Evaluation of scrotal circumference and testosterone levels at different ages of Gayo buffalo as local animal genetic resources

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Abstract. The purpose of this study was to evaluate the scrotum circumference and testosterone levels at various ages of the Gayo buffalo. This study involved 10 Gayo buffalo with four different age groups I: (1.0–2.0), II (2.1–3.0), III (3.1–4.0), IV (4.1–5.0). Blood serum sampling, measurement of scrotal circumference, and age were carried out in Central Aceh regency. The analysis of blood serum testosterone levels was carried out in the physiology laboratory of the Faculty of Veterinary Medicine at Universitas Syiah Kuala. Data on testosterone levels, scrotal circumference, and age were analyzed for variance and continued with Duncan's Multiple Range Test for data with significant differences. Simple regression analysis was used to determine the relationship between scrotal circumference, age, and testosterone levels. The results of the analysis in the age group and the concentration of the hormone testosterone showed significant differences (P <0.05). The age group of 3.1 to 4.0 years was significantly different from the age group of 1.0–2.0, 2.1–3.0 years, and 4.1–5.0 years. The results of the analysis on the scrotal circumference and testosterone concentration showed no significant difference (P >0.05) in various age groups. There was a significant relationship (P<0.05) between the concentration of the testosterone hormone with age and scrotal circumference, with a regression equation Y= 1.009-0.076X₁+0.002X₂, with a correlation coefficient (r) of 1 and a coefficient of determination (R²) of 1. These results indicated that scrotal circumference and age greatly influenced the concentration of the testosterone hormone of Gayo buffalo. The conclusion was that age affected the scrotal circumference, but it did not affect the concentration of the testosterone hormone. Gayo buffalo aged 3.1–4.0 and 4.1–5.0 years had a stronger correlation than Gayo buffalo aged 1.0–2.0 and 2.1–3.0 years to scrotal circumference and testosterone concentration.

1. Introduction
Buffalo is a livestock that is easily found in Indonesia as an agricultural country. There are various benefits of maintaining buffalo, including plowing in the fields, transportation, sources of meat and milk, and industrial raw materials. Preservation of genetic resources of local livestock as assets owned by an area is deemed necessary as an effort to preserve the natural wealth that exists in a place. Through the decision of the Minister of Agriculture of the Republic of Indonesia. Number: 302/Kpts/SR.120/5/2017 dated May 4, 2017, Gayo buffalo is officially designated as one of the buffalo clumps that have a characteristic shape and composition of genetic composition that is unique and different from other buffaloes that have been recorded throughout the world [1].
Gayo buffalo is local livestock that is raised and developed in a hereditary way, so it has historical, social value and has been integrated with the lives of the people in the highlands of Gayo. A local people use Gayo buffalo as domesticated animals which produce meat. For them, Gayo buffalo is the dominant commodity as a source of animal protein which is loved by the community. The utilization of genetic potential possessed by Gayo buffalo must also be applied through more extensive scientific studies. The spread of Gayo buffalo seeds as an attempt to maintain genetic purity and spread its potential nationally is considered necessary through ongoing research to address the decline in buffalo population caused by an imbalance in the number of cuts with relatively slow buffalo child production. Buffalo fertility is generally low with late puberty achieved, unclear estrus, and long postpartum ovarian inactivity [2–4]. Male buffalo sperm concentration is relatively lower than cattle sperm concentration. One contributing factor is the hormonal influence since hormones affect the volume and concentration of sperm produced by seminiferous tubular cells, thereby affecting libido in male buffalo [5].

The above description shows that the performance of male buffalo reproductive hormones is very necessary. However, information about the profile of male buffalo testosterone has not been fully presented, especially regarding the profile of male buffalo testosterone in Central Aceh. This is useful for increasing the buffalo population in Aceh Province or specifically in Central Aceh Regency.

2. Research method

This study involved 10 male Gayo buffaloes which were traditionally maintained by local people with natural grass feed and without using concentrates. The Gayo buffaloes involved were in the age range of 2–5 years. The sample was divided into three age groups: group I (1–2 years), group II (2.1–3 years), group III (3.1–4 years) and group IV (4.1–5 years). The buffalo criteria used in this study included proportional and healthy body condition, no disability, having a symmetrical pair of testes with a springy consistency, aged 1–5 years with a bodyweight of 125–500 kg. The equipment used was a testosterone kit (ELISA EIA-1559), EDTA (BD vacutainer, (K2E) 5.4 mg), needle (BD vacutainer®, PrecisionGlideTM, Multiple Sample needle 0.7x38 mm), holder, alcohol 70%, cotton, centrifuge (Hettich EBA20 Portable Centrifuge C2002, DJB Labcare Ltd, England), plasma tubes with a capacity of 2 ml, freezer (temperature -20°C), Shaker (model VRN-200, Gemmy Industrial Corp, Taiwan), Immuno wash (model 1575, Cat. No. 170-7009, Bio-Rad, California, USA), Benchmark Microplate Reader (Cat. No. 170-6850, Bio-Rad, California,USA), and a set of tools and materials of DRG Testosterone ELISA Kit [6]. The interpretation of the buffalo age used in the study was carried out through buffalo records, from the owner of the farm and the physical appearance of the buffalo. The measurements of scrotal circumference for each experimental animal according to the age group were carried out based on the Boyle method [7]. A sampling of 5 ml of buffalo blood was carried out in the morning (07.00–12.00 WIB) on the jugular vein using a vacutainer equipped with a venoject. Furthermore, the venoject tube was placed in an oblique position to facilitate the taking of serum. Parts of the blood that had undergone a separation (serum) were taken and inserted into a microtube and labeled. The serum was then put into a freezer at -20°C until testosterone concentration was tested. Moreover, the samples were diluted with aquadestilates in a ratio of 1:4. Standard solutions were prepared in concentrations of 0.2 ng/ml to 16 ng/ml. A total of 25 μl of sample and solution were put into the ELISA microplate. The conjugate enzyme was then added to each ‘well’ except the blank well, and covered with cling film and homogenized by slowly shaking for 10 seconds. The next step was to incubate them for 60 minutes at room temperature. After the incubation, each microplate well was washed with a washing solution of 300 μl each for 3–4 washing. After washing, 200 μl substrate solutions were put into each well and covered with cling film, and incubated for 15 minutes at room temperature. The enzymatic reaction was stopped by adding 100 μl of 0.5 M H₂SO₄ stop solution to each well. The absorbance readings were carried out using an ELISA reader at a wavelength of 450 nm [6]. Data were analyzed using one-way analysis of variance (ANOVA). If there were differences between treatments, the data were then tested by Duncan's multiple range tests. Simple regression analysis was used to determine the relationship between age and scrotal circumference with testosterone levels [8].
3. Results and discussion
The average circumference of the Gayo buffalo scrotum in this study ranged from 19.5 to 29.5 cm. This result was far greater than the study of Noviana et al (2006) that found the scrotal circumference of Simeulue buffalo at the age range of 2.1–5.0 years was 11.62–18.30 cm and Qadarsina (2019) who pointed out that the scrotal circumference of buffalo mud aged 36–60 months old was 21.35 cm [9,10]. The average circumference of the Gayo buffalo in different age groups is shown in table 1.

| Age groups (year) | Scrotal circumference (cm) |
|-------------------|-----------------------------|
| 1.0–2.0           | 20.0±3.0^a                  |
| 2.1–3.0           | 21.3±2.3^a                  |
| 3.1–4.0           | 29.5±0.7^b                  |
| 4.1–5.0           | 19.5±0.7^a                  |

Different superscripts in the same column show ‘significantly different’ (P<0.05).

Statistical analysis (Anova) results showed that there were significant differences (P<0.05) between the four buffalo age groups. These results prove that the age of the Gayo buffalo significantly influences the scrotal circumference. Furthermore, Duncan's multiple test results showed that the circumference of the Gayo buffalo age 3.1–4.0 years was significantly different than other age groups. These results prove that the greater the age group of the Gayo buffalo, the increasing the circumference of the Gayo buffalo scrotum. The size of the scrotal circumference would continue to increase with age and the size of the scrotal circumference reached a fixed number and did not change when the buffalo body was 'mature' [11] and [12]. This opinion is also supported by Luz (2013) that the bodyweight of the buffalo has a very significant effect on the size of the scrotal circumference [13]. Affirmed that a high body weight score could directly affect the performance of male buffalo reproductive organ development [14]. Moreover, the scrotal circumference of the Gayo buffalo of the age groups from 1.0 to 3.0 years and 4.1 to 5.0 years were not significantly different (P>0.05). It was allegedly caused by feed management which still relied on the field grass as the main feed without the addition of concentrate booster feed. The reproductive ability of male animals could be determined by libido, semen quality, and spermatozoa concentration. The results of the average measurement of the concentration of testosterone of buffalo Gayo in each age group are presented in table 2.

| Age groups (year) | Testosterone levels (ng/ml) |
|-------------------|-----------------------------|
| 1.0–2.0           | 0.97±0.7                    |
| 2.1–3.0           | 0.88±0.64                   |
| 3.1–4.0           | 0.90±0.57                   |
| 4.1–5.0           | 0.72±0.57                   |

The same superscripts show no significant difference (P> 0.05).

The average concentration of Gayo buffalo testosterone in various age groups in this study showed that the results were not significantly different. Status in the population plays a role in influencing low levels of testosterone and psychic livestock in which other males are more dominant in the group which can trigger weaker males experiencing stress. It inhibits the development of the reproductive system including the hormonal system in the body. Sexual behavior in mammals highly depends on social...
status, and daily activities [1]. Another factor that can trigger high and low levels of hormones is the palpable factor or stimulation factor given to the reproductive organs. Stimulation can trigger the central nerve to produce higher or lower testosterone levels [15]. Fluctuations in testosterone levels in livestock are determined by the physiological conditions of livestock. High and low hormones are caused by sensory stimulation, which is a stimulus received by the sensing system through the central nerve. These stimuli include light through the eyes, sounds captured by the ears, smell through the nose, levels of food, while physical stimuli include cold and heat and the amount of work, stress due to the release of glucocorticoid hormones from the adrenal glands, sensation as caused ascension, and intromission of the penis into the vagina [15].

Table 3 shows the correlation coefficient and determinant coefficient between serum testosterone concentration and scrotal circumference of buffalo Gayo aged 1.02 years with a correlation coefficient \( r = 0.866 \) and a determinant coefficient \( (R^2) = 0.750 \). The correlation coefficient \( (r = 0.866) \) is strong, which means that the close relationship between scrotal circumference \( (Y) \) and serum testosterone concentration \( (X) \) is strong. The value of the determinant coefficient \( (R^2=0.750) \), meaning that 75.50% concentration of serum testosterone \( (Y) \) Simeulue buffalo aged 1.0–2.0 years is influenced by scrotal circumference. This condition is likely due to the young Simeulue buffalo (aged 2.1–3.0 years) having genital organs and complementary genital glands which are in the stage of growth. Samsudewa et al (2012) stated that the development of reproductive organs is influenced by testosterone as the age to a certain age [16].

Table 3. The correlation coefficient \((r)\), determinant coefficient \((r^2)\) between testosterone concentrations and scrotal circumference of Gayo buffalo in various age groups

| Age groups(year) | Correlation coefficient \((r)\) | Determinant coefficient \((R^2)\) |
|------------------|---------------------------------|-------------------------------|
| 1.0–2.0          | 0.866                           | 0.750                         |
| 2.1–3.0          | 0.999                           | 0.997                         |
| 3.1–4.0          | 1                               | 1                             |
| 4.1–5.0          | 1                               | 1                             |

The results of this study proved that in buffaloes aged 2.1–3.0 years, the testosterone concentrations and scrotal circumference show a correlation coefficient \((r)\) of 0.999 and a determinant coefficient \((R^2)\) of 0.997. The correlation coefficient value \((r=0.999)\) is strong, which means that the close relationship between scrotal circumference \( (Y) \) and serum testosterone \( (X) \) concentration of Simeulue buffalo aged 2.1–3.0 years is also very strong. The determinant coefficient value \((R^2=0.997)\) illustrates that 99.70% of the concentration of buffalo serum testosterone at the age of 2.1–3.0 years is influenced by scrotal circumference. This result is because the genitals and genital glands of the Gayo buffalo aged 2.1–3.0 years have developed and have begun to function to produce testosterone and spermatozoa. The results of this study also prove that in male Gayo buffalo aged 3.1–4.0 and age 4.1–5.0 years, the concentration of testosterone and scrotal circumference show the same value, namely the correlation coefficient \((r)\) was 1 and the coefficient value the determinant \((r^2)\) is 1. The correlation coefficient \((r = 1)\) is very strong which means that the close relationship between scrotal circumference \( (Y) \) and serum testosterone concentration \( (X) \) is very strong. The value of the determinant coefficient \((r^2 = 1)\) illustrates that 100% of serum testosterone levels in the Gayo buffalo aged 3.1–4.0 years is influenced by scrotal circumference. These results indicate that the genital organs and complementary sex glands have developed perfectly and are functioning to produce testosterone and spermatozoa. The results of multiple regression analysis were used to find out how big the relationship between age and scrotal circumference to the serum testosterone concentration of Gayo buffalo in all age groups. Table 4 shows that there is no significant difference \((P>0.05)\) between serum testosterone concentration with age and scrotal circumference, with a regression equation \( Y = 1.009 - 0.076 \times X1 + 0.002 \times X2 \), where \( X1 \) is age...
and X2 scrotal circumference, with values the correlation coefficient (r) of 0.171 and the determinant coefficient (r²) of 0.029. The determinant coefficient value (r²) of 0.029 shows that 2.90% of the concentration of serum testosterone is influenced by age (X1) and scrotal circumference (X2), while the remaining 97.10% is another factor not observed. The multiple correlation coefficient (r) obtained was 0.171 or 17.10%, indicating that age and scrotal circumference had a very low relationship with serum testosterone concentrations.

Table 4. Regression equation of the relationship between age and scrotal circumference with Gayo buffalo testosterone concentration

| Independent variable (Y) | Correlation coefficient (r) | Determinant Coefficient (R²) | Linear line equation | Sig. |
|--------------------------|------------------------------|-----------------------------|----------------------|------|
| Testosterone             | 0.171                        | 0.029                       | Y = 1.009–0.076 X1 + 0.002 X2 | P>0.05 |

The results of this study are supported by the results of the study of Sajjad et al. (2007) on 14-year-old Pakistani Ravi buffalo who found a low relationship (r = 0.414) between age and testosterone levels with a regression equation Y = 14.353-0.436 X [17]. The difference in the results of this study with the research of Toelihere (1993) was likely caused by differences in buffalo species and age, patterns of maintenance, and sexual activity of a male used [17].

4. Conclusion
The average buffalo scrotal circumference aged 3.1–4.0 had the largest size than other age groups that was 29.5±0.7 cm, having a stronger correlation than other age groups on the scrotal circumference and testosterone concentration in the age group 3.1–4.0 and 4.1–5.0 years. Age and scrotal circumference of Gayo buffalo showed a low association of r = 0.171 and r² = 0.029 to testosterone levels and there was no significant difference between male Gayo buffalo testosterone levels in various age groups.

Acknowledgments
Authors wishing to acknowledge assistance or encouragement from the team in Aceh Tengah subdistrict.

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