing sound should be complete and consist of first syllable, second syllable, and third syllable (Figure 2). Furthermore, the crowing sound should be deep (hold breath like sound), *bitu* (increased intonation), *kebat* (prolonged sound), and *nanyer* (rhythmic sound). Each section had variations within and among other breeds. In this study, the differences will be discussed. The average duration of the entire Pelung chicken crowing sound was 8.435 ± 1.647 s, consisting of first syllable (1.1128±0.218 s), second syllable (5.532±1.274 s), and third syllable (1.858±0.969 s) (Figure 3). A formant is the concentrated sound energy detected in a spectrogram as a darker colored area. Humans and birds produce several formants such as the first formant (F1), second formant (F2), third formant (F3), fourth formant (F4), and fifth formant (F5), but F1 and F2 are adequate for distinguishing sound.

Table 2 Description of qualitative and quantitative characters of standard Pelung chicken breed issued by the Ministry of Agriculture of Indonesia with modifications

Parameters	Description
Comb	Single (*rprp*), red, ridged, and bigger in males than in females
Eye	Darker than other local chicken breeds
Wattle	Bigger and more extensive in males than in females
Lateral body appearance	Oval, cylindrical or round, and bigger in males than in females
Plumage color	Variative, dominantly combination of red and black (Wild type; e^{wt}/e^{wt}) then barred (e^{B}), brown (e^{b}), white dominant heterozygous with red and black spotted ($I/i^{+};e^{wt}$), and solid white (dominant [*I/I*] or recessive [i/i])
Shank color	Variative, dominantly black (W/-;Z^{d}/Z^{d}) then willow (w/w;Z^{d}/Z^{d}), yellow (w/w;$Z^{D}/-$), and white (W/W;$Z^{D}/-$)
Body weight	Male: 3.7–5.85 kg; female: 2.7–4.15 kg
Egg production	23–84 within 147 days of intensive rearing
Egg weight	45.03–57.03 g
Feed consumption	130 g/day
Maturity	5–6 month old
First laying	5.5–6 month old
Distribution	West Java Province, concentrated in Cianjur district

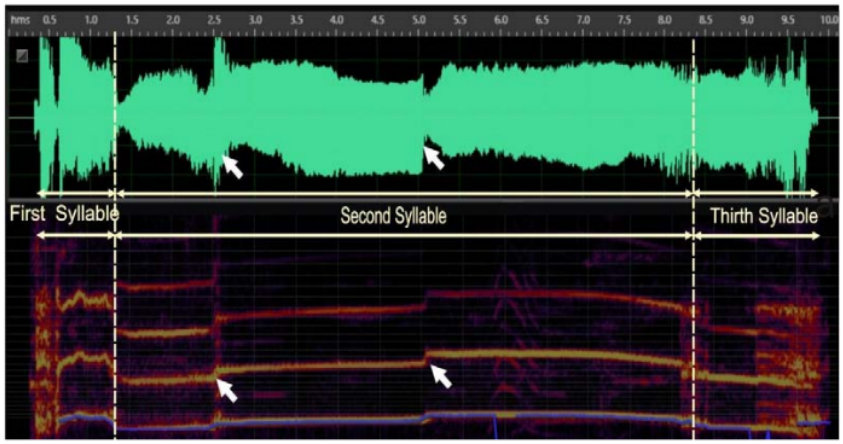

Figure 2 The Pelung chicken crowing sound consists of first, second, and third syllables
Arrows indicate the increased intonation (Indonesian local language: *bitu*)

Total crowing duration	First syllable duration	Second syllable duration	Third syllable duration
8.435 ± 1.647	1.1128 ± 0.218	5.532 ±1.274	1.858±0.969

The Component of Fisrt Syllable (Sec.)					
Element duration	Silent intervals	Initial Syllable	Peach Syllable (Hz)		
			Mean	Min	Max
0.1973±0.059	0.1213±0.038	0.7924 ±0.20	243.875 ±64.26	188.43 ±66.119	295.37 ±81.972

Formant (Hz)			
Fundamental freqwencies / Peach (F0)			
Mean	Min.	Max.	Second Syllable
278.310 ± 56.662	97.961 ± 38.559	416.060 ± 49.755	287.713 ± 64.407

Formant 1		Formant 2		Formant 3	
mean	2nd syllable	mean	2nd syllable	mean	2nd syllable
700.808 ±71.954	681.154 ±112.977	1253.583 ±137,724	1240.252 ±173.021	2563.275 ±205.928	2576.515 ±230.149

Figure 3 Pelung chicken crowing sound characteristic for each section of sound (first, second, and third syllables)

Each vowel can be diffentiated by each note. Each formant depicts resonation in the vocal tract. Each vowel has three formants or three high notes. These parameters can be observed by the PRAAT software. The first formant (F1) is the concentrated sound energy that depicts the height of the wave, whereas the second formant (F2) depicts the backness of the vowel. The F1 value is in contrast to the height of the vowel.

The first syllable (first wave) is the sound that starts the crowing retention. The first syllable consists of one element and one initial syllable, which are separated by a silent interval (Figure 4). The initial syllable is a part of the main vocalization, including the second and third syllables.

The Pelung chicken produces a loud sound at the initial syllable that is spelled "Ku" as element. The syllable "Ku" has 84.849 ± 1.758 dB amplitude, which then decreases to 83.174 ± 0.754 dB at the end of the syllable before it stops and continues by silent intervals. This decrease in amplitude was found to be statistically significant.

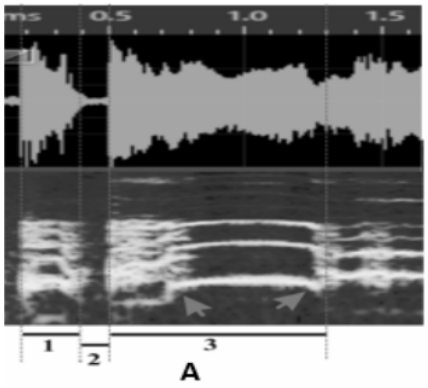

Figure 4 The first syllable consists of element (1), silent interval (2), and an initial syllable (3)
Arrows indicate the increase and decrease in amplitude

The element is a piece of a short sound wave that is approximately 0.1973 ± 0.059 s in duration. The element and the initial syllable were separated by a silent interval of about 0.1213 ± 0.038 s. The initial syllable was 0.7924 ± 0.20 s in duration. The initial syllable has F0 (fundamental frequency/pitch) of approximately 243.875 ± 64.260 Hz, which is located at vowel sound vibration. The minimum F0 of Pelung chicken was 188.43 ± 66.119 Hz, and the maximum F0 was 295.37 ± 81.972 Hz.

After the first syllable, the chicken crowing sound sequence is followed by the second syllable. During chicken exhibition, middle wave (second syllable) sound is one of the important criteria. A longer second syllable indicates that the voice is getting better, but it must have fulfill certain criteria such as bitu or pressure intonation, stability, and clearness.

Bitu is an intonation jump during crowing vocalization. Based on the type of *bitu*, the Pelung chicken second syllable can be divided into three groups, without *bitu*, single *bitu*, and double or more *bitu* (Figure 5).

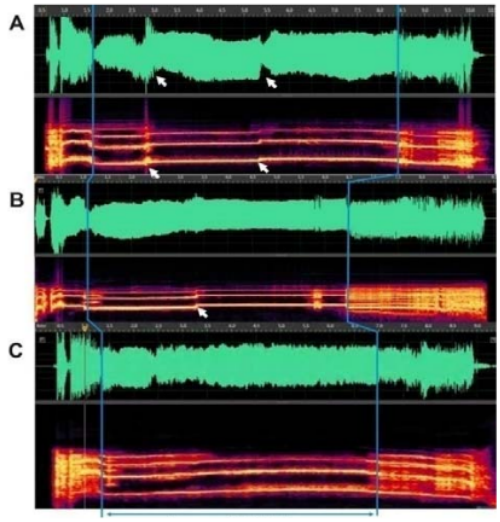

Figure 5 The variation of second syllable A) double *bitu*, B) single *bitu*, and C) without *bitu*
Bitu position is indicated by the arrow

The double *bitu* sound is often noticed in the young chicken, which is still in the learning phase. The double *bitu* sound often changes to the single *bitu* sound when the chicken reaches the adult phase. In contrast, the straight sound (no *bitu*) is recognized by the flattened second syllable without rhythm; the sound tends to the same from the beginning to the end. In general, the best second syllable should have a single *bitu*.

The last syllable, the third syllable, is an important factor that must be owned by a good Pelung chicken in the crowing contest. The end syllable sounds like the Pelung chicken is out of breath, causing hoarseness. However, this voice must play regularly so that it sounds melodious. The third syllable can be categorized into two types, graded and narrow sound. Figure 6 shows the visualization of the third syllable. The graded sound is recognized by the voice endings, which decrease regularly from large to small. The decreasing sound is like form tiers and down regularly. Another third syllable type, the tip pen sound, generally has a shorter duration and sounds as if the chicken is out of breath, which is like a reflection wave and sudden voice break.

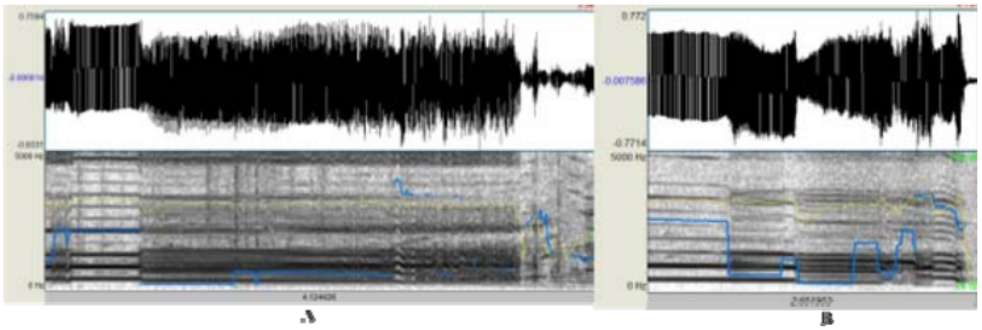

Figure 6 Variation in the third syllable of crowing sound, A) Graded sound and B) Tip pen sound
Blue lines indicate the *bitu* sequence

The third syllable is considered as the most excellent sound suffix. At the end of the third syllable, the chicken sound has a lower sound energy than that at the initial syllable; however, a good chicken individual is capable of maintaining the sound till the end.

The amplitude of the champion (having win record) was found to be lower than the common Pelung chicken. Other criteria, the F2, F3, F5, F2-F1 values, were statistically different ($P<0.05$), but the pitch (F0), F1, F4, F3-F2, the entire formant, and the F4 + F2 value were highly statistically different ($P<0.01$). The sound duration was not statistically different between the champion and the common Pelung chicken.

Energy is sound hardness measured in decibel (dB). The Pelung chicken generally produces sounds with different energy in each syllable through crowing vocalization. The average sound energy is approximately 83.237 ± 1.780 dB. At the end of the initial syllable, the chicken produces a higher sound energy than that at the beginning and middle initial syllable. This energy is still lower than the initial syllable element.

Good crowing should have a clear sound through the first syllable to the third syllable. The crowing duration of approximately 8.435 ± 1.647 s consisted of 1.104 ± 0.210 s first syllable, 5.532 ± 1.274 s second syllable, and 1.858 ± 0.969 s third syllable. The primary difference between Pelung chicken crowing and that of other crowing-type chicken in Indonesia is the straight crowing from the start till the end without many silent syllables.

Chicken genotype

In this study, we included an abnormal crowing-type chicken that was unable to exhibit any vocalization, known as mute chicken, to identify the role of the *FoxP2* gene. To our knowledge, this is the first study to compare the *FoxP2* gene of mute chicken to that of normal crowing-type chicken.

Sanger sequencing produced 230 bp long sequences of *FoxP2* gene from each sample (Figure 7). The sequences were aligned and compared with online available FoxP2 sequences by the BLAST online software. The organisms that were used as control in this study were chicken (XM_001232321.3), *Pseudopodoces humilis* (XM_005519), *Taeniopygia guttata* (NM_00104826), *Papio anubis* (NM_001168922.1), *Pongo pygmaeus* (DQ_789573.2), and *Homo sapiens* (AF515050.1). Online analysis revealed that the *FoxP2* gene was conserved through the crowing-type and the non-crowing-type chicken samples compared with several organisms.

Although no variation was detected among the tested samples, multiple alligment analysis using ClustalX 2.1 revealed four differences in nucleotides spread in exon 7 FoxP2 sequence of our samples compared with online available organisms. Furthermore, the popular variant residing at exon 7, R328X, was unchanged in abnormal chicken (Figure 8). In KE family, C→T transition at position 328 of exon 7 of *FoxP2* gene (R328X) later forms a stop codon (MacDermot *et al.* 2005). The authors hypothesized that R328X in mute chicken was missing, thus resulting in another possibility of a causative mutation residing elsewhere in chicken genomes. This result indicates an independent effect of R328X on the long crowing trait in the Pelung chicken breed. The expression level of FoxP2 might further contribute to the crowing ability as demonstrated in a previous study (Wang *et al.* 2012). In the corpus striatum of chicken brain, male individuals showed a higher FoxP2 expression level than females, but it was not significant compared with other chicken breeds.

Translation analysis of chicken and human FoxP2 revealed a similar amino acid sequence (Figure 9). Two different amino acids in humans and chicken were caused due to threonine-to-asparagine at position 303 (T303N) and asparagine-to-serine at position 325 (N325S), which were caused by rs753394697 mutation (Oswald *et al.* 2017).

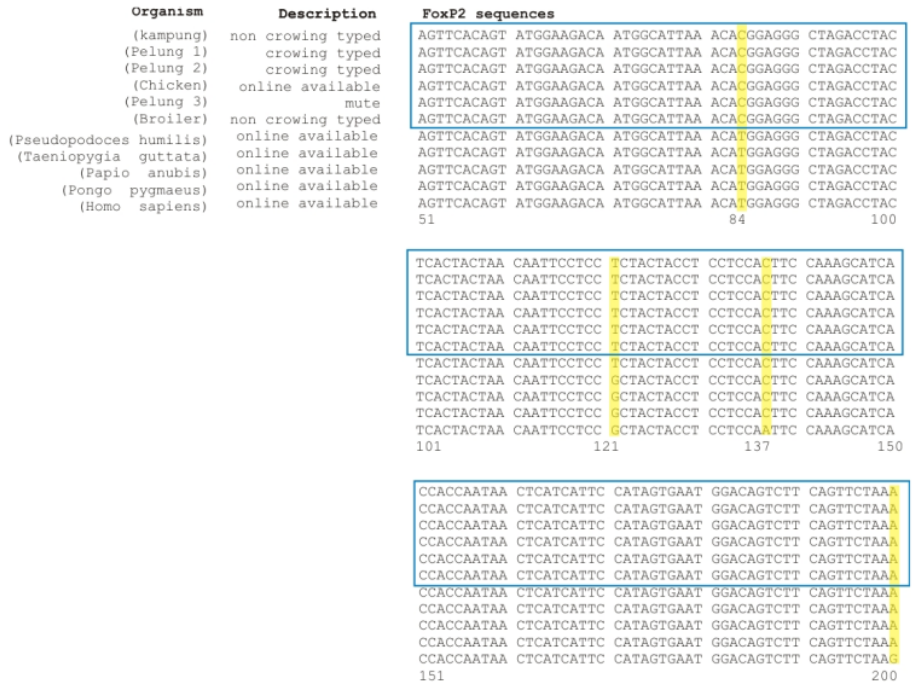

Figure 7 Nucleotide numbers 51–200 of *FoxP2* gene coding region of crowing-type chicken and several organisms Sanger sequencing results are placed inside blue boxes, whereas the nucleotide differences are highlighted in yellow

Both variations were located at exon 7 of *FoxP2* gene. The T303N variant is found only in humans, whereas N325S has been detected in several mammals (Zhang *et al.* 2002).

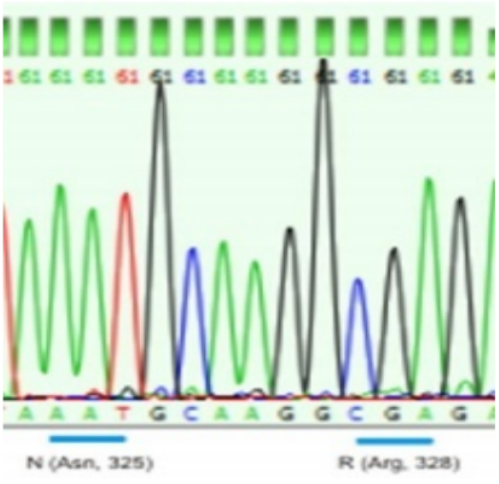

Figure 8 The amino acid sequence at position 325 to 328 of mute chicken exon 7 *FoxP2* gene

The inheritance of long crowing trait

The long crowing trait appears to be produced from the combination of internal (genetics) and external (nutrition and environment) factors, but it is not consistent throughout the available references.

In song bird species, *Zonotrichia leucophrys*, early ages learning by hearing adults sound was paramonts to develop ability to song typical song (Marler and Tamura, 1964; Soha and Marler, 2000). After reaching the adult phase, the sound exposure of other birds does not influence the song habits. Isolated hatching birds produced several normal song characters but deafened hatching resulted in quite a different song. Another independent study described that photoperiodization and circulating testosterone levels in *Z. leucophrys* affected the desire to exhibit song habits (Meitzen and Thompson, 2008). Researchers have also indicated the homolog between song bird learning process and human speech learning, which could open the possibility to use birds as an animal model (Brainard and Doupe, 2013; Webb and Zhang, 2005). These findings indicate the strong influence from external factors that alter the inner body hormonal homeostatis. However, the failure to mimic other species sound indicates that there are still internal (innate) factors limiting the mimicking.

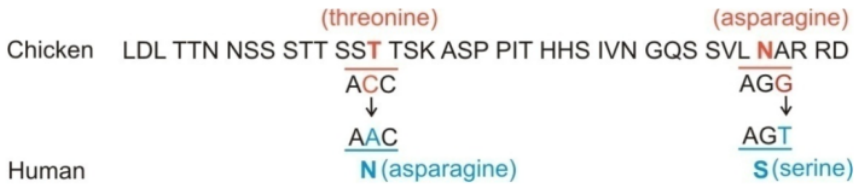

Figure 9 Translational analysis of exon 7 of *FoxP2* gene in humans and chicken revealing two different amino acids

Another study in domestic canaries (*Serinus canarius*), which had undergone selective breeding for song ability, reveal possible strain-specific changes in both song and hearing by a sex-linked inheritance mode (Wright *et al*. 2004). Similar to *Z. leucophrys*, the domestic canary is a song-learning bird species (Mori *et al*. 2018). Inheritance patterns for specific song traits through chromosome Z were observed in the Belgian Waterslager canary strains (specific song character) mated to the common canary. A more recent study on canaries indicated an autosomal additive effect on sex-linked inheritance (Mundinger and Lahti, 2014).

In Indonesia, the Pelung chicken is mostly reared in the community or at least in a neighboring farm at some distance. The majority of chicken with a perfect crowing sound could have descended from the champion chicken, but they were not always exposed to their sound. In other words, not all siblings were able to produce a similar perfect sound even when exposed to adults' crowing (having a winner record) from the hatchling to the juvenile. These juveniles failed to mimic their father but were almost successful in mimicking the standard Pelung crowing sound (long crowing trait).

In the chicken breeding facility at Gadjah Mada University, Sleman, Yogyakarta Special Province, some authors have attempted to crossbreed male Pelung chicken with female broiler chicken lines and its reciprocal (Retnoaji *et al*. 2016; Saragih and Daryono, 2016). All the produced siblings were unable to exhibit the standard crowing sound (long crowing trait) of Pelung chicken. Similar results were obtained in their backcrossed siblings (F_1 cross female Pelung chicken) (Utama *et al*. 2018). These findings indicate that the long crowing trait of Pelung chicken appear to follow a multilocus autosomal recessive inheritance pattern.

The autosomal additive effect of sex-linked inheritance scenario might not fit to Pelung chicken. Based on recent crossbreeds among Pelung and broiler chicken lines, accumulation of certain genes was paramount to express the long crowing trait, and it was independent of sex chromosomes. The inbreeding coefficient among Pelung chicken with the winner record reached 0.89, which implies a high homozygosity of certain genes for the long crowing trait.

CONCLUSION

Based on our results, long crowing traits was resulted by multiple genes and inherited by recessive autosomal fashion. Crowing is an important character for determining pelung chicken breed purity, and our bio-acoustic analysis was able to differentiate pelung chicken crowing quality.

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