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PREFACE

In this edition, Volume 30 No 4, we proudly present articles from animal and veterinary sciences including genetic, reproduction; animal physiology; and veterinary from scientist all over the world. The articles published in this edition are:
"Characteristics of Pasundan Cattle Sperm Activated by Toll-Like Receptor 7/8 (TLR 7/8) for Sexing Purposes"; "Physiological Responses, Blood Profile and Performance of Local Beef Cattle to Short Period of Road Transport"; "The Impact of Sexual Rest and Scrotal Circumference on the Quality of Fresh and Chilled Semen in Local Ram"; "Moringa Leaf Extract Improves Spermatozoa Motility and Viability Through an Antioxidant Mechanism in Older Wistar Rats"; "Sustainability Assessment of KUB Native Chicken Breeding Stock Availability in Malang Raya: A RAP-MDS Analysis"; and "Influence of Different Rearing Systems and Sex on the Hematological Profile of IPB D3 Chickens".

We extent high appreciation to all peer reviewers who make this journal academically high value. Hopefully, these articles would offer any benefit to readers and the end-users of technological innovation, and attract interests from scientists to contribute their papers to the Indonesian Journal of Animal and Veterinary Sciences.

Chief Editor

Bogor, December 2025

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LIST OF CONTENT

Characteristics of Pasundan Cattle Sperm Activated by Toll-Like Receptor 7/8 (TLR 7/8) for Sexing Purposes

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ABSTRAK

Hastuti, Karja NWK, Arifiantini RI, Purwantara B, Prasetyaningtyas WE. 2025. Karakteristik sperma sapi pasundan dengan aktivasi Toll-Like Receptor 7/8 (TLR 7/8) untuk tujuan sexing. JITV 30(4):185-192. DOI:http://dx.doi.org/10.14334/jitv.v30 i4.3554.

Menentukan jenis kelamin sperma sangat penting untuk meningkatkan efisiensi produksi ternak. Metode konvensional masih sering mengalami kendala, seperti penurunan motilitas pada sperma setelah proses sexing. Penelitian ini bertujuan untuk mengkaji karakteristik sperma yang telah di-sexing pada semen sapi dengan mengaktifkan Toll-Like Receptor 7/8 (TLR 7/8) menggunakan resiquimod 848 (R848). Penelitian telah dilakukan selama tujuh bulan di Unit Pelaksana Teknis Daerah Pusat Pembibitan dan Pengembangan Inseminasi Buatan (UPTD-BPPIB) Sapi Potong di Ciamis. Penelitian ini menggunakan tiga ekor sapi jantan pasundan umur produktif. Sperma diinkubasi dalam berbagai konsentrasi R848 (0, 0,03, 0,3, dan 0,6 µM) selama 30 dan 45 menit. Sperma pada lapisan atas diencerkan dan dibekukan. Parameter yang dievaluasi meliputi motilitas progresif, viabilitas, dan integritas membran plasma sperma, baik sebelum maupun setelah thawing. Hasil penelitian menunjukkan bahwa kualitas semen segar bervariasi antar individu, dan kualitas semen setelah inkubasi juga berbeda secara individu. Data menunjukkan bahwa konsentrasi R848 sebesar 0,03 µM selama 30 menit menghasilkan kualitas semen setelah pembekuan yang hampir sama dengan kontrol. Konsentrasi tertinggi (0,6 µM) secara signifikan menurunkan semua parameter kualitas semen. Aktivasi TLR 7/8 dengan konsentrasi 0,03 µM R848 selama 30 menit terbukti sesuai untuk proses sexing sperma pada sapi pasundan.

Kata Kunci: Resiquimod 848, Motilitas Sperma, S*exing* Sperma, Viabilitas Sperma, Toll-Like Receptor 7/8

ABSTRACT

Hastuti, Karja NWK, Arifiantini RI, Purwantara B, Prasetyaningtyas WE. 2025. Characteristics of pasundan cattle sperm activated by Toll-Like Receptor 7/8 (TLR 7/8) for sexing purposes. JITV 30(4): 185-192. DOI:http://dx.doi.org/10.14334/jitv.v30 i4.3554.

Determining the sex of sperm is vital for boosting the efficiency of livestock production. Conventional methods, however, often struggle with issues like reduced motility in sexed sperm. This study set out to examine the characteristics of sexed sperm in cattle by activating Toll-Like Receptor 7/8 (TLR 7/8) with resiquimod 848 (R848). Conducted over a period of seven months at the Regional Technical Implementation Unit of the Artificial Insemination Breeding and Development Center (UPTD-BPPIB) for Beef Cattle in Ciamis. Three productive-age Pasundan bulls were used in this study. The study involved incubating sperm with different concentrations of R848 (0, 0.03, 0.3, and 0.6 µM) for 30 and 45 minutes. The study evaluated parameters such as progressive motility, viability, and the integrity of the sperm plasma membrane, both before and after thawing. The results indicated that the quality of fresh semen varied among individuals, and the quality of semen after incubation also differed individually. Overall, the data demonstrated that a concentration of 0.03 µM R848 for 30 minutes resulted in semen quality after freezing that was nearly identical to the control. In contrast, the highest concentration (0.6 µM) significantly decreased all semen quality parameters. The activation of TLR 7/8 with 0.03 µM R848 for 30 minutes is suitable for sperm sexing in Pasundan cattle.

Key Words: Resiquimod 848, Sperm Motility, Sperm Sexing, Sperm Viability, Toll-Like Receptor 7/8

INTRODUCTION

Pasundan cattle are a local cattle breed and become the germplasm of the pride of West Java province, which has been maintained for generations and is an important part of the lives of farmers. Pasundan cattle are designated as a cattle breed that needs to be preserved based on the Decree of the Minister of Agriculture Number 1051/Kpts/SR.120/10/2014. The development of Pasundan cattle is very important in order to fulfill meat needs, especially in West Java. The Pasundan cattle breed is notable for its use in the production of beef, which leads to a comparatively higher demand for bulls than cows. This phenomenon can be attributed to the growth efficiency of bulls in beef cattle production systems, which results in a higher market preference for bulls compared to cows (Almakmum et al. 2021). Concurrently, the demand for bull cattle experiences a marked increase, driven by the need for sacrificial animals in preparation for Eid al-Adha. Sex selection,

otherwise known as sperm sexing, is a necessary procedure that can be conducted at artificial insemination centers (AICs). These facilities produce frozen semen for artificial insemination (AI) in the field.

Sperm sexing technology began in the 1980s (Moore & Hasler 2017). This technology has become one of the most important innovations in the field of animal reproduction. A good sexing method can improve the fertilization ability of sexed sperm (Moore & Hasler 2017). Sperm sexing methods can be categorized into two distinct classifications: conventional and advanced. Conventional sexing methodologies encompass Percoll gradient, bovine serum albumin (BSA), density gradient sedimentation, and free-flow electrophoresis (Yata 2022). The utilization of flow cytometry has been demonstrated to achieve a separation of X sperm with a success rate exceeding 95% (Balao da Silva et al. 2016 ; Diskin 2018). The cost of this technology is exorbitant due to the requirement for the AIC to pay royalties for each dose of sexed frozen semen produced, resulting in a significantly elevated selling price for farmers. In Indonesia, AICs currently utilize two methods: the Percoll gradient (Susilawati 2014) and BSA, which has been developed since 2012. However, the consistency of the desired gender of the offspring remains unsatisfactory. The accuracy of sexing dairy cows in Bogor and Bandung using the BSA method was 58.91%. A report by dairy farmers in Pujon, East Java, who utilized the Percoll gradient method, yielded a result of 58.14% (Achjadi et al. 2022). The inconsistent accuracy of current sperm sexing methods, specifically Percoll gradient and BSA, in Indonesia, where the success rates for producing offspring of the desired gender remain below 60%, despite decades of technological advancement and the availability of more precise methods like flow cytometry.

The most recent method of sex determination is the immunological approach. This method utilizes scFv antibodies present in Y chromosome sperm (Sringarm et al. 2022). The swim-up method is one of the alternative methods that is easy to perform in evaluating X and Y sperm. This method involves the activation of Toll-like receptors (TLRs), which utilize a group of pattern recognition receptors (PRRs). Toll-like receptor 7/8 (TLR7/8) is one of the TLRs expressed on the tail of X sperm (Wen et al. 2023). TLR7/8 is a receptor that is closely associated with TLR and is activated by resiquimod 848 (R848) (Hemmi et al. 2002). As demonstrated in prior research, the activation of TLR signaling has been observed to induce mitochondrial dysfunction, resulting in a decline in sperm motility in both murine and human subjects (Zhu et al. 2016). Recent studies in mice have demonstrated that TLR7/8 is localized on X sperm and responds to a specific agonist (R848) that only affects X sperm motility (Umehara et al. 2019). Research on TLR has led to the development of methods for sexing X and Y sperm in cattle. The development of a cost-effective sperm sexing method that minimizes cell damage and requires minimal equipment is imperative. The objective of this study is to characterize sexed sperm in Pasundan cattle semen through TLR 7/8 activation with R848 and to determine the optimal R848 concentration and incubation time in the sexing process.

MATERIALS AND METHODS

Ethical approval

The present study was approved by the Animal Ethics Committee of the School of Veterinary Medicine and Biomedical Sciences, Bogor Agricultural University, with the approval number 194/KEH/SKE/III/2024.

Research replication and location

The research was conducted with three replicates, using three bulls, each of which underwent semen collection three times as replicates, resulting in a total of nine ejaculations. The research was conducted at two locations: the Regional Technical Implementation Unit, Artificial Insemination Breeding and Development Centre (UPTD-BPPIB) for Beef Cattle in Ciamis, and the Primate Study Centre, IPB University.

Preparation of diluent

The diluent utilized in this study was a commercial (Andromed, Minitube Germany). The Andromed diluent was diluted with distilled water at a ratio of 1:4. The diluent was then divided into four separate tubes, with each tube containing a specific concentration of resiquimod 848 (R848): 0 µM, 0.03 µM, 0.3 µM, and 0.6 µM, respectively. Subsequently, the diluent was stored at 34°C.

Semen collection

Semen samples were collected from three Pasundan cattle with 3-5 years of age. Semen was collected using an artificial vagina twice a week. The semen underwent a thorough assessment to determine its quality. This evaluation was carried out in two stages: initially through a macroscopic examination, followed by a microscopic analysis (Arifiantini 2012).

Fresh semen analysis

A macroscopic analysis of the semen was conducted, encompassing parameters such as volume, color, pH level, and consistency. Microscopic analysis included sperm motility, concentration, sperm viability, and sperm plasma membrane integrity. Sperm motility was assessed using computer-assisted sperm analysis

(CASA) (Del Prete et al. 2022). The concentration of sperm is calculated using a photometer SDM 6 (Minitube, Germany). The evaluation of sperm viability is conducted through the utilization of eosin nigrosin staining, as previously outlined by Pardede et al. (2020). The sperm plasma membrane integrity is evaluated using the hypoosmotic swelling test (HOST) (Santoso et al. 2021).

Semen dilution

Semen is composed of four distinct tubes. Each semen tube was diluted in Andromed according to the resiquimod (R848) treatment with different concentrations (0 µM, 0.03 µM, 0.3 µM, and 0.6 µM). Each specimen was meticulously divided into two separate tubes and subjected to an incubation period of 30 minutes and 45 minutes at a temperature of 37°C. Following the designated incubation period, the sperm layer was meticulously separated into three distinct layers: the upper layer, which contained Y chromosomes; the middle layer, which was discarded; and the lower layer, which contained X chromosomes (Wen et al. 2023). The sperm motility from the upper layer was evaluated.

Semen freezing after incubation

Subsequently, semen from the upper layer is packaged into 0.25-ml straws and equilibrated for a period of four hours. Semen is cryopreserved in liquid nitrogen vapor for a period of 10 minutes, following which it is stored in a liquid nitrogen container maintained at a temperature of -196°C.

Frozen-thawed sperm sexing testing

The evaluation encompassed assessments of sperm motility, sperm viability, and sperm plasma membrane integrity. The testing method employed was analogous to that utilized for fresh semen, with certain modifications.

Data analysis

The analysis of research data was conducted using analysis of variance (ANOVA) with SPSS 20 software. In instances where substantial disparities were identified ($P<0.05$), the Duncan test was implemented, with a 95% confidence interval.

RESULTS AND DISCUSSION

Characteristic of Pasundan cattle fresh semen

The characteristics of fresh Pasundan cattle semen are presented in Table 1. As indicated by the data presented in Table 1, the quality of Pasundan cattle semen is suitable for further processing. The standard for normal semen quality is established as sperm motility exceeding 70% (Santoso et al. 2021).

Progressive motility of Pasundan cattle sperm after incubation with R848

The progressive motility of sperm from the upper layer was evaluated. The mean progressive motility of sperm following incubation exhibited no significant variation among the different groups, including the interaction between male subject and R848 concentrations or incubation times. The mean progressive motility of sperm exhibited variability among individual bulls. The study's findings indicated that R848 concentrations ranging from 0 to 0.3 µM, with incubation times of 30–45 minutes, did not demonstrate a significant difference in bull 1, exhibiting progressive sperm motility ranging from 82.43±1.84% to 84.67±2.70%. These values were higher than those obtained with an R848 concentration of 0.6 µM and an incubation time of 30–45 minutes. Bull number 2 exhibited variability in the values of progressive sperm motility. The R0–30 and R0.03–30 treatments exhibited higher values compared to the other treatments. No significant variations in progressive sperm motility values were observed among the other treatments. The progressive sperm motility exhibited by bull number 3 demonstrated nearly identical values between the control, 0.03 µM, and 0.6 µM concentrations, with incubation times of 30 and 45 minutes, ranging from 73.60±7.15 to 80.13±3.62% (Table 2).

Frozen-thawed quality of Pasundan cattle Semen after sexing with R848

Pasundan cattle are beef cattle; therefore, it is expected that the semen used for insemination will be Y sperm. A Y sperm is in the upper layer because it is lighter than X sperm, therefore, semen quality testing is only performed on the upper layer. The progressive motility of Pasundan cattle semen frozen-thawed in the upper layer showed that R848 treatment at 0 to 0.3 µM did not differ significantly with incubation times of 30 minutes and 45 minutes ($P>0.05$). These values were higher ($P<0.05$) compared to other treatments. The concentration of R848 0.3 µM with an incubation time of 45 minutes and the concentration of R848 0.6 µM with an incubation time of 30 minutes were not significantly different. The R848 concentration of 0.6 µM with an incubation time of 45 minutes showed progressive sperm motility post-thawing of only 30.42±10.28%, the lowest compared to all treatments (Table 3).

The viability of frozen-thawed Pasundan cattle sperm of the upper layer showed that treatment with R848 0 to 0.3 µM did not differ with incubation times of

Table 1. Characteristics of fresh semen from Pasundan cattle

Variable	Bull 1	Bull 2	Bull 3
Color	Creamy white	Creamy white	Creamy white
Consistency	moderate	moderate	moderate
pH	6.4	6.4	6.4
Semen Volume (ml)		7.1±2.52a	

Different superscripts in the same row indicate significant differences (P<0.05)

Table 2. Progressive motility of Pasundan cattle sperm in the upper layer after the administration of R848 at different concentrations and incubation times.

Concentration and incubation time	Bull number			Mean±SD
	1	2	3	
R0-30	82.83±2.38$^{abcd\ A}$	78.33±0.90$^{ab\ A}$	74.80±6.55$^{ab\ A}$	78.65±3.28^{a}
R0-45	84.67±2.70$^{ab\ A}$	76.73±3.14$^{abc\ B}$	78.93±2.25$^{a\ AB}$	80.11±2.70^{a}
R0,03-30	84.47±3.15$^{ab\ A}$	78.50±3.73$^{ab\ A}$	79.20±7.62$^{a\ A}$	80.72±4.83^{a}
R0,03-45	83.03±4.24$^{abcd\ A}$	74.60±3.05$^{bcd\ B}$	80.13±3.62$^{a\ AB}$	79.25±3.64^{a}
R0,3-30	82.43±1.84$^{abcde\ A}$	75.70±1.39$^{abc\ B}$	76.80±0.80$^{a\ B}$	78.31±1.62^{a}
R0,3-45	83.63±1.63$^{abc\ A}$	71.70±4.20$^{cd\ B}$	75.93±5.75$^{ab\ AB}$	77.09±3.86^{a}
R0,6-30	77.67±7.67$^{de\ A}$	74.43±2.45$^{bcd\ A}$	75.03±5.10$^{ab\ A}$	75.71±5.07^{a}
R0,6-45	78.70±4.95$^{cde\ A}$	73.03±7.10$^{bcd\ A}$	73.60±7.15$^{ab\ A}$	75.11±6.40^{a}

R0-30= R848 concentration 0 µM (control) incubated for 30 minutes; R0-45= R848 concentration 0 µM (control) incubated for 45 minutes; R0.03-30= R848 concentration 0.03 µM incubated for 30 minutes; R0.03-45= R848 concentration 0.03 µM incubated for 45 minutes; R0.3-30= R848 concentration 0.3 µM incubated for 30 minutes; R0.3-45= R848 concentration 0.3 µM incubated for 45 minutes; R0.6-30= R848 concentration of 0.6 µM incubated for 30 minutes; R0.6-45= R848 concentration of 0.6 µM incubated for 45 minutes. Different lowercase superscript letters following the same column number indicate significant differences (P<0.05). Different uppercase superscript letters following the same row number indicate significant differences (P<0.05)

Table 3. Progressive sperm motility of post-thawed Pasundan cattle sperm in the upper layer after the administration of R848 at different concentrations and incubation times.

Concentration and incubation time	Bull number			Mean±SD
	1	2	3	
R0-30	58.39±5.83$^{a\ A}$	57.32±5. 54$^{abc\ A}$	44.27±1.39$^{ab\ B}$	53.33±4.25^{a}
R0-45	54.18±9.40$^{abc\ A}$	56.33±5.00$^{abc\ A}$	39.54±10.26$^{abc\ A}$	50.02±8.22^{a}
R0,03-30	59.24±4.82$^{a\ A}$	60.98±0.51$^{a\ A}$	48.36±4.06$^{a\ B}$	56.19±3.13^{a}
R0,03-45	53.41±3.81$^{abc\ A}$	49.97±1.59$^{cde\ A}$	39.08±13.90 $^{abc\ A}$	47.49±6.43^{ab}
R0,3-30	57.03±9.62$^{ab\ A}$	47.63±5.96$^{de\ AB}$	29.33±15.50$^{bcde\ B}$	44.66±10.3^{ab}
R0,3-45	53.43±8.61$^{abc\ A}$	38.34±3.13$^{fg\ AB}$	26.63±9.57$^{cde\ B}$	39.47±7.10^{b}
R0,6-30	48.36±13.11$^{bcd\ A}$	42.98±0.55$^{ef\ A}$	26.39±12.04$^{cde\ A}$	39.24±8.57^{b}
R0,6-45	37.79±11.06$^{e\ A}$	33.93±4.02$^{g\ AB}$	19.54±15.75$^{e\ B}$	30.42±10.28^{c}

R0-30= R848 concentration 0 µM (control) incubated for 30 minutes; R0-45= R848 concentration 0 µM (control) incubated for 45 minutes; R0.03-30= R848 concentration 0.03 µM incubated for 30 minutes; R0.03-45= R848 concentration 0.03 µM incubated for 45 minutes; R0.3-30= R848 concentration 0.3 µM incubated for 30 minutes; R0.3-45= R848 concentration 0.3 µM incubated for 45 minutes; R0.6-30= R848 concentration of 0.6 µM incubated for 30 minutes; R0.6-45= R848 concentration of 0.6 µM incubated for 45 minutes. Different lowercase superscript letters following the same column number indicate significant differences (P<0.05). Different uppercase superscript letters following the same row number indicate significant differences (P<0.05).

30 minutes and 45 minutes (P>0.05) with values ranging from 53.96 7.77% to 64.04±4.26%. These values differed significantly (P<0.05) from other treatments. The concentration of R848 at 0.3 µM with an incubation time of 45 minutes, and the concentration of R878 at 0.6 µM with an incubation time of 30 minutes did not differ significantly. The concentration of R878 at 0.6 µM with an incubation time of 45 minutes was significantly different from the other treatments (P<0.05) with a value of 39.53±11.47%. This value was the lowest among all post-incubation sperm viability values (Table 4).

The integrity of the upper layer of after frozen-thawed Pasundan bull sperm membranes showed that treatment with R848 at concentrations ranging from 0 to 0.3 µM did not differ significantly between incubation times of 30 minutes and 45 minutes (P>0.05), with values ranging from 47.38±7.14% to 58.02±4.67%. These values differed significantly (p<0.05) from other treatments. The concentrations of R848 at 0.3 µM and R878 at 0.6 µM, with incubation times of 45 minutes and 30 minutes, respectively, showed no significant difference. The concentration of R878 at 0.6 µM with an incubation time of 45 minutes was the lowest compared to the other treatments (P<0.05), with a value of 34.18±11.52% (Table 5).

DISCUSSION

The results demonstrated that the progressive motility of Pasundan bull sperm following incubation in the upper layer did not exhibit any significant differences between the various treatments. However, as illustrated in Table 2, significant variations in response to concentration and incubation time were observed among individual bulls. The progressive motility of sexed Pasundan bull sperm exhibited a maximal response at a concentration of R848 0.03 µM, with an incubation time of 30 minutes, and a minimal response at a concentration of R848 0.6 µM, with an incubation time of 45 minutes. These findings corroborate earlier reports in the field of dairy goat research, indicating that concentrations greater than 0.4 µM, when incubated for 40 minutes, result in a decline in sperm motility (Huang et al. 2022). Sperm exhibit optimal motility at a temperature of 37°C. Prolonged incubation has been demonstrated to result in a decline in sperm motility (Gong et al. 2017).

It is important to understand that progressive sperm motility after thawing is key to successful fertilization because sperm must be able to move toward the egg (Byeun et al. 2024). The results of the study demonstrated that progressive sperm motility in the upper layer exhibited nearly identical results across all treatments. The administration of Resiquimod 848 at a concentration of 0.03 µM has been observed to induce a stimulatory effect on progressive sperm motility, which is associated with sperm cell activity. Conversely, a concentration of Resiquimod at 848 µM has been observed to exert an opposite effect. Furthermore, Wen et al. (2023) observed a substantial decline in sperm motility when dairy bull sperm was incubated with R848 at a concentration of 0.6 µM for a duration of 30 minutes. The optimal R848 concentration varies among different livestock species. The optimal concentration for goat semen has been determined to be 0.2 µM (Huang et al. 2022), while for sheep semen, it has been established as 0.3 µM (Setiawan et al. 2024).

The viability and plasma membrane integrity of prozen-thawed sexed Pasundan cattle sperm in the upper

Table 4. Sperm viability of post-thawed Pasundan cattle sperm in the upper layer after the administration of R848 at different concentrations and incubation times

Concentration and incubation time	Bull number			Mean ±SD
	1	2	3	
R0-30	68.44±3.79$^{ab\ A}$	63.31±7.04$^{abc\ B}$	52.06±3.06$^{ab\ C}$	61.27±4.63^{a}
R0-45	63.47±5.29$^{abcd\ A}$	63.55±8.24$^{abcd\ A}$	46.91±9.97$^{abc\ B}$	57.98±7.83^{a}
R0,03-30	69.44±3.27$^{a\ A}$	67.38±4.92$^{a\ A}$	55.29±4.58$^{a\ B}$	64.04±4.26^{a}
R0,03-45	62.44±02.49$^{abcd\ A}$	57.89±3.37$^{cde\ A}$	48.17±14.8$^{abc\ A}$	56.17±6.89^{a}
R0,3-30	65.63±8.26$^{abc\ A}$	58.57±1.03$^{bcd\ A}$	37.67±14.02$^{bcde\ B}$	53.96±7.77^{a}
R0,3-45	63.49±8.68$^{abcd\ A}$	45.79±2.58$^{fg\ B}$	35.64±10.61$^{cde\ B}$	48.31±7.29^{b}
R0,6-30	55.46±11.84$^{def\ A}$	50.81±6.11$^{ef\ A}$	34.66±13.04$^{cde\ B}$	46.98±10.33^{b}
R0,6-45	48.80±11.89$^{f\ A}$	42.65±6.66$^{g\ AB}$	27.13±15.86$^{e\ B}$	39.53±11.47^{c}

R0-30= R848 concentration 0 µM (control) incubated for 30 minutes; R0-45= R848 concentration 0 µM (control) incubated for 45 minutes; R0.03-30= R848 concentration 0.03 µM incubated for 30 minutes; R0.03-45= R848 concentration 0.03 µM incubated for 45 minutes; R0.3-30= R848 concentration 0.3 µM incubated for 30 minutes; R0.3-45 = R848 concentration 0.3 µM incubated for 45 minutes; R0.6-30 = R848 concentration of 0.6 µM incubated for 30 minutes; R0.6-45= R848 concentration of 0.6 µM incubated for 45 minutes. Different lowercase superscript letters following the same column number indicate significant differences (P<0.05). Different uppercase superscript letters following the same row number indicate significant differences (P<0.05)

Table 5. Sperm plasma membrane integrity of frozen-thawed Pasundan bull sperm in the upper layer after administration with R848 at different concentrations and incubation times

Concentration and incubation time	Bull number			Mean ±SD
	1	2	3	
R0-30	62.74±4.82$^{a\ A}$	57.58±9.40$^{ab\ A}$	45.82±3.09$^{ab\ B}$	55.38±5.77^{a}
R0-45	58.60±6.41$^{\mathbf{acde\ A}}$	58.40±6.86$^{\mathbf{ab\ A}}$	42.25±10.79$^{\mathbf{abc\ B}}$	53.08±8.02^{a}
R0.03-30	62.32±4.08$^{\mathbf{ab\ A}}$	62.32±5.06$^{\mathbf{a\ A}}$	49.43±4.87$^{\mathbf{a\ B}}$	58.02±4.67^{a}
R0.03-45	56.19±2.04$^{\mathbf{abcde\ A}}$	52.77±2.92$^{\mathbf{bc\ A}}$	42.44±13.77$^{\mathbf{abc\ A}}$	50.47±6.24^{ab}
R0.3-30	59.68±6.72$^{\mathbf{abcd\ A}}$	52.01±2.52$^{\mathbf{bcd\ A}}$	30.45±12.18$^{\mathbf{bcd\ B}}$	47.38±7.14^{ab}
R0.3-45	56.52±8.22$^{\mathbf{abcde\ A}}$	42.02±3.27$^{\mathbf{ef\ AB}}$	31.13±10.31$^{\mathbf{bcd\ B}}$	43.22±7.27^{b}
R0.6-30	50.71±11.14$^{\mathbf{ef\ A}}$	45.07±6.31$^{\mathbf{de\ A}}$	28.69±11.03$^{\mathbf{cd\ B}}$	41.49±9.49^{b}
R0.6-45	41.99±12.082$^{\mathbf{g\ A}}$	36.52±6.73$^{\mathbf{f\ AB}}$	24.03±15.75$^{\mathbf{d\ B}}$	34.18±11.52^{c}

R0-30= R848 concentration 0 µM (control) incubated for 30 minutes; R0-45= R848 concentration 0 µM (control) incubated for 45 minutes; R0.03-30= R848 concentration 0.03 µM incubated for 30 minutes; R0.03-45= R848 concentration 0.03 µM incubated for 45 minutes; R0.3-30= R848 concentration 0.3 µM incubated for 30 minutes; R0.3-45= R848 concentration 0.3 µM incubated for 45 minutes; R0.6-30= R848 concentration of 0.6 µM incubated for 30 minutes; R0.6-45= R848 concentration of 0.6 µM incubated for 45 minutes. Different lowercase superscript letters following the same column number indicate significant differences (P<0.05). Different uppercase superscript letters following the same row number indicate significant differences (P<0.05)

layer exhibited variation across treatments. Sperm viability in the upper layer at R848 concentrations ranging from 0 µM to 0.3 µM with a 45-minute incubation time was nearly identical. Conversely, an R848 concentration of 0.6 µM, with a 45-minute incubation time, exhibited an opposing effect. The integrity of the plasma membrane of sexed sperm in the upper and lower layers of goat semen at R848 concentrations of 0.3 µM, 0.6 µM, and 0.9 µM did not differ, ranging from 79.84% to 84.76% (Maulana et al. 2024).

The integrity of the sperm plasma membrane is imperative for maintaining sperm motility and viability. The plasma membrane fulfills the role of a barrier, thereby protecting the internal components of sperm and regulating the exchange of substances between the cell and the external environment (Glazar & McCue. 2021; Prihantoko et al. 2020). Damage to the plasma membrane, particularly in the midpiece, which is abundant in mitochondria, can result in a variety of adverse consequences. Mitochondria, in particular, are the primary energy source for sperm, playing a crucial role in the production of ATP (adenosine triphosphate) through the process of cellular respiration. Prolonged exposure to external stressors can result in the disruption of the plasma membrane, thereby facilitating the release of enzymes that are typically confined within the cell.

These enzymes, which are vital for metabolic processes, are released into the extracellular environment when the plasma membrane becomes compromised. This enzyme release has been demonstrated to disrupt biochemical processes within the sperm, which can lead to apoptosis or cell death. Consequently, this can further reduce the number of healthy sperm. A decline in ATP production, resulting in plasma membrane damage, leads to diminished sperm motility. This phenomenon has been demonstrated to exert a substantial influence on the sperm's capacity to navigate towards the egg during the process of fertilization. Therefore, the integrity of the sperm's plasma membrane is indicative of sperm health and efficiency, ensuring optimal function (Prihantoko et al. 2020). Maintaining the integrity of the plasma membrane of sexed sperm is imperative for both physiological and reproductive purposes, ensuring the seamless progression of the reproductive process.

The TLR7/8 mechanism in X sperm is activated by specific ligands, such as Resiquimod 848, which binds to TLR7/8, which is only present in X sperm. In X sperm, TLR7 is positioned in the tail, while TLR8 resides in the midpiece. This activation is specific to X sperm and does not impact Y sperm, as these receptors are absent in Y sperm (Umehara et al. 2019). These binding triggers intracellular reactions that inhibit the Tumor Necrosis Factor Receptor-Associated Factor 6 (TRAF6) protein through the Phosphatidylinositol-3-Kinase (PI3K) and Nuclear Factor-Kappa B (NFκB) pathways. This inhibition has been shown to reduce the activity of hexokinase, an enzyme that plays a critical role in glucose metabolism. Consequently, this results in a decrease in the formation of adenosine triphosphate (ATP), which is the primary energy source for sperm motility (Umehara et al. 2019; Wen et al. 2023).

Other studies have shown that TRAF6 activation triggers two main pathways. The TRAF6 → PI3K → GSK3α/β → inhibition of hexokinase activity pathway, and the TRAF6 → NF-κB → regulation of gene expression and mitochondrial activity pathway. Inhibition of hexokinase reduces the conversion of glucose to ATP, thereby decreasing energy production in X sperm (Ren et al. 2021). This reduction in hexokinase

activity leads to decreased ATP production in X sperm. Since ATP is the primary energy source required for sperm motility, the decrease in ATP directly results in reduced motility or sperm movement speed (Wen et al. 2023). X sperm move more slowly than Y sperm, whose motility is not affected by TLR7/8 activation. Slow X sperm typically remain at the bottom during sperm sexing using the swim-up method, while Y sperm remain at the top.

The present study demonstrated the individual effects on all variables of fresh semen quality, post-incubation with R848, or post-thawing. This observation was corroborated by Baharun et al. (2025), who reported similar findings in the same cattle. A concentration of R848 at 0.03 µM, with an incubation time of 30 minutes, yielded values that closely resembled the control values. Therefore, this concentration and incubation time can be employed in future studies. This study constitutes an inaugural investigation into the development of a sperm sexing method employing the swim-up principle with R848 in Pasundan cattle, with a primary focus on the quality of sexed semen. The necessity of validation is twofold: first, to confirm the suitability of X-chromosome-carrying and Y-chromosome-carrying sexed sperm through rt-PCR analysis; and second, to ascertain the success of artificial insemination using the sexed semen.

The limitations of this study lie in the evaluation of the effects of Resiquimod 848 (R848) concentration and incubation time on the quality of sexed Pasundan bull semen, specifically regarding progressive motility and plasma membrane integrity of spermatozoa in the upper layer during the swim-up method. This research focuses on optimizing incubation conditions—namely, an R848 concentration of 0.03 µM and 30 minutes of incubation—which showed the best sperm response, consistent with control values, without diminishing motility or damaging membrane integrity. However, higher R848 concentration (0.6 µM) and longer incubation time (45 minutes) resulted in decreased sperm performance, aligning with previous findings in other livestock species such as goats and sheep. Additionally, the study acknowledges general limitations that optimal responses to R848 vary across species and individuals, making the results specific to the Pasundan population and not broadly validated. Major limitations also include the absence of molecular validation (rt-PCR) to confirm accurate sex separation and the lack of functional testing via artificial insemination to demonstrate reproductive success, which are critical aspects for advancing sex separation methods based on swim-up and R848.

CONCLUSION

The present study concluded that the characteristics of Pasundan cattle sperm are influenced by individual factors. A resiquimod 848 concentration of 0.03 µM and an incubation time of 30 minutes have been determined to be optimal for activating TLR 7/8 in the sexing process of Pasundan cattle sperm. Further research is needed to validate the accuracy of sexing results in vitro using PCR and in vivo through artificial insemination.

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Physiological Responses, Blood Profile and Performance of Local Beef Cattle to Short Period of Road Transport

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ABSTRAK

Aditia EL, Priyanto R, Abdullah L, Karti PDMH, Manalu W. 2025. Respon Fisiologis, Profil Darah dan Performa Sapi Lokal pada Transportasi Darat Jarak Dekat. JITV 30(4):193-201. DOI:http://dx.doi.org/10.14334/jitv.v30 i4.3598.

Transportasi dapat menimbulkan dampak negatif terhadap performa dan aspek kesejahteraan ternak. Penelitian ini bertujuan untuk menganalisis pengaruh transportasi jarak dekat pada sapi Peranakan Ongole (PO) dan Silangan Lokal (SL) terhadap respon fisiologis, profil darah dan performa selama rekondisi, Penelitian ini menggunakan 16 ekor sapi jantan berumur I_1 (18-24 bulan), yang tediri atas 8 ekor sapi PO dan 8 ekor sapi SL, dengan rataan bobot sapi PO dan SL adalah 289,8±27,2 dan 285,5±31,7 Kg. Peubah yang diamati yaitu susut bobot, suhu rektal, laju respirasi, *panting score*, kandungan glukosa, kreatinin, *packed cell volume* (PCV), haemoglobin (Hb), eritrosit, leukosit dan diferensiasi leukosit, serta peubah performa selama rekondisi meliputi konsumsi dan konversi pakan, pertambahan bobot badan (PBB) dan pertambahan bobot badan harian (PBBH). Data susut bobot, respon fisiologis dan profil darah dianalisis menggunakan analisis sidik ragam dengan pengukuran berulang. Selanjutnya data performa selama rekondisi dianalisi menggunakan analisis kovarian dengan bobot awal yaitu bobot pasca transportasi sebagai kovariabel. Hasil penelitian menunjukkan sapi PO dan SL mengalami stress selama transportasi jarak dekat. Kadar glukosa dan PCV sapi PO pre-transportasi nyata lebih tinggi (P<0,05) dibandingkan pada sapi SL. Selanjutnya kadar glukosa dan PCV sapi PO dan SL nyata lebih rendah pasca transportasi (P<0,05), meskipun tidak berbeda nyata antar bangsa. Selain itu rasio neutrophil:limfosit meningkat pasca transportasi jarak dekat ini. Lebih lanjut sapi PO memiliki performa yang lebih baik selama pemeliharaan rekondisi, Dimana bobot akhir pasca rekondisi, PBBH dan PBB, serta konsumsi pakan dan bahan kering ransum yang nyata lebih tinggi dibandingkan pada sapi SL (P<0,05).

Kata Kunci: Profil Darah, Respon Fisiologis, Sapi Lokal, Performa, Transportasi

ABSTRACT

Aditia EL, Priyanto R, Abdullah L, Karti PDMH, Manalu W. 2025. Physiological Responses, Blood Profile and Performance of Local Beef Cattle to Short Period of Road Transport. JITV 30(4): 193-201. DOI:http://dx.doi.org/10.14334/jitv.v30 i4.3598.

Transporting livestock poses a risk to animal performance and welfare. The objective of the study was to evaluate the impact of short transport periods on physiological responses, blood profile and performance during recondition period of local beef cattle. A total of 16 bulls consisting of eight Ongole Grade (PO) and local crossbred (SL) were used in this study. Parameters observed were weight loss, rectal temperature, respiratory rate, panting score, glucose, creatinine, packed cell volume/PCV, hemoglobin (Hb), erythrocyte, leucocyte, and leucocyte differentiation, as well as data for performance during recondition period namely feed consumption and conversion, average daily gain and final weight after reconditioning. Data for body weight loss, physiological parameters, and blood profile were analyzed with analysis of variance (ANOVA) with repeated measurement. Whereas data for performance during recondition was analyzed by analysis of covariance with initial weight post transportation as covariable. Results showed that PO cattle have significantly higher (P<0.05) glucose and PCV concentrations during pre-transportation than SL cattle due to the more excited character of PO cattle. Glucose and PCV of PO and SL cattle were significantly decreased (P<0.05) during post transportation. It might be that cattle were exhausted due to transport, change in energy utilization, and fluid balance changes during and after transport. However, the concentration of glucose and PCV were similar for both breeds during post transportation. In addition, short periods of road transport increase neutrophil: lymphocyte ratio. Furthermore, PO cattle have better performance during recondition period compared to SL cattle. Final weight, average daily gain, feed consumption and dry matter intake after 10 days of recondition for PO cattle was significantly higher (P<0.05) compared to SL cattle

Key Words: Blood Profile, Physiological Responses, Local Beef Cattle, Transportation

INTRODUCTION

Transportation is one of the key elements in the livestock logistics chain, where the mobilization of livestock occurs from producers to consumers (Miranda-de la Lama et al. 2014). Mainly for local beef cattle that majorly transported both short and long transport conditions. Short transport conditions are mainly done

within the same region, usually within a single district or province (Saptana and Ilham 2018). Several local breed cattle which mainly transported were Ongole grade (PO) and Local crossbreed either Simmental-PO cross (SimPO) or Limousin-PO cross (LimPO). According to Cardoso et al. (2015a), cattle belong to *Bos indicus* has better adaptation to tropical conditions compared to *Bos taurus.* Therefore, crossbreeding between both species will have benefit impact for the offspring in adaptation to tropical conditions. Even though transportation is crucial, this process poses risks causing various negative impacts due to the many stressors that arise, such as microclimate and weather conditions, solar radiation intensity, vibration, mixing, as well as lack of feed and water. The negative impacts include weight loss (Coffey et al 2001), wounds, injuries, increased mortality (Malena et al. 2007; Minka and Ayo 2009a), and damage to carcasses and meat (Strappini et al. 2009), which result in high economic losses. Stress assessment during transportation can be observed through various indicators, including changes in physiological status and blood profiles (Avila-Jaime et al. 2021), and post-transportation performance (Coffey et al 2001).

A better understanding of cattle transportation in Indonesia, which has a tropical climate where high temperature and humidity represent major environmental stressors, is therefore essential to design mitigation programs to reduce the negative impacts caused. This study aims to evaluate the physiological status, blood profiles, and post-transportation performance of Ongole Crossbred (PO) and Local Crossbred (SL) cattle during short-distance transportation (short duration).

MATERIALS AND METHODS

Materials

A total of 16 bulls consisting of 8 bulls of PO and SL with average age was I_1 (18-24 months) were used for the trial. The average body weight of PO and SL bull were 289,8±27,2 kg and 285,5±31,7 kg respectively. the feeds during recondition periods were combination of commercial concentrate, forage and fermented feed (Sorinfer). Furthermore, the vehicle to transport the bulls was Mitsubishi Fuso Fighter 220 PS truck with floor space 19,25 m^2, which provide space allowance for the bulls 1,13 m^2. In addition, Data logger REED R6030 was used to record temperature and humidity real time during trial. 10 ml blood vacutainer, 10 ml syringe, medical alcohol 70% were used for blood sample preparation and digital thermometer was used for rectal temperature measurement.

Methods

The research was complied with ethical commission from The School of Veterinary Medicine and Biomedical Sciences (SKHB) IPB University. Cattle weighing, physiological parameters measurement, namely rectal temperature and respiratory rate as well blood sample collection was carried out a day before transportation in the evening with empty stomach condition. Whereas blood sample collection was carried out from *Vena Coccigea* using 10 ml of disposable syringe, then collected into *vacutainer* containing EDTA and put into cooler box for further measurement of glucose, creatinine, *packed cell volume* (PCV), hemoglobin (HB), erythrocyte, leukocyte dan leucocyte differentiation. On a day of transportation, the bulls were loaded into the truck with space allowance of 1.13 m^2/head and transported to beef cattle housing at Faculty of Animal Sciences, IPB University. In addition, data logger was adjusted into the truck to record temperature and humidity every hour.

The distance and duration of transport was approximately 102 km and 6 hours and 58 minutes, comprises of 4 hours and 23 minutes of travel (voyage) and a resting period of 2 hours and 12 minutes. During the resting period, the cattle were not provided with feed and drinking water. The average transportation speed during the journey was 23.2 km h^{-1}.The transportation was categorized into short transport duration according to (Nielsen et al. 2022). Road conditions during transportation varied from well-maintained asphalt roads to uneven surfaces. The journey was relatively smooth, with moderate traffic congestion at several points. Weather conditions were initially cloudy and overcast, while heavy rainfall occurred toward the end of the journey near the rearing facility.

Immediately after arriving at beef cattle housing facility cattle were unloaded, then the bulls were weighing to determine the weight loss (shrink), blood sample collected and measured for rectal and respiratory rate. The blood sample was analyzed at Laboratory of Meat, Draught and Sport Animal Nutrition, Faculty of Animal Sciences, IPB University. Immediately after transport, the bull fed with roughage, then after two hours they have access to clean water. Feeding trial was conducted on the previous day post transportation with different feed namely combination of commercial concentrate and forage with ratio 80:20 and Sorinfer (completed fermented feed based on sorghum and Indigofera plants), with daily feed requirement was 3% from body weight based on dry matter content. Feed was fed three times daily from 6-7 AM, 1-2 PM and 5-6 PM, with the ratio of feed given was 35:25:45. Feed intake was measured by subtracting the feed given and leftover. The bulls then reweigh after 10 days post transport to examine recovery weight.

Parameters observed

The parameters observed were weight loss, physiological responses namely rectal temperature and

respiratory rate. Whereas blood profile was glucose, creatinine, packed cell volume (PCV), haemoglobin (HB), erythrocyte, leukocyte and leukocyte differentiation. Temperature and humidity were measured to calculate temperature humidity index (THI) to support stress examination of cattle during transportation (Table 1). In addition, performance production parameters of the bull were initial weight pre-transport (kg), average daily gain (ADG), final weight after reconditioning (kg), as feed consumption (kg/day), dry matter intake (kg/day), as feed conversion and as feed dry matter conversion. The formula to calculate THI was described below:

$$THI = 0.8Tab + RH (Tab - 14.4) + 46.4$$

THI = Temperature Humidity Index
Tab = Temperature (^oC)

RH = Relative Humidity (%)

The analyses for glucose and creatinine were conducted using commercial assay kits with catalogue numbers 112191 and 112192. The hematocrit value was determined using a microcapillary hematocrit reader (microhematocrit method). Haemoglobin concentration was determined by the Sahli method. Erythrocyte and leukocyte counts were analyzed using a Hemocytometer (Neubauer chamber. Then, Leukocyte differential counts were determined using the Giemsa staining method.

Data analysis

Data of weight loss, physiological responses and blood profile were analysed with repeated measurement analysis of variance. Whereas production performance was analyzed with analysis of covariant 2x2 factorial with initial weight pre-transportation as co-variable.

RESULTS AND DISCUSSION

Physiological responses and weight loss

Average value of THI during transportation was 80.3±5.6, and its categorized as mild stress for the cattle (Bulitta et al. 2015). According to (Barnes et al. 2004), cattle undergo heat stress will increase respiratory rate and rectal temperature. The result of the study shows that the respiratory rate and rectal temperature of the PO and SL pre transportation were similar as well as post transportation. However, respiratory rate of PO and SL cattle were significantly decreased ($P<0,05$) post transportation (Table 2). In addition, rectal temperature of PO and SL bull pre- and post-transport were in normal range. However, the respiratory rate of PO and SL bull pre transportation was out of normal range than post transportation according to Jackson and Cockroft (2002). Higher respiratory rate of PO and SL bull pre transportation indicated that both cattle were stressed during handling before transport. In the other hand, declining value of rectal temperature and respiratory rate post transportation might be caused by microclimate condition during transportation in which heavy rainfall occurring in the final stage of transportation. Rainfall can significantly reduce ambient temperature, thereby lowering the heat load experienced by the animals. Several studies have reported that under cooler environmental conditions caused by rainfall, cattle tend to reduce their respiration rate and core body temperature as part of physiological mechanisms to maintain homeostasis. In addition, the presence of water droplets on the skin and hair due to rain exposure may influence body heat exchange with the environment. The water layer on the body surface can alter heat dissipation processes through conduction and evaporation, contributing to changes in thermoregulatory responses. Similarly, water spraying on cattle is often applied as a management practice to alleviate heat stress under high temperature conditions (Brouk et al. 2003; Gaughan et al. 2005; Legrand et al. 2011; Brown-Brandl 2018).

Result shows that cattle breed has no significant effect on weight loss post transportation. The average body weight loss of PO and SL bull were 7,0±4,0 kg (2,5±1,5 %) and 9,3±5,3 kg (3,1±1,7 %) respectively (Tabel 1). It is indicated that PO and SL cattle has capabilities to adapt with environmental condition during short period of transport. Even though the weight loss were not significant between breeds, the SL cattle produce higher loss. It might be that SL more susceptible to heat stress compare to PO cattle. in tropical conditions zebu cattle which carry *Bos indicus* blood are relatively more susceptible to heat stress (Cardoso et al. 2015). therefore, PO cattle tha belongs to *Bos indicus* has lower weight loss, compare to SL cattle that containing *Bos taurus* genetic proportion.

Table 1. Table 1 Heat stress categories based on THI values

THI Value	Heat stress categories
≤ 74	Normal
75 – 78	Stress ringan
79 – 83	Stress sedang
≥ 84	Stress berat

Bulitta et al. (2015)

Table 2. Mean values of physiological responses parameters and body weight Loss of PO and SL bull pre- and post-transportation

Parameters	Cattle breeds	Measurement time		Standard value*
		Pre-transportation	Post-transportation	
Rectal temperature (°C)	PO	39.1±0.1	38.8±0.5	38.0–39.5
	SL	39.2±0.4	38.5±0.6	
Respiratory rate (breathe/minute)	PO	46.5±9.8[a]	24.0±4.3[b]	15-30
	SL	43.5±6.2 [a]	24.0±3.0[b]	
Body weight (kg)	PO	289.8±27.2	283.0±29.9	
	SL	285.5±31.7	276.3.0±27.0	
Weight loss (kg)	PO	7.0±4.0		
	SL	9.3±5.3		
Weight loss (%)	PO	2.5±1.5		
	SL	3.1±1.7		

Different superscript ini similar coloum shows significant different (P<0.05) *)Jackson and Cockroft (2002)

Result study shows that PO bull has significantly (P<0.05) higher value of glucose and PCV than SL bull before transportation. Nevertheless. the average glucose and PCV value of both breeds were similar after transportation and the value was on normal range (Table 3). Increasing blood glucose levels on both PO and SL bull is closely related to stress responses induced during transportation. (Belhadj Slimen et al. 201), mentioned that in stressful situations, animal requirement of energy is highly relied on glucose as primary energy source. In addition, (Brunel et al. 2018) stated loading and unloading process during transportation were critical for animals. so that energy requirement will significantly increase. thus, blood glucose levels will increase.

Creatinine level was similar for PO and SL bulls pre- and post-transportation. even though the value was higher than standard (Table 3). This indicates that both PO and SL bulls were experienced muscle fatigue and dehydartion as response to stress induced by transportation, including handling process. Increasing level of creatinine induced by physical activity or exercise of cattle during loading and unloading. as well as standing activity of the cattle to maintain their balance during motion in the truck that causes muscle fatigue (Escalera-valente et al. 2021). Increasing concentration of PCV is strongly related to dehydration conditions of the cattle due to physical activity during handling and transportation, which exacerbated by thermal stress form the environment (Minka and Ayo 2009). In addition, Das et al. (2016) mentioned that increasing concentration of PCV is part of adaptation mechanisms of the body to compensate for water loss during the recovery process following dehydration due to evaporative fluid loss. Furthermore, *Bos indicus* cattle (Scheffler 2022), in this research represent by PO cattle have aggressive temperament than *Bos taurus* or *Bos taurus crossbred* (SL cattle). Therefore, PO bull has significantly higher glucose and PCV content pre-transport than SL bull.

Result shows that Hb concentration was increase post transportation for both breeds. The increasing concentration of Hb were similar among treatments both pre- and post transportation. however the values were above the standard (Weiss and Wardrop 2010) (Table 3). Furthermore. erytrocyte was decrease post transportation. eventhough the result were similiar both breeds pre- and post transportation (Table 3). Cattle that expose to stress and dehydration during transportation will lead to significant loss of body fluids (Brunel et al. 2018). In addition, the loss of body fluids occurs during respiration and through sweating (Barnes et al. 2004). Data analysis shows that leucosite consentration was increase post transportation. However the value was similar pre- and post transportation for both breeds and the value was in normal range (Weiss and Wardrop 2010). Change in leucosite concentration describes imune responses of the animal due to stress, physical activity and fear (Roland et al. 2014)., in this research stres was triggered by handling procedures and during transportation. Stress during handling and transportation will activate the hypothalamic–pituitary–adrenal (HPA) axis, resulting in increased secretion of catecholamines (adrenaline and noradrenaline) and cortisol. These hormones subsequently influence various physiological and hematological parameters, including erythrocyte concentration in the blood. In addition, variations in erythrocyte counts may be related to changes in body fluid balance. During transportation, animals may experience considerable fluid loss, leading to

dehydration. However, after transportation, when cattle regain access to drinking water, plasma volume increases, which can dilute circulating erythrocytes and consequently reduce their concentration in the blood. This mechanism likely explains the decrease in erythrocyte concentration observed after transportation (Minka and Ayo 2009; Rakib et al. 2016; Alam et al. 2018a; Brunel et al. 2018). Furthermore, prolonged stress conditions can elevate cortisol secretion, which may influence erythropoiesis and the lifespan of erythrocytes in circulation (Maheshwari et al. 2013; Zulkifli et al. 2019; Jung et al. 2022).

Result shows that limfosit concentration was similar between breeds both pre-and post transportation. However limfosit concentration post-transportation for both breeds was significantly lower ($P<0.05$) compare to pre-transportation. Furthermore, limfosit concentration was higher than standard value (Table 4). These means that PO and SL bulls were stressed due to physical activity during handling before and after transportation. Stress during handling for tranportation will stimulate secretion of catecholamines and glucocorticoids from cortex adrenal and medulla. So that elevation of glucocorticoid can causes damage on limfosit in tymus cortex and prolongs neutrofil lifespan, ultimately lymphocyte depletion will enhance neutrophil concentration in blood (Ishizaki & Kariya 2010). In addition, elevation of neutrophils concentration in blood may result from a shift in leukocyte proportions due to lymphopenia, it is a common condition observed in animals under stress condition (Alam et al. 2018).

Monocytes concentration of PO bull pre-transportation was significantly higher ($P<0.05$) compare to SL bull. However monocytes concentration of both breeds were similar post-transportation, and the average value of monocytes for both breeds were above standar value (Table 4). Elevation of monocytes count in PO bulls may reflects stress reponses which associated with handling procedures, in which PO bull that belongs to *Bos indicus* has more excitable temperament compare to *Bos taurus* and their crossbreed (Scheffler 2022). In addition, Roland et al. (2014), mentioned that enhancement monocytes concentration on blood circulation closely related to accute stress and infection condition on the animal.

Eosinophil and basophil concentration were similar for PO and SL bulls both pre- and post-transportation. Even though the value was above the standard (Table 4). This indicates both cattle were stress during handling and transportation, however, no evidence the bull has health disorder or infectious diseases during transportation. Acute stress following transportation may induce alterations in leukocyte dynamics, including changes in eosinophil and basophil concentrations. Transportation is recognized as a major stressor in cattle production systems, triggering activation of the hypothalamic–pituitary–adrenal (HPA) axis and increased secretion of cortisol and catecholamines. These stress hormones can modulate immune function and influence the redistribution of leukocyte populations in the bloodstream. Several studies have reported that transportation stress may increase the total leukocyte count and certain leukocyte subpopulations, including eosinophils and other granulocytes, as part of the physiological stress response in cattle (Hong et al. 2019). The increase in eosinophils and basophils observed after transportation may also reflect inflammatory or immunological responses associated with tissue irritation, dehydration, and environmental challenges during transport. Transportation conditions such as heat stress, prolonged standing, and feed and water deprivation may stimulate inflammatory mediators that influence granulocyte mobilization into circulation. In addition, stress-induced endocrine responses can alter leukocyte trafficking between blood and tissues, resulting in temporary changes in leukocyte distribution during the post-transport recovery period (Avila-Jaime et al. 2021).

Results shows that neutrofil: lymphocyte ration were similar between pre- and post transportation among the breeds However, the bulls were indicated stressed during short period of transportation due to elevation of neutrofil: lymphocyte ratio (Table 4). Neutrofil: Lymphocyte ratio describes stress condition on the animal (Viswanathan & Dhabhar 2005). Furthermore, elevation of cortisol on the blood was followed by elevation neutrofil concentration and the destruction of limfosit. Therefore neutrofil: lmyphocyte ratio was increase (Kim et al. 2018).

However, during transportation over relatively short distances or durations, changes in the neutrophil-to-lymphocyte (N:L) ratio do not always show a significant increase. This condition may be influenced by the physiological adaptive capacity of the animal, environmental conditions during transportation, and management practices applied before and after the journey (Earley & O'riordan 2006; Minka & Ayo 2009; Maheshwari et al. 2013; Romero et al. 2014).

Data analysis shows that there was significant correlation ($P<0.05$) between glucose and PCV, Hb and creatinine, erytrocyte and creatinine respectively on pre-transportation condition (Table 5). These indicated that the bulls were already stressed due to handling procedures during transportation. Stress will stimulates glycogensis, thereby blood glucose concentration will increase as part of adaptive physiological mechanism to supply energy during stress exposure (Tadich et al. 2013; Brunel et al. 2018). In addition, during stress exposure substantial body fluid losses will occur due to dehydration, and it will lead to reduction in blood plasma volume, while the erythrocyte concentration remains relatively constant. As a result,

Table 3. Mean values of leucosite differentiation of PO and SL bulls pre- and post-transportation

Blood parameters	Cattle breeds	Measurement time		Standard value*
		Pre-transportation	Post-transportation	
Glucose (mg/dl)	PO	51.504±3.889[b]	76.730±3.737[a]	45-75
	SL	39.963±3.287[c]	74.276±3.158[a]	
Creatinine (mg/dl)	PO	4.310±0.393	4.851±0.521	1.0-2.0
	SL	4.604±0.297	4.755±0.394	
PCV (%)	PO	10.629 ±0.461[a]	12.371±0.950[a]	21-30
	SL	9.000±0.498[b]	11.867±5.90[a]	
Hb (g%)	PO	28.429 ± 2.268	31.000±2.231	8.4-12
	SL	30.417±2.449	32.667±2.410	
Erytrocyte (10^6/ul)	PO	6.591±0.615	5.779±0.691	4.9-7.5
	SL	6.883±0.665	6.312 ± 0.747	
Leucosite (10^3/ul)	PO	5.936±0.645	6.893±0.691	5.1-13.3
	SL	6.400±0.696	7.250±0.746	

Different superscript ini similar coloum shows significant different (P<0.05) *)Weiss and Wardrop (2010)

Table 4. Mean values of leucosite differentiation of PO and SL bulls pre- and post-transportation

Leucosite differentiation	Cattle breeds	Measurement time		Standard value*
		Pre-transportation	Post-transportation	
Lymphocyte (%)	PO	60.414±3.647[b]	46.129±3.129[a]	1.8-8.1
	SL	59.145±3.939[b]	51.685±3.379[a]	
Neutrophil (%)	PO	30.457±3.757[b]	46.199±3.221[a]	1.7-6.0
	SL	33.310±4.058[b]	43.310±3.479[a]	
Monocytes (%)	PO	2.399±0.304[a]	1.370±0.274[a]	0.1-0.7
	SL	1.192±0.328 [b]	1.408±0.296[ab]	
Eosinophils (%)	PO	5.787±0.703	5.299±1.016	0.1-1.2
	SL	5.660±0.759	2.653±1.098	
Basophils (%)	PO	0.950±0.007	0.970±0.210	0-0.2
	SL	0.910±0.005	0.940±0.040	
Neutrophil: Lymphocyte (N:L)	PO	0.568±0.358	0.976±0.192	
	SL	0.548±0.218	0.932±0.544	

Different superscript ini similar coloum shows significant different (P<0.05). *Weiss and Wardrop (2010)

PCV and Hb concentration, and erythrocyte count will increase significantly (Avila-Jaime et al. 2021). Furthermore, very significant correlation (P<0.01) between erythrocyte and Hb were detected (Table 5). Hemoglobin concentrations will significantly increase during stress conditions. This mechanism was result from the release of adrenaline and epinephrine/norepinephrine, consequently it will induce the elevation of blood pressure thereby promoting the erythrocyte counts (Brunel et al. 2018a; Avila-Jaime et al. 2021).

Significant correlation (P<0.05) between leucocyte and Hb concentration, then very significant correlation (P<0.01) between erythrocyte and Hb concentration were detected post-transportation (Table 5). These phenomena may result from dehydration conditions of

the bull during transportation, whereas excessive body fluids loss occurred during thermal stress. Thereby blood plasma concentration was reduced, yet red blood cell concentrations were relatively constant. Hemoglobin, a blood protein, serves as the primary constituent of erythrocytes (Avila-Jaime et al. 2021). In addition, increasing leukocyte concentration as part of the acute stress response, fear and physical exercise (Roland et al. 2014).

Data analysis shows that PO cattle have significantly higher body weight (P<0.05) after 10 days recondition than SL cattle. The result corresponds with higher (P<0.05) feed consumption, dry matter consumption and ADG of PO than SL cattle (Table 6). Stress during transportation will reduce voluntary feed consumption, therefore it will decrease performance during initial feedlot period (Marques et al. 2012). In addition, stress associated with transportation will alter the physiological status of livestock, which consequently impacts the control of feed intake (Gouvêa et al. 2022). Furthermore, increasing body weight post-transportation is mainly caused by a shift of a lower to higher energy diet during recondition period that facilitated compensatory growth to the animal (Meléndez et al. 2021).

Table 5 Pearson's correlation coefficient of blood profiles of PO and SL bulls pre- and post-transportation

Blood profiles	Glucose	Creatinine	PVC	Hb	Erytrocyte	Leucocyte
			--- Pre-transportation ---			
Glucose	1.000	0.014	0.691*	-0.195	-0.149	-0.558
Creatinine	0.014	1.000	0.493	0.672*	0.671*	-0.065
PCV	0.691*	0.493	1.000	0.328	0.350	-0.265
Hb	-195	0.672*	0.328	1.000	0.870**	-0.017
Erytrocyte	-0.149	0.671*	0.350	0.870**	1.000	-0.228
Leucocyte	-0.558	-0.065	-0.265	-0.017	00.228	1.000
			--- Post-transportation ---			
Glucose	1.000	0.245	-0.092	-0.103	-0.311	-0.177
Creatinine	0.245	1.000	0.395	0.520	0.275	0.091
PCV	-0.092	0.395	1.000	0.030	0.141	-0.225
HB	-0.103	0.520	0.030	1.000	0.805**	0.669*
Erytrocyte	-0.311	0.275	0.141	0.805**	1.000	0.448
Leucocyte	-0.177	0.091	-0.225	0.669*	0.448	1.000

**very significant correlation (P<0.01). *Significant correlation (P<0.05)

Table 6. Impact of breed on the mean value of performance parameters of local beef cattle fed with different concentrate feed (commercial concentrate and Sorinfer) within 10 days recondition

Performance	Cattle breeds	
	PO	SL
Initial weight pre-transportation (kg)	289.80±27.20	285.50±31.70
Weight post-transportation (kg)	283.00±29.90	276.30±27.00
Recondition weight after 10 days (kg)	298.80 ±2.12^{a}	291.60±2.27^{b}
ADG (kg/day)	1.668 ±0.212^{a}	0.948±0.227^{b}
Feed consumption (kg/day)	15.837 ±0.864^{a}	11.149±0.925^{b}
Dry matter consumption (kg/day)	5.320 ±0.373^{a}	3.726±0.399^{b}
Feed conversion	10.904 ±1.396	11.760±1.494
Dry matter conversion	3.633±0.356	3.984±0.381

Different superscrip in similar rows shows significant difference (P<0.05)

Better performance of PO than SL cattle during recondition after short period of transportation indicated that PO cattle are more adaptive to novel environmental conditions and concentrate feed. In addition, PO cattle that belong to *Bos indicus* exhibit greater tolerance to thermal stress. *Bos indicus* species is more tolerant of thermal stress compared to *Bos taurus* species. Therefore, *Bos indicus* species can maintain a higher feed intake under stress conditions, with a slower rate of feed intake reduction during stress compared to *Bos taurus* cattle (Hansen 2004; Pereira et al. 2014; Beatty et al 2004).

CONCLUSION

Short period of road transportation (< 8 hours) induces stress on PO and SL cattle. Indication of stress can be observed from body weight loss and change in blood metabolite sucah as increase in blood glucose, as well as increasing lmphocyte and decreasing of neutrofil post transportation. In addition, recovery for body weight of PO and SL cattle were achieved after 10 days post transportation. Furthermore, PO cattle has better performance during recondition period with higher body weight, ADG, feed consumptioan and dry matter consumption the SL cattle.

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The Impact of Sexual Rest and Scrotal Circumference on the Quality of Fresh and Chilled Semen in Local Ram

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ABSTRAK

Kurniati RNS, Mulyadi RK, Yudi, Wientarsih I, Achmadi B. 2025. Kualitas semen cair domba lokal yang diistirahatkan dari program perkawinan. JITV 30(4):202-211. DOI:http://dx.doi.org/10.14334/jitv.v30.i4.3525.

Kualitas semen dipengaruhi oleh berbagai faktor, salah satunya istirahat kawin. Tujuan penelitian ini adalah mengevaluasi kualitas semen cair domba lokal Indonesia yang diistirahatkan kawin. Semen dikoleksi dari empat ekor domba menggunakan vagina buatan. Semen segar dievaluasi secara makroskopis (volume, warna, konsistensi, pH, dan gerakan massa) serta mikroskopis (konsentrasi, motilitas progresif, viabilitas, dan abnormalitas spermatozoa). Semen kemudian diencerkan menggunakan pengencer Tris-fruktosa-kuning telur (Tris) atau Na sitrat-fruktosa-kuning telur (Na-sitrat) hingga konsentrasi 50x106 sel/0,2 mL. Semen cair dikemas dalam tabung sentrifugasi tertutup dan disimpan dalam refrigerator bersuhu 4−5◦C. Motilitas progresif dan viabilitas spermatozoa semen cair dievaluasi setiap 24 jam selama 4 hari. Pengaruh lingkar skrotum (LS) terhadap semen segar maupun semen cair juga dievaluasi. Hasil penelitian menunjukkan bahwa semen segar memiliki volume 1,08±0,12 mL, warna putih susu, konsistensi encer hingga kental, pH 6,42±0,13, gerakan massa 2,94±0,13, konsentrasi 4941±926,84x106/mL, motilitas progresif 79,38±2,17%, viabilitas 87,74±3,48%, dan abnormalitas 7,99±3,14%. Istirahat kawin meningkatkan konsentrasi dan abnormalitas spermatozoa; sedangkan LS memengaruhi motilitas progresif, viabilitas, konsentrasi, dan abnormalitas spermatozoa semen segar. Istirahat kawin tidak memengaruhi kualitas semen cair. Media Tris mempertahankan motilitas dan viabilitas spermatozoa semen cair lebih baik (P< 0,05) dibandingkan dengan Na-sitrat. Motilitas dan viabilitas spermatozoa semen cair dipengaruhi oleh LS. Istirahat kawin meningkatkan beberapa parameter kualitas semen segar, namun perlakuan ini tidak berdampak pada kualitas semen cair domba lokal. Peningkatan abnormalitas spermatozoa setelah istirahat kawin dapat berdampak negatif pada efisiensi reproduksi, sehingga kualitas semen perlu dioptimalkan kembali sebelum digunakan dalam inseminasi buatan.

Kata Kunci: Domba, Istirahat Kawin, Lingkar Skrotum, Pengencer Semen, Semen Cair

ABSTRACT

Kurniati RNS, Mulyadi RK, Yudi, Wientarsih I, Achmadi B. 2025. The impact of sexual rest and scrotal circumference on the quality of fresh and chilled semen in local ram. JITV 30(4):202-211. DOI:http://dx.doi.org/10.14334/jitv.v30.i4.3525.

Semen quality is influenced by various factors, including sexual rest. This study aimed to evaluate the quality of chilled semen from Indonesian local rams subjected to sexual rest. Semen was collected from four rams using an artificial vagina and evaluated macroscopically (volume, colour, consistency, pH, and mass movement) and microscopically (sperm concentration, progressive motility, viability, and abnormality). Semen was then diluted using either Tris-fructose-egg yolk (Tris) or sodium citrate-fructose-egg yolk (Na-citrate) extenders to a concentration of 50×10^6 cells/0.2 ml. The diluted semen was stored in sealed centrifuge tubes at 4–5°C. Progressive motility and viability of spermatozoa in chilled semen were assessed every 24 hours for four days. The influence of scrotal circumference (SC) on both fresh and chilled semen quality was also evaluated. Results showed that fresh semen had a volume of 1.08±0.12 mL, milky white colour, thin to thick consistency, pH of 6.42±0.13, mass movement score of 2.94±0.13, concentration of $4941\pm926.84\times10^6$/mL, progressive motility of 79.38±2.17%, viability of 87.74±3.48%, and abnormality of 7.99±3.14%. Sexual rest increased sperm concentration and abnormality, while SC significantly influenced progressive motility, viability, concentration, and abnormality in fresh semen. However, sexual rest had no significant effect on chilled semen quality. The Tris extender could maintain sperm motility and viability significantly better ($P < 0.05$) than the Na-citrate extender. Additionally, chilled semen quality was influenced by SC. In conclusion, sexual rest improved certain parameters of fresh semen quality but did not significantly affect the quality of chilled semen in local Indonesian rams. An increase in spermatozoa abnormalities after a period of sexual rest can negatively affect reproductive efficiency; therefore, semen quality needs to be re-optimized before being used in artificial insemination.

Key Words: Chilled Semen, Ram, Scrotal Circumference, Semen Extenders, Sexual Rest

INTRODUCTION

Sheep (*Ovis aries*) are among the domesticated livestock species that have been widely cultivated in Indonesia for centuries. These animals are recognized for their high fertility rates, numerous benefits, and ease of management. They serve as an essential source of protein and animal fat through their meat, a valuable alternative to cow's milk through their milk production, and as suppliers of wool and hides for the textile industry. Sheep farming is particularly popular in Indonesia due to its cost-efficiency and minimal land requirements. Additionally, sheep are also one of the national food sources, ranking fifth in meat production, with a population reaching 17,902,991 in 2021 (BPS 2021). Thus, sheep farming holds significant potential for economic development.

One strategy to enhance sheep productivity is through the application of artificial insemination (AI) technology. Gibbons et al. (2019) define AI as a key biotechnology used to enhance the population and genetics of livestock. AI can utilize fresh, chilled, or frozen semen. Approximately 95% of AI procedures in livestock employ preserved semen (Bustani & Baiee 2021). The use of chilled semen in AI offers several advantages, including practicality, simplicity, cost-effectiveness, and reliability (Gibbons et al. 2019). Although unsuitable for extended storage or long-distance transport, chilled semen mitigates the physiological stress associated with cryopreservation processes, such as freezing and thawing, which can induce substantial damage to sperm (Richardson et al. 2017, Schoelerman et al. 2026). Reports indicate that sperm motility in chilled semen is superior to that observed in frozen semen after storage (Acharya et al. 2020). Furthermore, chilled semen exhibits lower rates of morphological damage, reduced fertilization failure, and decreased embryonic loss compared to frozen semen.

The quality of semen is a critical determinant of fertility and AI success. High-quality chilled semen relies on the use of superior fresh semen, extenders capable of maintaining sperm viability outside the male reproductive tract, and appropriate preservation techniques. Ejaculation frequency is a key factor influencing fresh semen quality, with prolonged sexual rest shown to significantly affect sperm physiology. Research on evaluating the chilled semen of local ram rested from breeding programs has been limited, thus prompting this research. The primary objective is to evaluate the quality of chilled semen from local rams following a rest period from breeding activities. Furthermore, the study investigates the effects of scrotal circumference and the use of two yolk-based extenders on the quality of chilled semen from ram undergoing breeding rest, providing a foundation for this research.

MATERIALS AND METHODS

Determination of age and scrotal circumference

The ram used in this study was thin-tailed. Each ram was manually restrained, ensuring proper immobilization by securely gripping the ram's body. The age of each ram was determined by assessing dental eruption and wear patterns, following standard age estimation protocols. Scrotal circumference (SC) was measured using a flexible measuring tape, following the procedures outlined by Tibary et al. (2018). The neck of the scrotum was gently grasped to ensure the testes were fully descended to the base of the scrotum. The measuring tape was then carefully wrapped around the widest circumference of the scrotum, and the measurement was recorded.

Fresh semen collection and evaluation

Fresh semen was collected biweekly using an artificial vagina (AV) designed for sheep. Adult ewes were used as teasers to stimulate ejaculation. The semen was collected into a tube and immediately protected from light exposure by placing it in a plastic cup wrapped with opaque paper to prevent light-induced sperm damage. The semen samples were then transported to the laboratory for evaluation. Both macroscopic and microscopic assessments were performed to analyse the quality of the semen.

Macroscopic evaluation

Macroscopic evaluation of semen was conducted according to the protocols described by Susilawati (2011). The evaluation included assessments of volume, colour, consistency, and pH. The volume of semen was measured directly using the collection tube. The colour of the semen was observed directly after collection in the collection tube. Consistency was determined by gently tilting the collection tube and then returning it to its original position, observing the rate at which the semen flowed back to the bottom of the tube. The pH of the semen was measured by applying a small drop of the sample onto narrow-range pH indicator paper (6.4–8.0). The pH was determined by comparing the colour change of the pH indicator strip to a standardized colour chart.

Microscopic evaluation

Microscopic evaluation of semen was conducted according to the protocols described by Susilawati (2011), which involved assessing several key parameters, including sperm mass movement,

concentration, progressive motility, viability, and total morphological abnormalities. Mass movement was evaluated by placing a drop of semen on a pre-warmed microscope slide and observing it under a microscope at 100x magnification (10x10).

Sperm concentration was measured using a Neubauer chamber. The semen sample was diluted 500 times (2 µL semen + 998 µL buffered formol saline), homogenized, and loaded into the Neubauer chamber, which was then covered with a special cover slip. The sperm were counted under a microscope at 400x magnification (40x10) across five large squares, measured diagonally.

Sperm progressive motility was evaluated by mixing fresh semen with 0.95% physiological saline at a ratio of 1:10, which was then placed on a pre-warmed microscope slide, homogenized with a cover glass, and a portion was transferred onto another slide and covered with a cover glass. The sample was examined under a microscope at 400x magnification (40x10) for a minimum of five fields of view. The percentage of progressive motility was assessed subjectively by comparing the total number of progressively moving sperm to the total number of sperm observed.

Sperm viability was assessed using differential eosin-nigrosine staining. To evaluate viability, semen was mixed with eosin-nigrosine stain at a ratio of 1:10 on a microscope slide, and a portion of the mixture was spread onto another slide to create a smear. The smear was air-dried on a heated stage for fixation. The sample was then observed under a microscope at 400x magnification (40x10). Viable sperm appeared with a clear or white head, while dead sperm had a purple head. At least 200 sperm or five fields of view were examined, and viability was calculated by dividing the number of live sperm by the total number of sperm observed, multiplied by 100%.

Total sperm morphological abnormalities were evaluated by observing the head, neck, and tail of the sperm. Abnormality assessment was also performed using eosin-nigrosine differential staining, with at least 200 sperm or five fields of view observed.

The preparation of chilled semen

The extenders used were Tris-fructose-egg yolk (Tris) and sodium citrate-fructose-egg yolk (Na-citrate), as referenced by Rather et al. (2017). The Tris buffer consisted of 3.028 g tris(hydroxymethyl)aminomethane, 1.70 g citric acid monohydrate, 1.25 g fructose, and distilled water to a final volume of 100 ml. The Na citrate buffer consisted of 2.9 g sodium citrate dihydrate, 1.25 g fructose, and distilled water to a final volume of 100 ml. Each extender was prepared by mixing the buffer with egg yolk (EY) in a 4:1 ratio. The extender was then supplemented with antibiotics, penicillin, and streptomycin, at concentrations of 1000 IU and 1000 µg per mL of solution. Both extenders had a pH of 6.4.

Fresh semen, once evaluated, was diluted by mixing with either the Tris or Na-citrate extender. The dilution of semen followed the protocol for artificial insemination (AI) in sheep using chilled semen, at a concentration of 50×10^6 sperm/0.2 mL. The diluted semen was then packaged into sealed centrifuge tubes and stored in a refrigerator (4–5°C) using a water-jacketed container. The refrigerator used for semen storage was designated exclusively for this purpose and was only opened during semen evaluation.

Chilled semen evaluation

The evaluation of chilled semen included the assessment of sperm progressive motility and viability, conducted every 24 hours at time points 0, 24, 48, 72, and 96 hours. The evaluation of progressive motility was performed by placing approximately 50 µL (one drop) of chilled semen on a pre-warmed microscope slide, which was then covered with a cover glass. The sample was observed under a microscope at 400x magnification (40x10) for a minimum of five fields of view. The motility was recorded as a percentage from 0 to 100%.

For sperm viability evaluation, 50 µL or one drop of chilled semen was placed on a microscope slide, and a differential eosin-nigrosine stain was added in a 1:1 ratio. A smear was prepared on another microscope slide and air-dried on a heated stage for fixation. The sample was examined under a microscope at 400x magnification (40x10). A minimum of 200 sperm or five fields of view were observed.

Data analysis

This study used 16 samples from 4 local rams with 4 repetitions. Data were analysed to assess fresh semen characteristics based on SC, and the motility and viability of chilled semen using Tris and Na-citrate extenders. Effects of SC, extender type, and storage time on semen quality were evaluated. Means and standard deviations were tabulated in Excel 2013 and analysed with SPSS 16.0. Independent t-tests assessed differences in fresh semen based on SC, as well as motility and viability of chilled semen in the two extenders. One-way ANOVA tested SC effects on chilled semen. The Independent t-test was used because SC was only compared between two groups, whereas the one-way ANOVA was applied to the other parameters, as they involved four groups, making it more appropriate for comparing more than two groups simultaneously.

RESULTS AND DISCUSSION

Age and scrotal circumference

The four rams were approximately 4 years old. Rams 1 and 2 had a scrotal circumference (SC) of 29 cm,

while rams 3 and 4 had a SC of 23 cm. Age is a key factor influencing semen quality, as it is closely linked to the development of the male reproductive system.

Variation in SC size may be influenced by individual factors. Pugh et al. (2020), in their evaluation of breeding soundness exams (BSE), categorized rams aged 8–14 months with a SC greater than 28 cm as satisfactory, while those with a SC smaller than 28 cm were considered unsatisfactory. The Indonesian National Standardization Agency (BSN 2015) mandates a minimum SC of 25 cm for Garut ram aged 18–24 months. Due to the differences of SC, SC was selected as one of the variables for this study.

Characteristics of fresh semen

Based on both macroscopic and microscopic evaluations, the fresh semen used in this study was of good quality (Table 1). The results of the fresh semen evaluation are necessary to determine the suitability of the semen for further processing. The analysis of fresh semen in this study showed characteristics that were similar to those previously reported. The volume of fresh semen in adult ram has been reported as 1.20 mL (Solihati et al. 2018), 0.5–2 mL (Hafez & Hafez, 2013), and 0.7–1.0 mL (Nubatonis et al. 2022). Normal ram semen is typically cream-colored (Solihati et al. 2018) or milky white (Hafez & Hafez 2013). The consistency of local ram semen has been described as slightly watery (Solihati et al. 2018) or ranging from thick to very thick (Hafez & Hafez 2013). The normal pH range of ram semen is 5.9–7.3 (Hafez & Hafez 2013).

The semen volume obtained in this study was considered normal, despite the extended sexual rest period (no semen collection for one year). Normal ram semen ejaculates typically have a low volume but high concentration (Hafez & Hafez 2013). Semen volume can be influenced by the plasma content of the semen and the collection method. Ram semen collected using an artificial vagina has been reported to have a volume range of 0.5–1.5 mL (Abott 2018).

The microscopic evaluation of sperm mass movement in this study was similar to the results of Solihati et al. (2018), which was 2–3. The average sperm concentration was higher than that reported by Solihati et al. (2018) and Nubatonis et al. (2022), which were 3487.1x106/mL and 2940x106/mL, respectively. The sperm motility obtained in this study was lower than that of Solihati et al. (2018), which was 87.72%, but still within the normal range (70–90%) according to Hafez & Hafez (2013). The sperm viability was higher compared to Nubatonis et al. (2022), who reported 81.5±4.54%. The total sperm abnormalities in this study were higher than those reported by Solihati et al. (2018) at 1%, but still within the normal range, which is <15% (Hafez & Hafez 2013). Differences in the quality of ram semen can be influenced by breed, age, environmental temperature, season, and frequency of collection (Benmoula et al. 2018).

The high sperm concentration in this study was likely due to the long sexual rest period. Sperm are continually produced by the testes, regardless of the frequency of ejaculation. Sperm that are not ejaculated for a long time will accumulate in the cauda epididymis, vas deferens, and ampulla vas deferens, leading to a higher sperm concentration when ejaculated (Abott 2018). A long sexual rest period has also been reported to be associated with the secretion of the epididymis in providing a conducive environment for sperm. Sperm undergo various biochemical and physiological modifications in the epididymis to acquire fertilizing

Table 1. The quality of fresh ram semen

Characteristics	Mean±SD	References
Macroscopic		
Volume (mL)	1.08±0.12	1.20[(a)], 0.5–2[(b)]
Colour	Milky-white	Cream[(a)], Milky-white[(b)]
Consistency	Thin to thick	Think[(b)]
pH	6.42±0.04	5.9–7.3[(b)]
Microscopic		
Mass movement	2.94±0.13	2.00–3.00[(a)]
Sperm concentration (10^6/mL)	4941±926.84	3487.1[(a)], 2940±0.50[(c)]
Sperm motility (%)	79.38±2.17	87.72[(a)], 70–90[(b)]
Sperm viability (%)	87.74±3.48	81.5±4.54[(c)]
Total abnormality (%)	7.99±3.14	1[(a)], <15[(b)]

[(a)]= Solihati et al. (2018); [(b)]= Hafez & Hafez (2013); [(c)]= Nubatonis et al. (2022)

ability. These modifications may include chromatin condensation, changes in plasma membrane composition, and the ability of sperm to withstand hypoosmotic stress (Koziol & Armstrong 2022).

The epididymis is also responsible for processing degenerative sperm. Dead sperm release enzymes that may harm nearby live sperm. Cauda epididymal cells are believed to secrete fibrinogen-related proteins that coat damaged sperm. A long sexual rest period reduces the epididymis capacity to provide a conducive environment for sperm (Dcunha et al. 2022). In this study, the slightly lower sperm progressive motility and higher total sperm abnormalities may be related to the decreased capacity of the epididymis.

Sperm stored in the cauda epididymis after a long sexual rest period may undergo degeneration or be passively transported to the vas deferens. Hafez & Hafez (2013) reinforced the idea that sperm have a limited lifespan and, if not ejaculated due to a long sexual rest period, will gradually be eliminated via urine or undergo aging and disintegration. Besides decreased motility, prolonged storage of sperm in the cauda epididymis leads to the loss of fertilizing ability. Other factors affecting sperm motility include breed, body weight (BW), environment, physical activity, diet, and heat stress (Hafez & Hafez 2013).

Sperm viability did not seem to be affected by the long sexual rest period in this study. Sperm viability is more dependent on an intact plasma membrane, which is influenced by pH, osmolarity, and sperm metabolism (Prastika et al. 2018). Sperm abnormalities have been reported to be related to epididymal dysfunction caused by long sexual rest periods (Bhosrekar 1990). In this study, the most common abnormality found was secondary abnormalities in the tail, while primary abnormalities such as pear-shaped (pyriform head) and macrocephalus heads were also found in small numbers. Other factors causing sperm abnormalities include nutrition, body weight, accessory gland activity, season, and staining treatment (Koziol & Armstrong 2022). However, the total abnormality of 7.99% observed in this study wass still within the Indonesian national standard, which sets a maximum limit of 20%.

The results of this study indicate that, although the total sperm abnormalities were slightly higher and progressive motility was somewhat lower, the semen quality could still be considered good. This suggests that local ram have their own mechanisms to adapt and produce high-quality semen after a prolonged sexual rest period. Koziol & Armstrong (2022) also reported that ram that have had prolonged sexual rest periods adapt to produce high-quality sperm.

Characteristics of fresh semen based on SC

Based on scrotal circumference (LS), the local ram with an LS of 29 cm had better semen characteristics compared to the local ram with an LS of 23 cm, both macroscopically and microscopically (Table 2).

The results of both macroscopic and microscopic evaluations of fresh semen from local ram with scrotal circumference (LS) of 29 cm and 23 cm can be considered good. However, the sperm concentration, progressive motility, and viability of semen from ram with an LS of 29 cm were significantly higher ($P<0.05$) compared to those with an LS of 23 cm. The total sperm abnormalities in fresh semen from ram with LS 29 cm

Table 2. The quality of fresh ram semen based on scrotal circumference

Characteristics	Scrotal circumference (cm)	
	29	23
Macroscopic		
Volume (mL)	1.14±0.26[a]	1.02±0.36[a]
Colour	Milky white	Milky white
Consistency	Thick	Thin to thick
pH	6.40±0.00[a]	6.44±0.11[a]
Microscopic		
Mass movement	3.00±0.00[a]	2.88±0.35[a]
Sperm concentration (106/mL)	5480.63±737.64[a]	4399.38±792.85[b]
Sperm motility (%)	81.25±3.54[a]	77.50±2.67[b]
Sperm viability (%)	90.51±2.16[a]	84.96±2.96[b]
Total abnormality (%)	5.37±0.89[a]	10.61±1.90[b]

*Numbers followed by different lowercase letters (a, b, c, d) in the same row indicate significant differences at the significance level of $\alpha=0.05$.

were significantly lower ($P<0.05$) compared to those with LS 23 cm. LS is an indirect measurement of testicular size (Chaudari et al. 2018). Testicular size has been used as a standard for fertility and reproduction in ram. LS can estimate the number of healthy testicular parenchymal cells, which are related to sperm production (Tibary et al. 2018). This underscores the importance of measuring LS in the selection of males for breeding programs.

The testes consist of seminiferous tubules (ST), which are the only site for sperm production. ST is made up of peritubular myoid cells (PTM), Sertoli cells, and germ cells, and there are Leydig cells between the tubules (interstitial) that influence spermatogenesis (Choudhary & Choudhary 2022). Significant correlations between LS and semen quality have been reported for sperm motility (Susilawati et al. 2020; Rashid et al. 2015).

Normal testicular function depends on endocrine, autocrine, and paracrine control (Koziol & Armstrong 2022). The coordinated interaction of hormones from the hypothalamus, anterior pituitary, and gonads regulates spermatogenesis (Campbell et al. 2020). The hypothalamus secretes hormones that stimulate the release of gonadotropins (gonadotropin-releasing hormone/GnRH), which in turn stimulate the secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) by the anterior pituitary. FSH promotes Sertoli cell activity, while LH stimulates Leydig cells.

Testosterone is the main androgen in the testes that regulates spermatogenesis. Testosterone is converted into a more active form, dihydrotestosterone, by 5α reductase and oestradiol by aromatase (Davey and Grossmann, 2016). Testosterone's actions are mediated by androgen receptors (AR), which are transcription factors activated by ligands (ligand-dependent nuclear transcription factors).

Testosterone in the testes interacts with AR in Leydig cells, PTM cells, arteriolar smooth muscle cells, and endothelial cells. Testosterone works synergistically with FSH to regulate Sertoli cells (Koziol & Armstrong 2022). Sertoli cells secrete androgen-binding protein (ABP), which binds testosterone and is responsible for maintaining high levels of testosterone in the testes and carrying it to the epididymis. Furthermore, testosterone regulates the levels of GnRH, FSH, and LH through feedback inhibition on the hypothalamus and anterior pituitary (Campbell et al. 2020).

Testosterone concentration has been reported to have a positive correlation with LS in bulls (Dasrul et al. 2020), buffaloes (Qadarsina et al. 2019), and ram (Maksimović et al. 2016). Testosterone stimulates the growth and development of spermatogenic tissue, influencing testicular size. Testosterone concentration has also been shown to have a positive correlation with sperm concentration, motility, and viability in cattle (Dasrul et al. 2020). Moreover, Dasrul et al. (2020) found a negative correlation between testosterone concentration and sperm abnormalities in cattle. Low testosterone levels may lead to an increase in sperm abnormalities due to impaired epididymal function (Azizunnesa et al. 2013). In this study, the total sperm abnormalities in rams with LS 23 cm were twice as high as in those with LS 29 cm, which may be related to testosterone levels.

Characteristics of chilled semen

Quality of chilled semen in Tris and sodium citrate

The study results indicated that the progressive motility and viability of local ram sperm in Tris were higher than in Na citrate. Progressive motility was significantly higher ($P<0.05$) in Tris compared to Na citrate at 72 and 96 hours of storage (Table 3). The viability of sperm was also significantly higher ($P<0.05$) in Tris compared to Na citrate at 0, 24, and 48 hours of storage (Table 4).

The type of extender is a crucial factor determining the success of sperm during chilled storage. Tris was able to maintain the motility and viability of ram sperm better than Na citrate during chilled storage at 4–5°C. Tris is a type of extender that has been proven to be superior to other extenders due to its ability to stabilize biomolecular structures and its low toxicity. Tris acts as a buffer against pH changes, electrolyte balance, and tonicity (osmotic pressure) during chilled storage (Soltanpour & Moghaddam 2013). Progressive motility (72 and 96 hours) and sperm viability (0, 24, and 48 hours) in Tris were significantly higher compared to Na citrate due to Tris's superior ability to maintain sperm plasma membrane integrity.

Tris is believed to form two hydrogen bonds that help stabilize protein bases (Rasad et al. 2019). These hydrogen bonds interact with the polypeptide chains in egg yolk, positively affecting the maintenance of the sperm plasma membrane. Sperm are considered viable if they have an intact plasma membrane (Glazar & McCue 2021). Sperm that can maintain their membrane integrity are better able to protect mitochondrial function. Intact mitochondrial function facilitates ATP production, which is essential for sperm motility and metabolic activity.

Both progressive motility and sperm viability in Tris and Na citrate extenders decreased gradually every 24 hours of chilled storage. The total decrease in progressive motility over 96 hours in Tris was 34.07% (from 79.38% to 45.31%), while in Na citrate it was 40.94% (from 79.38% to 38.44%). The total decrease in sperm viability over 96 hours in Tris was 15.51% (from 87.74% to 72.23%), while in Na citrate it was 18.71% (from 87.74% to 69.03%).

Table 3. Sperm progressive motility of chilled semen preserved with Tris and sodium citrate extenders

Storage (h)	Extenders	
	Tris	Sodium citrate
0	77.81±4.46^{aE}	75.63±4.03^{aE}
24	71.56±5.07^{aD}	68.75±4.65^{aD}
48	63.75±5.32^{aC}	60.00±5.16^{aC}
72	55.00±5.48^{bB}	50.00±5.16^{aB}
96	45.31±4.99^{bA}	38.44±5.07^{aA}

*Numbers followed by different lowercase letters (a, b, c, d) in the same row and different uppercase letters (A, B, C, D) in the same column indicate significant differences at the significance level of α=0.05

Table 4. Sperm viability of chilled semen preserved with Tris and sodium citrate extenders

Storage (h)	Extenders	
	Tris	Sodium citrate
0	83.91±3.42^{bD}	81.14±3.81^{aD}
24	81.09±3.67^{bCD}	77.80±4.13^{aC}
48	78.56±3.98^{bC}	75.20±4.58^{aBC}
72	75.66±4.28^{aB}	72.64±4.57^{aB}
96	72.23±4.6^{aA}	69.03±4.56^{aA}

*Numbers followed by different lowercase letters (a, b, c, d) in the same row and different uppercase letters (A, B, C, D) in the same column indicate significant differences at the significance level of α=0.05

The decline in sperm quality caused by storage of semen at 4–5°C does not completely halt the metabolic activity of sperm. Oxygen remains in the centrifuge tubes (the storage container for chilled semen), allowing for aerobic metabolism to occur. The accumulation of free radicals, the enzyme aromatic amino acid oxidase (AAAO), and lactic acid over prolonged storage becomes toxic and disrupts the intracellular balance of sperm, leading to a decline in their function (Khalifa & Khalil 2016).

The accumulation of free radical results in oxidative stress (Aitken & Drevet 2020). The enzyme AAAO, originating from dead sperm, has been shown to increase the production of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), leading to oxidative stress (Aitken et al. 2015). Oxidative stress induces lipid peroxidation in sperm. The ram sperm membrane is particularly susceptible to lipid peroxidation due to its high content of polyunsaturated fatty acids (PUFAs). Lipid peroxidation inhibits respiration and fructo-lysis, making it toxic to sperm. Similarly, excess lactic acid lowers pH, disrupting sperm motility.

Sperm viability should be higher compared to progressive motility, as not all viable sperm exhibit progressive movement. The decline in viability occurs gradually due to the presence of KT in the extender, which protects the sperm membrane from cold shock. KT has been reported to prevent oxidation of PUFAs and degenerative changes in the acrosome (Quan et al. 2016).

A prolonged sexual rest did not affect sperm during chilled storage in this study. Although the progressive sperm motility was slightly lower in fresh semen, the chilled semen exhibited good initial progressive motility (79.38±2.17%), providing a relatively high starting point for the subsequent decline during storage. This is in line with the initial decline in sperm viability, which started from a high percentage (87.74±3.48%).

Quality of chilled semen based on SC

The observations based on scrotal circumference (SC) indicated that the progressive motility and viability of sperm from chilled semen with a scrotal circumference of 29 cm were significantly higher ($P<0.05$) than those from a scrotal circumference of 23 cm. Within the same SC group, the progressive motility and viability of sperm in Tris were significantly higher ($P<0.05$) compared to those in sodium citrate (Na citrate) (Tables 5 and 6). The progressive motility of sperm in both Tris and Na citrate at an SC of 29 cm began to show significant differences ($P<0.05$) from 0 to 96 hours of storage, while at an SC of 23 cm, differences became significant ($P<0.05$) from 24 to 96 hours of storage

(Table 5). The viability of sperm from both SC 29 cm and 23 cm showed significant differences ($P<0.05$) from 0 to 96 hours of storage (Table 6). The highest progressive motility and viability of sperm during storage were found in Tris-SC 29 cm, followed by Na citrate-SC 29 cm, Tris-SC 23 cm, and Na citrate-SC 23 cm.

Sperm from ram with an SC of 29 cm were more resistant to detrimental effects during chilled storage in both Tris and Na citrate compared to sperm from ram with an SC of 23 cm in this study. In addition to predicting sperm production, testicular size can be used to predict sperm resistance during storage. Testicular size is a good indicator for estimating the quality of fresh semen, chilled semen, and frozen semen (Saurabh et al. 2018).

The estimation of a higher number of seminiferous tubules (ST) in larger scrotal circumferences (SC) suggests that the quantity and quality of the fresh semen produced are better. Rams produce approximately 21–25 $\times 10^6$ sperm per gram of testicular parenchyma per day (Montes-Garrido et al. 2023). Normal mitosis and meiosis processes, supported by components in the ST and testicular interstitial cells, determine the production of fertile sperm (Gordon 2017). Good quality fresh semen leads to semen that can better maintain its quality during chilled storage.

Sperm resistance during chilled storage is also influenced by the composition of seminal plasma. SC in ram and cattle has been positively correlated with testosterone, which affects the activity of the seminal plasma-producing organs (Maksimović et al. 2016; Dasrul et al. 2020). Rams with an SC of 29 cm have higher semen volume compared to those with an SC of 23 cm, suggesting that the semen from the SC of 29 cm contains more seminal plasma. Semen consists of one-third sperm and two-thirds seminal plasma (Kaushish 2019). Rams with an SC of 29 cm are better able to maintain sperm quality, both fresh and after dilution, compared to ram with an SC of 23 cm due to the higher content of seminal plasma and its biological components.

Lipids, cholesterol, and proteins in seminal plasma are reported to be related to the function, structure of membranes, and motility of ram sperm (Benmoula et al. 2017; Swelum et al. 2018). The main inorganic components in seminal plasma act as buffers to maintain an environment conducive to sperm metabolism, and the content of fructose has been shown to correlate with sperm fertility (Swelum et al. 2018).

Table 5. Sperm progressive motility in Tris and sodium citrate extenders based on SC

Storage (h)	Scrotal circumference (cm)			
	29		23	
	Tris	Sodium citrate	Tris	Sodium citrate
0	80.00 ± 3.78^{bE}	77.5 ± 3.78^{abE}	75.63 ± 4.17^{aE}	73.75 ± 3.54^{aE}
24	74.38 ± 4.17^{cD}	71.25 ± 4.43^{bcD}	68.75 ± 4.43^{abD}	66.25 ± 3.54^{aD}
48	66.88 ± 4.58^{cC}	63.13 ± 3.72^{bcC}	60.63 ± 4.17^{abC}	56.88 ± 4.58^{aC}
72	58.13 ± 4.58^{cA}	53.13 ± 4.58^{bA}	51.88 ± 4.58^{bA}	46.88 ± 3.72^{aA}
96	48.13 ± 5.30^{cB}	41.25 ± 3.54^{bB}	42.50 ± 2.67^{bB}	35.63 ± 4.96^{aB}

*Numbers followed by different lowercase letters (a, b, c, d) in the same row and different uppercase letters (A, B, C, D) in the same column indicate significant differences at the significance level of $\alpha=0.05$

Table 6. Sperm viability in Tris and sodium citrate extenders based on SC

Storage (h)	Scrotal circumference (cm)			
	29		23	
	Tris	Sodium citrate	Tris	Sodium citrate
0	86.23 ± 1.97^{cE}	82.81 ± 3.05^{bE}	81.60 ± 3.00^{abE}	79.48 ± 3.94^{aE}
24	83.00 ± 3.07^{cD}	79.83 ± 3.54^{bcD}	79.19 ± 3.34^{abD}	75.78 ± 3.81^{aD}
48	80.61 ± 3.41^{cC}	77.71 ± 3.64^{bcC}	76.51 ± 3.56^{bC}	72.69 ± 4.16^{aC}
72	77.90 ± 3.54^{cA}	75.35 ± 3.85^{bcA}	73.43 ± 3.90^{abA}	69.93 ± 3.62^{aA}
96	74.78 ± 4.03^{cB}	71.99 ± 4.11^{bcB}	69.69 ± 3.81^{abB}	66.08 ± 2.77^{aB}

*Numbers followed by different lowercase letters (a, b, c, d) in the same row and different uppercase letters (A, B, C, D) in the same column indicate significant differences at the significance level of $\alpha=0.05$

The data on sperm motility from the 29 cm SC group showed a significant difference (P<0.05) 24 hours earlier between Tris and Na citrate, compared to sperm from the 23 cm SC group. This difference may be due to the influence of seminal plasma composition on the extenders (Table 5). Seminal plasma has been reported to mitigate the effects of high dilution and even to improve sperm resistance to oxidative stress and cold shock (Swelum et al. 2018). However, prolonged exposure to seminal plasma is ineffective for maintaining sperm quality during dilution (Rajabi-Toustani et al. 2020). Seminal plasma is a complex biological fluid that is assumed to interact with the type of extender.

CONCLUSION

The quality of local ram semen was affected by the sexual rest period. A sufficiently long sexual rest period increased the concentration and abnormality of sperm in fresh semen, but did not affect chilled semen. Scrotal circumference significantly influenced the motility, viability, concentration, and abnormality of sperm in fresh semen, as well as the motility and viability of sperm in chilled semen. The Tris extender was more effective at maintaining the motility and viability of local ram sperm compared to Na-citrate when stored at 4–5°C for 96 hours. It is suggested that semen be collected approximately twice per week with an interval of around 3 days to support optimal sperm quality and sufficient replenishment of sperm reserves.

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Moringa Leaf Extract Improves Spermatozoa Motility and Viability Through an Antioxidant Mechanism in Older Wistar Rats

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ABSTRAK

Widiastini LP, Karuniadi IGAM. 2025. Ekstrak daun kelor meningkatkan motilitas dan viabilitas spermatozoa melalui mekanisme antioksidan pada tikus Wistar tua. JITV 30(4):212-218. DOI: http/dx.doi.org/10.1443/jitv.v30i3.3405.

Proses penuaan reproduksi ditandai dengan penurunan secara progresif integritas fisiologis yang dapat memicu kerusakan testis, epididimis maupun organ reproduksi lainnya. Stress oksidatif berkaitan erat dengan penuaan serta infertilitas pada pria yang menyebabkan penurunan volume semen, abnormalitas morfologi, penurunan viabilitas serta motilitas sperma. Penanganan terhadap stress oksidatif salah satunya dilakukan dengan menggunakan senyawa antioksidan seperti tanaman kelor. Tujuan penelitian ini, adalah untuk mengetahui mekanisme antioksidan ekstrak etanol daun kelor terhadap motilitas dan viabilitas spermatozoa pada tikus Wistar tua. Jenis penelitian ini adalah randomized post-test only control group design. Jumlah sample sebansyak 36 tikus Wistar tua (umur 18-19 bulan), yang dirandomisasi menjadi 2 kelompok, kelompok perlakuan (18 tikus) diberikan ekstrak etanol daun kelor (EEMO) 50 mg/kgBB + CMC 0.5%, pada dosis 0,5 ml/hari. Kelompok kontrol (18 tikus) diberikan CMC 0,5% sebanyak 0,5 ml/hari, selama 30 hari. Terminasi tikus menggunakan metode cervical dislocation. Selanjutnya dilakukan pemeriksaan motilitas dan viabilitas spermatozoa. Analisa data menggunakan independent sample t-test dan Mann Whitney dengan nilai signifikansi P= 0,05. Hasil penelitian menunjukkan bahwa kandungan antioksidan EEMO 50 mg/kg BB selama 30 hari pada tikus Wistar tua meningkatkan progressive motility, menurunkan non progressive motility dan immotility, serta meningkatkan viability spermatozoa, secara bermakna pada kelompok perlakuan dibandingkan kelompok kontrol dengan nilai P<0,05. Kesimpulan pada penelitian ini adalah, ekstrak daun kelor dapatmeningkatkan motilitas dan viabilitas spermatozoa melalui mekanisme antioksidan pada tikus Wistar tua.

Kata Kunci: Antioksidan, Daun Kelor, Motilitas, Tikus Tua, Viabilitas

ABSTRACT

Widiastini LP, Karuniadi IGAM. 2025. Moringa leaf extract improves spermatozoa motility and viability through an antioxidant mechanism in older Wistar rats. JITV 30(4):212-218. DOI: http/dx.doi.org/10.1443/jitv.v30i3.3405.

The reproductive aging is characterized by a progressive decline in physiological integrity that triggers in damaging to the testicle, epididymis and other reproductive organs. An oxidative stress is associated with aging and infertility in men which causes a decrease in semen volume, abnormal morphology, decreased sperm viability and motility. One of the treatments for oxidative stress is the use of antioxidant compounds such as Moringa plants. The purpose of this study was to determine the antioxidant mechanism of moringa leaf ethanol extract in sperm motility and viability in older Wistar rats. This study is a randomized post-test only control group design. The total sample was 36 older Wistar rats (age 18–19 months), which were divided randomly into 2 groups, including the treatment group (18 rats) was given ethanol extract of Moringa oleifera (EEMO) 50 mg/kg BW+CMC 0.5%, 0.5 ml /day, and the control group (18 rats) was given CMC 0.5%, 0.5 ml/day, for 30 days. Rats were terminated by cervical dislocation method. Motility and viability of spermatozoa were examined. Data analysis using an independent sample t-test and Mann Whitney with a significance value of P= 0.5. The results showed that the antioxidant content of EEMO 50 mg/kg BW+CMC 0.5%, for 30 days in old Wistar rats increased progressive motility, decreased non-progressive motility and immotility, and increased spermatozoa viability, significantly in the treatment group compared to the control group with a P<0.05. In conclusion, Moringa leaf extract could improves spermatozoa motility and viability through an antioxidant mechanism in old wistar rats.

Key Words: Antioxidant, Moringa Leaf, Motility, Old Rat, Viability

INTRODUCTION

Aging is a physiological process in the body that occurs due to increasing age (Han et al. 2017). The aging process is an accumulation of cellular damage that can be caused by stress, environmental influences, and poor nutrition. Aging is closely related to free radicals in the body, which can induce oxidative stress (Balin & Allen 2018; Liguori et al. 2018)

Some studies state that aging in male rats may induce a decrease in testicular size, the number of germ cells, Sertoli cells, and Leydig cells, as well as peritubular cells. Based on the ultrastructural analysis, sperm maturation in spermatogenesis stops at the

spermatogonium and spermatocyte stages in the testes and there is a decrease in the percentage of motility and viability of spermatozoa in the cauda epididymis (Lucio et al. 2013). Increased reactive oxygen species (ROS) can produce DNA damage and lead to decrease motility, viability and plasma membrane integration of spermatozoa (Lucio et al. 2013; Morielli & O'Flaherty 2015; Fatima 2018; Luceri et al. 2018; Alahmar 2019; Kurkowska et al. 2020)

Reactive oxygen species (ROS), which are free radicals produced from oxygen, cause increased cellular damage in a condition known as oxidative stress (OS). Reactive oxygen species (ROS) generation and the body's antioxidant capacity are out of balance under oxidative stress (Agarwal et al. 2014; Luceri et al. 2018). Oxidative stress is closely related to aging and male infertility, causing decreased semen volume, abnormal morphology, decreased sperm viability and motility (Lucio et al. 2013; Morielli & O'Flaherty 2015; Fatima 2018; Alahmar 2019; Kurkowska et al. 2020).

In the study carriedout by (Mehrotra et al. 2013), showing that an increased of ROS production was correlated to the decreased spermatozoa motility, due to lipid peroxidation of the spermatozoa membrane. It may then affect the fluidity and mobility of the spermatozoa axoneme, inactivation or reduction of enzymes on the membrane, fragmentation of structured DNA and cell death (Dias et al. 2020). The amount of sperm DNA fragmentation and sperm vitality are correlated each other. High sperm DNA fragmentation is linked to the decreased of sperm viability, whereas low sperm DNA fragmentation is linked to the increased of sperm vitality. Male fertility was decreased by high DNA fragmentation levels and low viability; DNA fragmentation levels >30% were an indicative of subfertility (Samplaski et al. 2015).

Sperm motility is a reflection of the normal development and maturity of spermatozoa in the epididymis, while viability is the vitality of spermatozoa (Carson & Kallen 2021). Sperm viability examination can be used as an indicator of the integrity of the spermatozoa membrane structure (Solihati et al. 2016), while motility is used as an indicator of the strength of the spermatozoa plasma membrane (Zhou et al. 2015).

One of the treatments for oxidative stress is the use of antioxidant compounds (Sumit et al. 2013; Avula et al. 2014; Webb et al. 2019), antioxidants that come from outside the body or from food and drink are called exogenous antioxidants (Parwata 2015). One of them is the Moringa plant (Razis et al. 2014). The results of the analysis of moringa leaf content have antioxidant capacity, including phenolics, flavonoids, tannins, ascorbic acid, alkaloids and saponins (Widiastini et al. 2021). The use of moringa leaf extract can prevent free radical damage to sperm and preserve the spermatozoa membrane, thereby increasing the viability and motility of spermatozoa (Wahjuningsih et al. 2019; Carrera-Chávez et al. 2020).

The purpose of this study was to determine the antioxidant mechanism of moringa leaf ethanol extract (EEMO) on sperm motility and viability in mature Wistar rats.

MATERIALS AND METHODS

Ethical clearance

This study was carriedout under an animal ethic clearance No 094/EA/KEPK-BUB-2023 approved by KEPK STIKES Bina Usada Bali.

Research design

This study used an experimental design, namely a randomized post-test only control group design (Pocock 2013). This study used 36 heads of older Wistar strain male rats (*Rattus norvegicus*), aged 18-19 months old of 200-250 grams body weight. The animals were then divided into two groups, including the control group given 0.5 ml CMC (Carboxy Methyl Cellulose) 0.5%, and the treatment group was given 0.5 mL of moringa leaf ethanol extract 50 mg/kg BW + CMC 0.5%, The administration of both control and treatment solutions was performed via oral gavage (sonde) once daily for 30 consecutive days

Preparation of Ethanol Extract Moringa Oleifera (EEMO)

Preparation of moringa leaf extract was followed a method described by (Cahyani and Sukadana 2017; Putra et al. 2017; Wasonowati et al. 2019). Approximately 50 grams of dried Moringa leaves were used to produce EEMO. The leaves were crushed in a blender, mixed with 96% ethanol solvent, placed in a jar, sealed, and dried for two days under of the sunlight. The macerate was obtained by filtering this combination. The same process was used to macerate the filtrates in 96% ethanol until a clear solution was obtained. The maceration process was performed three times. The macerate was evaporated by using a vacuum rotating evaporator at 40°C. Moringa leaf extract was then subjected to phytochemical screening to detect plant compounds based on their classification.

Research procedure

The animals were acclimatized for 7 days with 12 hours of light and 12 hours of darkness, at a temperature of 25±0.5°C and a humidity of 50–60%. The treatment

group was orally administered EEMO at a dose of 50 mg/kg BW combined with 0.5% CMC, in a total volume of 0.5 mL per day. The control group was administered 0.5% CMC (0.5 mL/day) orally. The administration was conducted between 08.00–09.00 WITA (Eastern Indonesian Time) for 30 days. At day-30 of the termination period, the animals were sacrificed by disclocation method of the cervical region of rats. It was then followed by an open orchidectomy. The testes were separated from the cauda epididymis and collected in a media container.

Spermatozoa motility examination

The examination is carried out immediately when the spermatozoa are taken from the caudal epididymis, by dripping a drop of spermatozoa on the glass object. Each droplets were approximately to be the same size for each examination. Observations were made under a microscope with a magnification of 400 times. The analysis was defined as (1) Progressive motility (PR): Spermatozoa move freely, either straight or in large circles, at any speed. (2) Non-progressive motility (NP): all types of spermatozoa that do not have progressive criteria, such as swimming in small circles, tail / flagellum that is difficult to move the head or only the tail moves. And (3) Immotility (IM) : does not move at all (World Health Organization (WHO) 2021)

Spermatozoa viability examination

The examination was carried out by making thin section preparations using eosin-negrosin staining which was then observed with an Olympus microscope at 1000X magnification. Live spermatozoa will not be stained by the dye, while dead spermatozoa will be stained.

Data analysis

The difference between the treatment and control groups was determined using the Statistical Program for Social Science (SPSS) 23 (IBM Corporation, Armonk, NY, USA) Independent Sample T Test and Mann Whitney test at the level of significance P= 0.05.

RESULTS AND DISCUSSION

Table 1 shows result of phytochemical screening of EEMO of moringa leaf ethanol extract in the South Denpasar area of Bali contains Antioxidant Capacity, including Phenol, Flavonoids, Tannin, Vitamin C, Saponins, and Alkaloids (Widiastini et al. 2021). Table 2 and Figure 1a show the treatment group that showed an average progressive motility of spermatozoa of 66.16±3.653% (mean±SD), while the control group showed an average of 45.55±6.073%. According to the Mann–Whitney test analysis, the results obtained a p-value of 0.000 ($P<0.05$), indicating a significant difference in the percentage of progressive motility between the group given Moringa leaf ethanol extract and the control group without Moringa extract. Therefore, it can be concluded that the ethanol extract of Moringa leaves has a significant effect on increasing the percentage of spermatozoa with progressive motility.

Table 2 and Figure 1b show that the treatment group has lower number of Non-Progressive Motility with an average of 21.54±3.393%. The independent sample T Test analysis was carried out and found that a P value was 0.000 ($P<0.05$), and shows that there is a significant difference in number of Non-Progressive Motility between the group given moringa leaf ethanol extract and the control group without moringa extract. This was then lead to a significant effects of moringa extract in the number of Non-Progressive Motility.

Table 1. Phytochemical screening of Ethanol Extract Moringa Oleifera (EEMO**)**

No	Phytochemical Test	Unit	Result
1	Antioxidant Capacity	mg/L GAEAC	1812.95
2	IC_{50}	Ppm	353.09
3	*Fenol*	mg/100 g	1556.52
4	*Flavonoid*	mg/100 g	23546.12
5	*Tannin*	mg/100 g	1300.34
6	Vit C	mg/100ml	23798.077
7	*Saponin*	-	Positif (+)
8	*Alkaloid*	-	Positif (+)

Table 2. Motility and viability of spermatozoa in older male Wistar rats (*Rattus norvegicus*) after Ethanol Extract Moringa Oleifera (EEMO) administration

Parameters	Treatment	Control	P-value
Progressive Motility (%)	66.16±3.653	45.55±6.073	0.000
Non-Progressive Motility(%)	21.54±3.393	29.02±3.380	0.000
Immotility (%)	12.30±1.184	25.44±5.829	0.000
Viability Spematozoa (%)	88.27±1.019	73.41±5.842	0.000

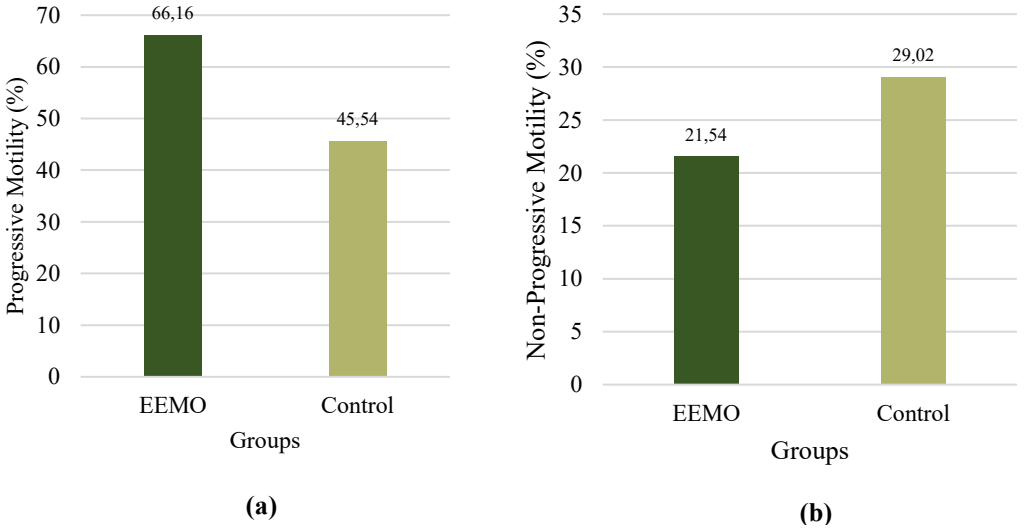

Figure 1. Progressive Motility (a) and non progressive motility (b) in older male Wistar rat (*Rattus norvegicus*) (n=18) (mean). EEMO= Ethanol Extract Moringa Oleifera

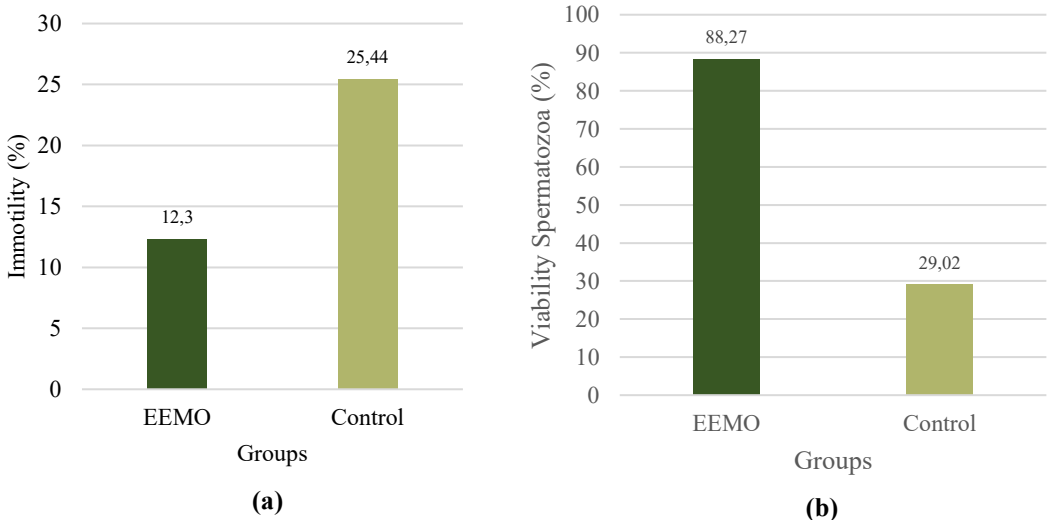

Figure 2. Immotility (a) and viability spermatozoa (b) in older male Wistar rat (*Rattus norvegicus*) (n=18) (mean). EEMO= Ethanol Extract Moringa Oleifera

Table 2 and Figure 2a shows that the treatment group has lower number of Immotility Spermatozoa with an average of 12.30±1.184% compares to control group. The independent sample T Test analysis shows that the P value was 0.000 (P<0.05), meaning that there is a significant difference in the number of Immotility

Spermatozoa between the group given moringa leaf ethanol extract and the control group without moringa extract. It appears that moringa leaf extract may have significant effect in the number of Immotility Spermatozoa.

Table 2 and Figure 2b show that the treatment group has better spermatozoa viability with an average of 88.27±1.019% compare to control group. Independent sample T Test analysis was carried out and the results obtained a p value of 0.000 (P<0.05), which means there is a significant difference in spermatozoa viability between the group given moringa leaf ethanol extract and the control group that is not given, so it can be said that moringa leaf extract can have a significant effect on spermatozoa viability.

Spermatozoa motility

The results showed that the administration of ethanol extract of moringa leaf (*Moringa oleifera*) at 50 mg/kg BW for 30 days to rats (*Ratus norvegicus*) of 18-19 months old increased significantly the progressive motility, decreased the non-progressive motility and the immotility in the treatment group compared to the control group with a value of P<0.05. The mean progressive motility in the treatment group was 66.16±3.653%, and the control group was 45.55±6.073%. The mean non progressive motility in the treatment group was 21.54±3.393% while the control group was 29.02±3.380%%, and the mean immotility of spermatozoa in the treatment group was 12.30±1.184% and the control group was 25.44±5.829%.

The antioxidants in moringa leaves (*Moringa oleifera*), including phenolic compounds, flavonoids, tannins, vitamin C, saponins, and alkaloids, neutralize free radicals, preventing oxidative damage to biomolecules and providing significant protection against oxidative damage. They have the ability to protect lipoproteins from peroxyl radicals, thereby increasing the progressive motility of spermatozoa. This is consistent with the findings of (Nayak et al. 2015), who showed that administering Moringa oleifera can increase sperm density and motility, reduce DNA damage, and significantly raise the levels of superoxide dismutase and catalase, along with a decrease in lipid peroxidation in testicular tissues. A study carried out by (Diao et al. 2019), showed that supplementation with Quercetin (Flavonoid) can protect Spermatozoa from sperm damage mediated by H_2O_2 (hydrogen peroxide) and increase spermatozoa motility. Vitamin C, as a key antioxidant, has been shown to play an important role in improving sperm quality. Bebas et al., (2015), demonstrated that vitamin C administration could maintain spermatozoa viability and motility, while the findings of Dimitriadis et al., (2023), further emphasized its role in enhancing sperm motility. Thus, vitamin C holds significant potential in improving male fertility parameters, particularly sperm motility. However, it should be noted that an excessive use of antioxidants may lead to pro-oxidants called antioxidant paradoxes, which can reduce endogenous oxidants that are important for the induction of physiological pathways, thereby inhibiting sperm capacitation, hyperactivation and acrosomal reactions of spermatozoa that can increase various pathological conditions such as aging and male infertility (Ali et al. 2020).

Spermatozoa viability

Viability of spermatozoa is the ability to live spermatozoa every time they are ejaculated. The normal percentage of spermatozoa viability is >58% regarded to be alive when spermatozoa do not absorb color, otherwise were died when spermatozoa absorb color (World Health Organization (WHO) 2021). Aging is also linked to decreased daily sperm production, total sperm count, and sperm viability as well as decreased semen volume brought on by diminished accessory gland activity (Gunes et al. 2016; Folorunsho et al. 2023).

The results showed that the administration of 50 mg/kg BW of moringa leaf ethanol extract (*Moringa oleifera*) for 30 days to older rats (*Ratus norvegicus*) of 18–19 months old may significantly increase spermatozoa viability when compared to the control group, with a value of P<0.05. The mean viability of spermatozoa in the treatment group was 87.70±1.183%, the control group was 74.57±5.828%, this is in line with the study of (El et al. 2022), that the administration of moringa extract, can improve sperm quality, and (Carrera-chávez et al. 2020), the supplementation of *M. oleifera* extract could increase antioxidant activity, sperm membrane integrity, viability, and progressive motility after thawing. Antioxidants contained in moringa leaves can react to and inactivate ROS, it is therefore the administration of moringa leaf ethanol extract may significantly increase the number and morphological quality of spermatozoa of older rats (Widiastini et al. 2022).

CONCLUSION

Ethanol extract *Moringa oleifera* (EEMO) has a significant effect as an antioxidant in improving male fertility. The results of phytochemical compounds from EEMO were effectively increasing the progressive motility of spermatozoa and spermatozoa viability, and reducing non-progressive motility and immotility of spermatozoa in older Wistar rats. To fully understand the impact of EEMO on the pathophysiology of male infertility brought on by aging, further study is required, particularly in regards to the role of EEMO in a number of molecular markers in testicular tissue.

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Sustainability Assessment of KUB Native Chicken Breeding Stock Availability in Malang Raya: A RAP-MDS Analysis

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ABSTRAK

Melinda PAR, Masyithoh D, Kalsum U, Halfi K, Setiasih, Rohaeni ES, Ariani M, Anggraeny YN. 2025. Penilaian keberlanjutan ketersediaan stok induk ayam KUB di Malang Raya: analisis RAP-MDS. JITV 30(4):219-228. DOI://dx.doi.org/10.1443/jitv.v30i4.3578.

Salah satu komoditas unggulan di subsektor peternakan adalah ayam khususnya ayam asli Kampung Unggul Balitbangtan (KUB), yang dikembangkan oleh Badan Litbang dan Pengembangan Pertanian Indonesia pada tahun 2014. Tujuan dari penelitian ini adalah untuk menganalisis status dan kondisi aspek keberlanjutan dalam hal ketersediaan stok pembibitan ayam KUB di Malang Raya (Kota Malang, Kabupaten Malang dan Kota Batu) pada bulan Maret - Juli 2025. Analisis RAP-Multi-Dimensional Scaling (MDS) digunakan dalam studi ini dengan melibatkan enam dimensi dan 29 atribut. Responden terdiri dari peternak, penyuluh, akademisi dan petugas dinas yang berpengalaman. Teknik pengumpulan data dilakukan dengan wawancara melalui kuisioner tersetruktur. Hasil analisis menunjukkan bahwa dimensi ekonomi memiliki nilai keberlanjutan tertinggi (70,77%) dibandingkan dengan dimensi lainnya. Hal ini secara berturut-turut adalah dimensi kesejahteraan hewan (56,31%), ekologi (55,42%), dan dimensi kelembagaan (50,24%). Dimensi sosial (49,92) dan teknologi (49,05) berada dalam kategori yang kurang berkelanjutan. Nilai indeks keberlanjutan dimensi secara keseluruhan berada dalam kategori hampir berkelanjutan (56,00), menunjukkan bahwa ketersediaan bibit ayam KUB di Malang Raya akan terus stabil dan berpotensi untuk pengembangan lebih lanjut. Atribut yang perlu diperkuat adalah dukungan masyarakat, peningkatan akses pelatihan, biosekuriti, program vaksinasi, dan adopsi teknologi seperti probiotik dan sistem pencatatan produktivitas ternak.

Kata Kunci: Bibit Ayam, Ayam Lokal, Multi-Dimensional-Scaling, Keberlanjutan

ABSTRACT

Melinda PAR, Masyithoh D, Kalsum U, Halfi K, Setiasih, Rohaeni ES, Ariani M, Anggraeny YN. 2025. Sustainability assessment of KUB native chicken breeding stock availability in Malang Raya: A RAP-MDS analysis. JITV 30(4): 219-228. DOI://dx.doi.org/10.1443/jitv.v30i4.3578.

One of the leading commodities in the poultry subsector is native chickens, especially the Kampung Unggul Balitbangtan (KUB) native chicken, which is developed by the Indonesian Agency for Agricultural Research and Development in 2014. The purpose of this study was to analyze the status and condition of the sustainability aspects related to the breeding stock availability of KUB native chickens in Malang Raya, examining it from six dimensions and 29 attributes. Respondents consisted of experienced farmers, extension workers, academics and service officers. The data collection technique was carried out by interviews through a structured questionnaire. The analysis uses the RAP-Multi-Dimensional Scaling (MDS) method to evaluate the sustainability indicators of breeding stock availability. Based on the study results, the dimensions that indicate an almost sustainable status are economic (70.77%), animal welfare (56.31%), ecological (55.42%), and institutional (50.24%). The dimensions social (49.92) and technological (49.05) are in the less sustainable category. The overall sustainability index value of the dimensions is in the almost sustainable category (56.00), indicating that the availability of KUB chicken seeds in Malang Raya will continue to be stable and has potential for further development. The attributes that need to be strengthened are community support, increasing access to training, biosecurity, vaccination program, and adopting technologies such as probiotics and productivity recording systems.

Key Words: Breeding Stock, Native Chicken, Multi-Dimensional-Scaling, Sustainability

INTRODUCTION

Native chickens have adapted to regional environments and traditional farming systems (Depison et al. 2022; Sumantri et al. 2020). These chickens are a local breed widely raised by Indonesian peoples. Due to their resilience and compatibility with conventional, low-input agricultural practices, native chickens serve as an essential genetic and economic asset within rural communities. These chickens make a significant

contribution to food security, rural livelihoods, and small-scale poultry development in Southeast Asia, including Indonesia (Abbasi, et al. 2024). In 2021, the population of native chickens in East Java reached 36.8 million (BPS 2023). One of the superior native chicken developed in Indonesia is the Kampung Unggul Balitbangtan (KUB). The KUB native chicken is the result of six generations of female-line genetic selection by the Indonesian Agency for Agricultural Research and Development (IAARD). KUB chickens have improved productivity traits compared to traditional native chickens, including 160–180 eggs per year, early laying age (22–24 weeks), low broodiness (10%), and an average slaughter weight of 800–900 grams at 10 weeks of age (Bakrie et al. 2021). Differences in egg and meat production potential are brought from different genetics, such as Sentul chickens are another potential local chicken, known as meat-producing chickens with good egg production (Sumantri et al. 2020).

Previous studies have highlighted the importance of breeding stock availability in ensuring the sustainability of the KUB chicken farming system (Antikasari et al., 2023). Sustainability is strongly influenced by the availability and quality of superior genetic stock (FAO 2010). Government institutions play a role in supporting this, the Balai Penerapan Modernisasi Pertanian (BRMP) East Java, located in Karangploso, Malang Regency, was established in 2017 as a regional center for producing and distributing certified KUB breeding stock. This institution plays a strategic role in preventing genetic degradation and ensuring the supply of Day-Old Chicks (DOCs) to farmers in East Java, including the Malang Raya area. Given the importance of breeding stock in the poultry production chain, maintaining both the quality and quantity of superior chicks is essential. The use of genetically superior breeding stock is directly linked to improved productivity and economic returns in poultry farming.

The development of a livestock commodity is highly determined by several factors, especially the availability of breeding stock, balanced feed, disease control, and post-harvest processing and marketing. The results of the study (Setiasih et al. 2021) in the province of East Java showed that the type of KUB chicken breeding business to produce DOC is the most economically feasible, this is because the DOC price of KUB chickens is still higher than other free-range chickens. The market demand for DOC KUB is much higher than other local chickens such as Sentul or Gaok. BRMP as a government institution that plays a role in producing KUB chicken breeding stocks is not able to satisfy market demand, so it has to foster breeders as partners. Therefore, research is needed on the sustainability of the provision of KUB chicken breeding stocks.

Therefore, this study aimed to evaluate the sustainability performance of KUB native chicken breeding stock availability in the Malang Raya, East Java, Indonesia. This research is expected to provide a comprehensive understanding of the current conditions and serve as a reference for BRMP East Java, the Regional Animal Husbandry Services, and other stakeholders in developing sustainable and competitive KUB breeding strategies.

MATERIALS AND METHODS

This study employed a descriptive mixed-method approach that combined quantitative and qualitative data. The research was conducted from March 3 to July 10, 2025, in 18 sub-districts located within Malang City, Batu City, and Malang Regency. These areas were selected purposively as central regions for KUB chicken breeding activities coordinated by the BRMP East Java.

A non-probability, purposive sampling technique was employed, involving 29 respondents selected based on their direct involvement in KUB breeding programs. The primary data were collected using structured questionnaires and face-to-face interviews with farmers, technical officers, academics, and institutional personnel. All respondents are people who are experienced with KUB chickens in their respective fields. Secondary data were obtained from relevant institutions, including BRMP East Java, BBPP Batu, the Central Statistics Agency (BPS), and the Ministry of Agriculture.

The classification of sustainability attributes is based on the dominant effect principle, although some attributes have cross-dimensional relationships (ecology, economic, and social), to avoid overlap between dimensions. This approach is commonly used in RAP-MDS analysis so that each dimension still represents its main sustainability function conceptually and operationally. The ecological dimension reflects the interaction of the KUB chicken breeding system with natural resources and environmental carrying capacity. The animal welfare dimension assesses the fulfillment of the biological needs and natural behavior of KUB chickens. The economic dimension represents the feasibility and stability of the breeding business. The social dimension emphasizes the contribution of the breeding system to the quality of life and social stability of the community. The institutional dimension describes institutional support for the KUB chicken breeding business, while the technological dimension reflects the level of innovation adoption in breeding activities for the sustainability of the breeding business. The analysis focused on six sustainability dimensions, comprising a total of 29 attributes showed in Table 1.

Sustainability analysis was conducted using the Rapid Appraisal for Poultry (RAP-Poultry) method, an adaption of the RAPFISH approach, based on non-metric Multidimensional Scaling (MDS) techniques (Pitcher & Preikshot, 2001; Kifaf et al., 2023). In RAP-

Tabel 1. Dimenssion and Atributes

No	Dimenssion	Atributes
1	Ecological	Availability of Local Feed and Water Sources
		Breeding productivity
		Feed Management Patterns
		The influence of climate conditions
2	Animal welfare	Cage System
		Expressing Natural Behaviors
		Biosecurity protocol
		Vaccination program
		Access to food and drink
3	Economic	Access to KUB breed
		Price Stability & Feed Quality
		Price stability and DOC supply
		Market Demand Stability
		The influence of DOC purity
4	Social	Community support
		Ratio of counseling and training
		Social conflict
		Level of local economic contribution
5	Institutional	The role of extension workers in Partnership institutions
		Regional regulations
		The Role of Extension Workers
		Partnership Institutions
		Capital Institutions
6	Technological	Technology for the use of probiotics
		Productivity system
		Alternative feed processing technology
		Adoption of Modern Technology
		Farm Waste Treatment Technology
		Recording System

MDS, sustainability attributes are transformed into ordination space using Euclidean distance in n-dimensional attribute space. The ordination process aims to minimize the discrepancy between the original dissimilarity matrix and the reduced-dimensional configuration. The squared distance between objects i and j in ordination space is expressed as:

$$d_{ij}^2 = \sum_{k=1}^{m} (x_{ik} - x_{jk})\ 2$$

where $x_{ik}\ and\ x_{jk}$ represent the scoresof object I and j ondimension k and m is the number of ordination dimensions.

The goodness-of-fit of the MDS configuration was assessed using S-stress, which measures the discrepancy between the squared distances in the ordination space and the squared distances of the original input data. Lower S-stress values indicate a better fit, with values below 0.25 considered acceptable. Additionally, the coefficient of determination (R^2) greater than 0.80 was used to assess how well the ordination represents the original data structure.

$$\text{S-stress} = \sqrt{\sum(d_{ij}^2 - d_{ij}^2)2\ / \sum d_{ij}^4}$$

Monte Carlo was used to evaluate the reliability and uncertainty levels of the RAP-MDS analysis results by repeatedly randomizing attribute scores within a predetermined range of values. Monte Carlo validity was evaluated by comparing the sustainability index values generated from the initial MDS ordination with the average sustainability index values from the Monte Carlo simulation results. A difference in index values of less than 5% indicates that the model has good stability and a low level of sensitivity to variations in attribute assessments.

The analysis steps included a) identifying 29 sustainability attributes across six dimensions, b) scoring each attribute using a Likert scale ranging from 1 to 5 (poor to good), c) conducting MDS to calculate the sustainability index values. Sustainability indices and status were categorized as follows: not sustainable (0.00−25.00), less sustainable (25.01−50.00), moderately sustainable (50.01−75.00), and highly sustainable (75.01−100.00), according to (Prabowo et al. 2024). Next step is e) validating the model through stress values and the coefficient of determination (R^2) >0.08, f) applying Monte Carlo simulation to evaluate uncertainty in a difference of less than 5% between the two results indicates acceptable model stability and low sensitivity to scoring errors, g) visualizing results using kite diagrams and classifying sustainability levels, and the last is h) conducting leverage analysis to identify the most sensitive attributes.

RESULTS AND DISCUSSION

The sustainability status indicators of breeding stock availability for KUB native chickens in Malang Raya were analyzed using RAP-Analysis, Monte Carlo, and the Coefficient of Determination (R^2). The Standardized Residual Sum of Squares (Stress) value was also used to determine the percentage of deviation from the initial characteristics, where the smaller the stress value, the smaller the level of deviation (Allaily et al. 2024).

Based on the analysis results shown in Table 1, a comparison of index values between the RAP-MDS and Monte Carlo methods shows a difference of less than 3% in all dimensions. This indicates that the analysis process is free from systematic bias uncertainty with a difference of less than 5%, and the results are stable despite random noise in the data (Kifaf et al. 2023). The Squared Correlation (R^2) value in all dimensions is above 0.91 (R^2 > 0.90) which is close to the maximum number of 1. This indicates that the RAP-MDS model has a very high explanatory ability of the initial data structure, in other words the results of the sustainability mapping are very representative of real conditions in the field. The results of the analysis can be said to explain the level of sustainability of breeding stock availability for KUB native chickens in Malang Raya. The level of deviation of the distance characteristics after ordination, compared to the distance before ordination, measured using stress, shows that all dimensions have a stress value <0.2, with an average ranging from 0.15 to 0.18, which indicates a very low level of data distortion. The stress value measures the extent of the difference between the distance configuration in the input and output of the MDS analysis. A standard stress value <0.25 indicates that the mapping result configuration is valid and can be interpreted, while a value <0.15 is considered very good (Pitcher & Preikshot 2001). Overall, the sustainability index status of breeding stock availability for KUB native chickens in Malang Raya, as depicted in each dimension, is illustrated using a kite chart (Figure 1).

Ecological dimension sustainability index

The ecological dimension obtained an index value of 55.42 (Table 2), placing it in the moderately sustainable category (score 50–75). This value indicates that the ecological aspects of the KUB livestock breeding business have been addressed but have not yet achieved optimal sustainability. Previous related research has been carried out; ecology is one dimension that needs to be considered in implementing sustainable development of KUB chicken businesses in Cianjur Regency (Antikasari et al. 2023).

Attributes considered sensitive to the ecological dimension's sustainability index are shown in Table 2. These include breeding productivity (3.19%), feed management patterns (3.06%), and the influence of climate conditions (3,00%). While the attribute that is not sensitive is availability of local feed and water sources (2,60). KUB chicken breeding productivity is the most sensitive attribute, meaning that breeding productivity has a significant impact on the stability of overall ecology sustainability. This finding aligns with previous research that emphasizes the role of productivity in ecology efficiency (Depison et al. 2022). Low productivity can indicate environmental pressures, such as poor feed quality, inadequate chicken health, or suboptimal rearing conditions. In the context of this study, feed management patterns are a contributing factor to ecological inefficiency. Feed management patterns play a crucial role in ecological impact. Inappropriate feeding frequency, inadequate ratio composition, and the use of environmentally harmful feed materials may increase waste and reduce sustainability. The dominant activity of KUB chickens is feeding; it was identified as the dominant activity among 20-week-old KUB chickens during both morning and evening periods. The proportion of feeding activity was 21.8% in the morning and 21.4% in the evening (Sutanto 2021). This consistent feeding pattern must be effectively managed to avoid feed wastage and negative ecological impacts in Malang Raya area.

Table 2. Sustainability Index Scores, Monte Carlo Validation, Coefficient of Determination (R^2), and Stress Values

Dimensions	Rap Analysis	Monte Carlo	R2	Stress
Ecological	55.42	54.99	0.92	0.19
Animal Welfare	56.31	56.03	0.93	0.17
Economic	70.77	68.77	0.94	0.15
Social	49.92	48.89	0.93	0.16
Institutional	50.24	49.91	0.93	0.17
Technological	49.05	48.88	0.94	0.16
Average	55.29	54.58	0.93	0.17

Source: Prepared by the researcher based on the questionnaire data

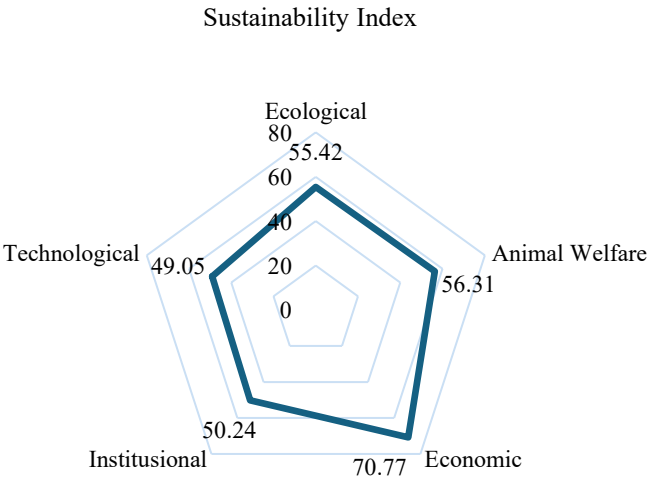

Figure 1. Sustainability Index Kite Diagram

Chicken feed patterns such as feed intake and FCR are affected by climate as chickens are highly sensitive to environmental changes, especially temperature and humidity as chickens have to adapt to heat stress (Kim et al. 2024). Farmers must make feed formulas according to the availability of local feed that changes according to the seasons so that egg production is not disrupted.

Moreover, climate change remains an undeniable factor. A range of previous studies have reported declines in livestock productivity due to rising temperatures, extreme humidity, or seasonal shifts (Osuji et al. 2024; Attia et al. 2024; Alhassan et al. 2024). Therefore, developing climate adaptation strategies remains a critical challenge in enhancing the ecological sustainability of KUB chicken breeding operations. The report from (Wibawa and Pamungkas 2022) on climate change in the Malang Raya area is mainly in terms of temperature, humidity, and rainfall per month during 2014 – 2020 which affects the time of fruit harvest, but there have been no reports of changes in chicken productivity. Climate change causes heat stress that affects the physiological of livestock, water and feed consumption, and immune system thereby reducing chicken production (Attia et al. 2024).

Animal welfare dimension sustainability index

The animal welfare dimension received a sustainability index score of 56.31 (Table 2), placing it in the moderately sustainable category (score 50-75). This score indicates that efforts to implement animal welfare principles have been made, but several challenges remain that need to be addressed to further support sustainability. Animal welfare is closely related to meeting the basic needs of living creatures. Welfare in poultry farming encompasses physical and mental health needs (Silvera et al. 2017; Pasteur et al. 2024).

Attributes considered sensitive are shown in Table 3. include biosecurity protocols (3.66%), vaccination programs (2.95%), and access to feed and water (2.87%). The non-sensitive attributes were Cage System (2.86) and Expressing Natural Behaviors (2.62). Biosecurity protocols rank highest as the most influential attribute.

Weak biosecurity implementation increases the risk of disease and a decline in the physiological condition of chickens, which directly impacts welfare aspects. Biosecurity can be implemented by starting with timely cage cleaning, as it can affect air Insensitive attributes were Cage System (2.86) and Expressing Natural Behaviors (2.62)quality and animal health. To support the sustainability of livestock businesses, monitoring and routine implementation of cage cleaning must be carried out promptly to help prevent the spread of disease, maintain environmental quality, and ensure animal welfare (Subedi et al. 2023; Khattak et al. 2025).

In addition to biosecurity attributes, vaccination programs are also one of the leverage attributes; this is due to the uneven or non-standardization of vaccination programs, which threatens the quality of chicken health and breeding results. Meanwhile, the vaccination program in the KUB chicken breeding farm environment in the Malang Raya area remains inconsistent, and information on its benefits is not fully understood by farmers, despite BRMP providing information and free ND vaccines to farmers who purchase day-old chicks (DOC). In Indonesia, Newcastle disease (ND) remains endemic, as indicated by the discovery of cases throughout the year, due to several factors, including poor vaccine quality, inadequate vaccine storage conditions, and vaccinator errors (Silvera et al. 2017).

Access to feed and water are environmental factors that affect the stress level of KUB chickens. Access to feed and water at KUB breeding farms continues to fall short of meeting the chickens' welfare needs, primarily due to the lack of automated cage systems. This limitation confines feeding and watering to fixed schedules, thereby restricting the chickens' ability to freely satisfy their nutritional and hydration needs. A recent ethological study on KUB chickens revealed that feeding and drinking are the predominant behaviors in both the morning and evening. These patterns reflect the animals' intrinsic drive to achieve welfare fulfillment and physiologically prepare for peak productivity (Sutanto 2021).

Economic dimension sustainability index

The economic dimension achieved a sustainability index value of 70.77 (Table 2). This value places the economic dimension in the category of moderately sustainable towards sustainable, or the upper spectrum of the moderately sustainable value range (50–75). This value indicates that, economically, the KUB chicken breeding business is relatively stable and has the potential to continue developing further. However, several challenges remain to be addressed to achieve optimal economic sustainability. The native chicken farming enterprise is expected to have a beneficial socio-economic impact, considering the relatively small flock size (Asnawi et al. 2023). In a recent study, the analysis of the sustainability of the KUB chicken business, the economic dimension has a high sustainability index of 52.89, which indicates that the economic dimension and its attributes are categorized as sufficiently sustainable, providing a higher opportunity for development to support the sustainability of the KUB chicken business (Antikasari et al. 2023). Attributes that are considered sensitive are shown in Table 3. They include the stability of the DOC price and supply (10.00%), market demand stability (7.37%), and the influence of DOC purity (5.33%). The non-sensitive attributes were price stability and feed quality (0.92) and access to DOC of KUB chickens (3.43).

The stability of the DOC price and supply is the most dominant economic attribute. Its fluctuations have a massive impact on the economic dimension. The breeding farming to produce DOC KUB is the most profitable in the Malang area (Setiasih et al. 2021). The price of DOC KUB is stable at Rp. 7000-7500 in Malang Raya. Supply instability or high prices will disrupt the cash flow of farmers, reduce profits, and increase the risk of loss. Stated that accessibility of breeding stock at stable prices is an essential factor in increasing farmer productivity (Kalangi et al. 2025). The high price of DOC was due to farmers not having direct access to the company, which set several requirements that they had not satisfied, such as deposits and minimum purchase requirements (Kalangi et al. 2025). BRMP has carried out efforts to stabilize the price and supply of KUB chicks through empowering KUB chick farmer groups or breeding centers in several areas of Malang Raya to meet the demand for KUB chicks. In Sukabumi Regency, the multiplication of KUB breeding stock can be done by building breeding centers in each region (Rusdiana 2019).

The stability of market demand also plays a key role. The demand for breeding stock of KUB in the Malang Raya area is relatively high. Farmers have to wait in a long queue to get a DOC. This is because there are not many business actors operating in the DOC breeding sector (Wantasen et al. 2024; Prawiranegara & Mulijanti 2024) The issue of DOC purity's influence is directly related to market perception and trust. As the market continues to doubt the purity of breeding stock, breeders tend to refrain from repeat purchases, switch to other breed stock suppliers, and spread negative perceptions that can systematically reduce demand. Factors influencing the sustainability of KUB chicken business development include the consumer's perspective on purity, variety, egg, and meat quality (Sirajuddin 2007).

Social dimension sustainability index

The social dimension obtained a sustainability index value of 49.92 (Table 2). This value is included in the less

sustainable category (score 26-49), All of attributes that are considered sensitive, as shown in Table 3. include community support (12.16%), social conflict (11.97%), the ratio of counseling and training (10.01%), and the level of local economic contribution (9.75%). Community support is a crucial factor, as community involvement in groups, collaboration between communities, and the exchange of information regarding KUB native chicken breeding have a significant impact on the sustainability status of the social dimension. Cooperation between farmers and groups within a forum or region can increase work productivity, as seen in the case of the KUB chicken breeding business, through the exchange of information, collaboration, and motivation (Prawiranegara & Mulijanti 2024).

Social conflict indicates that social dynamics, such as the presence of farms near residential areas, internal disputes within breeding groups, and conflicts between groups and government agencies, disrupt social cohesion and reduce mutual trust. Based on the questionnaire data, social strife is primarily related to the presence of farms near residential areas, as the breeding farm KUB is a small-scale operation. The negative impact of the KUB chicken business in Cianjur Regency was pollution in the business environment, which disrupted the comfort of the surrounding community (Antikasari, et al. 2023). The ratio attribute of extension and training for breeding reflects that although extension and training activities have been carried out, their coverage is not evenly distributed and has not reached all breeders. Meanwhile, the contribution to the local economy suggests that although breeding activities have benefited the local economy, the benefits have not been evenly distributed throughout the community.

Institutional dimension sustainability index

The institutional dimension achieved a sustainability index score of 50.24 (Table 2). This value is right at the bottom limit of the sustainable category (score 50-75). This score indicates that, although there is a functioning institutional framework, its effectiveness in ensuring the continuity of breeding activities remains weak. Attributes that are considered sensitive are shown in Table 3. They include partnership institutions (3.37%) and regional regulations (3.01%). Insensitive attributes include the role of extension workers in partnership institutions (0.29), the role of extension workers (0.49), and capital institutions (0.01). The availability and effectiveness of partnership institutions (such as the BRMP East Java partner farmer groups) greatly determine the sustainability of the institutional dimension. The productivity of KUB's breeding stock can be sustainable if suppliers or partner farmers are involved in providing DOC and feed at affordable prices through stable and sustainable partnerships (Prawiranegara & Mulijanti 2024). Some forms of partnership that can be carried out are Core plasma, Subcontract, Franchise, agency, or operational agribusiness cooperation in agriculture (Sirajuddin 2007). The regional regulatory institutions share similar values with partnership institutions; however, there is a lack of regional regulations or local policies governing the KUB native chicken breeding process (Apriliyanti et al. 2024). It is still considered insufficiently supportive or not yet implemented, resulting in suboptimal availability of chicks in Malang Raya. There is no pressure from other chicken breeding programs (such as broiler, layer, Arab chickens, etc.) but because KUB chickens are new innovative chickens, it still needs time to be widely disseminated in the Malang Raya area. The breeding of local chickens (Arab and joper chickens) is carried out by small, medium breeders while broiler and layer chicken breeds are carried out by companies (Nugroho 2023).

Technological dimension sustainability index

The technological dimension obtained a sustainability index value of 49.05 (Table 2). This value falls into the less sustainable category (score 26-49); technology in the KUB chicken breeding system is not optimal and has not been able to support overall sustainability. Attributes that are considered sensitive are shown in Table 3. They include technology for the use of prebiotics and probiotics (5.54%), and the productivity recording system (3.07%). Insensitive attributes are alternative feed processing technology (0.59), adoption of modern technology (0.36), farm waste treatment technology farm waste treatment technology (2.37) and recording system (0.36).

The low adoption of this technology has an impact on feed efficiency and makes it difficult to identify factors causing production declines, leading to a reliance on the subjective experience of farmers (Lokman Ali et al. 2020). This technology plays a vital role in increasing chicken endurance, reducing the need for antibiotics, and improving performance naturally (Putra & Humaidah 2022). Probiotics serve as an alternative to antibiotics in a variety of poultry feed ingredients. The provision of immune herbal probiotics in drinking water affects the economic value of KUB2 chicken feed rations (Younas et al. 2025).

The technological dimension in this study emphasizes the adoption of applicable farmer-level technologies, rather than upstream genetic improvement. Although genetic factors influence productivity, they are excluded because KUB native chickens are nationally standardized local chickens, and genetic management is carried out by official breeding centers. Therefore, technology attributes focus on operational practices, including probiotic application, productivity recording,

Table 3. Leverage analysis (The sensitive attributes)

No	Dimension	Attributes	RMS
1	Ecology	Breeding productivity	3.19
		Feed Management Patterns	3.05
		The influence of climate conditions	3.00
2	Animal Welfare	Biosecurity protocol	3.66
		Vaccination program	2.95
		Access to food and drink	2.87
3	Economic	Price stability and DOC supply	10.00
		Market Demand Stability	7.37
		The influence of DOC purity	5.33
4	Social	Community support	12.16
		Ratio of KUB chicken breeding, counseling, and training	10.00
		Social conflict	11.75
		Level of local economic contribution	9.97
5	Institutional	The role of extension workers in Partnership institutions	3.37
		Regional regulations	3.01
6	Technological	Technology for the use of probiotics	5.54
		Productivity recording system	3.07
		Alternative feed and waste processing technology	2.37

Source: Prepared by the researcher based on the questionnaire data

Table 4. The overall sustainability index

Dimensions	Sustainability Index		
	Weight	Dimension Status	Mark
Ecological	0.188	55.42	10.4
Animal Welfare	0.205	56.31	11.5
Economic	0.181	70.77	12.8
Social	0.13	49.92	6.49
Institutional	0.145	50.24	7.28
Technological	0.152	49.05	7.46
Overall Sustainability Index		56.00	

Source: Prepared by the researcher based on the questionnaire data

alternative feed processing, adoption of modern technologies, waste management, and record-keeping systems. Low sustainability scores on the technology dimension indicate that these technologies have not been widely adopted, likely due to limited technical assistance and inadequate training, rather than genetic improvement technologies.

The overall sustainability index value for the dimensions is in the sufficiently sustainable category (56.00), as shown in Table 3, indicating that the availability of KUB chicken seeds in Malang Raya will continue to be sustainable and has potential for further development.

The relatively high sustainability index value for the economic dimension reflects strong market demand, price stability, and the adaptability of KUB native chickens. This makes them economically attractive to farmers. Conversely, the social and technological

dimensions show lower sustainability index values due to limited farmer participation in collective activities, inadequate access to ongoing training, and low technology adoption. Furthermore, in general, farmers still operate with substandard technology. These results indicate that economic viability has developed more rapidly than social readiness and technological capacity. Without strengthening social capital and accelerating the adoption of appropriate technology, the long-term sustainability of KUB parent stock availability may be at risk, even despite favorable economic conditions.

CONCLUSION

Based on the analysis of the sustainability status of breeding stock availability, the KUB native chicken use in Malang Raya falls into a sufficiently sustainable category (56.00). The dimensions of a reasonably sustainable status of breeding stock availability for KUB native chickens in Malang Raya are the economic dimension (70.77%), animal welfare (56.31%), ecological (55.42%), and institutional dimension (50.24%). The dimensions social (49.92) and technological (49.05) are in the less sustainable category. Several sensitive attributes identified as priorities for improvement include biosecurity, vaccination, increasing access to training, and adopting technologies such as probiotics and productivity recording systems, as well as community support and considering regional regulations.

Based on these findings, the following recommendations are proposed to improve the sustainability of KUB chicken breeding systems in Malang Raya. The government should stabilize the market through formal partnerships and supportive regional regulations. Farmers should adopt modern technologies such as comprehensive recording systems, probiotics, prebiotics, and alternative feeds to enhance productivity and flock health. Extension services need to expand their training to focus on biosecurity, vaccination, and reproductive management, addressing social and technological limitations. BRMP can increase the number of satellite farm partners or community-based plasma breeders in the Malang Raya area and develop standardized certification and distribution systems to ensure the quality and availability of breeding stock. Suggestions for future research are the need to include the productivity attributes of nursery in the technological dimension rather than the ecological dimension.

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Influence of Different Rearing Systems and Sex on the Hematological Profile of IPB D3 Chickens

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ABSTRAK

Kuswandi W, Khaerunnisa I, Budiman C, Sumantri C. 2025. Pengaruh sistem pemeliharaan dan jenis kelamin yang berbeda terhadap profil hematologi ayam IPB D3. JITV 30(4):229-242. DOI:http:dx.doi.org/10.1443/jitv.v30i4.3519.

Sistem pemeliharaan berpengaruh signifikan terhadap kesejahteraan, produktivitas, dan status fisiologis ayam, termasuk respons hematologi yang mencerminkan kondisi kesehatan dan sistem imun. Penelitian ini bertujuan untuk menganalisis interaksi antara sistem pemeliharaan dan jenis kelamin terhadap profil hematologi IPB-D3 chicken. Sebanyak 60 ekor ayam umur 12 minggu dipelihara selama 12 minggu dalam dua sistem, yaitu intensif dan free-range, masing-masing terdiri atas 30 ekor (15 jantan dan 15 betina). Pada umur 24 minggu, sampel darah diambil melalui vena brakialis menggunakan tabung EDTA. Analisis hematologi dilakukan dengan metode standar meliputi penghitungan eritrosit dan leukosit menggunakan hemocytometer, pengukuran hemoglobin, penentuan hematokrit dengan metode mikrohematokrit, serta diferensial leukosit melalui preparat apus darah yang diwarnai menggunakan Giemsa stain. Hasil penelitian menunjukkan bahwa jumlah eritrosit, kadar hemoglobin, dan indeks eritrosit tidak berbeda nyata antara sistem pemeliharaan (P>0,05). Nilai hematokrit tidak dipengaruhi secara signifikan oleh sistem pemeliharaan, namun terdapat interaksi nyata antara sistem pemeliharaan dan jenis kelamin (P=0,038), di mana ayam jantan pada sistem free-range memiliki nilai hematokrit lebih tinggi dibandingkan sistem intensif. Jumlah leukosit total dipengaruhi secara signifikan oleh sistem pemeliharaan (P= 0,010), dengan nilai lebih tinggi pada sistem intensif (19,52×10³/µL) dibandingkan free-range (14,12×10³/µL), tanpa interaksi dengan jenis kelamin. Persentase leukosit diferensial dan rasio heterofil-limfosit tidak menunjukkan perbedaan nyata (P>0,05). Kesimpulannya, meskipun beberapa parameter hematologi dipengaruhi oleh sistem pemeliharaan dan interaksinya dengan jenis kelamin, ayam IPB-D3 menunjukkan kemampuan adaptasi fisiologis yang baik pada sistem intensif maupun free-range.

Kata Kunci: Sistem free-range, profil hematologi, Ayam IPB-D3, sistem pemeliharaan, interaksi jenis kelamin

ABSTRACT

Kuswandi W, Khaerunnisa I, Budiman C, Sumantri C. 2025. Influence of different rearing systems and sex on the hematological profile of IPB D3 chickens. JITV 30(4):229-242. DOI:http:dx.doi.org/10.1443/jitv.v30i4.3519.

Rearing systems significantly influence the welfare, productivity, and physiological status of poultry, including hematological responses that reflect immune and health conditions. This study analysed the interaction between rearing system and sex on the hematological profile of IPB-D3 chicken. A total of 60 twelve-week-old chickens were reared for 12 weeks under two systems: intensive and free-range, with 30 birds per system (15 males and 15 females). At 24 weeks of age, blood samples were collected from the brachial vein into EDTA tubes. Hematological analysis was performed using standard methods, including erythrocyte and leukocyte counts using a hemocytometer, hemoglobin determination, hematocrit measurement by the microhematocrit method, and differential leukocyte counts using blood smears stained with Giemsa stain. Results showed that erythrocyte count, hemoglobin concentration, and erythrocyte indices did not differ significantly between rearing systems (P>0.05). Hematocrit was not significantly affected by rearing system alone, but a significant interaction between system and sex was observed (P = 0.038), with free-range males exhibiting higher hematocrit than intensively reared males. Total leukocyte count was significantly influenced by rearing system (P = 0.010), with intensively reared chickens showing higher leukocyte levels (19.52×10³/µL) than free-range chickens (14.12×10³/µL), without interaction with sex. Differential leukocyte percentages and the heterophil-to-lymphocyte ratio were not significantly different (P>0.05). In conclusion, although certain hematological parameters were influenced by rearing system and its interaction with sex, IPB-D3 chicken demonstrated good physiological adaptability under both intensive and free-range conditions.

Key Words: Free-Range System, Hematological Profile, IPB-D3 Chickens, Rearing System, Sex Interaction

INTRODUCTION

The IPB-D3 chicken is a selectively bred strain developed from the seventh generation of IPB D1 chickens and was officially released as an Indonesian local chicken in 2019 (Sumantri et al. 2020). The IPB D1 population originated from a composite cross involving Pelung chicken × Sentul chicken and Kampung chicken × Broiler chicken, each contributing approximately 25% to the genetic composition, and was selected primarily for superior body weight. The parental lines exhibit distinct productive and adaptive characteristics. Pelung chickens are known for their large body size, with adult males reaching 2.5–3.5 kg and, in some cases, up to 5–6 kg (Asmara et al. 2019). Sentul chickens show moderate growth performance (1.5–2.0 kg) and good adaptability and disease resistance (Depison et al. 2019). Kampung chickens are characterized by high environmental adaptability but relatively slower growth, typically ranging from 0.8–1.2 kg at 12–24 weeks of age (Rahardja et al. 2015). In contrast, broiler chickens exhibit rapid growth, reaching 1.6–2.0 kg within 5 weeks under intensive management (Kuswandi et al. 2024). The combination of these parental traits through crossbreeding results in heterosis, which enhances growth rate and overall performance. Consequently, IPB D3 chickens demonstrate rapid growth, exceeding 1 kg before three months of age (Salsabila et al. 2022), with males reaching 1,256 g and females 1,042 g at 10 weeks. In addition, like other local chickens, IPB D3 exhibits strong environmental adaptability and improved feed efficiency, making it a promising genetic resource for sustainable poultry production (Gheyas et al. 2021). However, the expression of these superior genetic and growth characteristics may vary across rearing environments. Therefore, evaluating how different rearing systems influence physiological responses, particularly hematological parameters and productivity, is essential to optimize performance under diverse management conditions.

In addressing global challenges in the livestock sector, optimizing poultry-rearing systems is key to improving animal welfare and productivity. Various approaches, including intensive and free-range systems, offer distinct advantages and challenges, particularly their impact on chicken health. Environmental factors, feed quality, and stress levels due to stocking density and management practices significantly affect the physiological condition of chickens, including hematological parameters (Kim & Lee 2023). For instance, while the intensive system enhances production efficiency, it raises concerns about increased stress and disease risk. In contrast, the free-range system, with its more natural environment, has the potential to reduce stress but faces challenges such as feed variability and pathogen exposure (Sanchez-Casanova et al. 2020). Proper environmental and nutritional management is crucial to balancing productivity and poultry health. Therefore, an in-depth study of various rearing systems and their effects on hematological parameters is essential to developing sustainable, optimal poultry farming practices.

Blood hematology represents a critical indicator of poultry health status, physiological responses, and stress conditions. Key parameters, including leukocyte and erythrocyte counts, hemoglobin (Hb), hematocrit (Hct), and erythrocyte indices, provide insight into the internal physiological state of chickens and may vary in response to infections, stress, and nutritional imbalances (Griss et al. 2019). Elevated leukocyte counts are commonly associated with immune responses triggered by pathogenic challenges or environmental stressors. Normal Hb and Hct values indicate adequate oxygen-carrying capacity and the absence of anemia (Horhoruw & Rajab, 2024). Hematological profiles are also strongly influenced by management-related factors such as rearing systems, hygiene practices, and housing conditions (Gorelik et al. 2020; Tantikositruj et al. 2022). Moreover, suboptimal environmental conditions, including high stocking density and inadequate ventilation, can induce heat stress, thereby altering hematological parameters, particularly Hb and Hct levels (Dadgar et al. 2021). Therefore, hematological assessment serves as a reliable and integrative tool for evaluating poultry health and assessing the effectiveness of rearing systems and nutritional management in supporting optimal welfare and productivity.

Factors such as rearing systems, nutrition, growth, sex, breed, and age significantly influence chicken performance, with sex being a key factor in improving genetic traits (Cygan-Szczegielniak & Bogucka 2021). Blood parameter analysis is crucial for assessing chickens' physiological condition and overall health, and for providing insights into their responses to environmental and nutritional factors (Angoua et al. 2021). Sex differences can affect various hematological parameters through physiological and hormonal variations, thereby altering metabolism and immune responses across different rearing environments (Taha & Aljumaily 2021). However, the interaction between sex and rearing systems, such as intensive and free-range systems, in shaping hematological parameters remains underexplored. Previous studies have shown that strain and sex significantly influence several hematological parameters (Adewole et al. 2021), underscoring the importance of accounting for genetic factors and sex differences in poultry management to optimize health and productivity.

Studies on IPB-D3 chickens have primarily focused on growth performance and carcass quality, with limited attention given to their hematological responses under different rearing conditions (Ratnawati et al. 2024). In IPB-D3 chickens, total leukocyte counts have been reported to range from 39.9 to 51.07×10³/µL, while

hematocrit values were recorded at 27.25% (Romantis et al. 2024). However, these findings were obtained exclusively under intensive rearing systems. To date, no studies have specifically investigated the interaction between sex and rearing systems on hematological parameters in IPB-D3 chickens. Therefore, evaluating hematological responses under different rearing systems and sex variations is essential to better understand the adaptability and physiological resilience of this locally improved chicken line. This study provides new insight into the interaction between rearing system and sex on hematological responses in IPB-D3 chickens. The findings are expected to support the future commercialization of IPB-D3 chickens by improving their adaptability across diverse rearing environments and by contributing to the development of optimized, sex-specific poultry management strategies to enhance health and productivity.

MATERIALS AND METHODS

Ethical statement

The present study was conducted in accordance with animal welfare principles and received approval from the Animal Ethics Committee of the School of Veterinary Medicine and Biomedical Sciences, IPB University, Indonesia, under permit number 209/KEH/SKE/IV/2024.

Animal management and experimental design

The present study involved 60 twelve-week-old IPB-D3 chickens, a dual-purpose line developed by IPB University for both egg and meat production. The chickens were raised for 12 weeks under two rearing systems: intensive and free-range. Each system consisted of 30 birds, with equal numbers of males and females. The nearly equal number of males and females was chosen because the birds were in the growth phase rather than the breeding period, allowing a comparative evaluation of hematological responses between the sexes under different rearing conditions. At the start of the trial, chickens in both rearing systems were in the same growth phase to ensure initial uniformity. A commercial grower feed (B-12 L, PT. New Hope Indonesia, Tangerang, Indonesia) was offered twice daily. The diet contained approximately 14.0% crude protein, 3.0% crude fat, 8.0% crude fiber, 8.0% ash, 0.90–1.20% calcium, and 0.55–1.00% total phosphorus (with phytase enzyme). The amino acid composition included 0.70% lysine, 0.27% methionine, 0.45% methionine + cystine, and 0.17% tryptophan. This commercial feed was provided to chickens reared under both intensive and free-range systems. Ambient temperature and relative humidity in both the intensive (IN) and free-range (FR) housing systems were monitored three times daily (06:00, 12:00, and 17:00) using a digital thermo-hygrometer (HTC-1, Equipslab, China). In the FR system, the device was placed on a wooden platform positioned in a shaded area within the chicken environment to accurately capture the surrounding microclimate. Environmental conditions were maintained within the optimal comfort range for chickens, with temperatures between 20 °C and 30 °C and relative humidity levels of 60–70%. Housing facilities were equipped with feeders, drinkers, perches, and rice husk bedding. Chickens in the intensive system were kept fully confined, whereas those in the free-range system were released daily from 06:00 to 17:00 with access to shaded areas and natural vegetation, including *Indigofera* and wild grasses. Throughout the experimental period, no vaccinations were administered; health management focused on maintaining environmental hygiene, regularly replacing litter, and minimizing stress through gentle handling practices.

Preparation of the free-range and intensive rearing systems

The rearing experiment was carried out at the Laboratory of Animal Breeding and Genetics, Department of Animal Production and Technology, IPB University, Bogor, Indonesia. The free-range rearing area measured 20 m × 10 m (200 m²) and included a 2.5 m × 2.5 m enclosure for nighttime housing. A total of 30 chickens were allocated to the free-range system, resulting in a daytime stocking density of approximately 0.15 birds/m², well below the maximum limit of 1 bird per square meter recommended by the Australian free-range standards (McCormack, 2017). The area was enriched with natural vegetation, including banana trees (*Musa* spp.), *Indigofera*, and various native tropical grasses. These vegetation types, along with the presence of naturally occurring insects and earthworms, may contribute to additional nutrient intake and influence foraging behavior and activity patterns of the chickens. Approximately 4 meters of wooden perches were provided to facilitate roosting and support natural behaviors. The entire area was enclosed with 2-meter-high nylon netting to prevent escape and protect against predators. However, the contribution of natural feed sources, including vegetation, insects, and earthworms, was not quantitatively measured and was considered part of the free-range system conditions. The intensive housing unit measured 6x 1.5 m (9 m²) and accommodated 30 chickens, resulting in a stocking density of approximately 3.33 birds/m². The housing was designed with adequate ventilation and an easy-to-clean floor, and each enclosure was equipped with feeders and

drinkers to ensure animal welfare during the rearing period.

Sample collection

At 24 weeks of age, blood samples were collected from 60 IPB-D3 chickens, comprising 30 birds from each rearing system (intensive and free-range), with equal numbers of males and females per group. This sampling design ensured balanced sex representation within each system, thereby minimizing sex-related variation in hematological parameters. The 24-week sampling point was selected to represent a physiologically mature stage following 12 weeks of exposure to the respective rearing conditions, reducing age-related variability, and enabling standardized comparisons between systems and sexes. Additionally, selecting 30 individuals per group provided sufficient statistical power to detect biologically meaningful differences while adhering to ethical principles of animal use (3Rs: Replacement, Reduction, and Refinement). Approximately 2 mL of blood was collected from the brachial vein using a 3 mL syringe fitted with a 23-gauge needle (0.6×30 mm). The puncture site was disinfected with 70% alcohol prior to venipuncture, and no anesthesia was administered. Blood samples were immediately transferred into ethylenediaminetetraacetic acid (EDTA)-coated tubes to prevent coagulation, then gently inverted to ensure proper mixing with the anticoagulant.

Hematological analyses were performed to evaluate blood composition and cellular profiles according to the protocol described by Onunkwo et al. (2019). Parameters measured included packed cell volume (PCV), erythrocyte count (RBC), hemoglobin concentration (Hb), and total leukocyte count (WBC). Differential leukocyte counts, including lymphocytes (LYM), heterophils (HET), eosinophils (EOS), monocytes (MON), and basophils (BAS), were determined using a Neubauer improved hemocytometer (Marienfeld Superior, Germany) and standard blood smear staining techniques with Giemsa stain (Merck, Darmstadt, Germany). Collectively, these parameters provided comprehensive insights into the physiological status, stress response, and immune function of chickens under different rearing systems.

Variables observed

The variables observed in the present study encompass the following.

Erythrocytes

The number of Erythrocytes was counted using a hemocytometer (Improved Double Neubauer counting chamber, Germany). EDTA blood was diluted with Hayem's solution (France), homogenized using a figure-eight motion, and placed on a hemocytometer for observation under a 10× microscope (Pujiastuti et al. 2017).

Hemoglobin and hematocrit

Hemoglobin and hematocrit levels were measured using the Mindray BC-2800 Vet Auto Hematology Analyzer (Mindray, China), where the sample tube was placed under the nozzle, and results were automatically displayed on the screen.

Erythrocyte index calculation

The erythrocyte indices, including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), are used to evaluate red blood cell (RBC) characteristics and to detect anemia. These indices are calculated using standard formulas based on RBC count, hemoglobin concentration, and hematocrit values (Pujiastuti et al. 2017):

$$\text{MCV} = \frac{\text{Hematocrit (\%}\times 10)}{\text{Erythrocyte (million/}\mu\text{L)}} \quad \text{Formula (1)}$$

$$\text{MCH} = = \frac{\text{Hemoglobin (g/dL)}}{\text{Erythrocyte count (million/}\mu\text{L)}} \quad \text{Formula (2)}$$

$$\text{MCHC} = = \frac{\text{Hemoglobin (g/dL)}}{\text{Hematocrit (\%}\times 10)} \quad \text{Formula (3)}$$

Total leukocytes (leukocyte count)

The leukocyte count was performed using a leukocyte diluting pipette. Blood samples were mixed with Rees and Ecker's solution to stabilize leukocytes. After thorough homogenization of the mixture, the sample was loaded into a Neubauer counting chamber, manufactured by companies such as Marienfeld (Germany) or Thermo Fisher Scientific (USA). Leukocytes were manually counted under a microscope at 400× magnification in four large squares of the chamber. The observed count was multiplied by 50 to calculate the total leukocyte concentration per milliliter of blood. This multiplication factor represents a standard conversion factor for the chamber, accounting for its volume and dilution. (Maheshwari et al. 2017).

Leukocyte differentiation

Leukocyte differentiation was performed by preparing a blood smear, fixing it with methanol (Merck, Germany), staining it with Giemsa solution (Sigma-Aldrich, USA), and rinsing it with distilled water. The prepared slide was examined under a microscope

(Olympus, Japan) at 100× magnification. Leukocyte types, including lymphocytes, monocytes, heterophils, eosinophils, and basophils, were counted using the battlement method. At least 100 cells were counted per slide, and the percentage of each leukocyte type was calculated based on the total cell count (Nurhayati et al. 2019).

Heterophil/Lymphocyte (H/L) ratio

The H/L ratio is calculated by dividing the number of heterophils by the number of lymphocytes observed in the leukocyte differential count. The formula used is:

$$H/L = \frac{\text{Heterfophils (\%/dL)}}{\text{Limphocyte count (million/µL)}} \qquad \text{Formula (4)}$$

Data analysis

Hematological data from IPB-D3 chickens, along with environmental temperature and humidity, were analyzed using Student's t-test to compare mean differences between rearing systems (intensive versus free-range). To assess the effects of rearing system (intensive vs. free-range) and sex on hematological parameters, multiple regression analysis with dummy variables was performed, allowing the evaluation of potential interactions between rearing system and sex. Additionally, Pearson's correlation analysis was used to examine relationships among hematological parameters, providing insight into possible physiological interconnections. All statistical analyses were conducted at the 5% significance level ($P<0.05$), and the assumptions for each test were checked in RStudio 4.4.0. This approach ensured that each analysis was directly linked to the appropriate statistical method, clearly specifying the purpose and model used for each dataset.

RESULTS AND DISCUSSION

Environmental conditions during rearing

The temperature and humidity conditions in the rearing environments of IPB-D3 chickens under the intensive and free-range systems during the study showed significant variation throughout the day. The following table presents the temperature and humidity conditions observed during the study.

As presented in Table 1, midday temperature (12:00 p.m.) in the free-range system (34.56±3.06°C) was significantly higher ($P<0.05$; $P= 0.006$) than in the intensive system (33.46±2.63°C). Similarly, midday humidity differed significantly ($P<0.05$; $P= 0.006$), with higher values in the intensive system (67.69±11.89%) than in the free-range system (62.93±13.12%). No significant differences ($P>0.05$) were detected in temperature or humidity during morning (6:00 a.m.) and afternoon (5:00 p.m.) measurements across both rearing systems.

These findings indicate distinct microclimatic conditions between rearing systems, particularly during midday when the risk of heat stress is greatest. The observed temperature range (25–34°C) exceeded the thermoneutral zone for poultry (20–24°C), potentially impairing physiological functions and productivity (Ulupi & Afnan, 2016). Furthermore, the higher humidity observed in the intensive system is likely associated with limited ventilation in enclosed housing, which can increase thermal load and reduce evaporative heat loss. Humidity levels recorded (90.11%–90.43%) exceeded the optimal range for poultry (40%–60%), potentially increasing the risk of respiratory disorders and reducing productivity (Wolkoff et al. 2021; Li et al. 2023). The combined effect of elevated temperature and

Table 1. Environmental temperature and humidity conditions observed during the study

Parameter	Intensive ($\bar{x}$ ± SEM)	Free-range ($\bar{x}$ ± SEM)	P-value
Temperature (°C)			
Morning (6.00 a.m)	25.23±1.10	25.10±1.68	0.521^{ns}
Midday (12.00 p.m)	33.46±2.63	34.56±3.06	0.006^{*}
Afternoon (5.00 p.m)	28.39±2.20	27.98±2.08	0.169^{ns}
Humidity (%)			
Morning (6.00 a.m)	90.23±5.04	90.43±6.02	0.891^{ns}
Midday (12.00 p.m)	67.69±11.89	62.93±13.12	0.006^{*}
Afternoon (5.00 p.m)	81.70±6.65	81.17±6.86	0.575^{ns}

$\bar{x}$= mean value; SEM= Standard error of the mean; P-value= significance of the t-test between chickens raised under intensive and free-range systems; *= significantly different ($P<0.05$); ns= not significantly different. Temperature and humidity measurements were recorded three times daily throughout the rearing period

humidity may exacerbate heat stress and negatively affect poultry performance (Li et al. 2023). Therefore, these environmental variations highlight the critical importance of microclimate control in maintaining poultry welfare and minimizing heat stress under different rearing systems.

Hematological parameters of IPB-D3 chickens in different rearing systems

Hematological parameters are used to assess the physiological condition and health status of chickens, which are influenced by both genetic and environmental factors. This analysis provides an overview of the hematological profile of IPB-D3 chickens reared under different rearing systems. The indices are presented in Table 2.

The results indicated that none of the measured hematological parameters differed significantly (P>0.05) between IPB-D3 chickens raised under intensive and free-range rearing systems (Table 2). The erythrocyte count averaged 2.84±0.65 × 10^6/mm³ in the intensive system and 2.99±0.61 × 10^6/mm³ in the free-range system. Hematocrit values ranged from 28.60±3.89% in the intensive system to 29.77±4.16% in the free-range system, while hemoglobin concentrations were 9.06±1.22 g/dL and 9.69±1.46 g/dL, respectively. Similarly, no significant differences were observed in erythrocyte indices, including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), between the two rearing systems. MCV values were 104.35±22.15 fl in the intensive system and 103.12±23.02 fl in the free-range system, whereas MCH values were 33.15±6.49 pg and 33.55±7.63 pg, respectively. MCHC values were recorded at 32.08±3.80% for intensive rearing and 32.63±2.72% for free-range rearing.

All measured values fell within or close to the normal physiological ranges for healthy chickens, indicating that differences in rearing systems did not adversely affect the hematological status of IPB-D3 chickens. Erythrocyte count, hemoglobin concentration, and hematocrit are recognized as key indicators of oxygen-carrying capacity and physiological responses to environmental stress (Wang et al. 2018). The stability of these parameters in the present study suggests that IPB-D3 chickens maintained physiological homeostasis despite environmental variation. This finding is consistent with previous reports indicating that IPB chickens possess good adaptability to different rearing conditions (Sumantri et al. 2020). In addition, proper management practices, including adequate ventilation and balanced nutrition, play an important role in maintaining hematological stability in poultry (Fayzrakhmanov et al. 2020; Hollemans et al. 2020).

Leukocyte profile and immune response in different rearing systems

Leukocyte and differential leukocyte counts are essential parameters for assessing the immune response of chickens to environmental conditions and rearing systems. The results of leukocyte and differential leukocyte analysis, including the heterophil to lymphocyte (H/L) ratio, are presented in Table 3.

Table 2. Overview of total erythrocytes, hematocrit, hemoglobin, and erythrocyte indices of IPB-D3 chickens in different rearing systems

Parameter	IPB-D3 Chicken		P-value
	Intensive n=30 ($\bar{x}$ ± SEM)	Free-range n=30 ($\bar{x}$ ± SEM)	
Erythrocyte (10^6/mm ^{3})	2.84±0.65	2.99±0.61	0.374^{ns}
Hematocrit (%)	28.60±3.89	29.77±4.16	0.266^{ns}
Hemoglobin (g/dL)	9.06±1.22	9.69±1.46	0.103^{ns}
Erythrocyte Indices			
MCV (fl)	104.35±22.15	103.12±23.02	0.834^{ns}
MCH (pg)	33.15±6.49	33.55±7.63	0.829^{ns}
MCHC (%)	32.08±3.80	32.63±2.72	0.521^{ns}

N= number of samples; $\bar{x}$= mean value; SEM= standard error of the mean; P-value= significance of the t-test between chickens raised in intensive and free-range systems; *= significantly different (P<0.05); ns: not significantly different

Table 3. Total leukocytes, differential leukocytes, and H/L ratio in the blood of IPB-D3 chickens under different rearing systems

Parameter	IPB-D3 Chicken		
	Intensive n=30 ($\bar{x}$ ± SEM)	Free-range n=30 ($\bar{x}$ ± SEM)	p-value
Leukocyte (10^3 mm^{-3})	19.52±8.83	14.12±6.77	0.010*
Leukocyte Differential			
Lymphocyte (%)	49.55±7.60	46.97±8.07	0.207ns
Heterophil (%)	36.46±7.48	39.66±8.28	0.122ns
Eosinophils (%)	8.58±2.12	7.82±2.70	0.233ns
Monocytes (%)	4.57±2.14	4.71±1.64	0.775ns
Basophils (%)	0.83±0.07	0.83±0.06	0.688ns
Heterophil/Lymphocyte Ratio (H/L)	0.78±0.30	0.90±0.33	0.149ns

N= number of samples; $\bar{x}$= mean value; SEM= standard error of the mean; P-value= significance of the t-test between chickens raised in intensive and free-range systems; *= significantly different (P<0.05); ns: not significantly different

Table 4. Hematological parameters of IPB-D1 and IPB-D2 chickens compared with published reference ranges.

Parameter	IPB D1 Chicken	IPB D2 Chicken	Publish Range	Reference
Erythrocyte (10^6/mm^{-3})	1.95±0.38(1)	1.93-2.59(2)	2.5-3.9	Samour (2015)
Hematocrit (%)	22.40±5.04(1)	27.67-28.44(2)	24-43	Samour (2015)
Hemoglobin (g/dL)	7.25-8.03(1)	9.96-10.89(2)	10.2-15.1	Samour (2015)
Erythrocyte Indices				
MCV (fl)	-	-	90-140	Jain (1993)
MCH (pg)	-	-	33-47	Jain (1993)
MCHC (%)	-	-	26-35	Jain (1993)
Leukocyte (10^3 mm^{-3})	11.68±0.88(3)	15.29±4.28(4)	13-32	Hendro *et al*. (2013)
Leukocyte Differential				
Lymphocyte (%)	65.20±18.20(3)	50.96±11.91(4)	63-73	Hendro (2013)
Heterophil (%)	30.71±18.13(3)	42.76±12.23(4)	20-40	Lokapirnasari *et al*. (2016)
Eosinophils (%)	1-2(3)	1.61±0.9(4)	0-7	Lokapirnasari *et al*. (2016)
Monocytes (%)	4.00±4.48(3)	4.85±0.95(4)	2-8	Lokapirnasari *et al*. (2016)
Basophils (%)	-	1.40±0.61(5)	0-1	Lokapirnasari *et al*. (2016)
Heterophil/Lymphocyte Ratio (H/L)	0.58-0.64(3)	0.48±.39(5)	0.2-0.8	Hendro *et al*. (2013)

(1)Susanti et al. (2020),(2)Khairunnisa et al. (2021); (3)Al-Habib *et al.* (2020); (4)Lestari *et al.* (2025); (5)Falah et al. (2022). *Pu*blished range refers to reference values for normal hematological parameters in chickens from the cited literature

As shown in Table 3, the total leukocyte count differed significantly between rearing systems (P= 0.010). Chickens raised under the intensive system exhibited a significantly higher leukocyte count (19.52±8.83 × 10^3 mm^{-3}) compared with those raised in a free-range system (14.12±6.77 × 10^3 mm^{-3}), indicating a notable hematological response to the production environment. Elevated leukocyte counts in intensively reared chickens may reflect a physiological response to environmental pressure, such as restricted movement and limited air circulation, which can stimulate immune activity (Elsherbeni et al. 2024).

In contrast, the relative proportions of lymphocytes, heterophils, eosinophils, monocytes, and basophils did not differ significantly (P>0.05), suggesting that the distribution of immune cells remained stable across rearing systems. The average lymphocyte proportion was 49.55±7.60% in the intensive system and 46.97±8.07% in the free-range system, while heterophil proportions were 36.46±7.48% and 39.66±8.28%, respectively. Eosinophil, monocyte, and basophil values also remained within normal physiological ranges. The stability of differential leukocyte profiles indicates that, despite differences in total leukocyte counts, the overall immune balance was maintained under both management conditions, which may reflect adaptive physiological responses rather than pathological changes (Kamel et al. 2017).

The heterophil-to-lymphocyte (H/L) ratio did not differ significantly between systems, with values of 0.78±0.30 in the intensive system and 0.90 ± 0.33 in the free-range system (P>0.05). However, both values exceeded the typical physiological threshold of 0.5, indicating a mild stress response in chickens from both rearing environments. This elevation in H/L ratio may be associated with environmental challenges, including fluctuating microclimatic conditions and exposure to external stressors in free-range systems, as well as confinement-related stress in intensive systems (Wlazlak et al. 2023).

Hematology of male and female IPB-D3 chickens under different rearing systems

The hematological status of chickens is influenced by the rearing system and sex, reflecting their physiological condition and response to the environment. The hematological parameters of male and female IPB-D3 chickens under different rearing systems are presented in Table 5.

Table 5 shows that no significant differences (P>0.05) were observed between male and female chickens across all measured hematological parameters in both intensive and free-range systems. In the intensive system, females exhibited numerically higher leukocyte counts (22.64±2.63 × 10^3/mm^3) than males (18.11±2.25 × 10^3/mm^3), although the difference was not statistically significant (p > 0.05). A similar trend was observed in the free-range system, where females also showed slightly higher leukocyte counts (14.87±2.25 × 10^3/mm^3) compared to males (13.27±1.26 × 10^3/mm^3).

This consistent numerical pattern suggests potential sex-related variation in immune responses, despite the absence of statistical significance. Such differences may be influenced by physiological and hormonal factors, as estrogen is known to enhance immune function, whereas androgens tend to exert immunosuppressive effects (Sciarra et al. 2023) . This mechanism may explain the tendency for higher leukocyte counts in females across both rearing systems.

Furthermore, the slightly higher lymphocyte proportions and lower heterophil-to-lymphocyte (H/L) ratios observed in females, particularly under free-range conditions, may indicate a more balanced immune status. Previous studies have also reported that female poultry generally exhibit stronger humoral immune responses than males, although such differences are not always statistically significant (Sellau et al. 2022). In contrast, erythrocyte-related parameters, including erythrocyte count, hematocrit, hemoglobin concentration, and erythrocyte indices (MCV, MCH, MCHC), did not differ between sexes, indicating similar oxygen-carrying capacity in both males and females. Likewise, other differential leukocyte parameters, such as eosinophils, monocytes, and basophils, remained comparable, suggesting that overall hematological stability was maintained regardless of sex. Although the observed differences were not statistically significant, the consistent trend of higher leukocyte counts in females highlights the possibility of subtle sex-related differences in immune function. With a larger sample size, these differences may become statistically detectable, indicating the presence of sex-specific immune responses in IPB-D3 chickens.

Interaction of rearing systems and sex on the hematological profile of IPB-D3 chickens

To understand the interaction between rearing systems and sex on the hematological profile of IPB-D3 chickens, we analyzed hematological parameters. The results presented in Table 6 indicate an interaction between rearing systems, sex, and hematological values in IPB-D3 chickens, with two parameters showing significant effects. The following section provides a detailed analysis of the affected parameters.

The present study demonstrated that the rearing system significantly affected leukocyte count (P= 0.039), while a significant interaction between rearing system and sex was observed for hematocrit (P= 0.038). In contrast, erythrocyte count, hemoglobin concentration, erythrocyte indices, differential leukocyte percentages,

Table 5. Total erythrocyte, hematocrit, hemoglobin, and erythrocyte index parameters of IPB-D3 chickens under different rearing systems based on sex.

Parameter	IPB-D3 Chicken			
	Intensive		Free-range	
	Male n=15 ($\bar{x}$ ± SEM)	Female n=15 ($\bar{x}$ ± SEM)	Male n=15 ($\bar{x}$ ± SEM)	Female n=15 ($\bar{x}$ ± SEM)
Erythrocyte (10^6/mm^{-3})	2.77±0.17	2.80±0.19	3.08±0.18	2.89±0.16
Hematocrit (%)	27.80±1.10	29.33±0.93	32.25±1.14	28.67±0.91
Hemoglobin (g/dL)	8.92±0.40	9.09±0.24	10.55±0.45	9.29±0.28
Erythrocyte Indices				
MCV (fl)	104.19±5.96	108.37±6.24	107.70±5.83	103.38±6.53
MCH (pg)	33.29±1.86	34.04±1.69	35.20±2.09	33.45±2.04
MCHC (%)	32.33±1.25	31.59±0.68	32.76±0.93	32.52±0.67
Leukocyte (10^3 mm ^{3})	18.11±2.25	22.64±2.63	13.27±1.26	14.87±2.25
Leukocyte Differential				
Lymphocyte (%)	48.94±2.07	49.13±2.26	49.44±2.15	45.47±2.21
Heterophil (%)	36.78±1.96	37.84±2.19	37.31±2.21	41.32±2.31
Eosinophils (%)	8.63±0.50	8.25±0.72	8.04±0.76	7.54±0.72
Monocytes (%)	4.80±0.50	3.94±0.68	4.39±0.43	4.83±0.46
Basophils (%)	0.84±0.02	0.84±0.02	0.82±0.02	0.83±0.02
Heterophil/Lymphocyte Ratio (H/L)	0.80±0.08	0.81±0.09	0.79±0.09	0.97±0.09

N= number of samples; $\bar{x}$= mean value; SEM= standard error of the mean

and H/L ratio were not significantly influenced (P>0.05). These findings suggest that IPB-D3 chickens maintain hematological stability across different rearing systems, reflecting strong physiological resilience under varying environmental conditions. Erythrocyte-related parameters are closely associated with oxygen-carrying capacity and are highly sensitive to environmental stress such as temperature and humidity (Wang et al. 2018; Nwaigwe et al. 2020). However, the absence of significant differences indicates that IPB-D3 chickens can maintain stable erythropoiesis despite suboptimal environmental conditions, consistent with previous findings in IPB-derived chickens (Sumantri et al. 2020).

The significant effect of rearing system on leukocyte count likely reflects an adaptive physiological response to environmental conditions, particularly in intensive systems characterized by higher environmental pressure (Elsherbeni et al. 2024). Elevated leukocyte levels may be associated with increased microbial exposure, as reported by Kuswandi et al. (2025), although values remained within normal physiological ranges, indicating adaptation rather than pathological stress. The absence of significant differences in H/L ratio further supports that the chickens were not under severe chronic stress (Kamel et al. 2017). Additionally, the interaction between rearing system and sex on hematocrit suggests sex-specific physiological responses, likely influenced by hormonal differences affecting oxygen transport and immune regulation (Frerichs et al. 2022). Overall, these findings indicate that IPB-D3 chickens are well adapted to different rearing systems, with important implications for sustainable poultry production, although further studies on molecular and stress-related indicators are needed to confirm these adaptive mechanisms.

Relationship between hematological parameters, rearing systems, and sex in IPB-D3 chickens

A correlation heatmap (Figure 1) is presented for hematological parameters in IPB-D3 chickens, rearing systems, and sex. A positive correlation was observed between hemoglobin (Hb) and hematocrit (HCT),

Table 6. Interaction of blood hematology in IPB-D3 chickens under different rearing systems and sexes

Parameter Hematology IPB-D3 Chickens	Intensive and Free-range Housing	Sex	Interaction
Erythrocyte (106/mm 3)	0.834	0.283	0.221
Hematocrit (%)	0.527	0.065	0.038*
Hemoglobin (g/dL)	0.236	0.391	0.097
Erythrocyte Indices			
MCV (fl)	0.878	0.976	0.996
MCH (pg)	0.839	1.000	0.943
MCHC (%)	0.493	0.965	0.745
Leukocyte (103 mm 3)	0.039*	0.560	0.786
Leukocyte Differential			
Lymphocyte (%)	0.147	0.464	0.415
Heterophil (%)	0.091	0.311	0.383
Eosinophils (%)	0.192	0.275	0.492
Monocytes (%)	0.529	0.846	0.547
Basophils (%)	0.980	0.778	0.657
Heterophil/Lymphocyte Ratio (H/L)	0.099	0.320	0.354

The data presented above are derived from Table 5. Values with an asterisk (*) indicate a significant parameter difference (P<0.05)

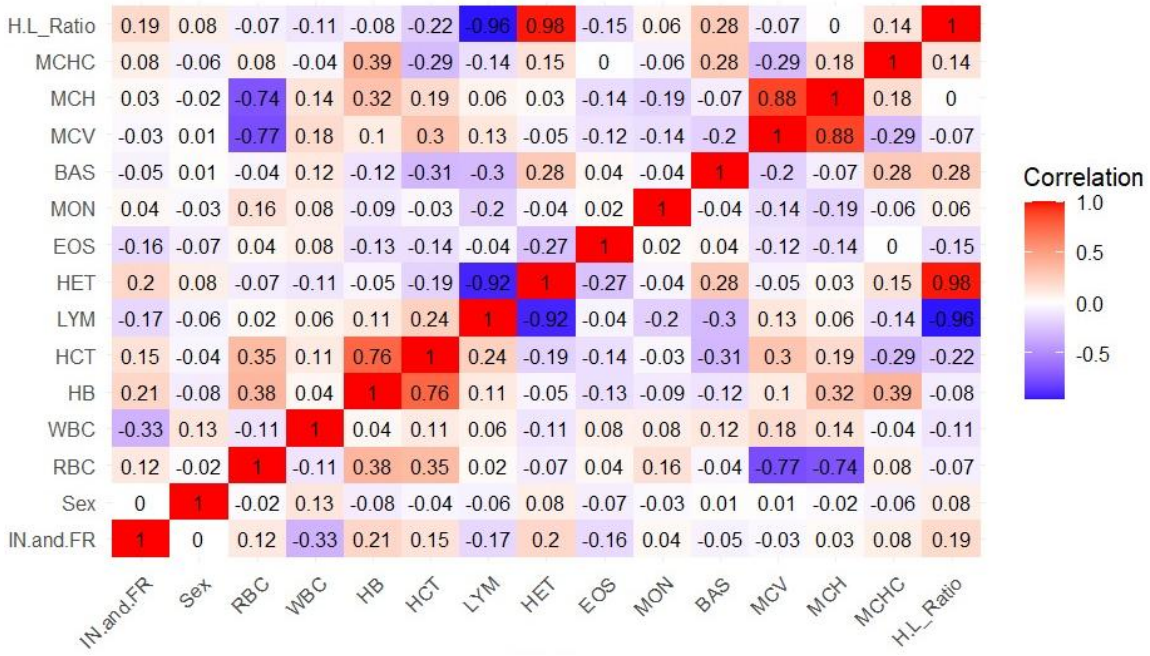

Figure 1: Pearson's correlation between hematological parameters in IPB-D3 chickens. IN and FR= Rearing system (Intensive and Free-range), RBC= Erythrocytes, WBC= Leukocytes, HB: Hemoglobin, HCT= Hematocrit, LYM= Lymphocytes, HET= Heterophils, EOS= Eosinophils, MON= Monocytes, BAS= Basophils, MCV= Mean Corpuscular Volume, MCH= Mean Corpuscular Hemoglobin, MCHC= Mean Corpuscular Hemoglobin Concentration, H.L_Ratio Heterophil/Lymphocyte Ratio.

indicating that higher red blood cell volume is associated with higher hemoglobin levels, reflecting their close physiological relationship in oxygen transport. Similarly, heterophil (HET) counts showed a positive correlation with the H/L ratio, indicating that an increase in heterophils directly increases the H/L ratio, which supports its use as an indicator of stress in chickens.

The correlation analysis revealed several significant relationships among hematological parameters in IPB-D3 chickens, reflecting coordinated physiological regulation between oxygen transport and immune function. A strong positive correlation was observed between hemoglobin (Hb) and hematocrit (HCT) (r= 0.76), indicating a close physiological association in oxygen-carrying capacity. This relationship is expected, as hematocrit represents the proportion of red blood cells in circulation and directly affects hemoglobin concentration and oxygen transport efficiency (Ivanov 2022).

Similarly, heterophils (HET) exhibited a very strong positive correlation with the heterophil-to-lymphocyte (H/L) ratio (r= 0.98), suggesting that variation in the H/L ratio is primarily driven by heterophil dynamics rather than lymphocyte fluctuation alone. Conversely, HET showed a strong negative correlation with lymphocytes (LYM) (r= −0.92), reflecting a classical leukocyte response pattern under physiological stress conditions. Heterophils function as the primary innate immune cells in poultry, responding rapidly to environmental stressors, whereas lymphocytes represent adaptive immune function. Therefore, a shift toward heterophil dominance indicates activation of stress-related immune pathways (Kamel et al. 2017; Thiam et al. 2021).

Erythrocyte count (RBC) was negatively correlated with mean corpuscular volume (MCV) (r= −0.77) and mean corpuscular hemoglobin (MCH) (r= −0.74), suggesting a compensatory regulatory mechanism between erythrocyte number and cell size or hemoglobin content. This inverse relationship may represent an adaptive strategy to maintain efficient oxygen transport under varying physiological demands, where increased erythrocyte numbers are balanced by reduced cell size to optimize blood viscosity and circulation efficiency (Udroiu 2023). Moderate positive correlations were also observed between Hb and MCH (r= 0.38) and between HCT and mean corpuscular hemoglobin concentration (MCHC) (r= 0.35), further supporting the coordinated regulation of erythrocyte characteristics.

In contrast, the correlation between rearing system and sex was weak (r= 0.19), and most other relationships ranged from weak to moderate. This indicates that hematological profiles in IPB-D3 chickens are predominantly regulated by intrinsic physiological mechanisms rather than external management factors. Such stability suggests the presence of effective homeostatic buffering systems that maintain hematological balance despite environmental variation. Although environmental temperatures in this study exceeded the thermoneutral zone for poultry, the stability of erythrocyte-related parameters indicates that IPB-D3 chickens possess efficient physiological adaptation mechanisms to cope with moderate heat stress. These mechanisms may involve regulation of erythropoiesis, blood viscosity, and oxygen transport efficiency to maintain metabolic homeostasis under fluctuating environmental conditions.

It is important to note that the absence of strong correlations involving rearing system and sex may also be influenced by biological variability among individuals, as reflected in the standard error values. Additionally, hematological responses are multifactorial and may require complementary indicators, such as hormonal stress markers (e.g., corticosterone), to fully elucidate physiological stress responses. Overall, these findings demonstrate that hematological parameters in IPB-D3 chickens are regulated by an integrated physiological network that involves erythrocyte dynamics and immune cell balance, enabling adaptive responses to environmental and internal challenges.

CONCLUCION

The rearing system and sex influenced hematological responses in IPB-D3 chickens, with most parameters remaining within normal physiological ranges and not significantly different, indicating strong adaptability across different management conditions. Free-range chickens showed higher erythrocyte-related parameters, while intensively reared chickens exhibited higher leukocyte counts. Females showed slightly higher leukocyte levels, suggesting potential sex-related differences in immune regulation. Overall, IPB-D3 chickens demonstrate good hematological stability across rearing systems.

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