

## Effects of seasons and environmental conditions on semen quality of Senduro goats reared under tropical climate

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**Abstract:** A study was carried out to examine the effects of seasons and environmental conditions (ambient temperature; AT, relative humidity; RH, temperature-humidity index; THI, and sunshine hours; SH) on semen quality of Senduro goats in the tropical climate. A total of 252 ejaculates semen were collected from 3 Senduro bucks from January to December 2017. The semen quality was then evaluated including semen volume (VOL), sperm concentration (CONC), total sperm output (TSO), individual motility (IMOT), before freezing motility (BFMOT), postthawing motility (PTMOT). The acceptance rate of fresh semen (ARFS), before freezing semen (ARBFS), and postthawing semen (ARPTS) were also calculated. The results showed the seasons had a significant effect ( $P < 0.05$ ) on CONC, IMOT, and PTMOT, with the higher value, was recorded during rainy than the dry season. It was observed that the Senduro buck was tolerable with the change in AT, RH, THI, and SH because most of the semen traits remain unchanged ( $P > 0.05$ ) among these groups. Moreover, it was interesting that the maximum PTMOT was achieved at high AT and THI. In conclusion, the rainy season has more preferred ejaculate semen than the dry season, but the semen collection is still acceptable to be carried out throughout the year. This study also suggests that Senduro bucks have good adaptability to tropical conditions.

**Keywords:** Artificial insemination, semen cryopreservation, Indonesian native goats, tropical area

### 1. Introduction

Goats have great potential to be developed as meat producers. In Indonesia, the mutton production in 2018 is 66.9 thousand tons, which is still much lower than beef production that reaches about 496.3 thousand tons [1]. One type of meat-producing goat in Indonesia is Senduro goat. This native goat has several advantages including high and long body postures, high productivity, high fertility rates, and have prolific births. However, the business scale of Senduro goat is still limited to the traditional management and breeding system, therefore advance technology such as artificial insemination (AI) is needed to increase productivity.

AI is a technology to improve genetic quality and increase the livestock population. AI could help farmers to get superior livestock that has high productivity. However, the success of AI will greatly depend on the semen quality of male sire. Several factors may affect semen quality of goats including seasons [2–4], and environmental conditions [5]. To date, information on the semen quality evaluation of Senduro bucks is still limited. Therefore, this study

aimed to evaluate the effects of seasons and environmental conditions on the semen quality of Senduro buck raised in the tropical climate.

### 2. Materials and methods

#### 2.1. Study location

The experiment was carried out at the Singosari National AI Center (Malang, Indonesia). The research farm is situated in a tropical region, with the coordinate between 7.84°S latitude and 112.65°E longitude on 816 m above mean sea.

#### 2.2. Environmental data

The records of environmental data were taken from Malang Climatological Station (Malang, Indonesia). The data show average daily rainfall, ambient temperature (AT), relative humidity (RH), and sunshine hours (SH) during the periods of the study (January to December 2017).

The average daily rainfall was used to determine the rainy and dry seasons. The rainy season is started when the cumulative rainfall for 10 days is greater than or equal to

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50 mm which is then followed by the next period. On the other hand, the dry season is begun when the cumulative rainfall for 10 days is lower than 50 mm which is then followed by the next period. Based on these prerequisites, January, February, March, April, November, and December were categorized as the rainy season, while the dry season includes May, June, July, August, September, and October.

To study the effect of AT, the average of  $23.94 \pm 0.07$  °C during the whole period was divided into  $< 23$  °C,  $23-24.5$  °C, and  $> 24.5$  °C. A similar model was also used to evaluate the effect of RH. Limits of relative humidity  $< 70\%$ ,  $70-80\%$ , and  $> 80\%$  were determined based on the average RH of  $79.98 \pm 0.38\%$ . The data of AT and RH were then used in the temperature-humidity index (THI) calculation with a formula =  $(0.8 \times AT) + [(RH / 100) \times (AT - 14.4)] + (46.4)$ . The average THI during the whole experimental period was  $73.18 \pm 0.11$  which then divided into limits of  $< 71$ ,  $71-73.5$ , and  $> 73.5$ . Next, for a purpose of SH effect evaluation, groups of  $< 3.5$  h,  $3.5-7$  h, and  $> 7$  h were determined from the average of  $5.5 \pm 0.20$  h.

### 2.3. Animals and diets

A total of 3 Senduro bucks aged at 2- to 4-years-old were included in this study. The bucks were kept under similar management conditions as per standard protocol in the breeding station. Each buck was placed in individual pens and fed 3 kg *Indigofera* sp. leaves, 3 kg *Pennisetum purpureum* cv. Mott, 0.25 kg *Gliricidia maculata* or *Calliandra calothyrsus* leaves, and 0.5 kg commercial concentrate feed daily. The concentrate feed contained 18% crude protein and 75% total digestible nutrient. The drinking water was provided *ad libitum*.

### 2.4. Semen collection, processing, and evaluation

This study has been performed as part of routine semen collection in the Singosari National AI Center so that ethical approval was not necessary. The semen was collected from each buck twice a week with an artificial vagina device (IMV Technologies, France), totaling 252 ejaculates semen throughout a year.

The semen volume (VOL) was measured directly upon collection using a scaled vial. After that, 8  $\mu$ l of semen was diluted in 4 mL physiological saline (0.9% NaCl) and then homogenized using a vortex for 15 s. The solution was then inserted into a cuvette and the sperm concentration (CONC) was measured using a spectrophotometer at 640 nm of wavelength. The total sperm output (TSO) was calculated by  $VOL \times CONC$ . Next, a drop of semen was placed on an object-glass using a micropipette and subsequently covered using cover glass. The individual motility (IMOT) of buck's sperm was then assessed under a light microscope at 400 $\times$  magnification. The semen with IMOT greater than or equal to 60% was accepted for further processing.

The accepted ejaculate was then diluted with Andromed® extender and adjusted to the final concentration of 50

$\times 10^6$  cell/straw. The semen was then filled into a mini straw (0.25 ml) using filling and sealing machine (IMV Technologies, France) followed by equilibration in cool top storage ( $3$  to  $5$  °C) during 3 to 4 h. After that, the semen sample was taken and before freezing motility (BFMOT) was evaluated using the same method as IMOT. At this step, the semen with BFMOT greater than or equal to 55% was accepted for freezing processing.

The approved batch was then frozen to  $-140$  °C using the freezer apparatus (IMV Technologies, France) and then placed into container storage with liquid nitrogen ( $-196$  °C). Shortly after frozen, the semen sample was taken, thawed at  $37$  to  $38$  °C for 15 to 30 s and evaluated for postthawing motility (PTMOT) using exactly the same method as IMOT. Only ejaculate batch with PTMOT greater than or equal to 40% was accepted.

### 2.5. Statistical analysis

The data were subjected to statistical analyses using SPSS 13.0 software. The semen variables were then assessed for normality using Kolmogorov-Smirnov test. The data of CONC was found normally distributed; therefore, it was analyzed using one-way analysis of variance. When the statistical significance was found, the group means were further compared using Duncan test. On the other hand, the data of VOL, TSO, IMOT, BFMOT, and PTMOT were not found normally distributed so that they were further analyzed using Kruskal-Wallis test and followed by Mann-Whitney test when statistical significance was found. The ARFS, ARBFS, and ARPTS were measured as the binary trait (0 = not accepted, 1 = accepted) and compared using chi-square test. After statistical analysis, the data of the acceptance rate were then presented as frequencies.  $P < 0.05$  and  $P < 0.01$  were considered to be statistically significant and highly significant, respectively.

## 3. Results

In this study, the overall mean VOL, CONC, TSO, IMOT, BFMOT, and PTMOT were  $1.86 \pm 0.04$  ml,  $2.53 \pm 0.05 \times 10^9$  cell/ml,  $4.78 \pm 0.15 \times 10^9$  cell/ml,  $51.69 \pm 1.37\%$ ,  $54.05 \pm 0.40\%$ , and  $38.20 \pm 0.52\%$ , respectively. In addition, the overall ARFS, ARBFS, and ARPTS were 60.32%, 53.17%, 39.29%, and 24.48%, respectively. The effect seasons and environmental conditions (AT, RH, THI, and SH) on semen characteristics and the acceptance rate of ejaculate are presented below.

### 3.1. Effect of seasons

This study shows that the seasons had a significant effect ( $P < 0.05$ ) on CONC, IMOT, and PTMOT with the higher value was recorded during rainy than the dry season (Table 1 and 2). Whereas, the VOL, TSO, and BFMOT were similar ( $P > 0.05$ ) between 2 seasons. The seasons of semen collection also did not indicate a statistically significant change ( $P > 0.05$ ) although the rainy season had 11.48% increases in the ARPTS (Figure).

**Table 1.** Values (mean  $\pm$  standard error) for semen volume, sperm concentration, and total sperm output of Senduro bucks as affected by the seasons and environmental conditions

Items	Parameters		
	VOL	CONC	TSO
<i>Seasons</i>			
Rainy	1.80 $\pm$ 0.06	2.67 $\pm$ 0.07 <sup>b</sup>	4.88 $\pm$ 0.21
Dry	1.94 $\pm$ 0.06	2.36 $\pm$ 0.06 <sup>a</sup>	4.66 $\pm$ 0.21
<i>AT</i>			
< 23	1.75 $\pm$ 0.10	2.38 $\pm$ 0.11	4.27 $\pm$ 0.35
23 - 24.5	1.96 $\pm$ 0.07	2.63 $\pm$ 0.06	5.16 $\pm$ 0.22
> 24.5	1.78 $\pm$ 0.06	2.45 $\pm$ 0.08	4.46 $\pm$ 0.22
<i>RH</i>			
< 70	1.66 $\pm$ 0.19	2.42 $\pm$ 0.18	4.12 $\pm$ 0.58
70 - 80	1.86 $\pm$ 0.05	2.43 $\pm$ 0.06	4.60 $\pm$ 0.18
> 80	1.89 $\pm$ 0.07	2.64 $\pm$ 0.07	5.04 $\pm$ 0.25
<i>THI</i>			
< 71	1.81 $\pm$ 0.11	2.31 $\pm$ 0.10	4.31 $\pm$ 0.36
71 - 73.5	1.99 $\pm$ 0.09	2.61 $\pm$ 0.08	5.24 $\pm$ 0.29
> 73.5	1.79 $\pm$ 0.05	2.53 $\pm$ 0.06	4.58 $\pm$ 0.17
<i>SH</i>			
< 3.5	1.93 $\pm$ 0.10	2.63 $\pm$ 0.09 <sup>b</sup>	5.17 $\pm$ 0.33
3.5 - 7	1.75 $\pm$ 0.06	2.68 $\pm$ 0.08 <sup>b</sup>	4.77 $\pm$ 0.24
> 7	1.93 $\pm$ 0.07	2.28 $\pm$ 0.06 <sup>a</sup>	4.44 $\pm$ 0.19

VOL: Semen volume (ml/ejaculate), CONC: Sperm concentration ( $10^9$  cell/ml), TSO: Total sperm output ( $10^9$  cell/ejaculate).

AT: Ambient temperature ( $^{\circ}$ C), RH: Relative humidity (%), THI: Temperature-humidity index, SH: Sunshine hours.

<sup>a-c</sup> Different superscript letters within the same factor show significant differences between the means ( $P < 0.05$ ).

### 3.2. Effect of ambient temperature

Table 1 shows that AT had no significant effect ( $P > 0.05$ ) on VOL, CONC, and TSO. The IMOT also did not significantly affect ( $P > 0.05$ ) by AT, however, the BFMOT and PTMOT were significantly influenced ( $P < 0.05$ ) (Table 2). It was observed that the peak BFMOT and PTMOT were achieved when AT  $> 24.5^{\circ}$ C. No significant effect ( $P > 0.05$ ) of AT was found on the acceptance rate of ejaculate, but the maximum value was achieved at high AT ( $> 24.5^{\circ}$ C) (Figure).

### 3.3. Effect of relative humidity

There were no significant effects ( $P > 0.05$ ) of RH on VOL, CONC, and TSO (Table 1). Similar results ( $P > 0.05$ ) were also observed on the motility traits (Table 2). The acceptance rate of ejaculate also did not differ ( $P > 0.05$ )

**Table 2.** Values (mean  $\pm$  standard error) for individual motility, before freezing motility, and postthawing motility of Senduro buck's sperm as affected by the seasons and environmental conditions

Items	Parameters		
	IMOT	BFMOT	PTMOT
<i>Seasons</i>			
Rainy	53.87 $\pm$ 1.83 <sup>b</sup>	54.71 $\pm$ 0.41	39.15 $\pm$ 0.62 <sup>b</sup>
Dry	49.09 $\pm$ 2.04 <sup>a</sup>	53.17 $\pm$ 0.74	36.84 $\pm$ 0.86 <sup>a</sup>
<i>AT</i>			
< 23	49.38 $\pm$ 3.69	52.20 $\pm$ 1.29 <sup>a</sup>	37.84 $\pm$ 1.01 <sup>a</sup>
23 - 24.5	50.08 $\pm$ 1.96	53.71 $\pm$ 0.58 <sup>a</sup>	37.08 $\pm$ 0.89 <sup>a</sup>
> 24.5	55.06 $\pm$ 2.19	55.26 $\pm$ 0.51 <sup>b</sup>	39.55 $\pm$ 0.69 <sup>b</sup>
<i>RH</i>			
< 70	45.45 $\pm$ 6.92	53.33 $\pm$ 1.67	35.40 $\pm$ 2.23
70 - 80	51.64 $\pm$ 1.92	54.31 $\pm$ 0.56	38.49 $\pm$ 0.72
> 80	52.35 $\pm$ 2.04	53.82 $\pm$ 0.60	38.10 $\pm$ 0.78
<i>THI</i>			
< 71	46.47 $\pm$ 4.01	52.11 $\pm$ 1.64	38.07 $\pm$ 1.21 <sup>ab</sup>
71 - 73.5	51.89 $\pm$ 2.26	53.70 $\pm$ 0.64	37.04 $\pm$ 0.87 <sup>a</sup>
> 73.5	52.93 $\pm$ 1.90	54.75 $\pm$ 0.48	38.93 $\pm$ 0.73 <sup>b</sup>
<i>SH</i>			
< 3.5	52.73 $\pm$ 2.44	53.23 $\pm$ 0.79	37.28 $\pm$ 1.09
3.5 - 7	52.98 $\pm$ 2.20	55.09 $\pm$ 0.47	38.69 $\pm$ 0.76
> 7	49.42 $\pm$ 2.49	53.72 $\pm$ 0.78	38.49 $\pm$ 0.87

IMOT: Individual motility (%), BFMOT: Before freezing motility (%), PTMOT: Post-thawing motility (%).

AT: Ambient temperature ( $^{\circ}$ C), RH: Relative humidity (%), THI: Temperature-humidity index, SH: Sunshine hours.

<sup>a-b</sup> Different superscript letters within the same factor show significant differences between the means ( $P < 0.05$ ).

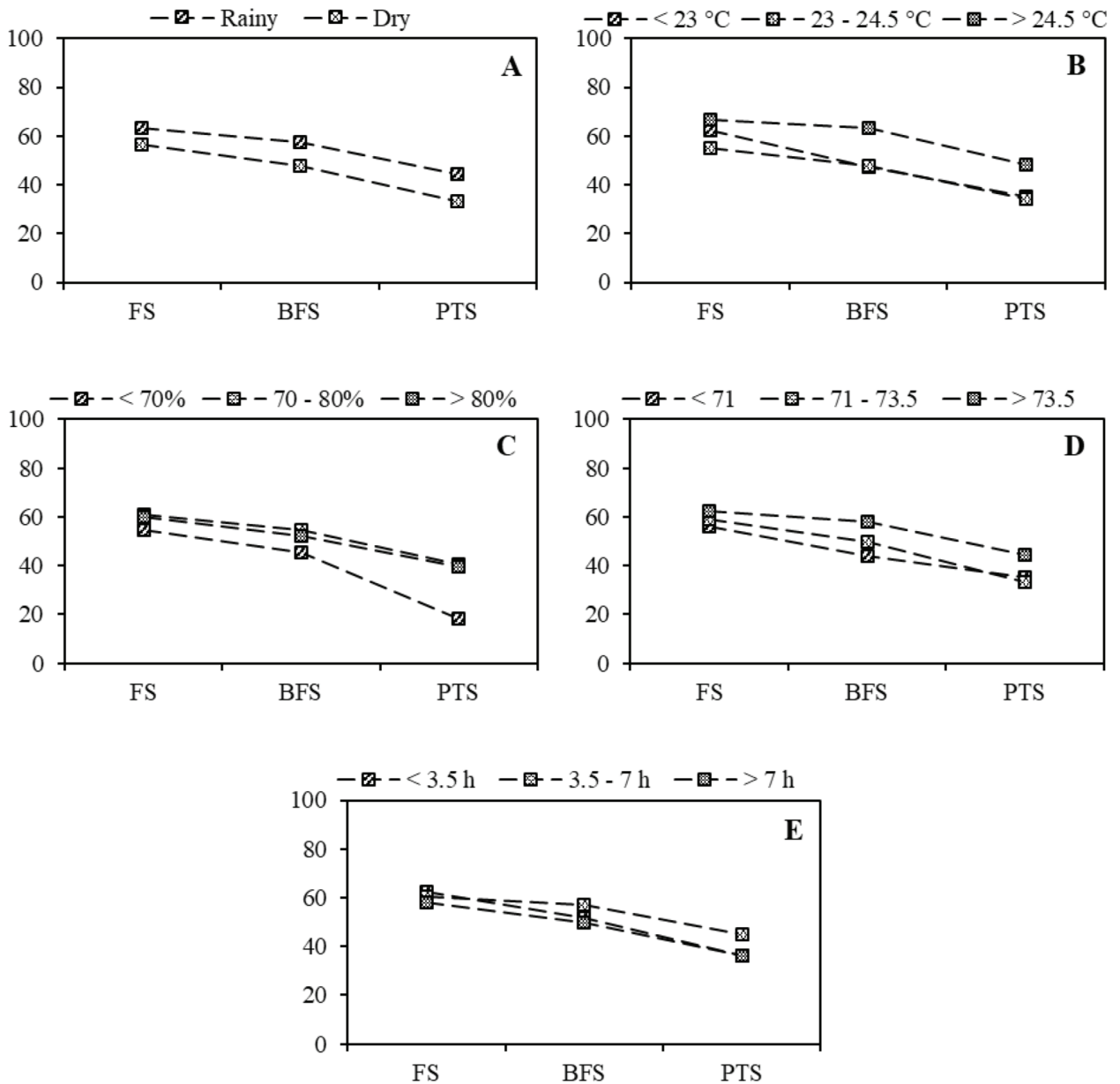
among the three groups of RH although ARPTS decrease by 22.04% at RH  $< 70\%$  (Figure).

### 3.4. Effect of temperature-humidity index

Table 1 shows that THI had no significant effect ( $P > 0.05$ ) on VOL, CONC, and TSO. The IMOT and BFMOT also similar ( $P > 0.05$ ) among THI groups, whereas, PTMOT was significantly differed ( $P < 0.05$ ) among groups, with the maximum value was recorded when THI  $> 73.5$  (Table 2). As can be seen in Figure, the THI did not influence ( $P > 0.05$ ) the acceptance rate of ejaculate. The peak value was observed at high THI ( $> 73.5$ ).

### 3.5. Effect of sunshine hours

This study showed that SH did not significantly affect ( $P > 0.05$ ) all semen variables (Table 1 and 2). No significant



**Figure.** Acceptance rate (%) of Senduro buck's ejaculate as affected by seasons (A), ambient temperature (B), relative humidity (C), temperature-humidity index (D), and sunshine hours (E). FS: Fresh semen, BFS: Before freezing semen, PTS: Postthawing semen.

difference ( $P > 0.05$ ) of acceptance rate of ejaculate was also observed among SH groups although the ARPTS was increased at SH 3.5–7 by 8.74% (Figure).

#### 4. Discussion

In this study, the semen collection of Senduro bucks seems to be more profitable when conducted during the rainy season compared to dry season as indicated by higher CONC, IMOT, and PTMOT. This current finding is in corresponds with other studies which also revealed that the bucks had higher sperm concentration and motility

in the rainy season than dry season [6,7]. The higher sperm concentration in the dry season as compared to dry season also previously reported in other livestock such as cattle and buffalo [8, 9]. In temperate areas, the change in semen quality was mainly affected by the difference in photoperiod among seasons [10–12]. Meanwhile, in tropical areas, the photoperiod remains unchanged throughout the year so that it could not be considered as an essential factor affecting seasonal variation in semen quality. Moreover, this current study expected that SH could provide alternative insight to explain the seasonal

change in semen quality. However, the results were negligible because no significant change was recorded among SH groups on all observed parameters.

In the previous report, it was elucidated that the decrease of sperm concentration and motility during the dry season was strongly affected by the elevated AT, RH, and THI [13–15]. In the case of AT, their elevation during a certain period could induce heat stress which led to testicular degeneration resulting in the reduction of sperm quality [16,17]. However, this current study shows that AT, RH, and THI exhibit no significant effect on most semen characteristics; even the PTMOT reached the maximum point at high AT (> 24.5 °C) and THI (> 73.5). This unexpected result indicates that Senduro bucks have good adaptability to the environmental change in the tropical climate.

Besides the environmental conditions, the forage availability also reported as another factor affecting the semen characteristics change between seasons [18]. The forage availability was abundant during the rainy season and become limited in the dry season, where the later will be followed by a reduction in the reproductive

performance [11]. However, since all of the bucks used in this study were reared in the breeding station with constant feeding throughout the year, the abovementioned mechanism also does not fit to explain this current finding. Therefore, it is still questioning why the rainy season had better sperm concentration and motility than the dry season. Nonetheless, this result did not limit the semen collection to be only conducted in the rainy season because the acceptance rate of ejaculate was similar between two seasons.

This current study clearly demonstrates that the rainy season has more preferred ejaculate semen than the dry season, but the semen collection is still acceptable to be carried out throughout the year. This study also highlights that Senduro bucks have good adaptability to the change in ambient temperature, relative humidity, temperature-humidity index, and sunshine hours in the tropical climate.

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