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Original article

Factors associated with repeat breeding in Holstein Friesian cows at the Tani Wilis Village Cooperative Unit, Sendang District, Tulungagung Regency, Indonesia

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ABSTRACT

This study aimed to evaluate the association of factors such as nutrition, thermal humidity index (THI), age, and lactation period with the occurrence of repeat breeding of dairy cows. The sample consisted of Holstein Friesian cows aged 1-11 years, with lactation periods ranging from 1 to 7 months, currently in lactation, and experiencing repeat breeding (inseminated three or more times without conception, despite exhibiting normal estrous cycles and intervals). The results showed that feeding practices by farmers did not significantly differ ($p > 0.05$) in relation to the incidence of repeat breeding. THI values recorded in several barns also showed no significant differences ($p > 0.05$), with the cows experiencing light to moderate heat stress. Similarly, the frequency of artificial insemination and the estrous cycle intervals remained within the normal range. The highest incidence of repeat breeding was observed in cows aged 2.5 to 5.5 years, accounting for 78.13%, and during lactation periods of 1-3 months, accounting for 81.26%. It can be concluded that there were no significant differences in the provision of forage and leguminous feed between normal cows and those with repeat breeding, and the crude protein content of forage did not influence its occurrence. Additionally, there was no indirect relationship between THI and repeat breeding. However, the incidence of repeat breeding tended to increase in cows aged 3-4 years, and during the second and third lactation periods.

Keywords: age, lactation period, protein intake, repeat breeding, thermal humidity index

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INTRODUCTION

Reproductive health is a critical determinant of dairy cattle productivity, as disturbances in reproduction can significantly reduce fertility rates and overall milk production efficiency (Bello *et al*., 2012). One of the most prevalent reproductive disorders with substantial economic implications in dairy farming is repeat breeding, a condition in which a cow with a normal estrous cycle fails to conceive after three or more artificial inseminations (Carbonari *et al*., 2024). Ideally, a reproductively mature cow that displays clear signs of estrus should conceive following insemination, maintain pregnancy, and calve approximately 270 days later, with subsequent insemination typically scheduled around 40–50 days postpartum (Syah *et al*., 2024). In practice, however, many cows require multiple inseminations across several cycles, with repeat breeding recognized as one of the major underlying causes of this inefficiency (Cardoso Consentini *et al*., 2021).

Nutritional status plays a pivotal role in reproductive performance and herd productivity. Among the modifiable factors influencing fertility, suboptimal nutrition is one of the most significant. Inadequate or imbalanced diets can impair ovarian function, disrupt hormonal balance, and hinder early embryonic development, thereby contributing to an increased incidence of repeat breeding (Shukla and Shrivastava, 2024). As global demand for milk rises and dairy production intensifies (Bojovic and McGregor, 2023), ensuring optimal reproductive success through proper nutrition is increasingly vital. In particular, understanding the impact of specific nutrients, especially protein, on repeat breeding may support the development of more targeted nutritional interventions to enhance fertility and herd sustainability (Sundrum, 2020).

Environmental stress, particularly heat stress resulting from high ambient temperatures and humidity, also plays a critical role in reproductive inefficiency. The thermal humidity index (THI) integrates temperature and humidity into a single metric to evaluate environmental comfort. Elevated THI values are associated with heat stress, which negatively affects physiological responses such as feed intake and metabolic regulation, leading to reduced milk yield and compromised reproductive function (Moore *et al*., 2024; Țogoe and Mincă, 2024). In Indonesia, dairy production is largely based on Holstein Friesian (HF) cattle, a breed developed in temperate regions under optimal conditions, approximately 18°C and 55% relative humidity. In tropical climates, FH cows often face suboptimal conditions, and efforts to mitigate heat stress include raising cattle at altitudes above 800 meters above sea level (Asmarasari *et al*., 2024). However, the ability of dairy cows to adapt to such environmental stressors remains a major challenge for smallholder farmers striving for sustainable dairy production in the tropics (Oloo *et al*., 2023).

In addition to environmental and nutritional factors, cow age is also believed to influence reproductive success. Age-related declines in fertility, as well as physiological immaturity in younger cows, may contribute to variation in conception rates (Kusaka *et al*., 2023). A more comprehensive understanding of the relationship between age and repeat breeding can improve reproductive management strategies and enhance cattle welfare (Pérez-Marín and Quintela, 2023). (Pérez-Marín and Quintela, 2023). Therefore, this study aims to investigate the relationships among cow age, lactation period, THI, and protein intake with the incidence of repeat breeding in HF cows.

MATERIALS AND METHODS

This study employed a survey-based observational approach. Primary data were collected through interviews with respondents and direct observation of farmer practices. Secondary data were obtained from farmers affiliated with the Tani Wilis Village Unit Cooperative in Sendang, Tulungagung. The research was conducted in three stages, 1) identification of the relationship between cow

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age and lactation period with the incidence of repeat breeding, 2) measurement of the THI and its association with repeat breeding, and 3) evaluation of the correlation between protein intake and the occurrence of repeat breeding.

Nutrition

The survey gathered data on the amounts of forage (*Pennisetum purpureum*), legumes (*Gliricidia sepium* and *Calliandra calothyrsus*), and concentrate feed (Wilis Feed) provided by farmers. Based on feeding patterns, cows were categorized into three groups: Group I received 50 kg of elephant grass, 5 kg of legumes, and 5 kg of concentrate; Group II received 35 kg of elephant grass, 14 kg of legumes, and 8 kg of concentrate; and Group III received 30 kg of elephant grass, 28 kg of legumes, and 7 kg of concentrate. The crude protein content and dry matter contents of the feed ingredients were determined using the Kjeldahl method (Table 1).

Table 1 Dry matter and **c**rude protein content of forages and Wilis Feed concentrate (Kjeldahl method)

feedstuff	dry matter (%)	crude protein (%)
Pennisetum purpureum	25.91	5.42
Calliandra calothyrsus	35.76	9.71
Gliricidia sepium	34.86	7.26
Wilis concentrate	90.16	14.95

Thermal humidity index

The study involved 15 pens housing dairy cows diagnosed with repeat breeding. All pens had comparable feeding practices, consisting of forage and concentrate, but differed in comfort levels as indicated by varying THI values. Sampling was conducted in stages, beginning with the identification of pens containing cows affected by repeat breeding. Prior to data collection, ambient temperature and relative humidity were recorded in each of the 15 pens. Measurements were taken every two days, with each pen assessed three times to obtain an average value. Data collection was performed between 10:00 am and 1:00 pm local time using a thermohygrometer, which was placed inside each pen for 7-10 minutes. Thermal humidity index was calculated using the formula described by Thom and Rhoades (1959): $THI = 0.8 \times T + (RH / 100) \times (T - 14.4) + 46.4$, where T is the ambient temperature in degrees Celsius (°C) and RH is the relative humidity (%).

Age and lactation period

Respondent selection was conducted using a random area sampling technique. Eligible participants were farmers who owned dairy cows diagnosed with repeat breeding, defined as cows that had failed to conceive after at least three artificial insemination (AI) attempts, despite exhibiting regular estrous cycles and intervals. This sampling method follows the recommendation of Cooper and Emory, as cited in Heraini *et al*. (2019), which suggests that a minimum sample size of 30 is sufficient to assume The final sample included 32 HF cows aged 1 to 11 years, all in lactation (1–7 months), and experiencing repeat breeding.

Data analysis

Associations between cow age, lactation period, and the incidence of repeat breeding were analyzed using the Chi-square test, followed by a binomial test. Path analysis was performed using SmartPLS4 software to examine the relationship between THI and repeat breeding. Nutritional intake data were analyzed using the independent samples Chi-square test in Statistical Product and Service Solution (SPSS). Statistical significance was set at $p < 0.05$ for all analyses.

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RESULTS

A total of 38 HF cows experiencing repeat breeding were included in this study. These cows were distributed across three feeding regimen patterns and housed in 15 different pens based on the THI. Among the cows, 32 had recorded ages ranging from 1 to 10.5 years, while the remaining six lacked age data.

Analysis of the feeding patterns used by farmers revealed no significant differences (p >0.05) in the incidence of repeat breeding (Table 2). Similarly, the recorded THI values across the pens showed no significant differences (p >0.05), corresponding to conditions of light to moderate heat stress (Table 3). The average frequency of artificial insemination (AI) and the interval between estrous cycles also remained within normal ranges. The highest prevalence of repeat breeding was found in cows aged >2.5 to 5.5 years, accounting for 78.13% of cases (Table 4). Additionally, 81.26% of repeat breeding cases occurred in cows within 1 to 3 months of the lactation period (Table 5).

Table 2 Distribution of repeat breeder cows based on feeding and protein consumption at the Tani Wilis cooperative

feeding pattern (EG : L : C)	protein intake (gram/cow/day)	repeat breeding
50 kg : 5 kg : 5 kg	53.49 ± 5.82 [a]	15.38% (2/13)
35 kg : 14 kg : 8 kg	73.38 ± 17.30 [a]	16.67% (2/12)
30 kg : 28 kg : 7 kg	109.38 ± 26.09 [ab]	15.38% (2/13)

EG: elephant grass; L: legume; C: concentrate; Chi-square analysis indicated that there was no significant difference (p >0.05) on the percentage of repeat breeding among protein consumption.

Table 3 Distribution of repeat breeder cows based on THI at the Tani Wilis cooperative

THI category	THI	frequency of AI	estrus interval (days)
light (75-78)	77.65 ± 0.39	3.33 ± 0.52	21.33 ± 1.37
moderate (79-83)	79.18 ± 0.97	4.10 ± 0.99	20.80 ± 1.32

THI: thermal humidity index; Path analysis using SmartPLS4 showed no significant relationship between THI and repeat breeding.

Table 4 Distribution of repeat breeding cases by cow age at the Tani Wilis cooperative

age (years)	repeat breeding
1 - 2.5	3.13% (1/32) [c]
>2.5 - 3,5	25.00% (8/32) [ab]
>3.5 - 4.5	37.50% (12/32) [a]
>4.5 - 5.5	15.63% (5/32) [abc]
>5.5 - 6.5	6.25% (2/32) [bc]
>6.5 - 7.5	3.13% (1/32) [c]
>7.5 - 8.5	3.13% (1/32) [c]
>8.5 - 9.5	3.13% (1/32) [c]
>9.5 - 10.5	3.13% (1/32) [c]

Different superscripts (a, b, c) indicate significant differences (p <0.05) using the Chi-square test.

Table 5 Distribution of repeat breeding cases by lactation period at the Tani Wilis cooperative

lactation period	repeat breeding (%)
1st	15.63% (5/32) [a]
2nd	31.25% (10/32) [a]
3rd	34.38% (11/32) [a]
4th	9.38% (3/32) [b]
5th	6.25% (2/32) [b]
7th	3.13% (1/32) [b]

Different superscripts (a, b) indicate significant differences between groups (p <0.05).

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DISCUSSION

Repeat breeding in dairy cows present a complex etiopathogenesis due to the multitude of contributing factors. Broadly, the causes of repeat breeding can be grouped into two categories, fertilization failure and early embryonic death (Carbonari *et al*., 2024). Interestingly, cows exhibiting repeat breeding often show better estrus expression, similar follicular diameters, and higher estrogen levels compared to normal cows, suggesting that the etiology may lie outside these parameters. However, a shortened proestrus phase and diminished luteinizing hormone (LH) secretion patterns, particularly preceding the LH surge, are potential contributors to reproductive failure in these animals (Sood *et al*., 2010). Several factors have been identified in association with repeat breeding, including elevated progesterone levels, abnormal follicular dynamics, delayed ovulation, and reduced oocyte quality. These factors are further influenced by a range of risk factors that contribute to hormonal imbalances. These include age, parity, body condition, milk production level, environmental stressors, as well as peripartum and postpartum physiological changes (Pérez-Marín *et al*., 2023).

In high producing dairy cows, there there is often a reduction in the secretion of gonadotropin hormones, trigerring a cascade of events that result in abnormal ovarian cycles (Hassanein *et al*., 2024). A concurrent decline in estrogen production contributes to weak or absent estrus behavior, leading to lower reproductive efficiency. High-yielding cows typically exhibit reduced plasma estradiol concentrations, which impairs stimulation of the neural centers regulating estrus behavior (Endo, 2022). Prolonged lactation further stimulates the pituitary gland to increase the production of prolactin (LTH) for milk synthesis. Elevated LTH inhibits the release of follicle-stimulating hormone (FSH), suppresses follicular development, reduces estrogen and LH secretion, and ultimately results in delayed or failed ovulation, key contributors to repeat breeding (Setyorini *et al*., 2023). Cows producing more than 38 liters of milk per day experience ovulation delays that are 2.6 times longer than those producing less than 29 liters per day (Lopez *et al*., 2005; Gross, 2023).

Nutrition

Nutrition plays a pivotal role in dairy cattle husbandry. The purpose of providing feed is essential not only for fulfilling basic metabolic requirements but also for supporting growth and optimizing milk production (Miller-Cushon and DeVries, 2017). Forage intake in dairy cows typically ranges between 30–50 kg per day. The amount of concentrate feed provided is proportional to milk yield, with a general ratio of 1 kg of concentrate per 3 liters of milk during lactation (Hansen *et al*., 2022). Supplementing rations with concentrates is aimed at meeting the increased nutritional demands of lactation, thereby enhancing productivity (Dineen *et al*., 2021). However, feed quality, particularly its nutritional content, is equally important. Inadequate nutrition can impair reproductive performance and contribute to fertility disorders (Nipane *et al*., 2021). In dairy cows, fat mobilization and protein catabolism in the absence of sufficient nutrient intake can lead to the depletion of essential fatty acids and amino acids required for reproductive function. These deficiencies may trigger endocrine disruptions in the pituitary–hypothalamic–ovarian axis, affecting the secretion of key hormones such as estrogen, gonadotropins, LH, and progesterone. Consequently, this results in alterations in the biochemical profiles of ovarian follicles, oocytes, the corpus luteum, and the uterus, ultimately leading to reduced conception rates and repeat breeding (Sammad *et al*., 2022).

According to BSN (2009), the recommended nutritional composition for dairy cattle are as follows, 1) pre-calving cows: 7% crude fat, 10% ash, and 15% crude protein; 2) lactating cows: 16% crude protein, 10% ash, and 7% crude fat; 3) dry cows: 14% crude protein, 10% ash, and 7% crude fat. Protein intake data collected from three farmer groups (each consisting of two

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farmers) revealed the following values, 1) 49.37 g/cow and 57.60 g/cow; 2) 85.61 g/cow and 61.15 g/cow; 3) 168.28 g/cow and 50.48 g/cow. These results suggest that protein consumption in all groups met basic nutritional needs. The concentrate feed used by Tani Wilis, contains 14.95% crude protein with a dry matter content of 90.16%. However, for high producing dairy cows, concentrate feeds should ideally contain at least 18% crude protein and approximately 4.75 Mkal/kg of dry matter (Sulistyowati *et al*., 2020).

Thermal humidity index

Based on the intervals between estrous cycles, the sampled cows generally exhibited normal reproductive cycles, averaging approximately 21 days. However, pregnancy did not occur even after three or more inseminations. The study results, derived from each test conducted, indicated no significant relationship between the THI and repeat breeding. Das *et al*. (2016) reported that an elevated THI can induce heat stress in cows, negatively affecting hormonal production associated with metabolism, such as cortisol. In stressed dairy cows, the hypothalamic-pituitary-adrenal (HPA) axis is activated in response to environmental stimuli perceived as threats. This response begins with nerve signals that stimulate the hypothalamus to release corticotropin-releasing hormone (CRH), which then promotes the secretion of adrenocorticotropic hormone (ACTH), ACTH subsequently stimulates the production of glucocorticoids, including cortisol (Koenneker *et al*., 2023).

Elevated cortisol levels can inhibit the secretion of gonadotropin-releasing hormone (GnRH), reducing the production of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in the ovaries (Hassanein *et al*., 2024). Suppression of FSH and LH can result in failure to fertilize immature ova. The repeated inability to conceive, followed by a return to estrus, is referred to as repeat breeding. Cortisol also impacts target organs by increasing catabolism of body fat and protein, thereby elevating blood glucose levels (Dovolou *et al*., 2023). Persistently elevated cortisol diminishes protein and fat reserves, which are crucial components for milk production and reproductive hormones. As a result, both milk yield and reproductive performance decrease in animals subjected to chronic stress (Grelet *et al*., 2022). Heat stress caused by uncomfortable environmental conditions can reduce feed intake, leading to an inadequate nutritional status (Chen *et al*., 2024). The relationship between reproduction and nutritional status in cows is closely interconnected. Nutritional deficiencies or insufficient nutrient intake can directly affect reproductive efficiency, resulting in poor reproductive performance and reduced productivity (Shukla and Shrivastava, 2024).

Age and lactation

The lactation period significantly influences seriivces per conception (S/C), days open (DO) and calving interval (CI) in dairy cows. As lactation progresses, the S/C value tends to increase (Ulfah *et al*., 2022). The normal S/C range is 1.6-2, while cows experiencing repeat breeding often show an elevated S/C value of 3 (Muhuruna *et al*., 2023). An S/C value below two indicates that a cow can still calve once a year. However, an S/C value above two can hinder the achievement of the ideal calving interval, and indicates reduced reproductive efficiency. This extended calving interval can be costly for breeders due to repeated artificial insemination procedures (Musriati *et al*., 2024).

Both younger and older cows generally exhibit lower reproductive success rates. In younger animals, the reproductive system may not yet be fully developed, lowering the chances of successful conception after insemination (Iwata, 2016; Butkiewicz *et al*., 2024). In contrast, older cows often experience reduced ovulation quality and quantity, along with hormonal imbalances that impair fertility (Alvarez *et al*., 2023). Recognizing how age impacts repeat breeding helps breeders and reproductive specialists adopt appropriate preventive and management strategies. These may include optimizing breeding schedules

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based on age, improving reproductive management, and performing regular health checks (Arningdiah *et al*., 2024). Such approaches can enhance the efficiency of artificial insemination programs and improve reproductive success, thereby supporting sustainable production and farm profitability.

CONCLUSIONS

There was no observed difference in the provision of forage and leguminous feed between normal dairy cows and those experiencing repeat breeding, and the crude protein content of forage did not appear to influence the occurrence of repeat breeding. An indirect relationship was noted between the temperature-humidity index and repeat breeding. The incidence of repeat breeding was higher in cows aged 3 to 4 years and during the second and third lactation periods. Further research is recommended to evaluate the levels of reproductive hormones involved in fertilization failure and early embryonic death, which are considered primary causes of repeat breeding.

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AUTHORS' CONTRIBUTIONS

Dimas Kunto Satrio (DKS), M' Izi Kumala Lazuardi Sultoni (MIKLS), Tasya Apritalia Putri (TAP), Dadik Rahardjo (DR), Djoko Legowo (DL), Gandul Atik Yuliani (GAY), Iwan Sahrial Hamid (ISH), Mohammad Anam Al-Arif (MAA), Pudji Srianto (PS), Soeharsono Soeharsono9 (SS), Sri Pantja Madyawati (SPM), Sunaryo Hadi Warsito (SHW), Tita Damayanti Lestari (TDL), Wurlina Wurlina (WW)

DKS, MIKLS, TAP: conceived the study, designed the framework of the manuscript, and conducted data acquisition, analysis and interpretation. DR, DL, GAY: contributed to manuscript drafting. IWS, MAA, PS, SS, SPM, SHW, TDL, WW: critically reviewed and revised the manuscript for important intellectual content. All authors read and approved the final version of the manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

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