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Grading performance of three-year-old Etawa Grade Buck based on semen quality at The Central Java Artificial Insemination Center, Ungaran

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ABSTRACT

This research aimed to evaluate and rank (grading) individual Etawa Grade buck with the best semen quality at three years of age based on volume, odor, color, viscosity, potential of hydrogen (pH), mass motility, individual motility, abnormality and concentration. The materials used were the semen of six three-year-old Etawa Grade buck at the Central Java Artificial Insemination Center, Ungaran. Semen collection equipment, as well as tools and substances for macroscopic and microscopic evaluation, were used in this research. Artificial vagina techniques were used for semen collection. Data analysis used the One Way ANOVA method and the Kruskal-Wallis method, then the grading of superior buck is determined by breeding value. The results of the semen quality evaluation namely, the average volume, pH and motility were significantly different ($P<0.05$), whereas color, viscosity, odor, mass motility, abnormality and concentration were not significantly different ($P>0.05$). The total breeding value based on the semen quality of each buck from the highest are Garjito (45), Luhung (44), Ardhani (44), Sahasika (44), Bayanaka (43), and Adinata (42). The most superior and best performing three-years-old Etawa Grade buck based on breeding value assessment judged by semen quality is Garjito.

Introduction

Etawa Grade goats are one of the goat breeds developed in Indonesia. Etawa Grade goats originated from a cross between Etawa goats and local goats. Etawa Grade goats are cultivated as dual-purpose livestock producing meat and milk (Syamsi *et al*., 2023). The potential of Etawa Grade goats to produce milk is very high as an alternative to cow's milk (Rokhayati *et al*., 2022). In addition, these animals can also adapt well and have a fairly good reproductive index. However, commercialization of Etawa Grade goats has not been widely carried out due to the limited availability of superior seeds.

The use of superior breeding stock is necessary to produce good quality of Etawa Grade goats. Superior breeding stock or genetic resources are factors that affect livestock performance (Khasanah *et al*., 2020). Therefore, selecting superior males is essential to ensure the inheritance of desirable genetic traits in their offspring. This selection can be performed by evaluating semen quality, which reflects their reproductive performance.

Semen quality is influenced by various factors such as age, health, nutrition, and maintenance management. In particular, the age of livestock has a significant role in determining semen quality (Nahdiyah *et al*., 2020). The quality of fresh semen from Etawa Grade Buck goats in terms of volume, color, pH, odor, viscosity, concentration, mass motility, individual motility and abnormality is as follows – namely 1.15±0.13, white, 6.70±0.29, typical, thick, 3.870±96.8, score 3 (+++), 80.00±0,00 and 7.50±2.52 (Ramadhan *et al*., 2023). Male goats have a sexually mature period usually starting around 8 months of age and mated at 12 months of age (Lubis, 2016). However, generally the peak of semen production with the best quality occurs at a certain age. Therefore, knowing the optimal age for high-quality semen production is important in efforts to improve reproductive efficiency.

Three years of age is considered as the maturity period for male goats, where males are in optimal physiological condition for reproduction. At this age, semen quality is expected to be at its peak. However, studies that specifically examine the semen quality of Etawa Grade buck at this age are still very limited. Previous studies have shown that age can affect various aspects of semen quality, but specific data at the age of three years in Etawa Grade buck still need to be obtained to validate this assumption. Thus, to determine the quality of semen, macroscopic and microscopic examinations of quantity and quality were conducted (Nubatonis *et al*., 2024). Etawa Grade buck studs with the best performance have good semen quality can be used for studs in natural mating and artificial insemination (AI), thus forming better offspring.

The grading of the most superior Etawa Grade bucks is determined by breeding value based on the order of very important to less important parameters related to fertilization. Individual motility is one of the important parameters to determine the ability of spermatozoa to fertilize (Suyadi *et al*., 2020). Good individual motility is around 80% (Laos *et al*., 2021). The breeding value of individual motility consists of five levels, namely 70%-80% is worth 10, 60%-70% is worth 9, 50%-60% is worth 8, 40%-50% is worth 7 and <40% is worth 6.

Mass motility shows the number of live spermatozoa and the forward movement of spermatozoa en masse. Good sperm mass motility in goats is (+++) (Barek *et al*., 2020). The breeding value of mass movement consists of three levels, namely (+++) worth 9, (++) worth 8, and (+) worth 7. Abnormality affects fertilization because abnormal sperm will have difficulty reaching and penetrating the ovum. A spermatozoa abnormality percentage of less than 20% can still be used for artificial insemination (Toelihere, 1993). The breeding value of abnormality consists of 5 levels, namely 1%-5% worth 8, 6%-10% worth 7, 11%-15% worth 6, 16%-20% worth 5, and >20% worth 0.

The higher the concentration of spermatozoa will increase the chances of fertilization because more spermatozoa can meet with the ovum. The normal concentration of goat spermatozoa is around 2.5 - 5.0 x 109

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spermatozoa cells/ml (Mugiyati *et al*., 2017). The breeding value of spermatozoa concentration consists of 6 levels, namely 7, 6, 5, 4, 3, and 2. The assessment of spermatozoa concentration is calculated from the results of the difference between the highest and lowest averages used in the study and divided into 6 groups. Large semen volume has an effect on Artificial Insemination (AI) mating because it is effective for mating more animals. Fresh semen volume of Etawa Grade bucks is about 1.18 ml (Ama *et al*., 2017). The breeding value of semen volume consists of 5 levels, namely 6, 5, 4, 3, and 2. Semen volume assessment is calculated from the difference between the highest and lowest averages used in the study and divided into 5 groups.

Potential of Hydrogen (pH) determines the environmental conditions in which spermatozoa live and move. Normal semen has a pH between 6.0 - 7.0 (Rokana *et al*., 2022). The breeding value of pH consists of 5 groups, namely pH 6.8 - 7.0 is worth 4, pH 6.6 - 6.8 is worth 3, pH 6.4 - 6.6 is worth 2, pH 6.2 - 6.4 is worth 1 and pH 6.0 - 6.2 is worth 0.

Color and viscosity have the same importance rating. Both parameters can indicate the concentration of spermatozoa in the semen. The thicker the semen, the higher the concentration of spermatozoa, resulting in a creamier semen color (Masyitoh *et al*., 2018). The breeding value of color consists of 3 levels, namely cream worth 3, milky white worth 2, and clear worth 0. The breeding value of viscosity consists of 3 levels, namely thick worth 3, medium worth 2, and thin worth 1. Semen odor determines whether or not the semen is suitable for use. Normal semen has a distinctive fishy odor (typical) accompanied by the odor of the animal itself (Ama *et al*., 2017). The breeding value of semen odor consists of 2 levels, namely the typical odor is worth 1 and the odor other than typical is worth 0.

This study aimed to evaluate and rank (grading) individual bucks who have the best semen quality at the age of three years based on volume, pH, individual motility, mass motility, abnormality, and concentration. The benefits of this study, namely to obtain information about the quality of Etawa Grade buck semen at the age of three years and select the best performing males.

Materials and methods

The research was conducted at the Central Java Artificial Insemination Center in Ungaran under the supervision of the Livestock and Animal Health Service Office of Central Java Province, and data analysis was conducted at the Genetics, Breeding and Reproduction Laboratory, Faculty of Animal and Agricultural Sciences Universitas Diponegoro.

Materials

The material used in this study was fresh semen collected from six three-year-old Etawa Grade bucks at the Central Java Artificial Insemination Center in Ungaran. The tools used for semen collection are artificial vagina (AV), tulip tube, and teaser. Tools used for macroscopic observations are scaled tulip tubes and pH indicators. The tools used for microscopic observation are object glass, cover glass, pipette, Minitube Photometer SDM 6 series spectrophotometer, and microscope. Other complementary tools used are tissue, towels, and stationery. The materials used were vaseline, 0.9% NaCl, 2% eosin, and warm water (±40°C).

Methods

The research method used an observational method. This research consists of three stages, namely pre-research stage, research stage, and post-research stage. The pre-research stage included the identification of three-year-old Etawa Grade bucks in Central Java Artificial Insemination Center in Ungaran to determine the scale of the research conducted. The research stage includes semen collection twice a week (Monday and Thursday) with 15 replicates, macroscopic and microscopic observations of semen, and the data collection stage includes macroscopic and microscopic semen quality parameters.

Macroscopic data collection

Volume

Volume measurement can be done by looking at the volume scale found on the tulip tube after collecting fresh semen. The volume of fresh semen of Etawa Grade bucks is about 1.18 ml (Ama *et al*., 2017).

Odor

Semen odor can be observed by smelling the fresh semen of Etawa Grade bucks on the tulip tube after collecting fresh semen. Good fresh semen has a characteristic fishy odor (typical) (Ama *et al*., 2017).

Color

Semen color can be observed by looking directly at the tulip tube after fresh semen collection. Criteria for fresh semen color are cream, milky white, and red or clear.

Viscosity

Semen viscosity can be observed by tilting the tube containing semen to form a 45° angle, then returning to its original position. Normal semen viscosity is thick with a texture slightly thicker than milk (Kusumawati *et al*., 2017).

Potential of Hydrogen (pH)

Measurement of pH can be done using a pH indicator. Semen is dripped on pH indicator paper. Reading the pH number is done every observation by matching the color with the pH color indicator on the package. Normal semen has a pH between 6.4-6.8 (Sekosi *et al*., 2016).

Microscopic data collection

Mass motility

Observation of mass motility by dripping a drop of semen using a pipette into a concave object glass, then the motility of the spermatozoa mass is observed using a microscope with a magnification of 100x for 5 fields of view. Mass motility can be categorized into +++ (large waves, fast, dense), ++ (medium waves, rather slow, sparse), and + (small waves, slow, very sparse).

Individual motility

Individual motility of fresh semen can be determined by dripping a drop of freshly collected semen and 0.9% NaCl using a pipette into an object glass, then closing the cover glass and the progressive movement of individual spermatozoa is observed using a 200x magnification microscope for 5 fields of view. Furthermore, progressive moving spermatozoa were counted using the Hafez formula (2000):

$$\%Motility = (Y-X)/Y \times 100\%$$

Description:

X = Non-motile spermatozoa

Y = Total spermatozoa observed

Abnormality

Abnormality observations were made by dripping a drop of semen

and a drop of 2% eosin and under a microscope with 400x magnification. Abnormality is seen from the abnormal shape of spermatozoa, such as large head shape, no head, broken tail, and circular tail. Furthermore, the percentage of abnormal spermatozoa can be calculated based on the formula of Barek *et al*. (2020):

%Abnormality = SA/Yx100%

Description:

SA = Abnormality spermatozoa

Y = Total spermatozoa observed

Concentration

Concentration observations can be known using the Minitube Photometer SDM 6 series spectrophotometer. The spectrophotometer is used by adding 8 µl (1 analytical pipette drop) of goat semen to the cuvette then adding 4 ml of physiological NaCl and homogenizing with a vortex. Then the cuvette was inserted into the available place on the spectrophotometer, then the livestock code was entered and the enter sign on the screen was pressed.

Quantitative data, such as volume, pH, individual motility, abnormality, and concentration were analyzed using the One Way ANOVA method through Statistical Package for the Social Science (SPSS) software version 26.0. The One Way ANOVA formula is based on the Riduwan (2008) formula listed in table 1.

Qualitative data, such as odor, color, viscosity, and mass motility were analyzed using the Kruskal-Wallis method through SPSS software version 26.0. The Kruskal-Wallis based on the formula of Steel and Torrie (1993):

$$\text{Kruskal-Wallis (K)} = (N-1)\frac{\sum_{i=1}^{g} ni\,(ri-r)^2}{\sum_{i=1}^{g}\sum_{j=1}^{n1} ni\,(rij-r)^2}$$

Description:

ni : Number of observations in the group

rij : Rank (among all observations) of observation j from group i

N : Number of observations in all groups

The references for breeding value assessment is based on a research study by Sutiyono *et al*., (2021), which included individual motility, mass motility, sperm abnormality, concentration of spermatozoa, semen volume, potential of hydrogen, color, viscosity, and semen odor.

Results

Macroscopic characteristics of fresh semen from three-year-old Etawa Grade bucks—including volume, pH, color, viscosity, and odor are presented in table 2. The results showed the significant differences ($P<0.05$) parameter in semen volume ($P=0.0001$) and pH ($P=0.026$) among three-year-old Etawa Grade bucks. The semen volume ranged from 0.85±0.22 mL to 1.81±0.45 mL. The average semen pH ranged from 6.68±0.15 to 6.83±0.07. Meanwhile, there were no significant differences ($P>0.05$) in the color ($P=0.066$), viscosity ($P=0.458$), and odor (1.00). The average semen color was cream, with thick viscosity and a typical odor.

The results of the microscopic evaluation of fresh semen quality from three-year-old Etawa Grade bucks, including individual motility, mass motility, abnormality, and sperm concentration, are presented in table 3. The results showed a significant difference ($P<0.05$) in individual sperm motility ($P=0.0001$), ranging from 52.67 ± 7.99% to 70.00 ± 0.00%. In contrast, no significant differences ($P>0.05$) were observed in mass motility ($P=0.085$), abnormality ($P=0.878$), and sperm concentration ($P=0.071$). The average mass motility of spermatozoa was consistent across all bucks (++). The percentage of abnormal spermatozoa ranged from 7.95±4.44% to 10.49±6.79%, while the sperm concentration ranged

Table 1. One Way Anova formula.

Source of Variance	Sum of Squares	Degrees of Freedom	Mean Square	F ratio
Between/group	SSB/SSG	k-1	SSG/k-1	F = MSG/MSW
Within	SSW/SSE	n-k	SSW/n-1	
Total	SST	n-1		

SSB= Sum of squares between; SSG= Sum of squares groups; SSW= Sum of squares within the groups; SSE= Sum of squares due to error; SST= Total sum of squares; k= Total number of populations; n= Total number of samples in a population; MSB= Mean square between; MSG= Mean square for groups; MSW= Mean square within.

Table 2. Macroscopic quality of fresh semen of three-year-old Etawa Grade Bucks.

Buck's name	Macroscopic Semen Quality				
	Volume*	pH	Color	Viscosity	Odor
Garjito	±0.47[a]	6.69±0.15[ab]	Cream	Thick	Typical
Luhung	1.47±0.28[a]	6.77±0.10[ab]	Cream	Thick	Typical
Adinata	0.85±0.22[b]	6.76±0.12[ab]	Cream	Thick	Typical
Bayanaka	1.81±0.45[a]	6.83±0.07[a]	Cream	Thick	Typical
Ardhani	1.65±0.45[a]	6.68±0.15[b]	Cream	Thick	Typical
Sahasika	1.44±0.20[a]	6.76±0.14[ab]	Cream	Thick	Typical

*Fresh semen volume in ml. [a, b]: Different superscripts indicate significant difference at 5% level.

Table 3. Microscopic quality of fresh semen of three-year-old Etawa Grade Bucks.

Buck's ame	Microscopic Semen Quality			
	Individual Motility	Mass Motility	Abnormality	Concentration
	(%)		(%)	(x 10^6 sel/ml)
Garjito	67.33±7.04[ab]	++	10.49±6.79	3410.07±915.76
Luhung	69.00±5.41[a]	++	9.45±5.81	3391.67±663.92
Adinata	60.00±10.00[bc]	++	10.26±4.76	3711.64±1230.87
Bayanaka	70.00±0.00[a]	++	7.95±4.44	2664.47±957.80
Ardhani	52.67±7.99[c]	++	10.47±6.39	3601.20±1024.56
Sahasika	61.43±12.92[ab]	++	9.87±7.08	3497.86±968.59

[a, b, c]: Different superscripts indicate significant difference at 5% level.

Table 4. Breeding value of three-year-old Etawa Grade Goats.

Semen Quality	Unit	Buck's name					
		Garjito	Luhung	Adinata	Bayanaka	Ardhani	Sahasika
Individual Motility	(%)	9	9	9	10	8	9
Mass Motility		8	8	8	8	7	7
Abnormality	(%)	7	7	7	7	7	7
Concentration	(x 10^6 sel/ml)	6	6	7	2	7	6
Volume	(mL)	5	5	2	6	6	6
pH		3	3	3	4	3	3
Color		3	2	2	3	2	2
Consistency		3	3	3	2	3	3
Odor		1	1	1	1	1	1
Total		45	44	42	43	44	44

from 2664.47±957.80×10^6 cells/mL to 3711.64±1,230.87×10^6 cells/mL.

The results of the study on breeding value based on semen quality in three-year-old Etawa Grade bucks are presented in table 4. The highest total breeding value was recorded for the Garjito buck, with a score of 45. The Luhung, Ardhani, and Sahasika bucks each obtained a score of 44, followed by the Bayanaka buck with a score of 43. The lowest score, 42, was recorded for the Adinata buck.

Discussion

The Bayanaka and Adinata bucks have relatively high average semen volumes, 1.81±0.45 mL and 1.65±0.45 mL. These results are similar to the study by Cahyani *et al.* (2020), which reported around 1.74±0.94 mL, while the lowest average semen volume for the Adinata buck was 0.85±0.22 mL. These semen volume results are still within the normal range according to the research by Zaenuri *et al.* (2021), which found the average ejaculate volume to be 1.0±0.2 mL. The differences in semen volume are due to genetic differences and health conditions among individual males. According to Saputra *et al.* (2019), body weight and scrotal circumference of bucks can affect semen volume. The physical condition of individual bucks can influence the differences in semen volume (Kusumawati *et al.*, 2017).

The Bayanaka buck had the highest average pH, while the Ardhani buck had the lowest (table 2). This research result is still within the normal range, in accordance with the study by Solihati *et al.* (2018), which was around 6.50±0.24, and by Sekosi *et al.* (2016), which was 6.4 - 6.8. The pH of goat semen is influenced by the internal factors of the individual's body. Semen pH is affected by the production of seminal plasma in the seminal vesicles and the ability of semen to control pH, which is related to spermatozoa metabolism. According to Stefanus *et al.* (2021), the more spermatozoa there are, the more lactic acid is produced from spermatozoa metabolism, making semen more acidic or lower in pH.

The average semen color of all Etawa Grade bucks was cream, indicating good semen quality. Normal semen has a cream or milky white color (Susilawati, 2011). Semen color is related to semen viscosity and spermatozoa concentrations. According to Pangestu *et al.* (2021), the more concentrated the semen color, the higher the spermatozoa concentration, and the thicker the semen consistency.

The overall average viscosity of Etawa Grade bucks semen is thick. According to Hastuti *et al.* (2020), normal semen has a thick consistency range. The viscosity of semen can determine the concentration of spermatozoa and color of the semen. Thicker viscosity affects semen color, making it more cream, and increasing the concentration of spermatozoa (Bette *et al.*, 2024).

On average, the semen odor obtained from each Etawa Grade buck was the characteristic smell of typical. As explained by Mugiyati *et al.* (2017), normal goat semen has a distinctive fishy odor. This characteristic fishy odor indicates that the semen is in a normal state and is not contaminated (Ramadhan *et al.*, 2023).

The highest average individual sperm motility was found in the Bayanaka and Luhung bucks, with percentages of 70.00±0.00% and 69.00±5.41%, respectively, which are lower than the average results reported by Hendri *et al.* (2017), where the sperm motility of Etawa Grade bucks aged 2-4 years ranged from 88.8±7.4%. However, these results are still relatively similar to those reported by Pahriadi *et al.* (2022), ranging from 72.5±2.50%. Meanwhile, the Ardhani buck had the lowest motility at 52.67±7.99%. The percentage of individual sperm motility in goats is approximately 70% (Rizal *et al.* 2016). This low motility percentage was caused by internal factors. Individual motility is related to the energy sources and metabolic activity of spermatozoa in the semen (Harissatria *et al.*, 2023).

The average mass motility of spermatozoa in all the males was (++). These data are consistent with those of Febriany *et al.* (2024), who stated that the mass motility of semen given only a basal feed ratio was (++). Mass motility indicates the progressive motility of individual spermatozoa. The more numerous and actively moving progressive spermatozoa, the better semen quality, with dense and rapid movement (Zulyazaini *et al.*, 2016).

The lowest average spermatozoa abnormality was found in Bayanaka at 7.95±4.44% and the highest in Garjito at 10.49±6.79%. These results are consistent with several studies, namely 7.50% (Ramadhan *et al.*, 2023), 11.5±2.16% (Nubatonis *et al.*, 2024), and 9.311±1.57% (Sumadiasa *et al.*, 2017). The percentage of abnormalities in this study was still considered good and was capable of fertilization. According to Hafez and Hafez (2000), a spermatozoa abnormal percentage of 8-10% does not affect fertility, but if it exceeds 25%, fertility is affected.

The average semen concentration in this study was lowest in Bayanaka, at 2664.47±957.80 x10^6 cells/mL, while the highest was found in Adinata, at 3711.64±1230.87 x10^6 cells/mL. These results are higher than those reported by Hendri *et al.* (2017), where the spermatozoa concentration in 2–4 year old Etawa Grade bucks was approximately 2074±262.2 x10^6 cells/mL, but lower than the average spermatozoa concentration reported by Rasad *et al.* (2019), which was 3968×10^6 cells/mL. The concentration in each individual was within the normal range. Evans and Maxwell (1987) stated that normal spermatozoa concentrations range from 2500 to 5000×10^6 cells/mL.

The results of total breeding value based on quality semen may indicate that Garjito has superior performance to other bucks for natural or artificial mating. Bucks that get the highest breeding value in the group indicate the potential for superior production performance that can be passed on to their offspring (Tribudi *et al.*, 2021). The selection process to obtain superior livestock needs to pay attention to the quality of spermatozoa from males. Hendri *et al.* (2017) stated that superior males have the performance to produce good quality semen so that they can fertilize females. Therefore, although Bayanaka bucks have a high majority of breeding value, the parameters of concentration and consistency get the

lowest value so that it affects the overall quality of the semen.

Although most semen parameters were within the normal range, there were significant individual variations that must be considered in the selection of males. For exanple, Bayanaka buck had high volume and pH but low concentration, resulting in a lower total breeding value than Garjito. This confirms that selection cannot rely on a single parameter, but rather on a combination of all semen aspects. However. This study was limited to a small sample size (six males) and a single location in Central Java Artificial Insemination Center, Ungaran, so the generalization of the results is still limited. Further research with a larger sample size, involving different ages and locations is needed to strengthen these findings.

Conclusion

Among the three-year-old Etawa Grade bucks, Garjito showed the best performance and semen quality, as indicated by the highest estimated breeding value. Further studies are needed to support buck selection strategies aimed at producing superior offspring.

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Conflict of interest

The authors have no conflict of interest to declare.

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