



Effect of Ketosis on Reproductive Efficiency and Delayed Conception in Friesian Holstein Heifers

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ABSTRACT

Background: This study aimed to determine whether ketosis during early lactation correlates with low reproductive efficiency in Friesian Holstein-fitted heifers on tropical dairy farms. An internationally acclaimed case, a group of 38 heifers aged 24-36 months from a dairy farm in a government farm in Banyumas, Central Java, Indonesia, was prospectively followed.

Methods: On day 7-30 postpartum, the BHBA levels were measured using a validated portable ketone meter operating on an electrochemical enzymatic principle and the animals were grouped into a standard (1.2 mmol/300 ml), subclinical ketosis (1.2-2.9 mmol/300 ml) and a clinical ketosis (3.0 mmol/300 ml) based on the results. The outcomes measured in reproduction were conception rate (CR), services/conception (SC) and days to conception (DC), obtained from records of insemination and pregnancy.

Result: Subclinical and clinical ketosis prevalences were 36.8% and 15.8%, giving a total ketosis prevalence of 52.6%. Pregnancy rate declined with increasing ketosis severity (77.8% in normal, 35.7% in subclinical, 16.7% in clinical) and heifers with normal BHBA showed markedly better reproductive performance (SC 1.5±0.5; DC 95±12 days) than ketotic animals (clinical: SC 3.4±0.9; DC 163±21 days; $p<0.01$). These findings indicate that elevated BHBA in early lactation is strongly associated with impaired fertility in replacement heifers, supporting the need for regular BHBA monitoring and optimized nutrition to reduce ketosis-related reproductive losses.

Key words: Breeding interval, Dairy replacement cattle, Fertility outcomes, Metabolic status, β -hydroxybutyrate.

INTRODUCTION

Among livestock commodities, dairy cows play a strategic role in meeting Indonesia's growing demand for milk. National fresh milk production in 2024 was estimated at approximately 808 thousand tons, which remains insufficient to meet domestic consumption needs (BPS, 2025; Fauzi *et al.*, 2020). To reduce dependence on imports, the Indonesian government has targeted domestic milk production to supply at least 60% of national demand (Kementan, 2022). Achieving this target requires not only increased milk yield per cow but also improved reproductive efficiency to ensure sustainable herd regeneration.

Reproductive performance is a key determinant of dairy farm productivity, influencing calving interval, herd replacement rate and overall economic efficiency (Berry *et al.*, 2014; Diskin and Kenny, 2014). Age at first calving is a critical indicator of heifer reproductive management, reflecting the effectiveness of growth, nutrition and health programs (Stevenson, 2002; Santos *et al.*, 2024). Although the optimal age for first calving is generally targeted at around 24 months, delayed conception remains a common issue under field conditions, particularly in heifers older than two years. This condition, often referred to as late breeding, increases non-productive periods, feed costs and negatively affects farm profitability (Blanc and Agabriel, 2008; Wathes *et al.*, 2014; Hufana-Duran and Duran, 2020).

One of the metabolic factors suspected to contribute to delayed conception is ketosis. Ketosis is characterized by negative energy balance and elevated blood ketone bodies, particularly β -hydroxybutyrate (BHBA) and is most

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prevalent during early lactation (Guliński, 2021). In dairy cows, ketosis has been associated with reduced feed intake, loss of body condition, decreased milk yield and impaired reproductive performance (Wathes *et al.*, 2008; Lei and Simões, 2021). Elevated BHBA levels have been shown to disrupt reproductive hormone secretion, follicular development and early embryo survival, thereby increasing the risk of conception failure (Mellado *et al.*, 2018; Melendez and Serrano, 2024).

Early detection of ketosis is essential for effective metabolic and reproductive management. Measurement of blood BHBA concentration is widely used as a practical biomarker to identify cows at risk of metabolic imbalance before the onset of severe clinical symptoms (Nielsen *et al.*, 2005). Factors such as early lactation stage, body

condition score at calving and previous metabolic disorders are known to increase susceptibility to ketosis and subsequent reproductive inefficiency (Jeong *et al.*, 2017). Although numerous studies have examined the relationship between ketosis and fertility in multiparous dairy cows, empirical data on Friesian Holstein (FH) heifers aged 24-36 months, particularly under tropical production systems in Indonesia, remain limited.

Therefore, this study aimed to evaluate the effect of early lactation ketosis on reproductive efficiency in 24-36-month-old Friesian Holstein heifers by examining the association between blood BHBA levels and key reproductive indicators, including conception rate, services per conception and days to conception. It was hypothesized that increased BHBA levels would be associated with reduced reproductive performance, reflected by lower conception rates, increased insemination requirements and prolonged time to conception. The findings of this study are expected to provide evidence-based insights for improving metabolic and reproductive management strategies in dairy heifers, thereby supporting the sustainability of dairy production systems in tropical environments.

MATERIALS AND METHODS

Ethical approval

All animal treatment procedures in this study have received ethical approval from the Perhimpunan Dokter Hewan Indonesia (PDHI), the Indonesian Veterinary Medical Association, Central Java II Branch, Indonesia, under number 023/EA/PDHI/II/2025.

Location and subject of the research

This study was conducted at the Center for Superior Livestock Breeding and Forage (BBPTU-HPT), Baturraden, Banyumas, Central Java, Indonesia, from February to July 2025. The research subjects were first-lactation Friesian Holstein (FH) heifers aged 24-36 months during the transition period between calving and early lactation (approximately 7-30 days postpartum). All cows included in the study were clinically healthy and had no history of infectious reproductive diseases based on farm medical records and veterinary examinations.

Exclusion criteria included heifers with reproductive anatomical abnormalities (ovarian hypoplasia or uterine aplasia) and heifers undergoing treatment for other metabolic diseases. Cows were selected using purposive sampling based on their suitability for inclusion and the availability of the population at the study site. Homogeneity of the rearing environment (housing, feed management and health management) was maintained because all animals came from a single farm unit managed under a uniform management system.

Cows were fed elephant grass (*Pennisetum purpureum*) as the primary source of crude fiber. Concentrates were formulated as a source of energy and protein sources to meet metabolic and milk production

requirements, in accordance with BBPTU-HPT standards. Drinking water was provided ad libitum to ensure adequate body fluids and support optimal physiological function.

All heifers were maintained under uniform housing, feeding and health management systems throughout the experimental period to minimize management-related variation.

Design of the study

This was an analytical observational study using a prospective cohort design. None of the experimental treatments and manipulations aimed at inducing or sustaining cows in a ketosis state. The status of ketosis was determined from measured b-hydroxybutyrate (BHBA) levels at a single time point during the initial lactation phase (7-30 days postpartum) and used to classify animals. Follow-up of the BHBA measurement was done according to the following circumstances:

1. Confirmed pregnancy was, or
2. At the time the observation period comes to an end (as long as the study period itself).

Therefore, exposure (ketosis status during lactation) was quantified at conception, whereas reproductive outcomes (pregnancy rate, inseminations per pregnancy and days to conception) were measured afterward. This design will enable the determination of the time effect on the relationship between early lactation status, metabolism and reproductive efficiency and one will not be required to ensure that animals remain in a given state of ketosis. Diagnosis of ketosis is usually done through testing of ketosis by measuring the blood concentration of BHBA (Guliński, 2021). As one of the key ketone bodies in circulation, BHBA is considered a key biomarker of ketosis.

Study variables

The independent variable was ketosis status, which was determined based on blood BHBA levels (Ahrens *et al.*, 2025) and classified as follows (Jeong *et al.*, 2017).

1. Normal: BHBA <1.2 mmol/L.
2. Subclinical ketosis: BHBA 1.2-2.9 mmol/L.
3. Clinical ketosis: BHBA ≥3.0 mmol/L.

Ketosis status was determined based on BHBA measurements from a single blood sample taken 7-30 days postpartum and used as an exposure category in the analysis. Dependent variables reflecting reproductive efficiency include.

1. Conception rate (CR): Percentage of heifers confirmed pregnant 50-60 days after artificial insemination (AI).
2. Services per conception (SC): Number of AI required to achieve conception.
3. Days to conception (DC): The interval (in days) from the first AI to confirmed pregnancy.

Blood sampling

Blood samples were collected once from each cow during the 7-30 days postpartum period, in the morning before feeding. Approximately 5 mL of blood was obtained from

the jugular vein using anticoagulant vacuum tubes and analyzed within 30 minutes after collection to ensure metabolite stability. Some whole blood was used immediately to measure BHBA levels in the barn using a portable blood ketone meter and these measurements were used as the basis for grouping ketosis status (normal, subclinical and clinical).

Assessment of ketosis

Ketosis status was assessed based on blood β -hydroxybutyrate (BHBA) concentrations measured using a portable blood ketone meter (FORA 6, ForaCare Inc., Taiwan). This method has been previously validated and shows a high correlation with laboratory-based enzymatic reference assays ($r>0.9$) (Ahrens *et al.*, 2025). Ketosis status was recorded at the time of measurement and used as an exposure variable in the statistical analysis (Iwersen *et al.*, 2009).

Data on reproductive status

Reproductive performance data, including artificial insemination records and pregnancy outcomes, were obtained from farm records at BBPTU-HPT. First-service conception rate (FSCR) and days to conception (DC) were used to evaluate time to conception, with pregnancy confirmed 50-60 days after insemination through routine veterinary examination.

Evaluation of reproductive performance

For each heifer, the number of SC (total AI until pregnancy) and final pregnancy status during the observation period were recorded. The DC value is calculated as the difference in days between the date of the first AI and the date of confirmed pregnancy, including all subsequent AI until conception occurs. Cows that are not pregnant by the end of the observation period are recorded as not pregnant in the CR and FSCR calculations. Pregnancy confirmation is performed 50-60 days after the last AI.

Data analysis

BHBA levels were categorized into three ketosis status groups: Normal (<1.2 mmol/L), Subclinical (1.2 - 2.9 mmol/L) and Clinical (≥ 3.0 mmol/L). The effect of ketosis status on conception rates (CR and FSCR) was analyzed using the Chi-square test. The mean SC and DC values between ketosis groups were compared using a one-way ANOVA test after the assumptions of normality and homogeneity of variance were met. All tests were two-tailed, with a

statistical significance level set at $p<0.05$. Results are presented as frequency distributions, mean $\pm$ standard deviation (mean $\pm$ SD), or median (interquartile range), depending on the data distribution.

RESULTS AND DISCUSSION

Ketosis status in heifers over two years old

The distribution of ketosis status based on β -hydroxybutyrate (BHBA) levels in Friesian Holstein heifers is shown in Table 1. Of the 38 heifers observed, 18 (47.4%) were in the normal metabolic category (BHBA <1.2 mmol/L), 14 (36.8%) were classified as having subclinical ketosis (BHBA 1.2 - 2.9 mmol/L) and 6 (15.8%) were classified as having clinical ketosis (BHBA ≥ 3.0 mmol/L). The proportion of heifers with normal status was the largest, followed by the subclinical group, while the clinical group was the smallest proportion in the study population.

When the subclinical and clinical groups were combined, the total incidence of ketosis (total ketosis) reached 52.6% of the heifer population examined. These results indicate that more than half of heifers aged >2 years at the study site had BHBA levels above the normal range. This distribution forms the basis for further analysis of the relationship between ketosis status and reproductive performance indicators in the same group of animals.

Relationship between ketosis status and conception rate

The relationship between ketosis status at the beginning of lactation and pregnancy success is presented in Table 2. Of the 38 heifers observed, 20 (52.6%) were confirmed pregnant during the observation period. The highest conception rate was found in the group with normal metabolic status, while the lowest was in the group with clinical ketosis. In sequence, the pregnancy rate decreased from 77.8% in heifers with normal BHBA to 35.7% in heifers

Table 1: Classification of ketosis status in heifers based on β -hydroxybutyrate levels.

Ketosis status	BHBA levels (mmol/L)	Number of heifers	Percentage
Normal	<1.2	18	47.4
Subclinical	1.2 - 2.9	14	36.8
Clinical	≥ 3.0	6	15.8
Total	-	38	100

Source: Data processing of research results (2025).

Table 2: Pregnancy rate of heifers based on ketosis status.

Ketosis status	Number of heifers	Pregnant (n)	Non-pregnant (n)	Pregnancy rate (%)
Normal	18	14	4	77.8
Subclinical	14	5	9	35.7
Clinical	6	1	5	16.7
Total	38	20	18	52.6

Source: Data processing of research results (2025).

Table 3: Relationship between ketosis status and SC and DC values in heifers.

Ketosis status	SC (Mean±SD)	DC (Days±SD)
Normal	1.5±0.5	95±12
Subclinical	2.1±0.7	121±15
Clinical	3.4±0.9	163±21

Source: Data processing of research results (2025).

with subclinical ketosis and only 16.7% in heifers with clinical ketosis.

The Chi-square test showed that the difference in the distribution of pregnant and non-pregnant animals between the three groups was statistically significant ($\chi^2 = 9.29$; $df = 2$; $p = 0.0096$). These results indicate a significant relationship between early lactation ketosis status and the chance of pregnancy, with a consistent pattern of decline in conception rate as the degree of ketosis increases.

Services per conception (SC) and days to conception (DC)

The relationship between ketosis status and pregnancy efficiency as measured by *services per conception* (SC) and *days to conception* (DC) is shown in Table 3. There is a consistent pattern of increased SC values and prolonged DC as metabolic status worsens: heifers with normal status require an average of approximately 1.5 inseminations with an interval of ± 95 days to become pregnant, while in subclinical and clinical ketosis, the need for insemination increases and the time to conception is prolonged to approximately 2.1 and 3.4 inseminations with DCs of ± 121 and 163 days, respectively. A one-way ANOVA test showed that the differences in SC and DC between ketosis status groups were statistically significant ($p < 0.01$), so it can be concluded that the degree of ketosis at the beginning of lactation is associated with a decrease in the efficiency of the pregnancy process in Friesian Holstein heifers.

The influence of Ketosis on reproductive efficiency and delayed conception in dairy

Results of this study clearly show that ketosis status at the beginning of lactation is closely related to a decrease in the reproductive efficiency of 24-36-month-old Friesian Holstein heifers. Heifers with normal metabolic status had a significantly higher conception rate (CR) and considerably lower services per conception (SC) and days to conception (DC) values compared to heifers with subclinical or clinical ketosis. The decrease in CR from 77.8% (normal) to 35.7% (subclinical) and 16.7% (clinical), accompanied by a significant increase in SC and prolongation of DC ($p < 0.01$), indicates that the degree of hyperketonemia at the beginning of lactation is an essential determinant of delayed conception in FH heifers in tropical environments.

The total ketosis prevalence of 52.6% in the heifer population in this study is relatively high when compared to a global meta-analysis of lactating dairy cows. This study reported an average subclinical ketosis prevalence of approximately 22.7% with regional variations (Loiklung *et al.*,

2022). Even when considering only subclinical ketosis (36.8%), the figure is still above the average. Compared with the report by Biswal *et al.* (2016) on dairy cows in India, the prevalence of clinical ketosis in this study (15.8%) was indeed lower than the 27.2% reported. However, the high proportion of subclinical cases indicates that negative energy balance disorders in the transition phase occur not only in multiparous cows but also in replacement groups (Zhou *et al.*, 2025).

This confirms that first-lactation heifers should be considered a high-risk group for metabolic disorders with important reproductive consequences. The observed decline in conception rate and increase in services per conception and days to conception in ketotic heifers are consistent with previous reports indicating that both clinical and subclinical ketosis impair estrus expression, ovulatory function and conception success (Mellado *et al.*, 2018; Rutherford *et al.*, 2016; Li *et al.*, 2020). Although most evidence originates from multiparous cows, the magnitude of reproductive impairment observed in this study suggests that similar metabolic–reproductive interactions also occur in young Friesian Holstein heifers under tropical production conditions.

The association between elevated BHBA levels and reduced reproductive performance is biologically plausible and supported by previous mechanistic studies. Hyperketonemia has been linked to impaired gonadotropin secretion, altered follicular development, reduced oocyte quality and compromised uterine environment, ultimately increasing the risk of conception failure (Bisinotto *et al.*, 2012; Mellado *et al.*, 2018; Lei and Simões, 2021). In addition, metabolic stress associated with ketosis may exacerbate oxidative imbalance, further disrupting ovarian and uterine function during early lactation (Verma *et al.*, 2025; Hildebrand *et al.*, 2023; Grzybowska *et al.*, 2025).

From a reproductive management perspective, the finding that a single BHBA measurement on days 7 to 30 postpartum is sufficient to predict subsequent reproductive outcomes supports the recommendation to use BHBA as a field screening tool for early identification of at-risk animals (Wang and Li, 2023). Integrating BHBA monitoring with management of body condition score, transition ration energy density and the use of glucogenic supplements or other functional nutrients (Wang *et al.*, 2025; Ali *et al.*, 2025) has the potential to reduce the incidence of subclinical ketosis in heifers. This approach may shorten DC, reduce the need for re-insemination and ultimately accelerate the achievement of optimal first calving age. Thus, scientifically, this study not only confirms the negative relationship between ketosis and fertility reported in multiparous lactating cows, but also expands the evidence that similar mechanisms have operated in young FH heifers in tropical environments, with direct implications for herd regeneration strategies and the sustainability of dairy farming.

However, this study has several limitations. The relatively small sample size and the use of a single-farm population

may limit the generalizability of the findings to broader dairy production systems. In addition, ketosis status was determined based on a single BHBA measurement during early lactation, which may not fully capture dynamic metabolic changes over time. Future studies involving larger sample sizes, multi-location herds and repeated metabolic measurements are recommended to further validate the relationship between early lactation ketosis and reproductive efficiency in dairy heifers (Serbetci *et al.*, 2024).

CONCLUSION

This research shows that early lactation ketosis plays a significant role in reducing the reproductive efficiency of 24-36 months old Friesian Holstein heifers in Banyumas. It can be seen based on the overall prevalence of ketosis at 52.6% (36.8% subclinical; 15.8% clinical) and its quantifiable effects on major reproductive indicators. There was a sharp decline in the conception rate in heifers with normal BHBA of 77.8 to 35.7 (subclinical) and 16.7 (clinical) and the number of inseminations required went up (1.5 to 2.1 and 3.4 times) and days to conception went up (95 to 121 and 163 days). These findings provide additional support that, despite the youth of young heifers in a tropical setting, BHBA values near or above 1.2 mmol/L are associated with a significant reduction in reproductive performance. One BHBA measurement conducted 7-30 days after parturition can be used as an effective screening tool to identify heifers with delayed conception. Therefore, by incorporating BHBA monitoring into heifer management, one can use it as a starting point to develop more specific, evidence-based approaches to ketosis prevention, thereby enhancing the efficiency of reproductive responses and the sustainability of dairy farming.

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Informed consent

All animal procedures for experiment were approved by Perhimpunan Dokter Hewan Indonesia (PDHI) or Indonesian Veterinary Medical Association, Central Java II Branch, Indonesia, number 023/EA/PDHI/II/2025.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding influenced the design study, data collection, analysis, decision to publish, or preparation of the manuscript.

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