Effect of Calving Time on Serum Total Protein Concentration and Passive Transfer Status in Holstein Calves

Nazari A.R.[1] ; Avarseji A.A.[1]; Abbasi J.[2*]; Kashi S.[3] ; Sheikhi A.[4]

Received: October 2025 Revised: December 2025 Accepted: February 2026

Abstract

Successful transfer of passive immunity (TPI) is essential for the health and long-term productivity of newborn calves. While colostrum quality and intake have been extensively investigated, the influence of calving time on TPI remains poorly understood. This study evaluated the effect of calving time on serum total protein (STP) concentration and passive immunity transfer in newborn Holstein calves. A total of 107 calves from a commercial dairy farm were assigned to four groups based on birth time: Group 1 (06:00–11:59 h), Group 2 (12:00–17:59 h), Group 3 (18:00–23:59 h), and Group 4 (00:00–05:59 h). Blood samples were collected 48 h after colostrum feeding, and STP concentrations were measured using a handheld optical refractometer. Data were analyzed using one-way ANOVA followed by Bonferroni post-hoc comparisons. Calving time significantly affected STP concentration ($P < 0.05$). Calves born during daytime hours had significantly higher STP concentrations (Group 1: 7.24 ± 0.51 g/dL; Group 2: 7.12 ± 0.72 g/dL) than calves born during nighttime hours (Group 3: 6.62 ± 0.66 g/dL; Group 4: 6.59 ± 0.47 g/dL; $P < 0.05$). No significant difference was observed between Groups 1 and 2 ($P > 0.05$). These findings suggest that calving time may influence passive immunity transfer in neonatal calves. Improved monitoring and standardized colostrum management during nighttime calving may help optimize calf health and passive immunity status.

Keywords: Holstein calves; Colostrum; Passive immunity transfer; Serum total protein; Calving time.

1-Department of Internal Medicine, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
2-Department of Animal and Poultry Health and Nutrition, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
3- Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
4-Department of Epidemiology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
*Corresponding author'a Email: jabbasi@ut.ac.ir

Introduction

The foundation of sustainable dairy farming is the long-term productivity and survival of replacement heifers. Newborn calves are born agammaglobulinemic due to the synepitheliochorial nature of the bovine placenta, which prevents the intrauterine transfer of maternal immunoglobulins. Consequently, the timely ingestion and absorption of high-quality colostrum are vital for successful transfer of passive immunity (TPI).

Failure of passive transfer (FPT) remains a significant challenge in the global dairy industry, directly linked to increased morbidity, mortality, and reduced growth rates in calves (Haggerty, 2022). Recent consensus guidelines have redefined the thresholds for TPI, emphasizing that even "fair" levels of immunity can lead to sub-clinical losses (Tyler *et al*., 1996; Lombard *et al*., 2020). While factors such as colostrum quality, volume, and cleanliness are well-documented, human and management factors—particularly during the "golden hour" after birth—are often overlooked (Uyama *et al.,* 2022).

In industrial dairy operations, management efficiency is not constant throughout a 24-hour cycle. Differences in labor availability, staff fatigue, and monitoring intensity between day and night shifts can lead to variations in the birth-to-feeding interval. Recent studies suggest that maternal and neonatal circadian rhythms may influence physiological readiness for immunoglobulin absorption (Piccione *et al.,* 2005; Frederick *et al.,* 2025).

Despite the importance of managing colostrum, there is little information about how the timing of birth—day versus night—affects serum total protein (STP) concentration in real-world farming conditions. Understanding this relationship is essential for optimizing farm protocols and ensuring calf welfare at all times. Therefore, the objective of this study was to evaluate the effect of calving time on STP concentration and the success of TPI in 107 Holstein calves. We hypothesized that calves born during nighttime intervals would exhibit lower STP levels due to potential delays in management and physiological diurnal variations.

Materials and methods

Study design and animal grouping

In this study, we monitored 107 Holstein calves from a commercial dairy farm. To assess the effect of birth timing on passive immunity, which is often influenced by management shifts and environmental factors (Robbers *et al.* 2021), we divided the calves into four groups based on their delivery time:

- Group 1: Born between 06:00 and 11:59 h (Morning to Noon).
- Group 2: Born between 12:00 and 17:59 h (Noon to Evening).
- Group 3: Born between 18:00 and 23:59 h (Evening to Midnight).
- Group 4: Born between 00:00 and 05:59 h (Midnight to Morning).

Sample collection and laboratory analysis

Blood samples were taken aseptically from the jugular vein of each calf 48 hours after colostrum administration, as this timing provides a stable estimation of immunoglobulin G absorption (Godden, 2008). Blood samples were collected in centrifuge tubes without anticoagulant. At the laboratory, the blood samples were allowed to clot and then centrifuged at 4000 RPM for 5 minutes. To assess TPI, serum total protein (STP) concentration was measured using a handheld optical refractometer (Fig. 1; Model HRB90-T, 0–90% Brix, accuracy ±0.2%, A. Krüss Optronic GmbH, Germany).

Figure 1: Handheld optical refractometer (Model HRB90-T, A.Krüss Optronic, Germany) featuring a 0–90% Brix scale and an integrated thermometer (6–36°C) used for analyzing serum samples. *Photograph by Saeed Kashi*

Optical refractometry is a validated and practical tool for monitoring calf health in field conditions (Deelen *et al.,* 2014). The interpretation of results followed the current consensus standards for calf immunity levels (Lombard *et al.,* 2020). Additionally, all calves underwent routine clinical examinations to monitor for symptoms of diarrhea.

Statistical analysis

The Kolmogorov–Smirnov test confirmed the normal distribution of the data. Given the normal distribution of the variables, a one-way analysis of variance (ANOVA) was employed to compare the mean STP concentrations among the four birth-time groups. In cases where significant differences were identified, the Bonferroni post-hoc test was utilized for pairwise comparisons. Statistical significance for all tests was set at $p<0.05$. All statistical analyses were performed using SPSS software.

Results

The Kolmogorov-Smirnov test confirmed that the STP concentrations followed a normal distribution (P=0.166). Based on the result of the Kolmogorov-Smirnov test, parametric statistical tests were performed. One-way ANOVA revealed a statistically significant effect of birth timing on STP concentrations ($p<0.05$). The descriptive statistics for each group, including 107 Holstein calves, are summarized in Table 1. Based on the Bonferroni post-hoc test, significant differences were observed between the daytime groups and nighttpime groups ($p<0.05$). The highest mean STP concentrations were associated with calves in Groups 1 and 2. Calves in Groups 3 and 4 had significantly lower concentrations than the first two groups. No statistically significant difference was found between Group 1 (Morning to Noon) and Group 2 (Noon to Evening) ($p>0.05$).

Table 1: Serum total protein (STP) concentrations (g/dL) in newborn calves based on birth time (n = 107).

Birth time group	Time interval	n	STP (Mean ± SD)[1]
Group 1	06:00 – 11:59	30	7.24±0.51[a]
Group 2	12:00 – 17:59	32	7.12±0.72[a]
Group 3	18:00 – 23:59	28	6.62±0.66[b]
Group 4	00:00 – 05:59	17	6.59±0.47[b]

[1]Note: Means within a column with different superscripts (a, b) differ significantly ($p<0.05$).

Discussion

The results of this study show that the timing of birth has a significant effect on STP concentration in newborn calves. This finding suggests a variation in the success of colostrum absorption and subsequent TPI based on the hour of delivery. Since STP concentration is widely recognized as a reliable indirect indicator of evaluating the absorption of colostral immunoglobulins (Tyler *et al.,*1996; Lombard *et al.,* 2020), the differences observed among birth-time groups likely stem from a complex interplay of managerial and physiological factors associated with the timing of parturition (Uyama *et al.,* 2022).

Effective colostrum absorption is strictly dependent on the timely administration of an adequate volume of high-quality colostrum within the first few hours of life (Godden, 2008). Any delay in feeding can lead to a marked reduction in immunoglobulin G (IgG) absorption (Zamuner *et al.,* 2024), as the permeability of the calf's intestinal epithelium to these large molecules rapidly declines soon after birth (Fischer *et al.,* 2018; Rabaza *et al.,* 2023). In the present study, the significant differences observed among birth-time groups may reflect variations in the efficiency of colostrum delivery or the physiological readiness of the neonate during those intervals.

One of the primary drivers of these differences is the variation in management practices between day and night shifts. During daylight hours, dairy farms typically benefit from higher staffing levels, more intensive monitoring of calving events, and stricter adherence to colostrum feeding protocols. Such conditions facilitate the rapid administration of colostrum, thereby increasing successful passive immunity transfer (Windeyer *et al.,* 2014). Conversely, during the night shift, reduced labor availability or delays in identifying the exact time of birth may increase the likelihood of delayed first feeding, which negatively impacts STP concentrations (Robbers *et al.,* 2021).

Beyond managerial influences, the circadian rhythm of both the dam and the calf may play a role in the observed variations. Physiological fluctuations driven by circadian rhythms—including maternal and neonatal hormonal profiles, the alertness of the newborn, and its suckling vigor—can significantly affect nursing behavior and immunological absorption efficiency. These biological factors may cause calves born at different times of the day to respond differently to colostrum administration (Piccione *et al.,* 2005; Frederick *et al.,* 2025).

In conclusion, the results of this study indicate that colostrum absorption and the success of passive immunity transfer are not only influenced by the timing of birth but are the result of a multifaceted interaction between management quality, feeding timing, and endogenous circadian rhythms. Therefore, implementing specialized management strategies for nighttime calvings, particularly regarding the timing and quality of the first colostrum meal, is essential for enhancing newborn calf health and immunity.

Conclusions

This study clearly demonstrates that birth timing is a decisive factor in the early life and health of Holstein calves. Our findings reveal a significant "immunity gap" between shifts: calves born during the high-activity daylight hours (06:00 to 18:00 h) consistently achieved higher STP concentrations than those born during the quieter evening and night shifts.

While biological circadian rhythms likely play a role in how the calf's body prepares for its first meal, the primary driver appears to be the human factor. The reality of industrial dairy farming is that nighttime shifts often face reduced staffing and increased fatigue, which can lead to critical delays in colostrum administration. Since the window for antibody absorption closes so rapidly after birth, even a short delay can have lifelong consequences for the calf's immunity.

To eliminate this disparity and prevent FPT, farm managers must treat nighttime calvings with the same clinical rigor as daytime ones, implementing stringent monitoring and standardized protocols across all 24 hours. Future research should focus on isolating and evaluating the effect of human labor versus biological rhythms to further improve evidence-based management practices.

Acknowledgements:

We thank the Institute of Biomedical Research, University of Tehran, Tehran, Iran procedures there.

Authors contribution:

Conceptualization: Alireza Nazari, Amirali Avarseji; Methodology: Javad Abbasi; Formal analysis and investigation: Javad Abbasi, Saeed Kashi, Ahoora Sheikhi; Writing - original draft preparation: Javad Abbasi, Alireza Nazari, Saeed Kashi, Writing - review and editing: Javad Abbasi, Amirali Avarseji, Alireza Nazari.

Data Availability The data that support the findings of this study are available within the manuscript and also are available from the corresponding author upon reasonable request.

Compliance with ethical standards

Funding

This study was not supported by any funding.

Conflict of Interest

The authors declare that they have no conflict of interest.

Ethical approval

The authors confirm that the ethical policies of the journal have been adhered to. All procedures performed in studies involving animals (107 Holstein calves) were in accordance with the ethical standards of the Faculty of Veterinary Medicine, University of Tehran, where the study was conducted. Blood samples were collected aseptically from the jugular vein by trained personnel to ensure minimal animal distress. This study was approved by the institutional ethical review committee.

Informed consent

For this type of study, formal consent is not required.

Consent for publication

For this type of study, consent for publication is not required.

Competing interest

The authors declare no competing interests.

References

Deelen, S.M., Ollivett, T.L., Haines, D.M. and Leslie, K.E., 2014. Evaluation of a Brix refractometer to estimate serum immunoglobulin G concentration in neonatal dairy calves. *Journal of Dairy Science*, 97, pp. 3838–3844. Available at: https://doi.org/10.3168/jds.2013-7163.

Fischer, A.J., Song, Y., He, Z., Haines, D.M., Guan, L.L. and Steele, M.A., 2018. Effect of delaying colostrum feeding on passive transfer and intestinal permeability in neonatal dairy calves. *Journal of Dairy Science*, 101, pp. 3099–3109. Available at: https://doi.org/10.3168/jds.2017-13440.

Frederick, G., Wieland, M., Singh, A., Ewing, R., Steele, M.A., Somula, H. and Mann, S., 2025. Effects of feeding colostrum volume at 6%, 8%, 10%, or 12% of birth body weight on efficiency of immunoglobulin G absorption, gastric emptying, and postfeeding behavior in Holstein calves. *Journal of Dairy Science*, 108, pp.13680–13690. Available at: https://doi.org/10.3168/jds.2025-27228.

Godden, S., 2008. Colostrum management for dairy calves. *Veterinary Clinics of North America: Food Animal Practice*, 24(**1**), 19 P. DOI:10.1016/j.cvfa.2007.10.005.

Haggerty, A., 2022. Failure of passive transfer and colostrum quality in Scottish dairy calves. Doctoral dissertation, University of Glasgow.

Lombard, J.E., Urie, N.J., Garry, F.B., Godden, S.M., Quigley, J.D., Earleywine, T.J., McGuirk, S.M., Moore, D.A., Branan, M.A., Chamorro, M.F., Smith, G.W., Shivley, C.B., Catherman, D.R., Haines, D.M., Heinrichs, A.J., James, R.E., Maas, J.P. and Sterner, K.E., 2020. Consensus recommendations on calf- and herd-level passive immunity in dairy calves in the United States. *Journal of Dairy Science*, 103, pp. 7611–7624. Available at: https://doi.org/10.3168/jds.2019-17955.

Piccione, G., Caola, G. and Refinetti, R., 2005. Daily rhythmicity of body temperature and heart rate in newborn calves. *Journal of Veterinary Medicine A*, 52, pp. 62–65. Available at: https://doi.org/10.1111/j.1439-0442.2004.00683.x.

Rabaza, A., Fraga, M., Mendoza, A. and Giannitti, F., 2023. A meta-analysis of the effects of colostrum heat treatment on colostral viscosity, immunoglobulin G and transfer of passive immunity in newborn dairy calves. *Journal of Dairy Science*, 106, pp. 7203–7219. Available at: https://doi.org/10.3168/jds.2022-22555.

Robbers, L., Jorritsma, R., Nielen, M. and Koets, A., 2021. A scoping review of on-farm colostrum management practices for optimal transfer of immunity in dairy calves. *Frontiers in Veterinary Science*, 8, 668639 P. Available at: https://doi.org/10.3389/fvets.2021.668639.

Tyler, J.W., Hancock, D.D., Parish, S.M., Rea, D.E., Besser, T.E., Sanders, S.G. and Wilson, L.K., 1996. Evaluation of 3 assays for failure of passive transfer in calves. *Journal of Veterinary Internal Medicine*, 10, pp. 304–307. Available at: https://doi.org/10.1111/j.1939-1676.1996.tb02065.x.

Uyama, T., Kelton, D.F., Winder, C.B., Dunn, J., Goetz, H.M., LeBlanc, S.J., McClure, J.T. and Renaud, D.L., 2022. Colostrum management practices that improve the transfer of passive immunity in neonatal dairy calves: A scoping review. *PLOS ONE*, 17, e0269824 P. Available at: https://doi.org/10.1371/journal.pone.0269824.

Windeyer, M.C., Leslie, K.E., Godden, S.M., Hodgins, D.C., Lissemore, K.D. and LeBlanc, S.J., 2014. Factors associated with morbidity, mortality, and growth of dairy heifer calves up to 3 months of age. *Journal of Dairy Science*, 97, pp. 3724–3737. Available at: https://doi.org/10.3168/jds.2013-7153.

Zamuner, F., Cameron, A.W.N., Carpenter, E.K., Arcos-Gómez, G., Kirkham, J., Leury, B.J. and DiGiacomo, K., 2024. Assessing the impact of colostrum feeding delay on serum immunoglobulin G and total protein in dairy goat kids. *Animal*, 18, 101246 P. Available at: https://doi.org/10.1016/j.animal.2024.101246.