p-ISSN 1410-5659 e-ISSN 2621-5144
Published by:
Unpad Press

Jurnal Ilmu Ternak Universitas Padjadjaran, Juni 2024, 24(1):93-102
DOI: 10.24198/jit.v24i1.51460
Available online at http://jurnal.unpad.ac.id/jurnalilmuternak

DIFFERENCES IN QUANTITY AND QUALITY OF FRESH SEMEN AND FROZEN SEMEN AFTER THAWING IN LIMOUSIN AND SIMMENTAL CATTLE AT LEMBANG ARTIFICIAL INSEMINATION CENTER

Benny Ahmat[1, a], Ervi Herawati[2], Tati Rohayati[2], Aisyatus Salamah[3]
[1]*Animal Husbandry Study Program, Faculty of Agriculture, Garut University*
[2]*Lecturers in the Animal Husbandry Study Program, Faculty of Agriculture, Garut University*
[3]*Lembang Artificial Insemination Center, Bandung*
[a]*email:* bennyahmad889@gmail.com

Abstract
The purpose of this study was to evaluate the differences in quantity and quality of fresh semen and post-thawing frozen semen of two different cattle breeds at the Lembang Artificial Insemination Centre (BIB). The method applied in this research was case study method and used secondary data. The research material consisted of semen production records obtained from 9 Limousin cattle and 9 Simmental cattle, which were collected in July 2023 at 08.00-09.00 WIB. The variables studied were colour, volume, consistency, pH, motility, mass movement, concentration, post-thawing motility, and individual spermatozoa movement. The results showed that Limousin cattle semen had milky white (88.89%) and cream (11.11%) colour, medium consistency (55.56%) and liquid (44.44%) and mass motility value (++). While Simmental cattle semen has a milky white colour, medium consistency and mass motility value (++). The breed of cattle significantly differed on the concentration, pH of semen and post-thawing motility, then the breed of cattle had no significant difference on the volume, motility and movement of individual spermatozoa. Simmental cattle have superior semen quality compared to Limousin cattle.
Keywords: quantity, quality, semen, limousin, simmental

PERBEDAAN KUANTITAS DAN KUALITAS SEMEN SEGAR DAN SEMEN BEKU PASCA THAWING SAPI LIMOUSIN DAN SAPI SIMMENTAL DI BALAI INSEMINASI BUATAN LEMBANG

Abstrak
Tujuan dari penelitian ini adalah mengevaluasi perbedaan kuantitas dan kualitas semen segar dan semen beku pasca thawing terhadap dua bangsa sapi yang berbeda di Balai Inseminasi Buatan (BIB) Lembang. Metode yang diterapkan dalam penelitian ini adalah metode studi kasus dan menggunakan data sekunder. Materi penelitian ini terdiri dari catatan produksi semen yang diperoleh dari 9 ekor sapi Limousin dan 9 ekor sapi Simmental, yang ditampung pada bulan Juli 2023 pada pukul 08.00-09.00 WIB. Variabel yang diteliti adalah warna, volume, konsistensi, pH, motilitas, gerakan massa, konsentrasi, motilitas pasca thawing, dan gerakan individu spermatozoa. Data yang didapatkan dianalisis secara deskriptif dan dilakukan uji-t tidak berpasangan dengan menggunakan SPSS versi 22. Hasil penelitian menunjukkan bahwa semen sapi bangsa Limousin memiliki warna putih susu (88,89%) dan cream (11,11%), konsistensi sedang (55,56%) dan cair (44,44%) dan nilai gerakan massa (++). Sedangkan semen sapi bangsa Simmental memiliki warna putih susu, konsistensi sedang dan nilai gerakan massa (++). Bangsa sapi secara signifikan berbeda terhadap konsentrasi, pH semen dan motilitas semen beku pasca thawing, kemudian bangsa sapi secara signifikan tidak memiliki perbedaan terhadap volume, motilitas dan gerakan individu spermatozoa. Sapi Simmental memiliki kualitas semen yang unggul dibandingkan sapi Limousin.
Kata kunci*: kuantitas, kualitas, semen, limousin, simmental*

INTRODUCTION

The livestock sector is one of the most important sectors in the Indonesian economy. This sector plays an important role in the provision of animal food. To improve this sector, the Directorate General of Animal Husbandry and Animal Health (PKH) of the Ministry of Agriculture launched a programme called SIKOMANDAN (Sapi Kerbau Komoditas Andalan Negeri) to encourage an increase in the population and production of cattle and buffaloes in Indonesia. Balai Besar

Inseminasi Buatan (BIB) Lembang also supports this programme by providing quality frozen semen. At BIB Lembang, the demand for frozen semen from imported cattle breeds, namely Limousin and Simmental cattle, is higher than other livestock.

Limousin and Simmental cattle are two types of exotic cattle that have been developed in Indonesia. According to Fikar & Ruhyadi, (2010) Limousin cattle are a type of beef cattle originating from France, while Simmental cattle originate from Switzerland, and are a dual-purpose type of cattle that produce good meat and milk. Limousin cattle have very fast body growth, where the body weight of adult males can exceed 1,000 kg, while Simmental cattle have very good body growth, with the ability to reach adult male body weights of up to 1,200 kg (Fikar & Ruhyadi, 2010). These two breeds of cattle have superior characteristics such as fast growth and good semen quality (Muada *et al.,* 2017). These two breeds of cattle have adapted well in Indonesia to be used as beef cattle. Apart from being used for meat production, these cattle are also often used as bulls to produce quality breeding stock through the Artificial Insemination (AI) process.

AI is a technique where male sperm is collected, processed, stored, then inserted into the female reproductive tract artificially *at* the right time for the purpose of fertilization (Patel *et al.*, 2018) . In Indonesia, AI technology has been introduced as a strategy to increase the population and improve the genetic quality of livestock. Patel *et al*., (2018) stated that AI technology can increase genetic progress, reduce the risk of disease transmission and increase the livestock population that can be bred from superior parents. The success of AI technology depends on the quality of semen produced by bulls. AI technology can improve the genetic quality of bulls by optimizing the quality and quantity of semen produced by bulls. (Widyawati, 2019) . Quantity is the amount of semen produced by a bull in one ejaculation. In the fertilization process, semen quality is an important factor that must be considered to determine the quality of spermatozoa. Parameters such as semen colour, volume, concentration, consistency, mass movement, pH, and motility of fresh spermatozoa from a bull are very different (Komariah *et al.,* 2013). Susilawati, (2011) and Seyoum *et al*., (2021) one of the factors that influences the quantity and quality of semen is the breed of cattle.

The quantity and quality of semen obtained by each breed of cattle and each livestock is different, so it can affect the quality of frozen sperm produced. According to the SNI 4869-1:2021 standard, frozen semen must meet certain qualifications before distribution. These qualifications include a minimum of 40% progressive spermatozoa motility, and a minimum of 70% spermatozoa motility in fresh semen. According to Yendraliza *et al.,* (2015) there are 2 methods that can be used to assess semen quality, namely macroscopic and microscopic examination. Macroscopic examination consists of volume, color, consistency and pH. Microscopic evaluation consists of concentration and motility (sperm mass motility, individual sperm motility). Based on this, this study aims to evaluate the differences in quantity and quality of fresh semen and frozen semen *after thawing* in Limousin cattle and Simmental cattle at the Lembang Artificial Insemination Center.

MATERIALS AND METHODS

This research was carried out at the Lembang Artificial Insemination Center (BIB) located in West Bandung Regency, West Java. The research material used was research data consisting of production records for Limousin and Simmental cattle accommodated at 08.00-09.00 WIB, totaling 9 Limousin cattle and 9 Simmental cattle in July 2023. The method used in this research was the case study method., which used secondary data obtained from BIB Lembang.

Research variable

The variables that were the focus of the research were fresh semen and frozen semen post thawing. Examination of fresh semen includes color, volume, consistency, pH, motility, mass motility and concentration. Evaluation of frozen semen includes assessment of post-thawing motility, and motility of individual spermatozoa.

Semen Collection

The method for storing semen uses the artificial vagina method. Before taking the semen, the parts of the artificial vagina, such as the rubber funnel, thin rubber sleeve, and

collection tube, are cleaned, sterilized and installed. The artificial vagina that has been installed is pumped until it expands, after which it is filled with water at a temperature of 42-44 ℃. Finally, the inner lining of the artificial vagina is lubricated with vaseline.

The semen storage process begins with preparing the bulls to be accommodated as well as the anglers (teaser). When the stud cattle mounts the angler for the first time, the collector (farm operator) does not immediately direct the male's penis into the artificial vagina. This is done three times with the aim of increasing libido and semen quality in male cattle (Chumairoh *et al.*, 2023). When the bull rides on the angler for the fourth time, the collector immediately directs the bull's penis into the artificial vagina, and then the bull gives the bull a push to ejaculate.

Fresh semen is immediately tested for quality in the laboratory, by evaluating semen volume, semen color, semen consistency, semen pH value, motility, mass motility, and sperm concentration. To produce frozen semen, semen was diluted using skim milk diluent, egg yolk, aquabides, glycerol, glucose, and antibiotic buffer. Next, post-thawing motility and individual spermatozoa movement tests were conducted.

Macroscopic Examination

The semen that has been collected is immediately evaluated, including volume, color, consistency and pH. Measure the volume of semen by observing the number scale on the semen storage tube. In the collection tube, the color of the semen can be directly assessed. Consistency (viscosity) can be determined by moving or tilting the semen through the wall of the container tube to observe the flow rate of the semen through the wall of the container tube. pH assessment using a pH meter.

Microscopic Examination

Fresh semen, the motility of frozen semen that has been thawed at a temperature of 37℃-38℃ for 30 seconds and the motility of spermatozoa are assessed using a microscope with a magnification of 10 x 20. Progressively motile spermatozoa will be seen moving forward. Assessment of semen motility is expressed in percent. The degree of motility of individual spermatozoa is expressed with a value of 0-4. Values 0 (none), 1 (slow), 2 (medium), 3 (fast), 4 (very fast) (BIB Lembang, 2022).

Mass motility was assessed using a microscope with a magnification of 4 x 10. Assessment of mass motility can be categorized with the following values: very good (+++), good (++), fair (+), and poor (0, Necrospermia) (Manehat *et al.*, 2021).

Concentrations were calculated via an SDM 6 photometer, a device designed to count the number of spermatozoa in each milliliter of semen. The spermatozoa concentration was checked by taking a semen sample of 0.1 ml. The semen sample was mixed with 0.9% NaCl in a cuvette and homogenized using a figure eight motility. Then the cuvette is inserted into a photometer to measure the concentration of spermatozoa.

Data Analysis

Descriptive analysis was applied to process the data (mean, standard deviation, and coefficient of variation) and conduct an unpaired t-test using SPSS version 22. The unpaired t-test was used to determine the differences in quantity and quality of fresh semen and frozen semen after thawing for two different breeds of cattle. The unpaired t-test is a statistical method that aims to compare the average values of two groups that are not related or are not paired with each other (Palupi *et al.*, 2021).

RESULTS AND DISCUSSION

Semen Volume

Volume refers to the amount of semen that a bull has produced in one ejaculation. The results of examining the volume of cattle semen are shown in Figure 1.

The volume of semen produced by Limousin cattle was 7.39 ± 2.56 ml, with a range of 5-9 ml and Simmental was 7.56 ± 1.33 ml with a range of 6-10 ml. The results of this research are higher than what was found in research conducted by Muada *et al.*, (2017) which stated that Limousin cattle semen had a volume of 7.17 ± 1.41 ml, while Simmental had a volume of 7.20 ± 1.49 ml. This research still shows semen volume results that exceed previous research conducted by Rahmawati *et al.*, (2015) which stated that Limousin cattle semen had a volume of 6.73 ± 1.11 ml and Simmental 6.60 ± 0.64 ml. Differences with

previous studies may be influenced by age, body weight, health status, collectors, and season so that semen production in stud cattle varies (Susilawati, 2011a).

Table 1 shows that there is no difference in semen volume (P>0.05) between cattle breeds. These results are in accordance with Budiyanto *et al.*, (2021) who found that semen volume did not differ significantly (P>0.05) between breeds of cattle (PO, Brahman, and Simmental). This is due to the similarity of the sub genus of the two breeds, namely the bos sub genus (Budiyanto *et al.*, 2021).

According to Gomes, (1985) the coefficient of diversity is classified into very high (>30%), high (20%-30%), medium (10%-20%), and low (<10%). The percentage coefficient of variation in semen volume for Limousin cattle was 34.63%, indicating very high diversity, while Simmental was 17.65%, indicating moderate diversity. The variation in semen volume may be caused by variations in age, and male libido level when collected (Saputra *et al.*, 2017). According to Rokhana, (2008) the higher the level of libido, the number of spermatozoa in males increases during ejaculation. According to Sio (2023), that selection can be carried out on individuals in a population if the diversity coefficient exceeds 15%.

Semen Color

Fresh cattle semen can be categorized based on color, such as milky white, cream, yellow, and abnormal (Muada *et al.*, 2017) . The results of semen color examination are shown in Figure 2.

The colour of semen produced by Limousin cattle is milky white dominant as much as 88.89% followed by cream colour as much as 11.11%, while that of Simmental cattle is milky white as much as 100%. In accordance with Bearden and Fuquay (1984) cited by Zulyazaini *et al.*, (2016) that the colour of semen produced from normal ejaculation in cattle is milky white and only 10% is creamy. With the increase in sperm count, the colour of the semen becomes cloudy, resulting in a cream colour (Alawiyah *et al.*, 2021). According to Muada *et al.*, (2017) Limousin and Simmental cattle have milky white semen, which indicates that the semen in these two cattle breeds is in the good and normal colour category. Sunami *et al.*, (2017) stated that semen that has good quality is milky white in colour, illustrating the high number of spermatozoa and the good quality of the semen.

Semen Consistency

Consistency is correlated with the level of spermatozoa concentration. Consistency is categorized into thick, medium, and thin. The results of the semen consistency test are shown in Figure 3.

The results of semen consistency examination in Limousin cattle showed the consistency level was in the medium category as much as 55.56% and dilute as much as 44.44%, while Limousin cattle had a medium consistency of 100%. Muada *et al.*, (2017) also found Limousin and Simmental cattle have moderate semen consistency. According to Susilawati, (2011), semen consistency assessment is categorised into liquid (<1000x10^6 spermatozoa cells/ml), medium (<1000x10^6-1500x10^6 spermatozoa cells/ml), and thick (>1500x10^6 spermatozoa cells/ml). Limousin cattle had a dilute semen consistency of 44.44%, which indicates a low concentration of spermatozoa. The low consistency of Limousin cattle semen is thought to be due to its low concentration (Figure 7). As described by Adhyatma *et al.*, (2013), the higher the concentration of spermatozoa, the more concentrated the semen consistency. Conversely, if the concentration is low, the consistency of the semen becomes watery.

Semen pH value

pH is the level of acidity or alkalinity of semen. The pH value in bull semen can influence a number of important parameters related to semen quality such as concentration, viability and motility (Manehat *et al.*, 2021) . The results of checking the pH value are shown in Figure 4.

The pH value for Limousin cattle semen was 6.65 ± 0.07, with a range of 6.56-6.75 and for Simmental was 6.53 ± 0.05, with a range of 6.5-6.6. Limousin cattle semen has a pH value of 6.65 ± 0.08 with a diversity coefficient of 1.20% and Simmental has a pH value of 6.66 ± 0.05 with a diversity coefficient of 0.75% (Muada *et al.*, 2017), meaning that these results are in accordance with previous research. Both breeds of cattle have normal semen pH levels. This is in line with Susilawati, (2011) that normal semen pH ranges from 6.2-6.8.

Table 2 shows that the pH value of semen has a statistically significant difference between cattle breeds (P<0.05). Cakti, (2022) also found differences between semen pH of local cattle breeds (Aceh cattle, Pasundan cattle, Peranakan Ongole cattle, and Madura cattle). The percentage coefficient of variation in the pH of Limousin and Simmental cattle semen is at low diversity, respectively 1.00 % and 0.77%.

Spermatozoa Motility

Spermatozoa motility plays an important role in male fertility and semen quality. According to Manehat *et al.,* (2021) fertility increases as individual spermatozoa motility increases. The results of the fresh semen motility evaluation are shown in Figure 5.

Spermatozoa motility in Limousin cattle was 70.00 ± 0.00% and Simmental was 71.67 ± 0.03% with a range of 70-75%. Priyanto *et al.*, (2015) reported that motility in Limousin and Simmental cattle was 70 ± 0.00 %. This shows that the motility of these two cattle breeds is in the good category, which is an important factor in increasing fertility success. The results of this research are in accordance with SNI Standard 4869-1:2021, which requires a minimum motility of 70% in fresh semen. According to Aerens *et al.*, 2012), differences in spermatozoa motility in semen between cattle breeds can occur due to variations in the availability of fructose, glycerylphosporilcholine (GPC), and sorbitol which ultimately affect the level of spermatozoa motility.

Table 3 shows that there is no statistical difference in motility (P>0.05) between cattle breeds. Likewise, Muada *et al.*, (2017) found no difference (P>0.05) in the motility of the Simmental and Limousin cattle breeds. Simmental cattle had a low percentage of motility diversity coefficient, namely 3.49%.

Mass Motility

Mass motility is the movement of spermatozoa forming a wave-like pattern in undiluted semen. The results of examining the mass motility of spermatozoa in Limousin and Simmental cattle are shown in Figure 6.

The spermatozoa mass motility value in each Limousin and Simmental cattle was (++). The value of mass motility in these two cattle breeds is relatively good. In line with Manehat *et al.,* (2021) stated that mass motilitys that have a value (++) are considered good with characteristics that include the presence of small, thin, rare, unclear waves and slow motilities. The value (++) can be obtained because the feed given to stud cattle has good nutritional value. Widhyari *et al.,* (2015) explained that nutritional factors or nutritional status of livestock and cattle breeds can affect mass motility.

Concentration

Sperm concentration refers to the number of spermatozoa present per milliliter of semen. The results of the concentration check are shown in Figure 7.

The concentration of spermatozoa from Limousin cattle was 932.22 ± 225.99x10^6 spermatozoa cells/ml, with a range of 600-1260x10^6 spermatozoa cells/ml and Simmental of 1232.78 ± 1047.71x10^6 spermatozoa cells/ml with a range of 1123-1410x10^6 spermatozoa cells/ml /ml. The results of this study show that Simmental cattle tend to have a greater spermatozoa concentration than Limousin cattle, this is in line with research by Rahmawati *et al.,* (2015) who reported that the spermatozoa concentration in Simmental cattle was 1184.16 ± 191.31x10^6 /ml which tends to be greater than Limousin cattle. as much as 1132.60 ± 177.46x10^6 /ml. Differences in spermatozoa concentration among various bulls are thought to be related to the genetic quality of each individual bull (Situmorang, 2002). Aerens *et al.,* (2012) reported that there is a genetic relationship between individuals with high spermatozoa concentrations in a breed of cattle.

Table 4 shows that there is a statistically significant difference in the concentration of cattle breeds (P<0.05). Rahmawati *et al.*, (2015) also reported significant differences in spermatozoa concentrations between various breeds of cattle, such as Acehnese, Angus, Brahman, Limousin, Madurese, Ongole, and Simmental cattle (P<0.05). The percentage coefficient of variation in spermatozoa concentration of Limousin cattle is at medium diversity, namely 24.24 % and Simmental is at low diversity, namely 8.49%.

Post-Thawing Motility

Post-thawing motility is a parameter to measure the level of sperm motility after the freezing and thawing process. The results of the post-thawing motility examination are shown in Figure 8.

Post-thawing motility results in Limousin cattle showed an average of 41.67 ± 2.50% with a range of 40-45% and in Simmental it was 44.4 ± 3.00% with a range of 40-50%. The results of this research are in accordance with SNI Standard 4869-1:2021, which requires a minimum motility of 40% in frozen semen that is suitable for distribution. Post-thawing motility percentage above 40% can be carried out artificial insemination (Savitri & Suharyati, 2014) and the pregnancy rate in livestock is high (Susilawati, 2011b).

Table 5 shows that there is a statistical difference in post-thawing motility (P<0.05) between cattle breeds. This research is in accordance with Nofa *et al.*, (2018) who found that post-thawing motility was different (P<0.05) for cattle breeds, including Limousin, Ongole, Simmental and Brahman cattle. The percentage coefficient of diversity in PTM in Limousin and Simmental cattle is at low diversity, respectively 6.00% and 6.76%.

Motility of Individual Spermatozoa

The motility of individual spermatozoa is the level of ability of spermatozoa to move to achieve fertilization. The results of examining the motility of individual spermatozoa in Limousin and Simmental cattle are shown in Figure 9.

The value of individual spermatozoa motility in Limousin cattle was 2.22 ± 0.44 and Simmental was 2.67 ± 0.50, meaning that the individual spermatozoa motility in these two breeds of cattle was between moderate to fast. The research results obtained are in accordance with the standards set by BIB Lembang, namely that the minimum individual spermatozoa motility value is 2 or (++).

Table 6 shows that spermatozoa motility has no difference (P>0.05) between cattle breeds. The percentage coefficient of diversity of spermatozoa motility in Limousin and Simmental cattle is at moderate diversity, respectively 19.84% and 18.75%.

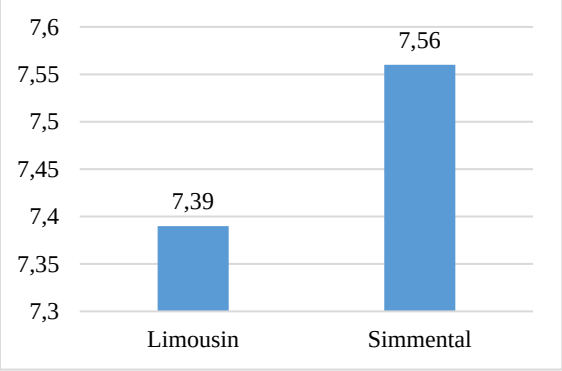

Figure 1. Semen Volume

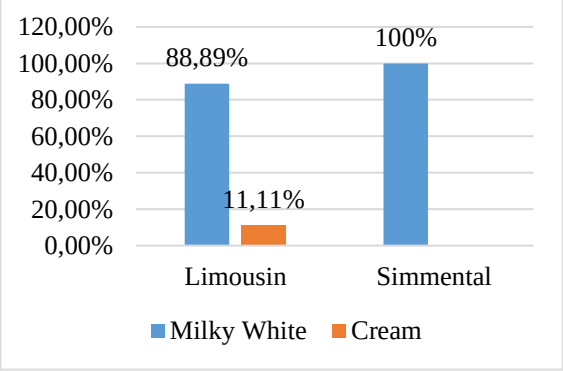

Figure 2. Semen Color

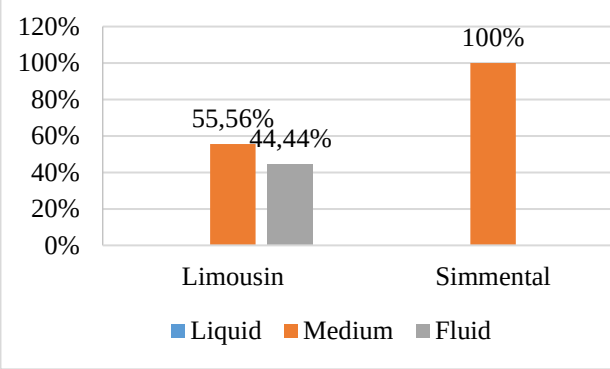

Figure 3. Semen Consistency

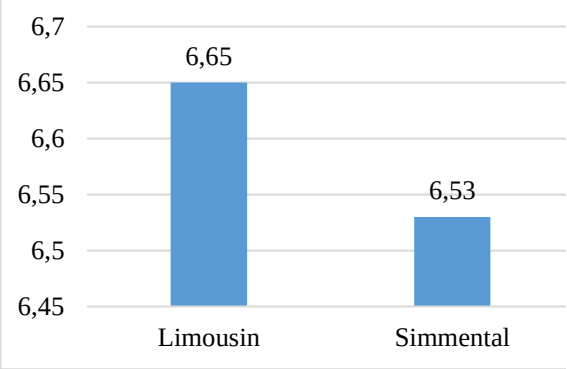

Figure 4. Semen pH Value

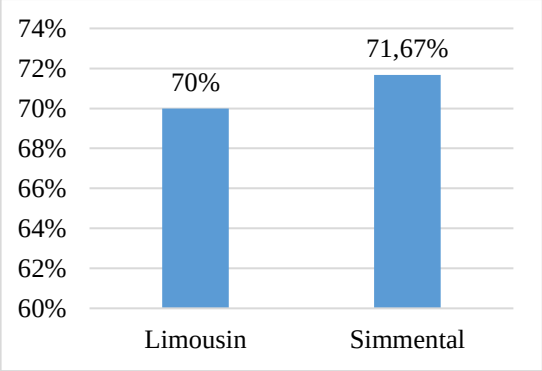

Figure 5. Spermatozoa Motility

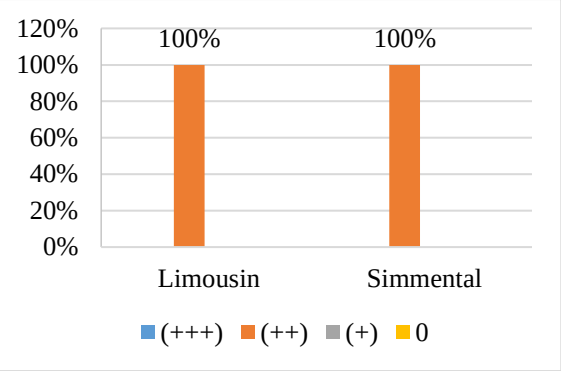

Figure 6. Mass Motility

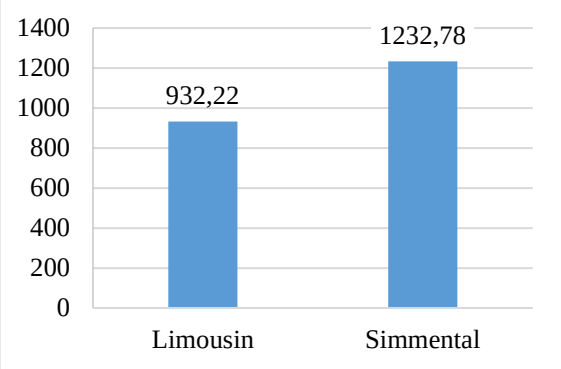

Figure 7 Spermatozoa Concentration

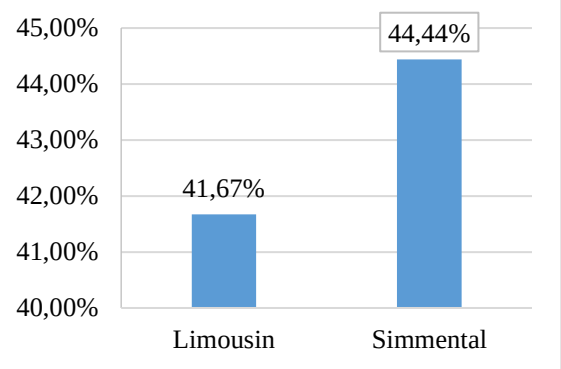

Figure 8. *Post-Thawing* Motility

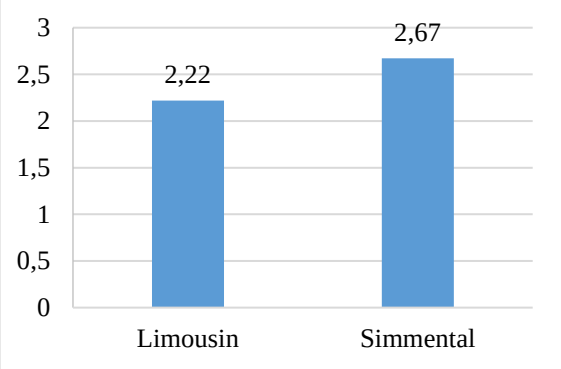

Figure 9. Motility of Individual Spermatozoa

Table 1. Unpaired t-test results on semen volume

Breed	Mean ± SD	Diversity Coefficient (%)	P-value
Limousin	7.39 ± 2.56	34.63	0.86
Simmental	7.56 ± 1.33	17.65	

Note: There is no significant difference (P> 0.05)

Table 2. Unpaired t-test results on semen pH

Breed	Mean ± SD	Diversity Coefficient (%)	P-value
Limousin	6.65 ± 0.07	1.00	0.001
Simmental	6.53 ± 0.05	0.77	

Note: There is a significant difference (P< 0.05)

Table 3. Unpaired t-test results on sperm motility

Breed	Mean ± SD	Diversity Coefficient (%)	P-value
Limousin	70.00 ± 0.00	0.00	0.063
Simmental	71.67 ± 0.03	3.49	

Note: There is no significant difference (P> 0.05)

Table 4. Unpaired t-test results on sperm concentration

Breed	Mean ± SD	Diversity Coefficient (%)	P-value
Limousin	932.22 ± 225.99	24.24	0.002
Simmental	1232.78 ± 104.71	8.49	

Note: There is a significant difference (P< 0.05)

Table 5. Unpaired t-test results on *post-thawing motility*

Breed	Mean ± SD	Diversity Coefficient (%)	P-value
Limousin	41.67 ± 2.50	6.00	0.049
Simmental	44.4 ± 3.00	6.76	

Note: There is a significant difference (P< 0.05)

Table 6. Unpaired t-test results on individual spermatozoa motility

Breed	Mean ± SD	Diversity Coefficient (%)	P-value
Limousin	2.22 ± 0.44	19.84	0.063
Simmental	2.67 ± 0.50	18.75	

Note: There is no significant difference (P>0.05)

CONCLUSION

Based on the analysis and discussion, it was found that the quantity and quality of fresh semen and frozen semen after thawing from Limousin and Simmental cattle were classified as good, meeting the Indonesian National Standards (SNI) 4869-1:2021. The results showed that Limousin cattle semen has a milky white colour (88.89%) and cream (11.11%), medium consistency (55.56%) and liquid (44.44%) and mass movement value (++). While Simmental cattle semen has a milky white colour, medium consistency and mass motility value (++). Cattle breeds significantly differed in concentration, semen pH and post-thawing motility, then cattle breeds did not significantly differ in volume, motility and individual spermatoza motility. Simmental cattle have superior semen quality compared to Limousin cattle.

ACKNOWLEDGMENT

The researcher would like to express his gratitude to BIB Lembang, West Java, for the cooperation and assistance provided in providing access and data for the purposes of this research.

REFERENCES

Adhyatma, M., Isnaini, N., & Nuryadi. (2013). Pengaruh Bobot Badan terhadap Kualitas dan Kuantitas Semen Sapi Simmental. *TERNAK TROPIKA Journal of Tropical Animal Production*, *14*(2), 53–62.

Aerens, D. C. C., Ihsan, N., & Isnaini, N. (2012). Perbedaan Kuantitatif dan Kualitatif Semen Segar pada Berbagai Bangsa Sapi Potong. *Universitas Brawijaya*, 1-10. https://fapet.ub.ac.id/wp-content/uploads/2013/04/Perbedaan-

Kuantitatif-Dan-Kualitatif-Semen-Segar-Pada-Berbagai-Bangsa-Sapi-Potong.pdf diakses 11 Agustus 2023.

Alawiyah, A., Rohayati, T., & Hadist, I. (2021). *Analisis Hubungan Bobot Badan Dengan Karakteristik Kualitatif Dan Kuantitatif Semen Sapi Brahman Di Balai Inseminasi Buatan Lembang Bandung*. *5*(2). https://doi.org/10.52434/janhus.v5i2.1350

BIB Lembang. (2022). *Insruksi Kerja: Laboratorium Pengujian Mutu Semen Balai Inseminasi Buatan Lembang*. Jawa Barat, Indonesia.

BSN (Badan Standarisasi Nasional). (2021). *Standar Nasional Indonesia SNI 4869-1:2021: Semen Beku Sapi*. BSN. Jakarta, Indonesia.

Budiyanto, A., Arif, M., Alfons, M. P. W., Fani, R. T., Hafid, A. F., Wicaksono, B., Insan, K. M., & Herdinta, M. (2021). The The Effect of Age and Breed on The Quality of Bull Semen in The Regional Artificial Insemination Centre. *Acta Veterinaria Indonesiana*, *2021*, 132–136. https://doi.org/10.29244/avi...132-136

Cakti, N. W. (2022). *Pengaruh Bangsa Sapi Lokal terhadap Kualitas Semen di Balai Inseminasi Buatan Lembang*. Universitas Gadjah Mada.

Chumairoh, Z., Mubarakati, N. J., & Jayanti, G. E. (2023). Analisis Kualitas Spermatozoa Segar Pada Sapi Limousin (Bos Taurus) Terhadap Berbagai Variasi Jumlah False Mounting. *Journal of Comprehensive Science (JCS)*, *2(5)*, 1031–1042. https://doi.org/10.59188/jcs.v2i5.314

Fikar, S., & Ruhyadi, D. (2010). *Beternak dan Bisnis Sapi Potong*. PT Agro Media. https://www.google.co.id/books/edition/Beternak_Bisnis_Sapi_Potong/R_loZAc5uKcC?hl=id&gbpv=0 diakses 15 Oktober 2023

Gomes, F. P. (1985). *Curso de estatística experimental*. https://repositorio.usp.br/item/000773318 17 Agustus 2023

Komariah, Arifiantini, L., & Nugraha, F. W. (2013). Kaji Banding Kualitas Spermatozoa Sapi Simmental, Limousin, dan Friesian Holstein terhadap Proses Pembekuan. *Buletin Peternakan*, *37*(3), 143. https://doi.org/10.21059/buletinpeternak.v37i3.3078

Manehat, F. X., Dethan, A. A., & Tahuk, P. K. (2021). Motility, Viability, Spermatozoa Abnormality, and pH of Bali Cattle Semen in Another-Yellow Water Driller Stored in a Different Time. *Journal of Tropical Animal Science and Technology*, *3*(2), 76–90. https://doi.org/10.32938/jtast.v3i2.1032

Muada, D. B., Paputungan, U., Hendrik, M. J., & Turangan, S. H. (2017). Karakteristik Semen Segar Sapi Bangsa Limousin dan Simmental di Balai Inseminasi Buatan Lembang. *ZOOTEC*, *37*(2), 360. https://doi.org/10.35792/zot.37.2.2017.16156

Nofa, Y., Karja, N. W. K., & Arifiantini, R. I. (2018). Status Akrosom dan Kualitas Post-Thawed Spermatozoa pada Beberapa Rumpun Sapi dari Dua Balai Inseminasi Buatan. *Acta VETERINARIA Indonesiana*, *5*(2), 81–88. https://doi.org/10.29244/avi.5.2.81-88

Palupi, R., Yulianna, D. A., & Winarsih, S. S. (2021). Analisa Perbandingan Rumus Haversine Dan Rumus Euclidean Berbasis Sistem Informasi Geografis Menggunakan Metode Independent Sample t-Test. *JITU : Journal Informatic Technology And Communication*, *5*(1), 40–47. https://doi.org/10.36596/jitu.v5i1.494

Patel, G. kumar, Haque, N., Madhavatar, M., Chaudhari, A. kumar, Patel, D. kumar, Bhalakiya, N., Jamnesha, N., Patel, P., & Kumar, R. (2018). Artificial insemination: A tool to improve livestock productivity. *Journal of Pharmacognosy and Phytochemistry*, *6(6S)*, 307–313.

Priyanto, L., Arifiantini, R. I., & Yusuf, T. L. (2015). Deteksi Kerusakan DNA Spermatozoa Semen Segar dan Semen Beku Sapi Menggunakan Pewarnaan Toluidine Blue. *Jurnal Veteriner Maret*, *16*(1).

Rahmawati, M., Susilawati, T., & Ihsan, M. N. (2015). Kualitas semen dan produksi semen beku pada bangsa sapi dan bulan penampungan yang berbeda. *Jurnal Ilmu-Ilmu Peternakan*, *25*(3), 25–36. https://doi.org/10.21776/ub.jiip.2015.025.03.04

Rokhana, E. (2008). *Hubungan Antara Jumlah False Mounting dengan Produksi Semen Pejantan Sapi Madura*. 37-43. https://www.academia.edu/download/45197480/HUBUNGAN_ANTARA_JUMLAH_FALSE_MOUNTING_DG_PRODUKSI_SEMEN_PJANTAN_SAPI_MADURA.pdf. diakses 17 Desember 2023.

Saputra, D., Ihsan, M., & Isnaini, N. (2017). Korelasi Antara Lingkar Skrotum Dengan Volume Semen, Konsentrasi Dan Motilitas Spermatozoa Pejantan Sapi Bali. *TERNAK TROPIKA Journal of Tropical Animal Production*, *10*(2), 59–68. https://doi.org/10.21776/ub.jtapro.2017.018.02.9

Savitri, F. K., & Suharyati, S. (2014). Kualitas Semen Beku Sapi Bali dengan Penambahan Berbagai Dosis Vitamin C pada Bahan Pengencer Skim Kuning Telur. *Jurnal Ilmiah Peternakan Terpadu*, *2*(3), 30–36.

Seyoum, K., Alemayehu, L., & Asrat, T. (2021). Effect of Breed, Age and Period of Production on Bovine Semen Quality Used for Artificial Insemination. *International Journal of Livestock Production*, *12*(1), 43–48. https://doi.org/10.5897/IJLP2020.0739

Sio, S. (2023). *Sapi Bali di Pulau Timor*. PT. Pusat Literasi Dunia. https://books.google.com/books?hl=id&lr=&id=BbvFEAAAQBAJ&oi=fnd&pg=PR5&dq=Sio,+S.+2023.+Sapi+Bali+di+Pulau+Timor.+Majalengka,+Jawa+Barat:+PT.+Pusat+Literasi+Dunia.&ots=6BOZiI7pHb&sig=5XQVqC8hEz_9k4WcgNL5JP0zQ7A diakses 8 September 2023

Situmorang, P. (2002). The Effects of Inclusion of Exogenous Phospholipid in Tris-Diluent Containing a Different Level of Egg Yolk on the Viability of Bull Spermatozoa. *Indonesian Journal of Animal and Veterinary Science*, *7*(3), 181–187.

Sunami, S., Isnaini, N., & Wahjuningsih, S. (2017). Kualitas Semen Segar dan Recovery Rate (RR) Sapi Limousin pada Musim yang Berbeda. *TERNAK TROPIKA Journal of Tropical Animal Production*, *18*(1), 36–50. https://doi.org/10.21776/ub.jtapro.2017.018.01.6

Susilawati, T. (2011a). *Spermatologi*. Universitas Brawijaya Press. https://fapet.ub.ac.id/wp-content/uploads/2017/10/Buku-Spermatologi-bu-trinil.pdf diakses 8 September 2023

Susilawati, T. (2011b). Tingkat keberhasilan inseminasi buatan dengan kualitas dan deposisi semen yang berbeda pada sapi Peranakan Ongole. *TERNAK TROPIKA Journal of Tropical Animal Production*, *12*(2), 15–24.

Widhyari, S. D., Esfandiari, A., Wijaya, A., Wulansari, R., Widodo, S., & Maylina, L. (2015). Tinjauan Penambahan Mineral Zn dalam Pakan Terhadap Kualitas Spermatozoa pada Sapi Frisian holstein Jantan. *Jurnal Ilmu Pertanian Indonesia (JIPI)*, *20*(1), 72 77.

Widyawati, E. A. (2019). *Hubungan Lingkar Skrotum Dengan Kualitas Semen Segar Pada Sapi Simmental Di Balai Besar Inseminasi Buatan Singosari* [Thesis]. Universitas Brawijaya.

Yendraliza, Anwar, P., & Rodiallah, M. (2015). *Bioteknologi Reproduksi*. Aswaja Pressindo.

Zulyazaini, Z., Dasrul, D., Wahyuni, S., Akmal, M., & Abdullah, Mohd. A. N. (2016). Karakteristik Semen dan Komposisi Kimia Plasma Seminalis Sapi Aceh yang Dipelihara di BIBD Saree Aceh Besar. *Jurnal Agripet*, *16*(2), 121–130. https://doi.org/10.17969/agripet.v16i2.5803