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Correlations between age of Bali bull and semen production at National Artificial Insemination Center, Singosari - Indonesia

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ABSTRAK

Penelitian ini bertujuan untuk menganalisis hubungan umur, berat badan dan kualitas semen pejantan sapi Bali. Materi yang digunakan dalam penelitian ini terdiri data rekording dari 50 pejantan sapi Bali dari tahun 2012 - 2018. Data tersebut termasuk catatan produksi semen (n = 1304) dan bobot badan (n = 1544). Observasi dilakukan untuk menganalisis hubungan antara umur dan bobot badan, volume semen, motilitas individu dan konsentrasi. Data yang diperoleh dianalisis dengan metode One-Way ANOVA menggunakan program SPSS 24. Korelasi antara umur sapi Bali (variabel independen) dan kualitas semen (variabel dependen) dihitung dengan menggunakan metode korelasi pearson dan disajikan dalam model regresi linier. Hasil penelitian menunjukkan bahwa umur mempengaruhi bobot badan (P<0,05) dari umur 1 hingga 10 tahun. Umur menunjukkan pengaruh yang signifikan (P<0,05) pada volume, motilitas individu dan konsentrasi. Umur berkorelasi secara signifikan (P<0,05) dengan bobot badan (r = 0,658), volume (r = 0,386), motilitas individu (r = 0,134) dan konsentrasi semen (r = 0,086). Disimpulkan bahwa umur sapi Bali dapat digunakan sebagai indikator untuk mengukur bobot badan dan kualitas semen pada sapi Bali.

Kata kunci: korelasi, umur, bobot badan, kualitas semen, sapi bali

ABSTRACT

The aim of this research was to analyze the correlation of age of Bali bull to the body weight and semen qualities. The materials were 50 Bali bulls data collected from 2012 to 2018. The recorded data were consisted of semen productions (n = 1304) and body weight (n = 1544). The observation was conducted to analysis the interrelationship between the Bali bulls age and body weight (BW), to the semen volume (SV), individual motility (IM) and sperm concentration (SC). The obtained data was analyzed with One-Way ANOVA analysis by using SPSS 24 and followed with Duncan's Multiple Range Test to determine any significant differences. The correlation between Bali bulls age (the independent variable) and semen quality (the dependent variable) was computed by using Pearson correlation method and presented in linear regression models. The results showed that the Bali bulls age significantly

affect (P<0.05) the BW from the age of 1 to 10 years old. The age also showed a significant effect (P<0.05) on the semen qualities. The age correlated significantly (P<0.05) with BW, SV, IM, SC were 0.658, 0.386, 0.134 and 0.086, respectively. In conclusion, the age of Bali bull could be used as an indicator to determine its BW and semen qualities.

Keywords:correlation value, age, body weight, semen quality, Bali bull

INTRODUCTION

Bali cattle (*Bos Sondaicus*) is one of the indigenous cattle breeds which have the highest population among other indigenous breeds in Indonesia. This breed was known to have high resistance to the tropical parasites and environment (Pribadi *et al.,* 2014). Furthermore, the breed is also resistant to poor feed quality and has better reproductive efficiency compared other indigenous breeds (Pribadi *et al.,* 2015). Based on data from Indonesian Central Bureau of Statistics (BPS, 2011), the population of Bali cattle in Indonesia reached 4,789,521; which consisted of 1,493,213 bulls and 3,296,308 cows. The data thus indicates that Bali breed has an important role in the supplying meat in Indonesia. Bali bulls in Indonesia has very important roles in fulfilling a source of breeding cattle and providing agricultural labor, source of income of farm households (Kapa *et al*., 2018). Lindell (2013) showed that the body weight of Bali bull was varied on different locations, with Bali bulls raised in Bali and Lombok were weighted at 466 kg, and 262 kg, respectively. However, the report did not present the observed bull's age in detail.

One of the efforts to support the continuous Bali cattle breeding is by improving reproductive performance for female and male, since reproduction aspect is one of most important factors determining the success of genetic improvement, especially supported by artificial insemination program (Suyadi *et al.,* 2014). The bull as the source of semen production should have fit performance to ensure producing continuously semen with standardized volume and quality. The age and body of cattle are known to affect the semen production and quality, especially during puberty phase, although the high effect of the birth weight on the semen production was also reported (Gupta *et al.,* 2016).

For achieving success of artificial insemination, the minimum criteria of semen quality should be met. For better result of artificial insemination, the semen at least contains 60% to 75% sperm motility (Ax *et al.* (2008a), sperm mass movement minimum value 2+ (Argiris *et al.,* 2017) and sperm abnormality ≤ 20% (Menon *et al*., 2011). In cattle, the normal volume was 7 to 10 mL per ejaculate with sperm concentration was 1000×10^6 to 1500×10^6 sperm/mL (Ax *et al.,* 2008b).

The accurate prediction of semen volume per ejaculate and semen characteristics is important to determine whether the male cattle will be selected as bull for semen production or not. The easy method should be established so could be useful and valuable tools for pre-determination of bull candidate by practitioner in farm. The high correlation between semen volume and sperm motility was reported in Sahiwal bulls (Ahmad *et al.,* 2011), where body weight, scrotal circumference, and number of pixels of testicular ultrasonograms were positively correlated with age from birth until >100 months (r = 0.91, 0.87, and 0.40, respectively).

In Cholistani Bull Pakistan, Mahmood *et al.* (2014) reported that age of bull had significantly correlatation with semen volume (r = 0.93), negative correlation with sperm concentration (r = -0.94), and with sperm viability (r = -0.87). In Indonesian Ongole Grade cattle, Muthiapriani *et al.* (2019) reported no significant correlation between body weight with semen volume, sperm concentration and sperm motility (r = 0.48, 0.33 and 0.65, respectively) might be due to limited number of sample (n = 9).

The aim of this research was to analyze correlations between age and body weight, semen volume, individual motility, sperm concentration of Bali bulls at National Artificial Insemination Center, Singosari, Indonesia.

MATERIALS AND METHODS

Animals

This research was conducted at National Artificial Insemination Center (NAIC) at Singosari, Malang, East Java, Indonesia. NAIC is a government agency that produces frozen semen for many regions in Indonesia, especially East Java, and during the latest years it expands to overseas market in ASEAN countries. The material was ejaculate records of 50 Bali bulls

collected from 2012 to 2018. The bulls included in this study aged 1 to 10 years old, 260 to 784 kg body weight. The animals were kept individually in the house, and were daily fed with 10 kg forage crop, 2.5 kg silage, 2.5 kg concentrate, 3.0 kg hay and 0.05 kg commercial minerals/animal. While drinking water was served ad libitum.

Data Collections

The method of this study was observation by collecting secondary data of the records of individual bull including age (AG), body weight (BW), semen volume (SV), sperm motility (SM) and sperm concentration (SC). While semen was collected from the Bali bull by artificial vagina (AV) from the age of 2 years until 10 years old, twice a week of each. The body weight was measured monthly from 1 years until 10 years old. Semen volume (SV) was determined directly after semen collection, while the sperm motility (SM) was observed through a microscope without a cover glass with 400x or 100x magnification at a stable temperature (Susilawati, 2011). The sperm concentration ($SC \times 10^6$/mL) was observed by using a spectrophotometer. According to this criterion, it was collected 1544 records BW, 1304 for SV, 1304 for SM and 1304 for SC.

Data Analysis

The age of animal was grouped according yearly age, and then was analyzed by using computer statistical package (SPSS 24) with One-Way ANOVA analysis. The result was further analyzed with Duncan's Multiple Range Test (DMRT) to determine any significant differences. The correlation between age and semen parameters was analyzed by using Pearson correlation method (r). Linear regression analysis was done to determine the correlation between Bali bull's age and semen production by using computer statistical package (SPSS 24). Age (the independent variable presented as X) and body weight, semen volume, individual motility or sperm concentration (the dependent variable presented as Y) in the produced linear equation. However, if the results of regression analysis did not show a linear regression, the data would then analyzed with cubic polynomial model as nonlinear equation (Isaac *et al.,* 2012).

RESULTS AND DISCUSSION

Effect of Age on the Body Weight

Table 1 showed that age gave significant effect ($P<0.05$) on the body weight of Bali bull. The highest body weight of Bali cattle was obtained at the age of 10 years, while the lowest body weight was obtained at the age of 1 year. The average body weight of Bali bull was 212 ± 46.2 kg to 660 ± 17.9 kg, with the highest increase was occurred at the age of 1 to 2 years. The result is similar to research by Silveira *et al.* (2018) which showed that the average body weight increases with age. Furthermore, Controlled feed management is considered as a supporting factor, noting that Singosari Artificial Insemination Center is specifically aimed to produce bull semen with high quality. Garcia *et al.* (2008) stated that feed affects body weight in the form of compensatory growth as a form of the body response. However, the average body weight from this study is different from other studies. Haque *et al.* (2012) showed 24 months Brahman cross bull weighted at 222 kg; while Tahuk *et al.* (2017) showed that 24 to 30 months Bali bull weighted at 229 kg.

The highest growth occurs at the age of 1 to 4 years. This is regarding that during this age Bali bull would enter the rapid growth phase as the growth hormone which would affect body weight increased rapidly. Lawrence and Fowler (2002) stated that growth is the process of an increasing the number of cells in the tissue (hyperplasia) and increasing the size of cells in the tissue

Table 1. Average Body Weight of Bali Bulls at Age 1 to 10 Years

Age	Number of Data	Mean (kg) ± sd
1	9	212 ± 46.2^{a}
2	78	441 ± 90.3^{b}
3	188	468 ± 66.1^{c}
4	189	579 ± 61.9^{d}
5	228	591 ± 73.9^{de}
6	331	583 ± 67.7^{d}
7	288	597 ± 50.9^{de}
8	130	610 ± 36.9^{e}
9	72	636 ± 66.1^{f}
10	31	660 ± 17.9^{f}

Different superscripts shows significant differences ($P<0.05$)

(hypertrophy). Stimulation of the hypothalamus in anterior pituitary to produce Growth Hormone (GH). GH stimulates skeletal muscle cells, adipose tissue and bone. GH increases muscle deposition and reduces fat content. Moreover, the growth pattern does not resemble a sigmoid ('S') pattern as the Bali bull still experienced a growth phase even at the age of 10 years. This is regarding that bulls aged more than 8 years old accumulation of fat in the body. Hossner (2005) stated that the establishment of fat occurs in the nutritional energy in the feed exceeds the body's needs so that the energy expended in the form of fat.

Effect of Age on the Semen Quality

Table 2 showed that age gave significant effect ($P<0.05$) on the semen production of Bali bull (volume, individual motility and sperm concentration). The average semen volume from the age of 2 to 10 years tends to increase, however at the age of 3 years there is a slight decrease in the average semen volume compared to the age of 2 years old. The volume of Bali bulls from the age of 2 to 10 years ranged from 4.43 ± 1.12 mL to 6.40 ± 0.85 mL with the average at 5.47 ± 1.36 mL. The average semen volume produced by Bali bull in this research is higher with the study of Haryani *et al.* (2016) which was 3.62 mL, but lower than Lemma and Shemsu (2015) on Borana bull which produced 6.51 ± 2.69 mL semen. From the result, it can be seen that Bali bull at 3 years showed a decreasing semen production. It is allegedly caused by the young and still developing reproductive organs of the Bali bull. This is supported by the statement of Bhakat *et al.* (2011) that semen collected from mature bulls is of better quality than young bulls caused mature bulls showed better libido than young bulls.

The spermatozoa are formed in the seminiferous tubules located within the testicles. The semen production will increases along with the bull's age regarding to the positive correlation between bulls' age and the testicles size. The bigger testicles resulted in more seminiferous tubules which will increase the semen production. This is supported by Azenabor *et al.* (2015) that the process of spermatogenesis takes place within the seminiferous tubules. spermatogenesis beginning with the formation of spermatogonia in the germinal epithelium, into primary and secondary spermatocytes and finally mature spermatozoa, It is highly dependent upon optimal conditions for the process to occur correctly. Susilawati (2011) stated that 90% of the testicles are composed of seminiferous tubules, while the other 10% are composed of intertitiel cells and connective tissues.

The average individual motility of Bali bulls tends to increase at the age of 2 to 7 years, then

Table 2. Average Semen Quality of Bali Bull at the Age of 2 to 10 Years Old

Age	n (ejaculate)	Volume (mL)	Individual Motility (%)	Sperm Concentration (10^6)
		---------- Mean ± sd ----------		
2	35	4.74 ± 1.41^{ab}	56.7 ± 15.6^{a}	1088 ± 239^{ab}
3	137	4.43 ± 1.12^{a}	60.1 ± 11.6^{b}	1124 ± 306^{b}
4	157	4.90 ± 1.09^{bc}	62.5 ± 10.2^{bc}	1068 ± 270^{ab}
5	185	5.21 ± 1.16^{c}	62.5 ± 9.38^{bc}	1004 ± 289^{a}
6	278	5.67 ± 1.24^{d}	62.5 ± 10.1^{bc}	1026 ± 278^{ab}
7	271	5.82 ± 1.44^{d}	64.5 ± 8.93^{c}	1092 ± 284^{ab}
8	151	5.98 ± 1.26^{de}	62.9 ± 11.2^{bc}	1086 ± 257^{ab}
9	69	6.29 ± 1.32^{e}	62.8 ± 11.9^{bc}	1032 ± 273^{ab}
10	21	6.40 ± 0.85^{e}	65.6 ± 7.66^{c}	1070 ± 173^{ab}

Different superscrip shows significant differences ($P<0.05$)

decreased at the age of 8 to 9 years, and increased again at the age of 10 years old. The lowest individual motility was found at the age of 2 years old (56.7 ± 15.6%) and the highest was found at the age of 10 years old (65.6 ± 7.66%). The average sperm motility in this researchis different compared to Ax *et al.* (2008a), who stated that bulls that are good for breeding should have a minimum motility value at 65%. However, research by Lemma and Shemsu (2015) in Borana cattle showed that sperm motility is at 79,2 ± 2.16%. Similarly, Lukman *et al.* (2014) stated that the semen motility of Bali bull aged 3 years is 56.0 ± 3.84%. The lowest sperm motility was found at the age of 2 years as the reproductive organs of the Bali bull were still growing. Furthermore, Bali bulls aged from 2 to 7 years showed an increasing sperm motility, but subsequently decreased due to reduced reproductive organ function. This is similar with Salisbury and Van Demark (1985) that showed bulls aged from 2 to 7 years would yield the best semen production compared to bulls outside the age interval.

Sperm motility is the progressive movement of an individual spermatozoa. The result of this research showed that age differences affected individual sperm motility in Bali bull. This is due to the motility and the viability of semen which requires an energy produced in the form of ATP. This energy is produced by the accessory glands, therefore the varying ATP production by the accessory glands caused different sperm motility. The energy used for semen movement came from the accessory gland which produced seminal plasma. Seminal plasma is a biochemical component which contains organic compounds such as fructose, citric acid, potassium, sorbitol, insitol, and glycerylphosphoryl-choline (GPC) (Susilawati, 2011). Seminal plasma plays an important role to maintain the spermatozoa motility, and also act as spermatozoa carrier and protector.

The average sperm concentration measured in this research is fluctuating from the ages of 2 to 10 years old. The average sperm concentration ranged from 1004 ± 289 $\times 10^6$/ML to 1124 ± 306 $\times 10^6$/mL. The lowest average sperm concentration was found at the age of 5 years old and the highest was found at the age of 3 years old. The results of this research were different from research by Sarsaifi *et al.* (2013) which stated that the average sperm concentration in Bali bull at 2 to 3 and 3 to 4 years were 731 ± 14.9 and 738 ± 14.7 million/ML. In Borana bull, Lemman and Shemsu (2015) found that the average sperm concentration was 1,350 ± 0.39 million/mL. In Raj-08 cattle (Local x Friesian x Friesian) the average sperm concentration was 1,225 ± 95.3 million/mL (Akhter *et al.,* 2013).

The sperm concentration is the number of spermatozoa contained in one ejaculation. Sperm concentration is related to the semen consistency used to determine the dilution level. The average sperm concentration in this research was very fluctuative. Salisbury and Vandemark (1985) showed that the sperm concentration will follow sexual development and maturity, reproductive health, size of the testes, age, quality of feed given and frequency of ejaculation. The average sperm concentration of Bali bull in this research is in accordance with Ax *et al.* (2008b) who stated that the sperm concentration is at least 1,000 million/mL.

Correlation between Age and Body Weight, Semen Quality

The results of the analysis showed that age (X) correlated with body weight, semen volume, individual motility and sperm concentration (Y) which can be seen in Table 3. The highest correlation value is found between age and body weight (r = 0.668) (P<0.05), while the lowest was found between age and sperm motility (r = 0.086) (P<0.05). The results of the correlation analysis showed that the coefficient of determination, meaning 43% of body weight; 15% of the volume; 1.8% of individual motility; 0.74% of the sperm concentration is explained by the age factor. The semen quality is also affected by body weight, healthy body condition, nutitional status, libido, frequency of ejaculation, skills of shelter operator (Susilawati, 2011; Nugraha *et al*., 2019). Different from Mahmood *et al.* (2014) that Cholistani bull at age to body weight (R^2 = 1.3%), volume (R^2 = 86%), individual motility (R^2 = 0.04%) and sperm concentration (R^2 = 88.9%). The higher the value of the coefficient of determination (R^2) shows the higher the accuracy of the regression line (Nugraha *et al*., 2019). Age can be used to estimate the body weight, semen volume, individual motility and sperm concentration of Bali bull with equations as follow:

a. Body weight, $Y = 61.469 + 235.213x - 34.978x^2 + 1.759x^3$

Table 3. Correlation between Age and Body Weight, Semen Quality of Bali Bull in 2012-2018

Correlation	r	R^2	Y	P Value
Age - BW	0.658	0.43	$y = 61.469 + 235.213x - 34.978x^2 + 1.759x^3$	0.000
Age - SV	0.386	0.15	$y = 3.8 + 0.285x$	0.000
Age - SM	0.134	0.018	$y = 47.454 + 6.483x - 0.884x^2 + 0.04x^3$	0.000
Age - SC	0.086	0.0074	$y = 1502.788 - 234.479x + 37.681x^2 - 1.878x^3$	0.022

BW = Body Weight; SV = Semen Volume; SM = Sperm Motility; SC = Sperm Concentration

b. Semen Volume, $Y = 3.800 + 0.285x$
c. Sperm motility, $Y = 47.454 + 6.483x - 0.884x^2 + 0.04x^3$
d. Sperm concentration, $Y = 1502.788 - 234.479x + 37.681x^2 - 1.878x^3$

CONCLUSION

Bali bull's age had a positive correlation with the average body weight, semen volume and sperm motility, while the sperm concentration tends to fluctuate over the bull's age. The age of Bali bull can be used as an indicator to measure its body weight and semen quality in terms of semen volume, and sperm motility.

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