Genetic and Non-Genetic Effects on Semen Characteristics of Bali Cattle (*Bos javanicus*)

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ABSTRAK

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Penelitian ini bertujuan mengevaluasi pengaruh faktor genetik dan non-genetik terhadap karakteristik semen meliputi volume semen, konsentrasi spermatozoa, jumlah total spermatozoa dan motilitas spermatozoa sapi Bali. Data semen (volume semen, konsentrasi spermatozoa, jumlah total spermatozoa dan motilitas spermatozoa) diperoleh dari Balai Besar Inseminasi Buatan Singosari, Malang, Jawa Timur, Indonesia. Sapi yang digunakan berjumlah 17 ekor dengan total 3.847 ejakulat pada tahun 2014 sampai 2016. Data dianalisis dengan metode *restricted maximum likelihood* (REML) menggunakan *mixed model* dengan individu sapi sebagai pengaruh acak, sementara umur, musim, frekuensi ejakulasi dan interval penampungan sebagai pengaruh tetap. Hasil studi menunjukkan bahwa umur berpengaruh nyata (P<0,01) terhadap semua karakteristik semen. Musim hanya berpengaruh terhadap motilitas spermatozoa (P<0,01). Frekuensi ejakulasi dan interval penampungan berpengaruh nyata terhadap semua karakteristik semen (P<0,01), kecuali motilitas spermatozoa. Ripitabilitas volume semen, konsentrasi spermatozoa, jumlah total spermatozoa dan motilitas spermatozoa masing-masing sebesar 0,43; 0,35; 0,32 dan 0,31. Disimpulkan bahwa umur, frekuensi ejakulasi dan interval penampungan sangat berpengaruh terhadap karakteristik semen sapi Bali. Ripitabilitas karakteristik semen termasuk sedang sampai tinggi.

Kata Kunci: Sapi Bali, Non-Genetik, Ripitabilitas, Karakteristik Semen

ABSTRACT

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The objective of this study was to evaluate effect of genetic and non-genetic factors on semen characteristics including ejaculate volume, sperm concentration, total sperm number and sperm motility of Bali cattle. Semen data were collected from the National Centre of Artificial Insemination at Singosari, Malang, East Java, Indonesia. A total of 3,847 ejaculates of 17 Bali bulls from 2014 to 2016 were collected and evaluated. Data were analyzed by restricted maximum likelihood (REML) method using mixed models which the bull was a random effect, while age of bull, season of collection, frequency of ejaculation and collection intervals were the fixed effects. Results showed that age significantly affected all semen characteristics (P<0.01). Season affected only on sperm motility (P<0.01). Effect of frequency of ejaculation and collection intervals was significant on all studied variables (P<0.01), except sperm motility. Repeatability of ejaculate volume, sperm concentration, total sperm number and sperm motility was 0.43; 0.35; 0.32 and 0.31, respectively. It is concluded that age, frequency of ejaculation and collection intervals were the most factors affected semen characteristics of Bali cattle. Repeatability estimations of semen characteristics were moderate to high.

Key Words: Bali Cattle, Non-Genetic, Repeatability, Semen Characteristics

INTRODUCTION

Bali cattle (*Bos javanicus*) is an Indonesian native cattle domesticated from direct descendants of wild Banteng (*Bibos banteng*) (Martojo 2012; Purwantara et al. 2012). The breed had been acknowledged by Food and Agriculture Organization as a world breed (FAO 2007). Bali cattle were spread in almost all Indonesian provinces. In 2011, the population of Bali cattle was recorded at 4.7 million heads (Badan Pusat Statistik 2011) and it represents 32% of the total Indonesian cattle population.

Bali cattle has several potential traits, such as the ability to survive in harsh environments (Panjaitan et al. 2014), high fertility and conception rate (Purwantara et al. 2012), high carcass percentage (Tahuk et al. 2018), and high meat quality (Jakaria et al. 2017). Hence, this breed has the potential for meat production and contributes to fulfill the national beef demand. The Indonesian government strives to increase the population and genetic quality of Bali cattle through artificial insemination (AI). The success or failure of AI is influenced by the quality of semen. The quality of semen itself is affected by genetic and non-genetic factors.

Repeatability evaluation on semen characteristics might be useful for selection of superior bulls. Repeatability estimations of semen characteristics such as ejaculate volume, sperm concentration, total sperm number and sperm motility were reported to vary from moderate (Karoui et al. 2011), high (Burren et al. 2019), and moderate to high (Atagi et al. 2017). The effect of non-genetic factors on semen characteristics is important to improve semen characteristics. Studies regarding the effect of non-genetic factors such as age of bull, season of collection, frequency of ejaculation and collection intervals on semen characteristics vary in different breeds and countries (Boujenane & Boussaq 2013; Snoj et al. 2013; Murphy et al. 2018). However, there is still less information in Bali cattle. Therefore, this study was done to investigate genetic and non-genetic effects influencing semen characteristics in Bali bulls.

MATERIALS AND METHODS

Semen data, animals and location

Data of semen characteristics were collected from 2014 to 2016. Those data were provided by the National Artificial Insemination (AI) Centre at Singosari, Malang, East Java, Indonesia. The AI Center has an ambient temperature between 16 and 22 ^{o}C, with humidity ranging from 70 to 90%. The AI Center was established in 1982.

A total of 3,847 ejaculates from 17 Bali bulls were evaluated. The average age of bulls was in a rank of 65 months, with the youngest of 25 months to the eldest of 171 months. The bulls were similarly maintained on feeding and management during the semen production period.

Semen collection and evaluation

In the AI Centre, semen was routinely collected twice a week at early morning by artificial vagina with the frequency of ejaculation 1-2 times with 15 minutes interval between each ejaculation. The bulls mounted a teaser animal, but a dummy was also available. Ejaculate volume was obtained through scale reading from the graduated collection tube (milliliters). Sperm concentration (10^6 per milliliters) was determined using Photometer SDM 6 (Minitube, Germany). Sperm motility was presented percentage (%) on dilution stage and measured by a microscopic examination with a warm stage at 200 × magnification (Olympus BX 53, Minitube, Germany). Total sperm number were calculated by multiplying of ejaculate volume to sperm concentration.

Statistical analysis

Non-genetic factors

The effect of non-genetic factors on semen characteristics was analyzed using the following generalized linear model (GLM).

$Y_{ijkl} = \mu + A_i + S_j + E_k + I_l + \varepsilon_{ijkl}$

Where, Y_{ijkl} is semen characteristics measured (ejaculate volume, sperm concentration, total sperm number and sperm motility); μ is overall means; A_i is fixed effect of i^{th} age of bull (i = > 2 to 4.5 years = 6 bulls, > 4.5 to 7 years = 6 bulls, > 7 to 9.5 years = 2 bulls, > 9.5 to 12 years = 2 bulls, > 12 to 14.5 years = 1 bull); S_j is fixed effect of j^{th} season of collection (j = rainy: October to March, dry: April to September); E_k is fixed effect of k^{th} frequency of ejaculation (k = first ejaculate, second ejaculate); I_l is fixed effect of l^{th} collection intervals (l = 3 days, 4 days); ε_{ijkl} is random residual effect.

Significant differences among treatment means were tested through Duncan's multiple range test at level of 1% (P<0.01).

Repeatability

Repeatability estimations for semen characteristics were calculated by using the following formula.

$$r = \frac{\sigma_s^2}{\sigma_s^2 + \sigma_e^2}$$

Where, r is repeatability; σ_s^2 is the sire variance; σ_e^2 is the error variance.

Repeatability estimations were determined using mixed model analysis where the bull as random effect variables and the fixed effects of age, season, frequency of ejaculation and collection intervals. All statistical analyses were performed using SAS software version 9.4 (SAS Institute 2013).

RESULTS AND DISCUSSION

The Bali bulls had an average ejaculate volume of 4.85 mL, sperm concentration of 990 × 10^6/mL, total sperm number of 4,759 × 10^6 and sperm motility of 60% (Table 1). Other studies reported that average values were in the range of 2.06-7.22 mL for ejaculate volume, 475-1,312 × 10^6/mL for sperm concentration, 3,023-8,352 × 10^6 for total sperm number and 55-85% for sperm motility (Bhakat et al. 2011; Karoui et al. 2011; Boujenane & Boussaq 2013; Burren et al. 2019; Yin et al. 2019; Olsen et al. 2020).

Non-genetic effect

Table 2 presents least square means along with their standard errors for ejaculate volume, sperm concentration, total sperm number and sperm motility of Bali bulls. Non-genetic effects were discussed as belows:

Effect of bull age

This study demonstrated that age of bulls had highly significant effects (P<0.01) on all semen characteristics traits (Table 2). Our results are in agreement with previous studies (Boujenane & Boussaq 2013; Sitanggang 2018) in Holstein and Ongole crossbreed bulls which reported that age of bull affects all semen traits (ejaculate volume, sperm concentration, total sperm number and sperm motility). However, these results are not in agreement with previous report (Bhakat et al. 2011) which determined that the influence of age on sperm concentration has no significant effect in Sahiwal bulls.

Results for total sperm number followed those for ejaculate volume. The present findings are consistent with other studies (Snoj et al. 2013; Murphy et al. 2018). Ejaculate volume and total sperm number improved with the increasing age of bulls, but decreased again for the oldest ages (> 12 to 14.5 years). In different pattern, sperm concentration and sperm motility increased with age, but it began to decline after 9.5 years of age. The increase of ejaculate volume, total sperm number, sperm concentration and motility in older bulls were due to physiological changes of body mass augmentation (Balić et al. 2012), and the testicular growth and maturity (Moura et al. 2011; Rajak et al. 2014). However, Snoj et al. (2013) reported that reproductive decline in bull sires starts at different ages in different breeds.

In the current study, the highest ejaculate volume was observed in bulls > 9.5 to 12 years of age. This is similar to the findings of Snoj et al. (2013), with optimum value for Holstein bulls over 84 months of age.

Effect of season of collection

The current study shows that season only affected sperm motility (P<0.01) (Table 2). Season did not affect ejaculate volume, sperm concentration and total sperm number. Similar to the results, season of collection is reported to show significant effect on sperm motility in Karan Fries bulls (Bhakat et al. 2014). This present study contradicts to other researchers (Boujenane & Boussaq 2013; Snoj et al. 2013) in Holstein bulls. As reported by Boujenane & Boussaq (2013), the season had effects on all semen traits (ejaculate volume, sperm concentration, total sperm number and sperm motility) at AI Centers located in Morocco. Meanwhile, Snoj et al. (2013) observed that season affected ejaculate volume and total sperm number in AI Centers located in Slovenia.

Ejaculate volume and sperm motility were the highest during rainy season (4.88 mL and 61%, respectively) and the lowest during dry season (4.82 mL and 59%, respectively). In this study, there were significant differences (P<0.01) in sperm motility, but insignificant differences in ejaculate volume between rainy (October to March) and dry (April to September) seasons. Moreover, sperm concentration and total sperm number were the greatest during dry season (994 million/mL and 4.76 billion, respectively) and the smallest during rainy season (987 million/mL and 4.75 billion, respectively), but without significant differences. Similarly, Murphy et al. (2018) reported that semen collected in summer (May, June, July) and autumn (August, September, October) was of greater semen characteristics in term of sperm concentration and total sperm number than winter (November, December, January) and spring (February, March, April). Meanwhile, Boujenane & Boussaq (2013) reported the highest semen characteristics (ejaculate volume, sperm concentration, total sperm number and sperm motility) during the winter (January to March) and spring (April to June) season, and the lowest value in summer (July to September) and autumn (October to December) period. No clear pattern could be found to explain the effect of season on semen characteristics, but seasonal effects may be due to various factors, such as temperature, humidity, photoperiod, feed composition and management (Boujenane & Boussaq 2013).

Effect of ejaculation frequency

In the present study, ejaculation frequency affected all studied semen traits (P<0.01) (Table 2), except for sperm motility. The same results were found by Fuerst-Waltl et al. (2006) where the influence of frequency of ejaculation on ejaculate volume, sperm concentration

Table 1. Least square means, standard error and coefficients of variation for ejaculate volume, sperm concentration, total sperm number and sperm motility of Bali bulls

Characteristics	Number of data	Mean	Standard error	Coefficient of variation (%)
Ejaculate volume (mL)	3,847	4.85	0.03	37.31
Sperm concentration ($\times 10^6$/mL)	3,847	990.95	5.36	33.54
Total sperm number ($\times 10^6$)	3,847	4,759.03	35.57	46.36
Sperm motility (%)	3,847	60.23	0.27	27.67

Table 2. Least square means and standard error (SE) for semen characteristics of Bali bulls

Non-genetic factors	Number of data	Ejaculate volume (mL)	Sperm concentration ($\times 10^6$/mL)	Total sperm number ($\times 10^6$)	Sperm motility (%)
Age (years)					
> 2–4.5	1,334	4.24 ± 0.04^d	982.92 ± 7.73^b	$4{,}180.45 \pm 51.58^d$	59.83 ± 0.46^b
> 4.5–7	1,501	4.92 ± 0.05^c	$1{,}022.72 \pm 9.59^b$	$4{,}968.27 \pm 60.45^b$	60.95 ± 0.42^b
> 7–9.5	487	4.77 ± 0.08^c	$1{,}093.12 \pm 13.03^a$	$5{,}206.33 \pm 100.76^{ab}$	66.97 ± 0.49^a
> 9.5–12	418	6.40 ± 0.07^a	851.19 ± 15.19^c	$5{,}391.18 \pm 101.78^a$	54.66 ± 0.86^c
> 12–14.5	107	5.90 ± 0.22^b	726.39 ± 28.13^d	$4{,}531.74 \pm 261.25^c$	46.07 ± 2.00^d
Season					
Rainy (October–March)	2,069	4.88±0.04	987.75±7.27	4,757.68±46.58	61.15 ± 0.35^a
Dry (April–September)	1,778	4.82±0.04	994.66±7.94	4,760.59±54.67	59.15 ± 0.41^b
Frequency of ejaculation					
First ejaculate	3,507	5.01 ± 0.03^a	$1{,}012.75 \pm 5.46^a$	$4{,}989.27 \pm 35.96^a$	60.30±0.28
Second ejaculate	340	3.18 ± 0.09^b	766.11 ± 18.53^b	$2{,}384.13 \pm 78.94^b$	59.42±0.93
Collection intervals					
3 days	1,950	4.74 ± 0.04^b	964.24 ± 7.21^b	$4{,}521.45 \pm 47.28^b$	59.89±0.38
4 days	1,897	4.97 ± 0.04^a	$1{,}018.41 \pm 7.91^a$	$5{,}003.24 \pm 52.74^a$	60.57±0.38

Means in the same column with different superscript highly significant (P<0.01)

Table 3. Repeatability estimations for semen characteristics of Bali bulls

Characteristics	Number of data	σ_s^2	σ_e^2	Repeatability
Ejaculate volume	3,847	1.48	1.94	0.43
Sperm concentration	3,847	40,207.93	72,764.36	0.35
Total sperm number	3,847	1,607,045.10	3,278,349.48	0.32
Sperm motility	3,847	89.38	197.67	0.31

σ_s^2, the sire variance; σ_e^2, the error variance and total sperm number in Simmental bulls was observed. On the contrary, Bhakat et al. (2011) reported that frequency of ejaculation had non-significant effect on ejaculate volume in Sahiwal bulls. Whereas Murphy et al. (2018) and Sitanggang (2018) found there was ejaculation frequency on all studied semen characteristics of ejaculate volume, sperm concentration, total sperm number and sperm motility in Holstein and Ongole crossbreed bulls.

In general, the highest values for semen characteristics were found in the first ejaculates. First ejaculates were about 50%, 30% and 100% greater than second ejaculates for volume, sperm concentration and total sperm number, respectively. In accordance with the present findings, many other researchers (Karoui et al. 2011; Boujenane & Boussaq 2013; Murphy et al. 2018) reported that first ejaculates were superior in semen characteristics (ejaculate volume, sperm concentration and total sperm number).

Effect of collection interval

Table 2 shows that collection intervals affected ejaculate volume, sperm concentration and total sperm number ($P<0.01$). There was no significant effect of collection intervals on sperm motility. This is consistent with the findings of Fuerst-Waltl et al. (2006) who found no significant influence of collection intervals on sperm motility in Simmental bulls. This result is different from that described by Boujenane & Boussaq (2013) in Holstein bulls. They reported that there was significant effect of collection intervals on all semen traits (ejaculate volume, sperm concentration, total sperm number and sperm motility). Meanwhile, Karoui et al. (2011) in Holstein bulls reported that collection intervals had significant effect on ejaculate volume and total sperm number.

Collection intervals of 4 days exceed intervals of 3 days for semen characteristics. The differences were about 0.23 mL, 50 million/mL, 480 million and 0.68% for ejaculate volume, sperm concentration, total sperm number and sperm motility, respectively. According to the results for collection intervals, these indicated that Bali bulls produce optimum semen characteristics in intervals of 4 days. This study agrees closely to estimations of several studies (Fuerst-Waltl et al. 2006; Boujenane & Boussaq 2013). In Holstein bulls, Boujenane & Boussaq (2013) observed intervals of 4 days greater than intervals of 3 days for sperm concentration and total sperm number. Whereas, Fuerst-Waltl et al. (2006) found maximum intervals of 4-6 days for sperm concentration in Simmental bulls.

Genetic effect

With regard to the genetic effect, repeatability estimation of ejaculate volume, sperm concentration, total sperm number and sperm motility were 0.43, 0.35, 0.32 and 0.31, respectively (Table 3). These moderate to high repeatability estimations in recent study indicated that the selection of Bali bulls based on early performance records is reliable.

The repeatability of ejaculate volume in Bali bulls is higher than the estimations in Norwegian Red bulls at 0.29 (Olsen et al. 2020), and also higher than in Holstein bulls (Karoui et al. 2011; Boujenane & Boussaq 2013) at 0.31 and 0.411, respectively. Nevertheless, higher estimations were reported by Atagi et al. (2017) and Burren et al. (2019) which were at 0.467 and 0.63 in Japanese Black and Simmental bulls, respectively.

For sperm concentration, repeatability in the present study is greater compared to previous reports (Karoui et al. 2011; Boujenane & Boussaq 2013) in Holstein bulls which were at 0.30 and 0.175, respectively. However, it is smaller than Simmental bulls at 0.50 (Burren et al. 2019). A higher repeatability than the present estimation was also reported for Norwegian Red bulls at 0.43 (Olsen et al. 2020).

Repeatability estimations for total sperm number in Bali bulls seem to be higher than in Holstein and Ongole crossbreed bulls (Karoui et al. 2011; Sitanggang 2018) at 0.27 and 0.28. However, repeatability value in this study is lower than shown by another research (Atagi et al. 2017) was at 0.425 in Japanese Black bulls.

The repeatability value of sperm motility in this study is higher than Japanese Black and Norwegian Red bulls at 0.265 and 0.21 (Atagi et al. 2017; Olsen et al. 2020), however was lower than those shown by some studies of Karoui et al. (2011) and Burren et al. (2019) in Holstein and Simmental bulls at 0.35 and 0.52, respectively.

The difference in repeatability estimations by other studies might be due to the model, measurement and size of data used in the analysis (Lee et al. 2015).

CONCLUSION

Non-genetic factors of bull age, frequency of ejaculation and interval collection had a large effect on semen characteristics in Bali cattle. The bulls of >7 to 12 years of age were able to produce the highest semen characteristics. In general, the first ejaculate was the best as compared to the second ejaculate for semen characteristics. Semen characteristic of interval collection of 4 days was better than interval of 3 days. With regard to the genetic effect, repeatability estimated for semen characteristics of Bali bulls was in moderate to high (0.31–0.43). The finding could help the AI Centre to optimize semen characteristics.

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