



Changes in Testosterone and Corticosterone Profiles in Stone Magpie Birds under Songbird Contest Condition

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Abstract | A previous study illuminated the influence of testosterone hormone and stress on bird songs, with the stress response being measured by their corticosterone levels. Since the data about Stone Magpie testosterone and corticosterone levels during songbird competition remains unreported, this study aims to investigate testosterone and corticosterone hormone levels in Stone Magpies before and after the songbird contest. Fecal samples were collected from twelve birds, distinguishing between amateur and experienced groups, both before and after the songbird contest, and preserved at -20°C. Corticosterone and testosterone hormone levels were assessed using an ELISA kit, and the acquired data were tested for normal distribution and homogeneity, followed by analysis through the T-test method. The results indicated an increment of testosterone and corticosterone hormones among experienced Stone Magpie birds after the contest; however, these changes did not reach statistical significance. The mean values, both before and after the contests, of testosterone levels in the amateur group did not show a significant difference. However, corticosterone levels in this group showed significant differences ($P < 0.05$), with post-contest levels being higher than pre-contest. In the experienced group of Stone Magpie birds compared to the amateur group, the values of testosterone hormone showed higher results both before and after the contest. On the other hand, the corticosterone levels were lower than the amateur group, both before and after the contest. However, the statistical test did not indicate the significance of this difference. From the findings, it can be concluded that amateur birds were more stressed under the songbird contest whereas the experienced group displayed better performance, which may be related to their increased testosterone levels after the contest. However, our study did not show a direct relation between testosterone and corticosterone levels in Stone Magpie birds with their contest experience.

Keywords | Contest, Corticosterone, Songbirds, Stone Magpie, Testosterone

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INTRODUCTION

Birds are one of many favored pet animals in Indonesia; one of the species is the Stone Magpie (Putranto *et al.*, 2018). Stone Magpies, known as Murai Batu, is native

to Sumatera and Borneo Island, Indonesia (Mutiaru *et al.*, 2020; Sobiatin *et al.*, 2019). Known for its impressive song, this bird has been recognized as the best songbird, according to Putranto *et al.* (2018). Its captivating and melodious vocalizations of this bird have contributed to

its popularity among 'Kicau Mania' as songbird lovers, and many Indonesians, establishing it as a prominent participant in bird contests (Jaya *et al.*, 2021; Mutiara *et al.*, 2020; Angguni *et al.*, 2020).

In their native habitats, Stone Magpies, characterized as thrushes, demonstrate marked territoriality and a pronounced defensiveness towards their surroundings. According to Basuni *et al.* (2005), the territorial size of individual birds in the Pangandaran forest is 0.79 hectares. In contrast, the area allocated for the songbird contest is considerably smaller than their natural habitat. The contest area may differ among organizers; for instance, one organizer provided 12 x 8 m² of land for 65 birds in Tangerang. Despite the unavoidable noise from shouting, whistling, and finger snapping during the contest, specific birds consistently perform the longest, loudest, and most melodious songs, earning them as the winner.

The different environments between the natural habitat and the contest arena may induce stress in birds. Stress is a state that can pose a threat to and disrupt the regulation of homeostasis in animals, leading to adverse effects on animal welfare. The most straight forward method to assess animal stress levels is through behavior observation (Titisari *et al.*, 2019). The limitation of this approach is that the observed behavior appears to be more dependent on individual factors and may not accurately represent the actual stress condition. Hence, an alternative approach for assessing stress levels, such as cortisol hormone assays, is recommended (Kantasa *et al.*, 2016).

Bird's songs or vocalizations serve important purposes as territory defense and attraction during mating rituals. Various factors, such as sexual activities, influence these vocalizations (Chiver and Schlinger, 2019). The testosterone hormone affects the sexual activities of male animals. A study by Strand *et al.* (2008) stated that testosterone is a catalyst for singing behavior and has impacted the size and neuroanatomy of the brain area governing this behavior in songbirds. In a past study by Alward *et al.* (2013), singing frequency was affected by testosterone but did not affect the quality of the song. Meanwhile, Madison *et al.* (2015) concluded that testosterone is ineffective for both male and female canaries singing activity improvements.

During the contest, birds trained for competitions or deemed experienced tend to sustain prolonged and high-quality singing. Earlier investigations on soccer athletes have demonstrated increased testosterone levels after winning a competition, observed in both professional and amateur players. Further, the findings of this study established that cortisol hormone levels were lower in professional athletes than in their novice counterparts

(Jimenez *et al.*, 2020). Schmidt *et al.* (2014) have shown that song complexity in birds was correlated with the glucocorticoid stress response. Stress can impair song complexity and birds' ability in song learning; therefore, copies of tutor songs become poor (Brumm *et al.*, 2009). However, currently, there is no reported information on testosterone and corticosterone levels in Stone Magpies during certain conditions.

Examining the stress-related hormone corticosterone in Stone Magpies during competition is the primary objective of this study. Additionally, it aims to evaluate male steroid hormone/testosterone levels in Stone Magpies during these contest conditions. The expected outcome of this research is to contribute to showcasing the potential influence of testosterone and corticosterone hormones in enhancing the quality of songbirds.

MATERIALS AND METHODS

ANIMAL ETHICS

The Committee on Research Ethics in the Faculty of Veterinary Medicine of the Universitas Gadjah Mada, Yogyakarta, authorized every procedure utilized in this study with the assigned code number 40/EC-FKH/Int./2023.

SAMPLE ANIMALS

Fecal samples were collected from twelve Stone Magpie birds which were categorized as experienced or amateur based on their experience in songbird contests. Birds were kept in the individual cages and provided proportional feed and ad libitum drinking water.

FAECAL SAMPLES COLLECTIONS AND HORMONE EXTRACTION

Fecal samples were collected before and after the contests from each bird and stored at -20°C. The frozen samples underwent lyophilization in a freeze dryer continuously for 3 x 24 hours each, followed by pulverization. Fifty milligrams of powder yielded were extracted using 3 mL of methanol 80%. After transferring the mixture to a 15 mL polypropylene tube and vortexing for 10 minutes, then the samples were centrifuged at 500 rpm for another 10 minutes. After being collected, the resulting supernatant was put into a 1.5 mL microtube and kept cold (-20°C) until further examination (Astuti *et al.*, 2006).

HORMONE ANALYSIS

The examination of fecal testosterone levels from each bird used DRG Testosterone ELISA Kit (Calbiotech, USA), while fecal corticosterone levels were examined using General Cortisol ELISA Kit (Cor) Abclonal. The competitive binding principle is utilized in the examination

of testosterone and corticosterone. The wells of the microtiter were coated with mouse antibodies sensitive to testosterone or corticosterone molecules. Testosterone or corticosterone samples will compete with testosterone or corticosterone horseradish peroxidase conjugate when reacting with antibodies. The level of peroxidase conjugate binding to the antibody is inversely proportional to the amount of testosterone or corticosterone in the sample. Color changes will occur as peroxidase reacts with the Substrate Solution, influencing the light absorbance. The absorbance measured during reading indicates the level of testosterone or corticosterone in the sample (Astuti *et al.*, 2006).

DATA ANALYSIS

The statistical analysis of testosterone and corticosterone levels data were analyzed using SPSS. An analysis of data distribution was performed using normality and homogeneity tests. Data exhibiting normal distribution and homogeneity will undergo further analysis using the T-test to assess potential differences in the average duration of tweets concerning testosterone levels and the impact of competition on corticosterone levels. Significant results indicate a P value <0.05. The T-test method was applied to statistically analyze the data on testosterone (ng/ml) and corticosterone (ng/ml) percentages, aiming to determine differences between treatments.

RESULTS AND DISCUSSION

Regarding the concentrations of testosterone and corticosterone hormones, the result data obtained in this study demonstrated a distribution in both normal and homogenous. Levels of testosterone and corticosterone hormones of Stone Magpie in the experienced group increased after the contests. However, the mean values obtained for the testosterone and corticosterone variables did not exhibit statistically significant differences. The results of the testosterone and corticosterone hormones analysis in the experienced group of Stone Magpie birds are presented in Table 1.

Table 1: Testosterone and corticosterone hormone levels in the experienced stone Magpies group.

Hormone	Sampling time		Significance value
	Before contest (ng/ml)	After contest (ng/ml)	
Testosterone	6.75 ± 4.55 ^a	10.46 ± 6.28 ^a	0.10
Corticosterone	192.34±123.76 ^a	215.42 ± 63.22 ^a	0.67

The mean values of testosterone in the amateur birds revealed no statistically significant difference in the pre- and post-contest results. However, significant differences (P<0.05) in corticosterone levels occurred in the amateur

group. The corticosterone levels after the contest were higher than before the contest. The results of the analysis of testosterone and corticosterone hormones in the amateur group of Stone Magpie birds are presented in Table 2.

Table 2: Testosterone and corticosterone hormone levels in the amateur stone Magpies group.

Hormone	Sampling time		Significance value
	Before contest (ng/ml)	After contest (ng/ml)	
Testosterone	5.25 ± 1.81 ^a	5.64 ± 1.74 ^a	0.66
Corticosterone	204.56 ± 50.91 ^a	287.85±32.12 ^b	0.03

^{a,b} Significant variations between the treatments before and after contest periods are shown by different superscripts within the same row, P<0.05.

The results of the comparison of testosterone and corticosterone hormone levels in different Stone Magpie groups during the contest are presented in Table 3. Testosterone hormone values in the experienced group of Stone Magpie birds showed higher results compared to the amateur group, both before and after the contest. Corticosterone level in the experienced group of Stone Magpie birds was lower compared to the amateur group, both before and after the contest. However, the statistical test was not able to show a significant difference.

Table 3: Comparison of testosterone and corticosterone hormones in different Murai Batu groups during contest.

Hormone	Sampling time	Group		Significance value
		Experienced (ng/ml)	Amateur (ng/ml)	
Testosterone	Before contest	6.75 ± 4.55 ^a	5.25 ± 1.81 ^a	0.76
	After contest	10.46 ± 6.28 ^a	5.64 ± 1.74 ^a	0.18
Corticosterone	Before contest	192.34±123.76 ^a	204.56±50.91 ^a	0.13
	After contest	215.42±63.22 ^a	287.85±32.12 ^b	0.23

This study marks the first to document the testosterone and corticosterone levels in Stone Magpie birds participating in a songbird contest. According to Alward *et al.* (2013), testosterone is a steroid hormone which regulates social behavior, as singing and reproduction. Our study, while not statistically significant, revealed an increase in testosterone levels among experienced Stone Magpies after the contest. It has been suggested that testosterone influences the song output in birds when facing specific challenges or competitive environments. Blocking testosterone brought impacts for birds' song production and structural measurements, according to Apfelbeck *et al.* (2012). However, the study identified a change in testosterone levels only during breeding seasons. These observations

may lead to the connection to the territorial characteristics of the Stone Magpie. Conversely, encountering another individual during a contest may serve as the challenge or stimulus triggering an increase in testosterone levels in the experienced group. A comparable pattern was observed in canaries, where even a low dose of testosterone treatment was found to enhance singing frequency (Madison *et al.*, 2015). Unexpectedly, the treatment group's circulating testosterone levels did not significantly rise above those of the control group.

Testosterone levels from the amateur group were lower compared to the experienced group, even after the contest, the increase in testosterone levels was considerably low. As a natural behavior, the birdsong is frequently emitted to deter other male competitors in a breeding context (Catchpole and Slater, 2008; Collins, 2004). However, it is crucial to highlight that during observation at the contest, the amateur birds in this study either did not produce any songs or presented delayed timing in song production. This phenomenon may have correlations between the subordinate to the competitors and avoiding conflict mechanisms, as explained in a study by Vergara and Martinez-Padilla (2012). Lower testosterone levels in the amateur group may have correlations with a lack of singing motivation. Ritschard *et al.* (2011) reported that testosterone levels have correlations with singing motivation and the song output in Bengales finches. This report aligned with the findings of Shevchouk *et al.* (2019) that in male canaries' motivation to sing increased with testosterone levels, primarily through the medial preoptic nucleus (POM). Acute susceptibility to steroid effect in adulthood is also influenced by prior song production or singing experience (Madison *et al.*, 2015), as it has been demonstrated to stimulate neuroplasticity in male songbirds (Alward *et al.*, 2013; Alvarez-Borda and Nottebohm, 2002).

Earlier research findings indicated that testosterone levels may stimulate rising song frequency, although it does not consistently lead to uniform songs, as evident in birds systemically exposed to testosterone. Additionally, the singing activity has stimulated neuroplasticity effects, which means singing activity is independent of global testosterone levels. The findings presented by Alward *et al.* (2013) imply that tweeting affects brain development and plasticity. According to Strand *et al.* (2008), the testosterone action mechanism, induced singing behavior, and brain regions, which control the singing behavior can be affected by various factors, including the social context. Alward *et al.* (2017) stated that, in generating songs, a coordinated modulation by testosterone is necessary across various brain regions.

The study outcomes indicate that experienced Stone

Magpie birds demonstrate superior singing activity compared to amateurs, likely influenced by their experience in crowded environments. These results align with earlier theories proposing that testosterone conversion can be affected by season and environment. The changes in androgen hormones depend on the match's seriousness, distinguishing between official matches and training sessions. Jimenez *et al.* (2020) reported that the condition implies in competitive matches where potential social status threats are possible.

During the sampling period, the birds were placed in a new environment and crowded atmosphere that may lead to stress and behavioral changes. Significant differences occurred in corticosterone levels in amateur Stone Magpies, before and after the contest, as reported that it was the bird's first time joining the contest. These findings align with the research conducted by Kim *et al.* (2005), which stated that apart from behavioral changes, stress may lead to increased cortisol levels in the bloodstream, followed by increased neutrophils and decreased lymphocytes. Goll *et al.* (2023) conducted another study reporting the interaction between cortisol levels and testosterone in a social behavior context. The authors found that testosterone with higher levels and low cortisol had better leadership with risk involvement, as aligned with Mehta and Joseph's (2010) study on males under competitive conditions.

Our findings showed the amateur Stone Magpies' corticosterone levels were higher than experienced birds. Consistent with past research, professional and semi-professional cortisol levels were lower if compared to amateur players (Jimenez *et al.*, 2020). According to our study, amateur Stone Magpie birds tend to delay singing in contrast to experienced birds. Schmidt *et al.* (2014) mentioned a correlation between birdsong complexity and the glucocorticoid stress response. The impact of stress and corticosterone treatment on song complexity and learning can result in reduced proficiency in song copying among birds (Brumm *et al.*, 2009; Boogert *et al.*, 2018).

This study also demonstrated a significantly higher corticosterone level in the amateur group after the contest, suggesting that this group has higher stress levels than the experienced birds group. Past research reported that professional soccer players tend to have higher testosterone levels and lower cortisol. On the other hand, the amateurs have lower testosterone levels and higher cortisol (Jimenez *et al.*, 2020). The impact of testosterone in animals is considered to be associated with cortisol levels. In the current research, the contest was identified as the environmental trigger, specifically within the competitive context, inducing hormonal changes in the birds and influencing their behavior and singing ability. The increased corticosterone levels observed in amateur Stone

Magpies in our study could also impact the outcomes of testosterone. Earlier research indicated that cortisol might suppress in the axis of hypothalamic–pituitary–gonadal (HPG), resulting in inhibition effects on testosterone and downregulation of androgen receptors (Tilbrook *et al.*, 2000). Interestingly, testosterone levels before exposure to stress inhibited the cortisol stress response (Zueger *et al.*, 2023), which explained the insignificance of corticosterone hormone in the experienced Stone Magpie based on our findings.

In the context of competitive interactions, Mehta and Josephs (2010) observed that dominance can be predicted by both testosterone and cortisol levels, specifically in scenarios of defeat or threat. Baseline testosterone levels and post-competition cortisol levels exhibited a significant inverse correlation in the losing group (Zilioli and Watson, 2013). In contrast, between the pre-competition testosterone and the post-competition cortisol in the winning group, a significant interaction was occurred, particularly with high pre-competition testosterone levels. The lack of consistency and inconclusive findings regarding testosterone and cortisol profiles may be influenced by factors including the competitive environment, sample size, time points, and treatment variables (Mangine *et al.*, 2018; Pante *et al.*, 2022).

CONCLUSIONS AND RECOMMENDATIONS

This study concluded that amateur Stone Magpie birds exhibited heightened stress responses during the songbird contest, as evidenced by a significant increase in corticosterone levels after the competition. The experienced Stone Magpie birds displayed higher testosterone levels than their amateur counterparts; however, they exhibited lower corticosterone levels than the amateur Stone Magpie birds. The findings presented in this research highlight the potential role and interaction of the corticosterone and testosterone hormones in Stone Magpies during songbird contests. Further investigations were warranted, particularly in assessing the song quality, duration, and different sampling time points that were not covered in the study. Additionally, the consideration of a larger sample size is essential.

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NOVELTY STATEMENT

Our study is the first report on the examination of testosterone and corticosterone profile in Stone Magpie under songbird contest. Our research demonstrated that amateur Stone Magpies' corticosterone levels were higher than experienced birds and significantly increased after songbird contest.

AUTHOR'S CONTRIBUTION

All authors participate in conceptualizing the research, sampling, investigating the result, and writing the publication.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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