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Seasonal effect on semen quality of Limousine and Simmental bulls

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ABSTRACT

This study aimed to determine the effect of breed and season on the semen quality of AI bulls. The material used was n= 78,648 records of ejaculated semen from 85 Limousine and 88 Simmental bulls collected between period 2010 and 2020. Semen qualities evaluated in the study were: ejaculate volume (Vol), pH, individual sperm motility (ISM), sperm concentration (SC), and post-thawing motility (PTM). The data was analyzed by using a MIXED model with breeds and seasons as fixed effects and animals as random effects. The seasons were significant ($p<0,05$) on Vol, ISM, SC, and PTM, then the interaction between season and breed was affected on Vol, SC, and PTM. Simmental bulls generally showed better semen qualities than Limousine bulls. Further, the same result showed significant differences in each season. The Vol, ISM, SC, and PTM of Simmental were 6.48 ± 1.26, 64.22 ± 1.16, 1,108 ± 0.46, and 42.19 ± 0.38, respectively. The increase of Vol and SC in the humid season has not proportionally affected ISM.

Keywords: Ejaculate volume, Individual sperm motility, Mixed model, Post-thawing motility, Sperm concentration

INTRODUCTION

Artificial Insemination (AI) is the first generation of biotechnology that plays an important role in improving the genetic quality of livestock, especially cattle. AI can maximize the use of superior bull and reduce the possibility of the spread of infectious reproductive diseases (Konenda *et al.,* 2020). The success of artificial insemination depends on the semen quality, which has been affected by genetic and environmental factors (Brito *et al.,* 2002; Isnaini *et al.,* 2021). The quality of semen could be different between individuals as well as between breeds of bulls (Söderquist *et al.,* 1991).

Frozen semen of Limousine and Simmental Bull is the most widely used in Indonesia. In one year, more than three million doses of frozen semen were distributed throughout the regions in Indonesia. Currently, almost Limousine and Simmental Bull used for the production of frozen semen are imported from a subtropical country. That can be the inhibiting factor for reproductive performance and impact the sustainability of frozen semen production due to seasonal and climatic differences between the origin. In temperate

regions, photoperiod (time interval between sunset and sunrise) is the most responsible factor affecting seasonal variation in semen quality (Snoj *et al.,* 2013). While in the tropical region, since the photoperiod is almost similar throughout the year, other factors such as temperature and relative humidity probably become contributors to the semen quality variation (Auvigne *et al.,* 2010).

Many factors can affect semen quality. It becomes a challenge for artificial insemination centers to produce frozen semen in a sustainable manner with a sufficient amount to fulfill the national demand. Therefore, to answer these challenges this study aims to determine the effect of season, breed, and their interaction on the semen quality of AI Bulls.

MATERIAL AND METHODS

Data Collections

Semen quality data of fresh and frozen semen (n= 78,648) was recorded from 173 bulls at Lembang artificial insemination center of Indonesia. The collection periods were from 2010 to 2020. The detail of the data used in the study is presented in Table 1. The seasonal data was carried out from the Indonesian Agency for Meteorological Climatological and Geophysics. The data consist of temperature, humidity, precipitation, and sunshine duration. Seasonal data are presented in Table 2. Breeds categories were Limousine (n=37,901) and Simmental (n=40,747). Seasons were categorized based on the precipitation: dry season (rainfall <100 mm/month; n=26,152 ejaculate); humid season (rainfall 100-200 mm/month; n=14,595 ejaculate) and rainy season (rainfall > 200; n=36,501 ejaculate). Semen quality observed in this study were ejaculate volume of semen (Vol), pH, individual sperm motility (ISM), sperm concentration (SC), and post-thawing motility (PTM).

Data Analysis

The data have been analyzed with the MIXED procedure of SAS On Demand for Academic (SAS, 2021). Breed, season, and their interaction were designed as fixed effects and bull as a random effect. The linear mixed model was as follows:

$$y_{ijklm} = B_i + S_j + BS_{ijk} + a_l + e_{ijklm}$$

Tabel 1. The descriptive statistic of semen quality data used in the study

Semen Quality	n	Mean	Max	Min	SD
Vol (ml)	78.648	6.47	20	0.25	2.12
pH	78.648	6.58	9.63	3.3	0.18
ISM (%)	78.648	64.2	90	5	14.39
SC (x10^6/ml)	78.648	1133.59	9	10	367.33
PTM (%)	45.304	42	85	4	3.27

Vol : volume; ISM: individual sperm motility; SC: sperm concentration; PTM: post thawing motility.

Tabel 2. Season information during period 2010 to 2020

Seasonal information	Dry season (63 months)	Humid Season (25 months)	Rainy season (44 months)
Rainfall (mm/month)	<100	100-200	>200
Relative temperature (°C)	23.73±0.13	23.55±0.08	21.72±0.11
Relative humidity (%)	71.25±0.89	77.04±0.81	79.80±0.48
Temperature-humidity index	71.25±0.89	72.28±0.15	70.93±0.14
Sunsine duration (%)	72.37±2.16	60.32±2.08	51.95±1.03

where y_{ijklm} is the observation of semen quality, B_i the ith fixed effect of breed, S_j the jth fixed effect of season, BS_{ijk} the kth effects of interactions between ith of breed with jth effect of season, a_l is the random effect of animal, and e_{ijklm} the random residual of y_{ijklm}. Tukey-Kramer multiple comparisons were used with a significant level 5%.

RESULTS AND DISCUSSION

The analysis of variance showed that breed, season, and their interaction have significantly affected sperm quality except for pH. The result agreed with a previous study reported for crossbred (Karan Fries) bulls in India (Bhakat *et al.,* 2014). pH was not affected by genetics and environment, it could be influenced by the protein involved in sperm. Kasimanickam *et al.* (2019) stated that sperm is a complex biological fluid containing proteins, amino acids, enzymes, fructose, lipids, major minerals, and other carbohydrates.

The average of Simmental bulls was slightly higher than that of Limousine bulls. Further, the same result showed significant differences in each season (Table 3). The opposite result by Isnaini *et al.* (2019) reported that Vol of Limousine bulls was higher than Simmental bulls. Genetic, environmental, and management previously reported affected Vol of bulls (Mathevon *et al*., 1998; Fuerst-Waltl *et al.,* 2006). Both Simental and Limousine Bulls indicated higher Vol (6.68 ± 2.09 and 6.77 ± 1.98, respectively) in the humid season than in other seasons. In contrast, Prastowo *et al.* (2019) reported that the season had not affected Vol of Bali bulls. Bulls produce a lot more Vol in the humid season could be due to heat stress.

The ISM average of Simental and Limousine bulls was similar. Breed had not significant but season has statistically significant on ISM (Table 4). The result agreed with the previous studies stating that season significantly affects ISM of AI bulls (Ax *et al.,* 2000; Brito *et al.,* 2002; Tiwari *et al.,* 2012; Perumal *et al.,* 2017; Konenda *et al.,* 2020). The highest ISM 64.94 ± 1.58 for Limousine and 64.49 ± 1.48 for Simmental was shown in the rainy season. The results indicated even though the bulls produce more ejaculate volume in the humid season, the semen had not better ISM. Heat stress makes the ISM on the semen decrease significantly. Sperm kinematics, viability, acrosome integrity, and reactive oxygen species were affected by season (Sabes-Alsina *et al.,* 2019; Morrell, 2020). In the subtropical condition, ISM of semen collected in winter or springs had better membrane integrity (Valeanu *et al.,* 2015). Heat stress reduces motility through the mechanism of increasing gene expression from heat shock protein (HSP) (Cheng *et al*.. 2016). Increasing temperature has an impact on the process of spermatogenesis.

The SC was significantly affected by breed and season. Simmental bulls have higher SC than Limousine bulls all over the seasons. The results agreed with a previous study by Sumeidiana *et al.* (2007) reported the average SC was 1,788 and 1,388 x106/ml, respectively for Simmental and Limousine bulls. Seasons significant effect on

Table 3. Interaction between breed and season for ejaculation volume (mean ± SE)

Season	Breeds	
	Limousine	Simmental
Mean	6.48 ± 1.26	6.54 ± 1.42
Dry	6.41 ± 2.17^{ax}	6.44 ± 2.06^{ay}
Humid	6.68 ± 2.09^{bx}	6.77 ± 1.98^{by}
Rainy	6.34 ± 2.17^{ax}	6.41 ± 2.12^{ay}

^{a,b}Values within a column without a common superscript are different (P < 0.05).
^{x,y}Values within a row without a common superscript are different (P < 0.05).

Table 4. Interaction between breed and season for individual sperm motility (mean ± SE)

Season	Breeds	
	Limousine	Simmental
Mean	64.22 ± 1.16	63.54 ± 1.02
Dry	64.04 ± 1.42[a]	63.74 ± 1.12[a]
Humid	63.69 ± 1.67[a]	63.43 ± 1.04[a]
Rainy	64.94 ± 1.58[b]	64.49 ± 1.48[b]

[a,b]Values within a column without a common superscript are different (P < 0.05).
[x,y]Values within a row without a common superscript are different (P < 0.05).

Table 5. Interaction between breed and season for sperm concentration (mean ± SE)

Season	Breed	
	Limousine	Simmental
Mean	1,108 ± 0.46	1,152.28 ± 0.51
Dry	1,088.24 ± 0.52[ax]	1,127.55 ± 0.65[ay]
Humid	1,126.55 ± 0.52[cx]	1,177.54 ± 0.54[cy]
Rainy	1,110.92 ± 0.64[bx]	1,151.75 ± 0.69[by]

[a,b]Values within a column without a common superscript are different (P < 0.05).
[x,y]Values within a row without a common superscript are different (P < 0.05).

Table 6. Interaction between breed and season for post-thawing motility (mean ± SE)

Season	Breed	
	Limousine	Simmental
Mean	42.19 ± 0.38	41.89 ± 0.25
Dry	42.13 ± 0.44[ay]	41.84 ± 0.37[ax]
Humid	42.32 ± 0.36[by]	42.12 ± 0.32[bx]
Rainy	42.12 ± 0.46[ay]	41.71 ± 0.33[ax]

[a,b]Values within a column without a common superscript are different (P < 0.05).
[x,y]Values within a row without a common superscript are different (P < 0.05).

SC with the lowest concentration found in the dry season and the highest concentration in the humid season (Table 5). The same results were for Limousine and Simmental bulls. In line with the studies reported the effect of season on SC in Limousine by Konenda *et al.* (2020), Ongole grade cattle by Suretno *et al.* (2018), and Isnaini *et al.* (2019) Simmental by Nichi *et al.* (2006). The increase of SC in the humid season has been shown directly proportional to the increase of Vol.

The last evaluation on sperm quality is PTM, PTM was also affected by breed and season. The PTM of Simmental was higher than that of Limousine. The highest PTM was shown in the humid season for both breeds (Table 6). Murphy *et al*. (2018) reported that PTM of Holstein Friesian was higher in winter. The result of the study could be due to the difference in freezer-thawing protocols among seasons.

CONCLUSION

Breeds and seasons have shown favorable interaction affected sperm quality. The increase in volume and concentration have not proportion-

ally affected to individual sperm motility. Need a focused study about the effect freezer-thawing process on post-thawing motility.

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