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Sperm Quality and Daily Fecal Testosterone Among Six Phenotypes of Kokok Balenggek Rooster

Ananda[1]*, Jaswandi[1], Rusfidra[1], Harif Gusdinal[2], Gusti Azones Abimanyu[3], Lusi Anggraini[3] and Firda Arlina[1]

[1]Department of Livestock Production Technology, Faculty of Animal Science, Universitas Andalas, Padang, 25163, West Sumatera, Indonesia
[2]Graduate Program, Faculty of Animal Science, Universitas Andalas, Padang, 25163, West Sumatera, Indonesia
[3]Undergraduate Program, Faculty of Animal Science, Universitas Andalas, Padang, 25163, West Sumatera, Indonesia
***Corresponding author:** ananda@ansci.unand.ac.id

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ABSTRACT

This study aims to know sperm quality and daily testosterone profile from fecal samples of the Kokok Balenggek rooster (KBR). This study used 10 KBR consisting of 1 Balang phenotype, 4 Biriang phenotypes, 1 Jalak phenotype, 2 Kinantan phenotypes, 1 Kuriak phenotype, and 1 Taduang phenotype. Semen was collected three times and evaluated for the quality of spermatozoa, including volume, pH, color, consistency, mass movement, motility, concentration, viability, and abnormalities. A fecal sample was carried out on the second day of semen sampling and was collected eight times within 24 hours at 01.00, 03.00, 07.00, 10.00, 13.00, 16.00, 19.00, and 22.00. The results showed that the Taduang phenotype had the lowest quality compared to the other phenotypes ($P<0.05$) with a mass movement value of 1.0±0.0 (+), spermatozoa concentration 154±69×10^6 cells/ejaculate, spermatozoa motility 50±10%, viability 88±7.0%, and spermatozoa abnormalities showed the lowest rate with a percentage of 4.6±2.1%. On the other hand, the highest mass movement (+++) and sperm motility of 90±0.0% was established in the Jalak phenotype. The Kuriak phenotype exhibited the highest concentration of sperm and the greatest sperm viability, with values of 2453±530×10^6 cells/ejaculate and 96±1.0%, respectively. The results of the ELISA analysis showed that the dried fecal testosterone concentration was 179.86±2.22ng/g and was not significantly different between phenotypes ($P>0.01$), with a testosterone peak occurring at 9-hour intervals in each phenotype. Meanwhile, an increase in testosterone has a positive correlation with an increase in pH and a negative correlation with the concentration, motility, and viability of KBR spermatozoa.

Key words: KBR, Sperm quality, Fecal testosterone.

INTRODUCTION

Kokok Balenggek rooster (KBR) is an Indonesian indigenous rooster from Tigo Lurah District, Solok Regency, West Sumatra. This rooster has even become an icon of the Solok Regency (Masfi and Mafardi 2022). KBR is known as a singing rooster because of its multilevel crow. The uniqueness of this crow makes KBR have a higher selling value than other local roosters. Apart from being a singing rooster, KBR can be selected to form superior broiler local roosters (Husmaini et al. 2022). The last KBR population was recorded in 2021, with as many as 1960 individuals with a male-female ratio of 1:1.3 (Husmaini et al. 2022). Meanwhile, previous reports of the male-female ratio were 1:3 for ex-situ conservation (Rusfidra et al. 2014) and 1:7 for in-situ conservation (Rusfidra et al. 2015). This makes it essential for conservation efforts to be carried out to increase KBR's productivity and population. Conservation efforts that have been carried out by Jaswandi et al. (2023) through artificial insemination showed the fertility rate of KBR spermatozoa is 50.87%. In general, this research has provided an overview of the reproductive quality of KBR. However, it is suspected that KBR, which has a variety of phenotypes, also influences its reproductive quality.

Six KBR phenotypes were developed at the Faculty of Animal Science, Universitas Andalas. According Muryanto and Pramono (2014), Balang has a tail that is a

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combination of black and white with white and black spots on the neck; Biriang has a red neck, back, and loin fur; Jalak has a yellowish shank and beak, black chest, wings, and tail feathers and Biriang has a red tail, tail feathers, and waist; Kinantan has white feathers on its neck, legs, beak, eyes, and wings; Taduang has black legs, a beak, eyes and Taduang has spotted plumage. The phenotypic difference also influences spermatozoa quality (Gee et al. 2004). Differences in reported characteristics can indicate differences in semen quality in roosters (Rahimpoor et al. 2016; Talebi et al. 2018). The growth of combs, spurs, and coat color patterns are closely related to testosterone secretion in roosters (Wingfield et al. 1987), which is thought to be related to the quality of rooster sperm. Changes in testosterone circulation are associated with changes in rooster feathers' level of black color (Bókony and Garamszegi 2008). This is related to their melanization and sexual dimorphism at the onset of molting. Giving testosterone to roosters can inhibit pigmentation on the back (Ros 1999). Roosters with black and blue feathers have lower semen volume and higher blood plasma testosterone levels than brown and maroon roosters. This is directly proportional to the sperm concentration of the rooster (Göger et al. 2018).

Rowe et al. (2010) reported that bright feather color could indicate sperm motility, but bright feather color negatively correlates with semen volume in *Malurus melanocephalus*. The bright color of the feathers was further explained that during the breeding season, it is also associated with androgen concentrations in *Malurus cyaneus* (Peters et al. 2000). Testosterone is one of the androgens crucial in sperm quality (Preston et al. 2012). Testosterone and FSH maintain spermatogonia by suppressing the events of apoptosis in male germ cells. Testosterone also forms secondary sex organs, libido, behavior against threats, mating behavior, and group formation (Queiroz and Cromberg 2006). Sun et al. (2019) also reported the importance of testosterone concentration. Low testosterone levels result in low sperm motility, related to testicular weight, affecting male fertility. Tabatabaei et al. (2010) reported that maximum rooster sperm motility was at 26 weeks of age, decreased at 34 weeks of age and continued to decrease until 45 weeks. The decrease in fertility in roosters is accompanied by a decrease in testosterone concentration which results in a decrease in the quality of the semen of the roosters (Fragoso et al. 2012).

Testosterone in several animals is a daily hormone where the hormone profile can be seen if blood, urine, or feces samples are taken every three hours (Senger 2005). However, blood sampling every three hours is an invasive method that can stress the roosters and affect the results. So a non-invasive method is used by using a fecal sample. Non-invasive methods are usually carried out based on the principle of identification and quantification. This hormone analysis can be obtained from biological material other than blood, for example, feces, urine, saliva, and hair (Heistermann 2010). Analysis of reproductive hormones such as testosterone can be done using a fecal sample. The results obtained from measuring hormone levels in the feces can be used to determine reproductive status (Kersey and Dehnhard 2014). Meanwhile, studies on the reproduction of KBR through hormones and its correlation to spermatozoa quality have never been reported so this study can provide new information on the reproductive status of KBR. The results of this study are expected to provide information for the development of better conservation programs, especially in supporting the increase in the number of births and new offspring with good genetic diversity.

MATERIALS AND METHODS

Ethical Approval

The present study was approved by the Animal Ethics Committee of Universitas Andalas, West Sumatera, Indonesia.

Experimental Birds

This study used 10 KBR aged 24 months weighing 1.84±0.3kg, consisted of 1 Balang phenotype, 4 Biriang phenotypes, 1 Jalak phenotype, 2 Kinantan phenotypes, 1 Kuriak phenotype, and 1 Taduang phenotype (Fig. 1). KBR is raised intensively using modified battery cages to accommodate feces. Each KBR is given a numbered anklet as a label. Commercial feed (Comfeed ABS Crumble, PT. Japfa Comfeed Indonesia) of 120g/head/day was given in the morning, afternoon, and evening (@40g). Drinking water is provided *ad libitum*.

Semen Collection and Evaluation

Semen was collected three times (the interval of each semen collection was three days.) in the morning at 08.00 – 09.00 using the dorsal massage method (Arifiantini 2012). Semen was collected using a 1.5mL microtube and immediately taken to the laboratory for evaluation.

Evaluation of the macroscopic quality of spermatozoa includes volume (µL), pH, color, and consistency. As for microscopic quality, it was carried out using an Olympus microscope (CX-23), including mass movement of spermatozoa, motility of spermatozoa (%), concentration of spermatozoa (10^6/ejaculate), viability of spermatozoa (%) and abnormalities of spermatozoa. The evaluation process was modified for use with rooster semen (Arifiantini 2012). Semen was diluted with Ringer's lactate solution, homogenized, and examined under a microscope to determine the motility of spermatozoa. The motility of spermatozoa was evaluated using five visual fields. Eosin-Nigrosin staining was used to detect abnormalities and the viability of spermatozoa. The concentration of spermatozoa was determined using a Neubauer chamber hemocytometer.

Fecal Collection

KBR feces was carried out on the second day of semen sampling (Fig. 2). Fecal samples were collected eight times within 24 hours, namely at 01.00, 03.00, 07.00, 10.00, 13.00, 16.00, 19.00, and 22.00. Each wet sample was then put into a plastic clip, weighed, and stored at -20ºC until further analysis.

Sample Preparation and Testosterone Analysis

The fecal samples were then thawed, placed in a petri dish, put in the oven, and dried at 50ºC overnight. Periodic weighing was carried out to ensure that the fecal samples were completely dried. After drying, the fecal samples

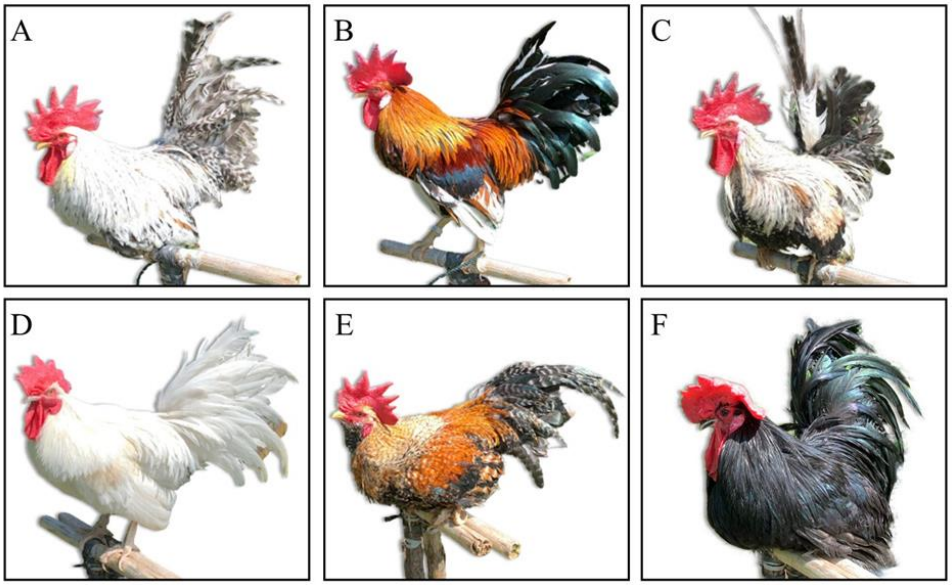

Fig. 1: The phenotypes of Kokok Balenggek rooster: (A) Balang; (B) Biriang; (C) Jalak; (D) Kinantan; (E) Kuriak; and (F) Taduang

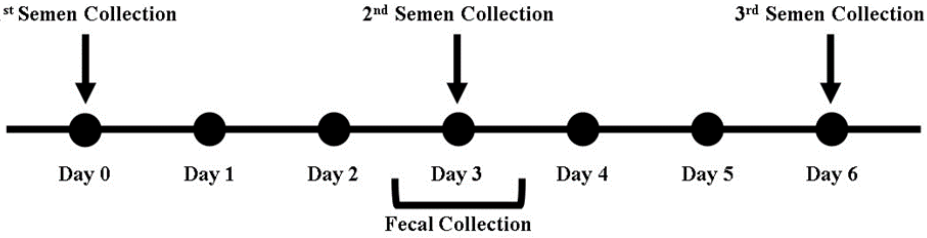

Fig. 2: Research design

were ground using a mortar and pestle. The fecal powder weighed as much as 200mg and was put into a centrifuge tube, then 2mL of absolute ethanol was added (100mg/mL). Each sample was vortexed for 1min and shaken overnight. Then the sample was centrifuged at 5000rpm for 15min. The supernatant (KBR fecal ethanol extract) was taken as much as 1mL and placed into a 1.5mL microtube which had been labeled. The samples were then stored at -20ºC until further analysis. KBR fecal ethanol extract was further analyzed using the DetectX® Testosterone ELISA kit, cat. K032-H (Arbor Assays™, USA), according to the manual book.

Data Analysis

Data on KBR spermatozoa quality and concentration of dried fecal testosterone were analyzed using a randomized block design with six replications and analyzed using the IBM SPSS Statistics 25. If there was a difference, a multiple comparative test of LSD and Duncan was carried out with a significance level of 95%. Meanwhile, the profile of dried fecal testosterone and its correlation to spermatozoa quality were analyzed descriptively-qualitatively.

RESULTS

Sperm quality of Kokok Balenggek Rooster

According to the findings of the observations on the fresh semen quality (Table 1), there was no significant difference in the volume of the semen for each phenotype ($P>0.05$). Similar to that, there was no difference between each phenotype's semen acidity (pH) level ($P>0.05$). The Taduang phenotype had the lowest quality compared to the other phenotypes ($P<0.05$) with a mass movement value of 1.0±0.0 (+), spermatozoa concentration 154±69×10^6 cells/ejaculate, spermatozoa motility 50±10%, viability 88±7.0%, and spermatozoa abnormalities showed the lowest rate with a percentage of 4.6±2.1%. On the other hand, evaluation of the microscopic quality of spermatozoa in different phenotypes showed varying results. The highest mass movement ($P<0.05$) was established in the Jalak phenotype with a value of 3.0±0.0 (+++). The Jalak phenotype demonstrated the highest motility ($P<0.05$) with a percentage of 90±0.0%, the highest viability ($P<0.05$) with a rate of 96±1.0%, and the highest spermatozoa concentration ($P<0.05$) was shown in the Kuriak phenotype with a total of 2453±530×10^6 cells/ejaculate. Apart from the Taduang phenotype, spermatozoa motility in all KBR phenotypes deserves cryopreservation because it shows a percentage of more than 75%. To date, standardization of rooster semen for cryopreservation has not been carried out so that all groups show characteristics suitable for cryopreservation (Junaedi et al. 2016).

Daily Fecal Testosterone of Kokok Balenggek Rooster

The results of the ELISA analysis showed that the average concentration of testosterone (Fig. 3) showed that the daily testosterone concentration in the KBR dry feces sample was 179.86±2.22ng/g, with the highest concentration in the Taduang phenotype (179.86±2.22ng/g) and the lowest daily testosterone concentration was found in the Kuriak phenotype (177.19±4.68ng/g). However, the statistical analysis results showed no significant difference in concentration between phenotypes ($P>0.01$).

The results of hormone analysis on dried KBR fecal samples showed that the testosterone profile had a fluctuating pattern within 24 hours, so the hormone profile

Table 1: Fresh semen quality of Kokok Balenggek rooster

Parameter	Balang	Biriang	Jalak	Kinantan	Kuriak	Taduang	Average±SD
Macroscopic							
Volume (µL)	400±26.4	397.9±31.8	216.7±76.3	200±13.2	378.3±16.6	216.7±76.4	338.2±23.9
pH	7.3±0.1	7.5±0.3	7±0.2	7.1±0.4	7±0.1	7.2±0.5	7.2±0.3
Color	white	white	white	white	white	cloudy	white
Consistency	thick	thick	thick	thick	thick	watery	thick
Microscopic							
Mass Movement	2.7±0.6a	2.3±1.0a	3.0±0.0a	2.0±1.0ab	2.8±0.4a	1.0±0.0b	2.3±0.9
Concentration (10^6 cell/ejaculate)	1150±597bc	1215±761bc	1417±827b	983±472bc	2453±530a	154±69c	1347±884
Motility (%)	83.3±11.5a	75.8±18.8a	90±0.0a	86.7±5.8a	88.3±4.1a	50±10b	79±16.9
Viability (%)	92.7±4.4ab	92.2±4.1ab	95.6±1.0a	95.4±0.7a	96±1.0a	88±7.0c	93.3±4.2
Abnormality (%)	16.6±2.3a	10.1±3.4ab	10.1±6.0ab	14.3±10.9a	12.5±3.1a	4.6±2.1c	11.1±5.2

Different letters on the same row indicates statistical difference (P<0.05) among phenotypes.

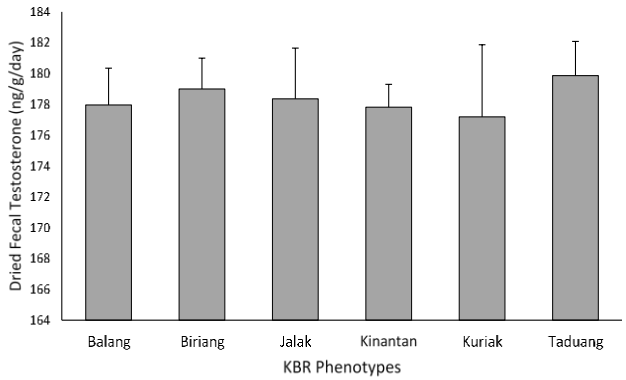

Fig. 3: Daily testosterone concentrations in KBR dried fecal samples.

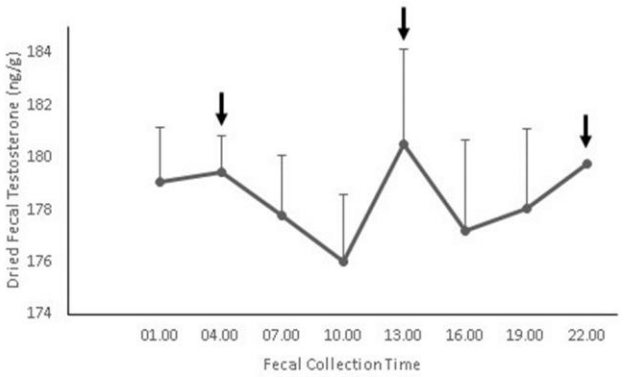

Fig. 4: Daily fecal testosterone level in KBR

obtained showed normal physiological reproductive function in KBR (Fig. 4). The resulting peaks in testosterone concentration are within 9-hour intervals, namely at 04.00, 13.00 and 22.00.

The profile of dried fecal testosterone for the six KBR phenotypes also illustrates a fluctuating pattern (Fig. 5). Although the pattern of fluctuations varies, each phenotype shows the number of testosterone peaks occurring thrice in 24 hours. In the Balang phenotype, testosterone peaks were detected at 04.00, 13.00, and 22.00; in the Biriang phenotype, testosterone peaks were detected at 04.00, 13.00, and 22.00; on the Jalak phenotype, testosterone peaks were detected at 04.00, 13.00 and 22.00; in the Kinantan phenotype, testosterone peaks were detected at 01.00, 13.00 and 19.00; in the Kuriak phenotype, testosterone peaks were seen at 01.00, 13.00 and 19.00; and in the Taduang phenotype testosterone peaks were detected at 01.00, 13.00 and 22.00.

The increase in testosterone hormone positively correlates with the rise in pH. With a higher concentration of testosterone, the pH of the semen increased, placing the semen in a base condition. Conversely, a negative correlation was observed in several parameters of sperm quality, where the higher concentration of testosterone led to a decrease in the concentration of sperm cells per ejaculate, sperm motility and sperm viability (Fig. 6).

DISCUSSION

Fresh Semen Quality of KBR

In West Sumatra, the male-to-female KBR ratio is just 1:1.3 (Husmaini et al. 2022). In order to increase the population, male KBRs must have good fertilization abilities, so it is crucial to assess the quantity and quality of fresh KBR semen (Table 1). In addition, the characterization of rooster semen is essential for selecting superior males. The average semen volume in this study was lower than that of Du Plessis et al. (2015), who reported that the semen volume of laying hens ranged from 0.38 to 0.41mL, IPB D-1 roosters ranged from 0.06 to 0.25mL (Setiadi et al. 2019) and Merawang roosters with a semen volume of 0.28 up to 0.59mL (Magfira et al. 2017).

According to Rusfidra (2006), the Indonesian singing roosters, such as the KBR, Pelung rooster, and Bekisar rooster, are all descended from the Green Jungle rooster. The average volume of KBR semen is higher when compared to other singing roosters, measuring up to 338±0.16µL/ejaculate, as opposed to the Pelung rooster reported by Junaedi and Husnaeni (2019), which measures 0.23±0.02mL/ejaculate, and lower than the evaluation results reported by Kusuma et al. (2018), which are as much as 0.46mL. Additionally, the outcomes of this study were better than the Green Forest rooster's semen, which was 0.15±0.05mL/ejaculate. The Taduang phenotype in this study had the lowest semen volume, at 216.7±76.4µL/ejaculate, whereas the Balang phenotype had a higher semen volume of 400±26.4µL/ejaculate. However, these differences were not statistically significant (P>0.05). Semen volume variations can vary depending on the country, type, storage method (Hijriyanto et al. 2017), age, body size, nutrition, and environmental temperature (Almahdi et al. 2014).

The color and consistency of KBR semen in this study were still classified as usual, namely white and thick. These results are the same as the color and consistency of semen in Pelung roosters (Junaedi dan Husnaeni 2019). However, of the six KBR phenotypes, the Taduang phenotype

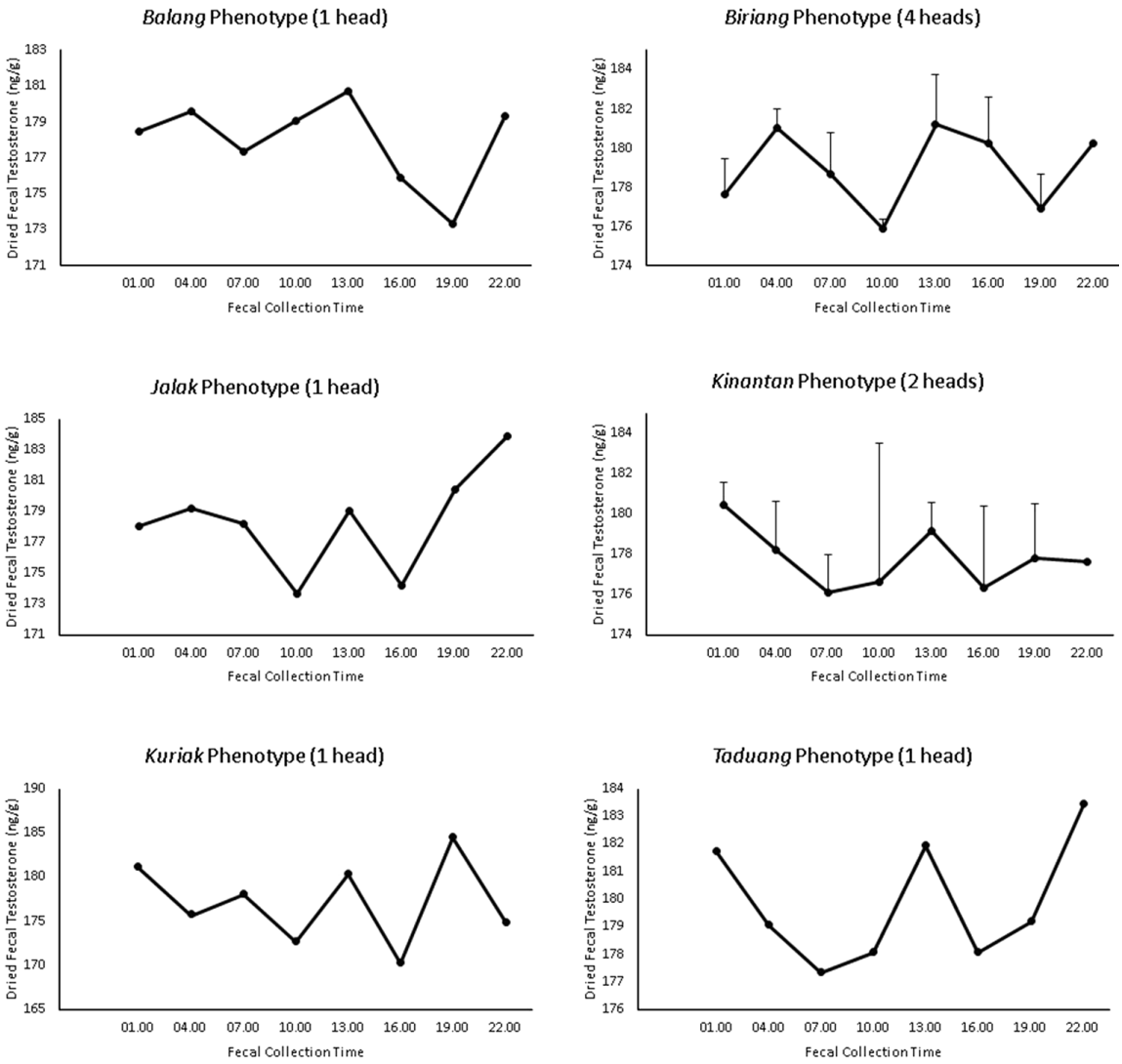

Fig. 5: Profile of dried fecal testosterone in each KBR phenotypes.

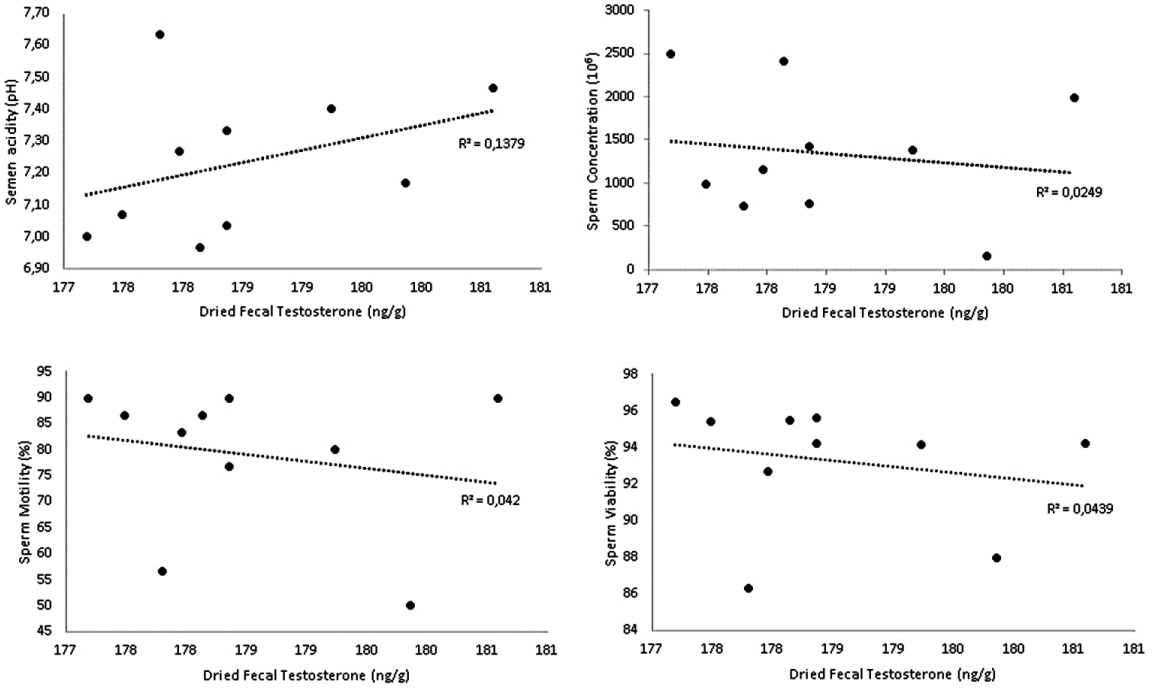

Fig. 6: Correlation between dried fecal testosterone with pH, concentration, motility and viability of spermatozoa in KBR

appeared to have a cloudy white color with a watery consistency, indicating poor quality of spermatozoa. Similar results were also found in Green Forest roosters reported by Andaruisworo and Yuniati (2021), who found a watery consistency and cloudy white color in semen. One of the parameters that is an indicator of semen quality is color and consistency (Almahdi et al. 2014). The average concentration of spermatozoa cells in KBR was 1347±884 million/ejaculate, with concentrations (10^6 cells/ejaculate) sequentially from highest to lowest, namely: Kuriak 2453±530; Jalak 1417±827; Biriang 1215±761; Balang 1150±597; Kinantan 983±472; and Taduang 154±69. The average concentration of spermatozoa cells in KBR was higher than that of the Pelung rooster, which was $5043.33±51×10^6$/mL or $1160±11.73×10^6$/ejaculate (Junaedi dan Husnaeni 2019) and the Green Jungle rooster, which was $898±4.24×10^6$/mL or $61.96±0.29×10^6$/ejaculate (Bebas and Laksmi 2013). As in other livestock, the concentration of spermatozoa also plays a role in the survival and success of reproduction in poultry. This was exacerbated by the report of Wishart and Staines (1999), which stated that most of the semen would be pushed out of the vagina and only 2% of spermatozoa managed to reach the sperm-storage tubules (SST), so the concentration of spermatozoa cells in roosters must be high to increase the chances of fertilization. Thus, the concentration of spermatozoa cells is closely related to the success of fertilization in poultry (Arifiantini 2012).

Measurements on the degree of acidity (pH) of KBR in this study were 7.2±0.3. This result is the same as the pH value of the Pelung rooster, which is 7.2 (Kusuma et al. 2018) and the Green Forest rooster, which is pH 7 (Andaruisworo dan Yuniati 2021). Various factors, including individuals, spermatozoa motility, viability, and metabolism, can cause differences in pH values. Activity can cause a decrease in the pH value of semen (Mphaphathi et al. 2016). The motility of KBR spermatozoa in this study was 79.63±16.9%, with motility from highest to lowest in sequence, namely, Jalak 90±0.0%; Kuriak 88.3±4.1%; Kinantan 86.7±5.8%; Balang 83.3±11.5%; Biriang 75.8±18.8%; and Taduang 50±10%. Junaedi and Husnaeni (2019) and Kusuma et al. (2018) reported that spermatozoa motility in Pelung roosters ranged from 84-86%. Meanwhile, the Green Forest rooster has a very low motility of 45% (Andaruisworo and Yuniati 2021). Spermatozoa motility is critical in evaluating spermatozoa quality because high spermatozoa motility will also increase fertilization success (Danang et al. 2012). In this study, Jalak and Kuriak phenotypes had the highest motility. Allegedly, Jalak phenotype has a higher concentration of carotenoids than the other phenotypes. Carotenoids act as antioxidants, which function to ward off free radicals. Yellow, orange and red pigmentation in most birds is also a marker of carotenoid content in their bodies (Koch et al. 2016). Carotenoids are also thought to protect spermatozoa from oxidative stress. Carotenoids are located in mitochondria and are thought to play a role in cell aerobic respiration (Hill et al. 2019). Carotenoids in the Jalak and Kuriak phenotypes are also thought to play a role in increasing spermatozoa cell viability and are in line with increasing spermatozoa motility.

Fresh KBR semen in this study had a sperm viability of 93.4±4.2%. Meanwhile, Pelung and Green Forest roosters have motility of 89.17±1.23% (Junaedi dan Husnaeni 2019), and 30% (Andaruisworo and Yuniati 2021), respectively. In this study, KBR with the Kuriak phenotype had the highest spermatozoa viability (96±1.0%), while the Taduang phenotype had the lowest spermatozoa viability (88±7.0%). Meanwhile, the Balang phenotype had the highest spermatozoa abnormality (16.6±2.3%). Viability and abnormalities are also important parameters for spermatozoa quality and are essential to fertilization success. In roosters, the minimum standard for spermatozoa motility is ≥40% to increase the chances of successful artificial insemination. The abnormalities obtained in this study were also almost the same in IPB D-1 roosters, namely 7-21% (Setiadi et al. 2019), and different in Merawang roosters, namely 2.0-3.03% (Magfira et al. 2017). Genetic differences are also thought to influence the level of spermatozoa abnormalities in roosters.

Fecal Testosterone Level

Testosterone levels are episodic, and the pattern will be seen in hours. Senger (2005) said that the LH profile occurs periodically and is followed by episodic testosterone that lasts for 0.5 to 1.5 hours. In this study, samples were taken every 3 hours and showed that within 24 hours, there had been an increase in testosterone three times with an interval of 9 hours. This pattern is similar to a study by Bachman et al. (1987), where an average of two to three times the testosterone peak was observed in the blood plasma of White Leghorn roosters exposed to light for 24 hours. The study showed that testosterone peak occurred between 21.00, 9.00, and 15.00, while in this study, the average peak in testosterone in the feces occurred at 04.00, 13.00, and 22.00. This indicates that testosterone is needed to metabolize from the blood and end up in the urine or feces. In contrast to the measurement of hormones in blood samples, which can describe hormone levels at the time of collection, measurements of steroid metabolites from feces result from accumulation excreted during excretion (Hirschenhauser et al. 2005). Therefore, there is a lag between the levels of hormone metabolites in the feces and the actual levels of hormones in the blood circulation (Schwarzenberger et al. 1996; Kumar et al. 2013). The time interval between hormone levels in blood plasma and levels in feces is determined by the gut passage time (GPT), which is the time from the release of excretion of hormone metabolism by the liver through the gallbladder to the digestive tract until it is excreted with feces and urine, which is also strongly influenced by the metabolic rate of these animal species (Hirschenhauser et al. 2005; Palme et al. 2005; Kumar et al. 2013). Depending on the species, activity, and season, steroid metabolites are excreted in fecal samples within 30 minutes to several days (Millspaugh and Washburn 2004; Palme et al. 2005; Ashley et al. 2011).

In general, steroid hormones are metabolized by many tissues in the body, including the liver, kidney, muscle, and blood (Schiffer et al. 2019). However, the primary metabolism occurs in the liver. Most testosterone excreted from the testes will be in the bloodstream bound to protein or other blood components, while the rest is unbound. After use, testosterone will be inactivated by the target organs, liver, and kidneys. To stop and regulate hormone activity

in target organs and body physiology, this inactivation is required. Additionally, steroid hormones like testosterone have a high level of bioactivity. They can be toxic if allowed to remain in the body in high concentrations so that the body carries out a deactivation process, including by conjugating these hormones in a form that is inactive and easily soluble in water (Möstl et al. 2005; Rohrmann et al. 2011). Testosterone is conjugated with glucuronide or sulfate during metabolism in the liver and is excreted in the urine. A certain amount is excreted into the bile, reabsorbed into the circulatory system via the enterohepatic pathway, and then excreted via the feces (Li et al. 2019). Further research is needed to determine how long it takes to metabolize testosterone, starting from its secretion process in the testes and excretion in the feces, especially in songbirds, and linking it to sexual behavior.

Testosterone profiles for each phenotype in this study showed different patterns and timing (Fig. 5). This shows that metabolic processes are not always the same in every individual. Heistermann (2010) revealed that each animal has different metabolites, even in closely related species, in terms of volume percentage and type of hormone metabolites excreted (Schwarzenberger et al. 1996; Kumar et al. 2013). The average testosterone in this study was 178.36ng/g dry feces. In other species, such as *Strigops habroptilus*, it has a testosterone metabolite concentration of 120-309.5ng/g (Cockrem dan Rounce 1995), *Pipile cumanensis* has a testosterone metabolite concentration of 709.5±39.2ng/g (Sterling et al. 2016). Preliminary studies also revealed that most of the unconjugated steroid metabolites in the feces of Japanese quail (*Coturnix coturnix japonica*) accounted for 31% of testosterone (Bishop and Hall 1991). Several testosterone concentrations were also reported by Kelemen et al. (2003) on several birds, such as Domestic cockerel 55.36 ng/g, Domestic fowl 23.02ng/g, Mallard drake 18.85ng/g, Muscovy drake 22.7ng/g, and Domestic gander 12.95ng/g. The 17-oxo androgen assay from Chinese quail (*Excalfactoria chinensis*) droppings yielded a biologically significant androgen pattern without deconjugated testosterone from the fecal extract. Unconjugated testosterone metabolites were also detected in chickens (Dehnhard et al. 2003; Rettenbacher et al. 2004; Cui et al. 2018) while geese mainly had conjugated testosterone metabolites (Hirschenhauser et al. 2005).

Correlation Between Fecal Testosterone and Sperm Quality in KBR

A negative correlation was shown in this study where high testosterone concentrations would have an impact on decreasing several sperm quality parameters such as concentration, motility, and sperm viability (Fig. 6). This result is not in line with the report of Sun et al. (2019) which showed that roosters with high testosterone concentrations produced high sperm motility but did not affect sperm concentration. Testosterone is known to play a role during spermatogenesis. Testosterone is produced in Leydig cells by stimulation of the LH, which together with FSH, triggers and maintains spermatogenesis (Oduwole et al. 2021; Shah et al. 2021). Walker (2011) asserts that Src kinase activation by testosterone can encourage Sertoli cells to maintain germ cells and release mature sperm. However, the negative feedback mechanism causes high testosterone levels to suppress FSH and LH secretion, inhibiting spermatogenesis (Senger 2005). Several studies have also shown that exogenous testosterone administration will lead to high testosterone concentrations in the blood, resulting in reduced testicular size and low sperm count (Cohen et al. 2020). Research by Schröcksnadel et al. (1971) reported that age also affects testosterone concentration. Blood plasma of 22-week-old roosters contains testosterone of 0.12µg/100mL and reaches a maximum concentration at 1 to 2 years of age ranging from 0.23 to 0.24µg/100mL. The increase in testosterone in poultry is closely related to sexual maturity and sperm production. Androgen hormones are also responsible for secondary sex characteristics (Queiroz and Cromberg 2006).

Several researchers have reported the relationship between testosterone concentration and various reproductive parameters in other livestock. Dasrul et al. (2020) reported that serum testosterone was positively correlated with scrotal circumference, semen pH, sperm concentration, and sperm motility and negatively correlated with sperm morphology in Aceh bulls. Testosterone was correlated with scrotal circumference and buffalo semen volume (Sajjad et al. 2007). However, it was negatively correlated with semen pH. Research on the semen quality of sheep conducted by Moghaddam et al. (2012) showed that testosterone is correlated with semen quality, but scrotal circumference is correlated with sperm concentration and viability. In this study, the testosterone concentration was positively correlated with an increase in pH. These varied results can be influenced by several factors, such as species, genetics, age, and the environment in which the animal is kept.

Conclusion

The Jalak and Kuriak phenotypes had the best spermatozoa quality, while the Taduang phenotype had the lowest quality. The testosterone concentration in all phenotypes did not show any significant difference, but the Taduang phenotype had a higher fecal testosterone concentration than the other phenotypes. The fecal testosterone profile in KBR showed three peaks in testosterone within 24 hours with an average interval of 9 hours. High testosterone concentrations are negatively correlated with spermatozoa concentration, viability, and motility and positively correlated with increased semen pH.

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Author's Contribution

Ananda analyzed the data and wrote the manuscript. Ananda, Jaswandi, and Rusfidra designed the concept, searched for funding, and compiled and reviewed the paper. Harif Gusdinal oversees field and laboratory work. Gusti Azones Abimanyu and Lusi Anggraini conducted field and laboratory work and data tabulation. Firda Arlina accommodates sample logistics and transportation.

REFERENCES

Almahdi AB, Ondho YS and Sutopo, 2014. Comparative studies of semen quality on different breed of chicken in poultry breeding center temanggung-central java. International Refereed Journal of Engineering and Science 3(2): 94–103. https://doi.org/10.183X/K030294103

Andaruisworo S and Yuniati E, 2021. Teknologi inseminasi buatan pada ayam hutan hijau jantan dengan yyam kampung betina dalam upaya pelestarian plasma nuftah ayam bekisar. Jurnal Ahli Muda Indonesia 2(1): 57–62. https://doi.org/10.46510/jami.v2i1.59

Arifiantini RI, 2012. Teknik koleksi dan evaluasi semen pada hewan. Bogor: Penerbit IPB Press.

Ashley NT, Barboza PS, Macbeth BJ, Janz DM, Cattet MRL, Booth RK and Wasser SK, 2011. General and comparative endocrinology glucocorticosteroid concentrations in feces and hair of captive caribou and reindeer following adrenocorticotropic hormone challenge. General and Comparative Endocrinology 172(3): 382–391. https://doi.org/10.1016/j.ygcen.2011.03.029

Bachman SE, Bachman JM and Mashaly MM., 1987. Effect of photoperiod on the diurnal rhythm of plasma testosterone, dihydrotestosterone and androstenedione in mature male chickens. Comparative Biochemistry and Physiology Part A: Physiology 87A(3): 775–779. https://doi.org/10.1016/0300-9629(87)90399-9

Bebas W and Laksmi DNDI, 2013. Konsentrasi spermatozoa dan motilitas spermatozoa ayam hutan hijau (Gallus varius). Buletin Veteriner Udayana 5(1): 57–62.

Bishop CM and Hall MR, 1991. Non-invasive monitoring of avian reproduction by simplified faecal steroid analysis. Journal of Zoology 224: 649–668. https://doi.org/10.1111/j.1469-7998.1991.tb03793.x

Bókony V and Garamszegi LZ, 2008. Testosterone and melanin-based black plumage coloration: a comparative study. Behavioral Ecology and Sociobiology 62: 1229–1238. https://doi.org/10.1007/s00265-008-0551-2

Cockrem JF and Rounce JR, 1995. Non-invasive assessment of the annual gonadal cycle in free-living kakapo (Strigops habroptilus) using fecal steroid measurements. Ornithology 112(1): 253–257. https://doi.org/10.2307/4088787

Cohen J, Nassau DE, Patel P and Ramasamy R, 2020. Low testosterone in adolescents & young adults. Frontiers in Endocrinology 10: 1–6. https://doi.org/10.3389/fendo.2019.00916

Cui X, Cui H, Liu L, Zhao G, Liu R, Li Q, Zheng M and Wen J, 2018. Decreased testosterone levels after caponization leads to abdominal fat deposition in chickens. BMC Genomics 19(344): 1–10. https://doi.org/10.1186/s12864-018-4737-3

Danang DR, Isnaini N and Trisunuwati P, 2012. Pengaruh lama simpan semen terhadap kualitas spermatozoa ayam kampung dalam pengencer ringer's pada suhu 4°C. Journal of Tropical Animal Production 13(1): 47–57.

Dasrul, Wahyuni S, Sugito S, Hamzah A, Zaini Z, Haris A and Gholib G, 2020. Correlation between testosterone concentrations with scrotal circumference, and semen characteristics in aceh bulls. E3S Web of Conferences 151: 4–8. https://doi.org/10.1051/e3sconf/202015101015

Dehnhard M, Schreer A, Krone O, Jewgenow K, Krause M and Grossmann R, 2003. Measurement of plasma corticosterone and fecal glucocorticoid metabolites in the chicken (Gallus domesticus), the great cormorant (Phalacrocorax carbo), and the goshawk (Accipiter gentilis). General and Comparative Endocrinology 131: 345–352. https://doi.org/10.1016/S0016-6480(03)00033-9

Fragoso JS, Díaz MP, Moreno JCA, Infesta PC, Rodriguez-Bertos A and Barger K, 2012. Relationships between fertility and some parameters in male broiler breeders (body and testicular weight, histology and immunohistochemistry of testes, spermatogenesis and hormonal levels). Reproduction in Domestic Animals 48(2): 345–352. https://doi.org/10.1111/j.1439-0531.2012.02161.x

Gee GF, Bertschinger H, Donoghue AM, Blanco J and Soley J, 2004. Reproduction in nondomestic birds: physiology, semen collection, artificial insemination and cryopreservation. Avian and Poultry Biology Reviews 15(2): 47–101. https://doi.org/10.3184/147020604783637435

Göger H, Demirtaş ŞE and Yurtoğulları Ş, 2018. Determination of semen characteristics and their effect on hatch results in white layer sire lines. Journal of Poultry Research 15(2): 40–46.

Heistermann M, 2010. Non-invasive monitoring of endocrine status in laboratory primates: methods, guidelines and applications. Advances in Science and Research 5(1): 1–9. https://doi.org/10.5194/asr-5-1-2010

Hijriyanto M, Dasrul and Thasmi CN, 2017. Pengaruh frekuensi penampungan semen terhadap kualitas spermatozoa pada ayam bangkok. Jurnal Ilmiah Mahasiswa Veteriner 01(1): 46–53. https://doi.org/10.21157/jim%20vet..v1i1.1817

Hill GE, Hood WR, Ge Z, Grinter R, Greening C, Johnson JD, Park NR, Taylor HA, Andreasen VA and Powers MJ, 2019. Plumage redness signals mitochondrial function in the house finch. Proceedings of The Royal Society B - Biological Sciences 286(20191354): 1–9. https://doi.org/10.1098/rspb.2019.1354

Hirschenhauser K, Kotrschal K and Möstl E, 2005. Synthesis of measuring steroid metabolites in goose feces. Annals of the New York Academy of Sciences 1046: 138–153. https://doi.org/10.1196/annals.1343.011

Husmaini, Putra RA, Juliyarsi I, Edwin T, Suhartati L, Alianta AA and Harmaini, 2022. Population Structure of Kokok Balenggek Chicken in In-situ Area as Indigenous Chicken of Indonesia. Advances in Animal and Veterinary Sciences 10(5): 993–998. https://doi.org/10.17582/journal.aavs/2022/10.5.993.998

Jaswandi, Ananda, Rusfidra, Subekti K, Gusdinal H, Wahyuni RS and Caniago FA, 2023. Fertility rate, fertility period, and doc sex ratio of Kokok Balenggek chicken after artificial insemination. Advances in Animal and Veterinary Sciences 11(5): 795–801. https://doi.org/10.17582/journal.aavs/2023/11.5.795.801

Junaedi, Arifiantini RI, Sumantri C and Gunawan A, 2016. Penggunaan Dimethyl Sulfoxide Sebagai Krioprotektan dalam Pembekuan Semen Ayam Kampung. Jurnal Veteriner 17(2): 300–308. https://doi.org/10.19087/jveteriner.2016.17.2.300

Junaedi and Husnaeni, 2019. Penggunaan dimethyl sulfoxide sebagai krioprotektan dalam pembekuan semen ayam kampung. Jurnal Veteriner 17(2): 300–308. https://doi.org/10.19087/jveteriner.2016.17.2.300

Kelemen K, Péczely P, Szoke Z and Ladjánszky V, 2003. A comparative methodical study of the faecal steroid analysis on birds: looking for a valid method of testosterone. Acta Biologica Hungarica 54(3–4): 285–298. https://doi.org/10.1556/ABiol.54.2003.3-4.7

Kersey DC and Dehnhard M, 2014. The use of noninvasive and minimally invasive methods in endocrinology for threatened mammalian species conservation. General and Comparative Endocrinology 203: 296–306. https://doi.org/10.1016/j.ygcen.2014.04.022

Koch RE, Mcgraw KJ and Hill GE, 2016. Effects of diet on plumage coloration and carotenoid deposition in red and yellow domestic canaries (Serinus canaria). The Wilson Journal of Ornitholog 128(2): 328–333. https://doi.org/10.1676/wils-128-02-328-333.1

Kumar A, Mehrotra S, Dangi SS, Singh G, Singh L, Mahla AS, Kumar S and Nehra K, 2013. Faecal steroid metabolites assay as a non-invasive monitoring of reproductive status in

animals. Veterinary World 6(1): 59–63. https://doi.org/10.5455/vetworld.2013.59-63

Kusuma PW, Bebas W and Budiasa MK, 2018. Motilitas dan daya hidup spermatozoa ayam pelung dalam pengencer kuning telur fosfat yang disimpan pada suhu 29°C. Indonesia Medicus Veterinus 7(2): 115–122. https://doi.org/10.19087/imv.2018.7.2.115

Li CY, Basit A, Anshul Gupta, Gáborik Z, Kis E and Prasada B, 2019. Major glucuronide metabolites of testosterone are primarily transported by MRP2 and MRP3 in human liver, intestine and kidney. The Journal of Steroid Biochemistry and Molecular Biology 191: 1–9. https://doi.org/10.1016/j.jsbmb.2019.03.027

Magfira, Arifiantini RI, Karja NWK and Darwati S, 2017. Efektivitas low density lipoprotein dan kuning telur ayam dan puyuh pada pengawetan semen ayam merawang. Jurnal Veteriner 18(3): 345. https://doi.org/10.19087/jveteriner.2017.18.3.345

Masfi R and Mafardi, 2022. The symbolization of the kukuak balenggek chicken statue, solok regency, west sumatra province: analysis of cultural mythology. International Journal of Science and Society 4(3): 251–264. https://doi.org/10.54783/ijsoc.v4i3.517

Millspaugh JJ and Washburn BE, 2004. Use of fecal glucocorticoid metabolite measures in conservation biology research: considerations for application and interpretation. General and Comparative Endocrinology 138: 189–199. https://doi.org/10.1016/j.ygcen.2004.07.002

Moghaddam G, Pourseif MM, Asadpour R, Rafat SA and Jafari-Jozani R, 2012. Relationship between levels of peripheral blood testosterone, sexual behavior, scrotal circumference and seminal parameters in crossbred rams. Acta Scientiae Veterinariae 40(3): 1–8.

Möstl E, Rettenbacher S and Palme R, 2005. Measurement of corticosterone metabolites in birds' droppings: an analytical approach. Annals of the New York Academy of Sciences 34: 17–34. https://doi.org/10.1196/annals.1343.004

Mphaphathi ML, Seshoka MM, Luseba D, Sutherland B and Nedambale TL, 2016. The characterisation and cryopreservation of Venda chicken semen. Asian Pacific Journal of Reproduction 5(2): 132–139. https://doi.org/10.1016/j.apjr.2016.01.009

Muryanto and Pramono D, 2014. Sukses budi daya ayam kampung. Sudaryanto B, Winarti Y, editor. Ungaran

Oduwole OO, Huhtaniemi IT and Misrahi M, 2021. The roles of luteinizing hormone, follicle-stimulating hormone and testosterone in spermatogenesis and folliculogenesis revisited. International Journal of Molecular Sciences 22(23): 12735. https://doi.org/10.3390/ijms222312735

Palme R, Rettenbacher S, Touma C, El-bahr SM and Möstl E, 2005. Stress hormones in mammals and birds: comparative aspects regarding metabolism, excretion, and noninvasive measurement in fecal samples. Annals of the New York Academy of Sciences 171: 162–171. https://doi.org/10.1196/annals.1327.021

Peters A, Astheimer LB, Boland CRJ and Cockburn A, 2000. Testosterone is involved in acquisition and maintenance of sexually selected male plumage in superb fairy-wrens, Malurus cyaneus. Behavioral Ecology and Sociobiology 47(6): 438–445. https://doi.org/10.1007/s002650050688

Du Plessis SS, Agarwal A, Mohanty G and Van Der Linde M, 2015. Oxidative phosphorylation versus glycolysis: What fuel do spermatozoa use?. Asian Journal of Andrology 17(2): 230–235. https://doi.org/10.4103/1008-682X.135123

Preston BT, Stevenson IR, Lincoln GA, Monfort SL, Pilkington JG and Wilson K, 2012. Testes size, testosterone production and reproductive behaviour in a natural mammalian mating system. Journal of Animal Ecology 81(1): 296–305. https://doi.org/10.1111/j.1365-2656.2011.01907.x

Queiroz SA and Cromberg VU, 2006. Aggressive behavior in the genus Gallus sp. Brazilian Journal of Poultry Science 8(1): 1–14. https://doi.org/10.1590/S1516-635X2006000100001

Rahimpoor M, Khosravinia H and Khaldari M, 2016. Rooster phenotypic traits influence on the reproductive performance of the broiler parents. Indian Journal of Fundamental and Applied Life Sciences 6(2): 7–12.

Rettenbacher S, Möstl E, Hackl R, Ghareeb K and Palme R, 2004. Measurement of corticosterone metabolites in chicken droppings. British Poultry Science 45(5): 704711. https://doi.org/10.1080/00071660400006156

Rohrmann S, Platz EA, Selvin E, Shiels MS, Joshu CE, Menke A, Feinleib M, Basaria S, Rifai N and Dobs AS, 2011. The prevalence of low sex steroid hormone concentrations in men in the Third National Health and Nutrition Examination Survey (NHANES III). Clinical Endocrinology 232–239. https://doi.org/10.1111/j.1365-2265.2011.04043.x

Ros AFH, 1999. Effects of testosterone on growth, plumage pigmentation, and mortality in Black-headed Gull chicks. IBIS International Journal of Avian Science. 141(3): 451–459. https://doi.org/10.1111/j.1474-919x.1999.tb04414.x

Rowe M, Swaddle JP, Pruett-Jones S and Webster MS, 2010. Plumage coloration, ejaculate quality and reproductive phenotype in the red-backed fairy-wren. Animal Behaviour 79(6): 1239–1246. https://doi.org/10.1016/j.anbehav.2010.02.020

Rusfidra A, 2006. Pengembangan riset bioakustik di indonesia: studi pada ayam kokok balenggek, ayam pelung dan ayam bekisar. National Conferences of Math and Natural Science, Yogyakarta State University, pp: 352–372.

Rusfidra, Gusrizal M, Gusrin Y, Abbas MH, Husmaini, Arlina F, Subekti K and Nova TD, 2015. Flock composition, effective population size and inbreeding rate of kokok balenggek chicken breed under in-situ conservation. International Journal of Poultry Science 14(2): 117–119. https://doi.org/10.3923/ijps.2015.117.119

Rusfidra, Marajo SDT, Heryandi Y and Oktaveriza B, 2014. Estimation of inbreeding rate in kokok balenggek chicken (kbc) population under ex-situ conservation. International Journal of Poultry Science 13(6): 364–367. https://doi.org/10.3923/ijps.2014.364.367

Sajjad M, Ali S, Ullah N, Anwar M, Akhter S and Andrabi SMH, 2007. Blood serum testosterone level and its relationship with scrotal circumference and semen characteristics in Nili-Ravi buffalo bulls. Pakistan Veterinary Journal 27(2): 63–66.

Schiffer L, Barnard L, Baranowski ES, Gilligan LC and Taylor AE, 2019. Human steroid biosynthesis, metabolism and excretion are differentially reflected by serum and urine steroid metabolomes: a comprehensive review. The Journal of Steroid Biochemistry and Molecular Biology 194: 1–25. https://doi.org/10.1016/j.jsbmb.2019.105439

Schröcksnadel H, Bator A and Frick J, 1971. Plasma testosterone level in cocks and hens. Steroids 18(3): 359–365. https://doi.org/10.1016/0039-128X(71)90049-3

Schwarzenberger F, Möstl E and Palme R, 1996. Faecal steroid analysis for non-invasive monitor & of reproductive status in farm, wild and zoo animals. Animal Reproduction Science 42: 515–526. https://doi.org/10.1016/0378-4320(96)01561-8

Senger P, 2005. Pathways to pregnancy and parturition. second rev. Washington DC: Washington State University Research & Technology Park

Setiadi DR, Hasibuan H, Indriastuti R, Arif AA, Rosyada ZNA, Arifiantini RI and Sumantri C, 2019. Karakteristik semen ayam IPB-D1. Jurnal Ilmu Produksi dan Teknologi Hasil Peternakan 7(2): 57–61.

Shah W, Khan R, Shah B, Khan A, Dil S, Liu W and Wen J, 2021. The molecular mechanism of sex hormones on sertoli cell development and proliferation. Frontiers of Endocrinology

(Lausanne). 12: 648141. https://doi.org/10.3389/fendo.2021.648141

Sterling LA, Clawitter H, Kozlowski CP, Macek M and Tieber A, 2016. Patterns of fecal progestagens, estrogens, and androgens associated with reproduction in blue-throated piping guans (Pipile cumanensis). Undergraduate Research Symposium Posters: 77.

Sun Y, Xue F, Li Y, Fu L, Bai H, Ma H, Xu S and Chen J, 2019. Differences in semen quality, testicular histomorphology, fertility, reproductive hormone levels, and expression of candidate genes according to sperm motility in Beijing-You chickens. Poultry Science 98(9): 4182–4189. https://doi.org/10.3382/ps/pez208

Tabatabaei S, Chaji M and Mohammadabadi T, 2010. Correlation between age of rooster and semen quality in iranian indigenous broiler breeder chickens. Journal of Animal and Veterinary Advances 9(1): 195–198. https://doi.org/10.3923/javaa.2010.195.198

Talebi A, Alimehr M, Alavi MH, Najafi G and Simaei N, 2018. Comparative study of semen traits and histomorphometric features of testes of broiler breeder males with different phenotypic traits. Veterinary Research Forum 9(1): 1–6.

Walker WH, 2011. Testosterone signaling and the regulation of spermatogenesis. Spermatogenesis. 1(2): 116–120. https://doi.org/10.4161/spmg.1.2.16956

Wingfield JC, Ball GF, Jr AM. D, Hegner RE and Ramenofsky M, 1987. Testosterone and aggression in birds. American Scientist 75(6): 602–608.

Wishart GJ and Staines HJ, 1999. Measuring sperm:Egg interaction to assess breeding efficiency in chickens and turkeys. Poultry Science 78(3): 428–436. https://doi.org/10.1093/ps/78.3.428