Research Article

Age-Specific Hematological Profiles of Holstein Friesian Cattle in Vietnamese Smallholder Farms

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Abstract | The aim of this study was to determine hematological parameters of Holstein Friesian (HF) cattle at different physiological stages of development (young calves, heifers, and lactating cows) that raised under smallholder farm conditions. A total of 162 individual blood samples from 35 young calves within 3-12 months of age (HF3-12), 44 heifers within 13-18 months of age (HF13-18) and 83 lactating cows (HF-Lact) were taken at smallholder dairy farms in Don Duong commune of Lam Dong province. The samples were then analyzed for blood hematological parameters using the Mindray BC-2800 Vet analyzer. The results showed that the differences were identified in three basic blood physiological indices among three groups of HF young calves, heifers and lactating cows. The WBC (10^9/L) value of HF3-12 group was lower than those in the HF13-18 or HF(Lact) group (8.12 vs 11.47 or 12.80, $P<0.05$). The RBC ($\times 10^{12}$/L) value was 8.75 in HF3-12, 7.20 in HF13-18 and 6.15 in HF-Lact groups, the significant difference ($P<0.05$) was found among grouped cows examined. The value of PLT ($\times 10^9$/L) was ranged from 361.80 to 397.50 and no significant difference was found among groups. Nonsignificant variation in blood parameters between pregnant or non-pregnant heifers was found. The significant variation of PLT was found between groups of HF(Lact-1) and HF(Lact-dry off) or HF(Lact-3) but no significant variation in WBC and RBC count among lactating cows was found. In conclusion, the basic hematological indices established in the current study could be useful for diagnosing of different diseases or nutritional disorders in HF dairy cattle, especially in determination of anemia in cows and calves, and the data are also meaningful for academic purposes. The young calves might be under risk of anemia due to under suboptimal feeding. More studies are required to better understand the variability of HF cattle hematological profiles for further applications in diagnostics, health, as well as productivity performance assessment.

Keywords | Calf, Heifer, Hematology, Lactating cow, Platelet cell, Red blood cell, White blood cell

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INTRODUCTION

As in humans, hematological profiles are of crucial importance for clinical technicians and scientists in determining the health status and/or nutritional disorders of animals. Validation of blood indices in certain status of animals is helpful in establishing suitable physiological baseline values for various breeds, which in turn could be used in the realistic evaluation of the status of practical management, feeding or diagnosis of health condition in the most critical periods of life (Gharban and Yousif, 2020; Zhang *et al*., 2022; Al-Eodawee *et al*., 2024; Almaliky *et al*., 2024; Solomon *et al*., 2024; Gonzalez-Garduno *et al*., 2025). From the view of animals, the measurement of hematological parameters in domestic animals in general or specifically in cattle have rarely been carried out as compared to pet animals up to date. There are several explanations for this, such as the costs associated with labor and laboratory testing, especially due to the low economic value of an individual animal and the limited availability of reference intervals for different age categories in animals required for accurate interpretation of laboratory results (Perri *et al.*, 2017; Jezek *et al.*, 2018; Oliveira *et al.*, 2019; Gonzalez-Garduno *et al.*, 2025). Since the ranges of most hematological values are broad scope, they vary depending on many factors, including gender, age, diet, season, biosecurity, physiological status different husbandry techniques, restraint and sample collection techniques, time of sample transportation or preparation, as well as the type of the analyzer used for hematological analysis (Herman *et al.*, 2018; Jezek *et al.*, 2018; Thorn *et al.*, 2022; Mekroud *et al*, 2023; Fanta *et al.*, 2024; Piotr and Marta, 2024). Beneficially, measurement of hematological profiles can contribute to early identification of diseases or poor growth performance (Perri *et al.*, 2017; Sanchez *et al.*, 2019) and may be highly valuable in the treatment or prognosis of many diseases in human (Eze *et al.*, 2010) or domestic animals (Walczak *et al.*, 2021; Gonzalez-Garduno *et al.*, 2023, 2025; Unal and Uslu, 2024). There are many factors such as breed, age, nutrition, physiological status or disease can impact on the variation of hematologic parameters (Farooq *et al.*, 2017; Astuti *et al.*, 2022; Mekroud *et al.*, 2023; Zhelavskyi *et al.*, 2024), indicating the reasonable status is required.

In Vietnam, for the last decade, several studies have been directed to the field of veterinary hematology in goat or sheep (Bui *et al.*, 2015; Nguyen *et al.*, 2023), native pig (Dao *et al.*, 2024) and cattle (Nguyen *et al.*, 2023) or HF young calves (Bùi *et al.*, 2025), however, there is limited information on Holstein Friesian dairy cattle at different stages of development in normal physiological conditions. The industry of dairy cattle production in Vietnam has been developing during last decades with the population about 350,000 heads in 2024, in which more than 60% of population are under smallholder farming system. So, this study aimed to present the differences between basic hematological parameters of HF young calves, heifers and lactating cows under field condition and establish threshold values for RBC parameters to predict the anemia in HF cattle.

MATERIALS AND METHODS

Animals and feeding

The growing female post weaned calves, heifers and lactating cows are raised at small holder farms of Don Duong Commune (11°43'19.9"N 108°27'16.1"E) - Lam Dong Province were used in this study. A total of 162 individual blood samples, which are collected within three days in June 2025 (summer season), were collected from 35 young calves within 3-12 months of age (HF3-12), 44 heifers within 13-18 months of age (HF13-18) and 83 lactating cows (HF-Lact). The representative image of sampled cattle are presented in the Figure 1. To reduce the variation of nutritional effects on blood profiles, the samples were collected at the farm with the herd size about 25-30 cows and total mixed ration was applied. The animals were fed twice daily with the standard TMR diet formulated in accordance with the NRC (2001), the main ingredients of ration are fresh grass, corn silage, rice straw, soya been husk and commercial feed. And, the mineral lick block, fresh water is available and without any clinical signs of diseases at the time of sample collection.

Figure 1: Representative images of cattle examined. A: HF 4 months of age; B: HF 15 months of age; C: second lactating HF cow.

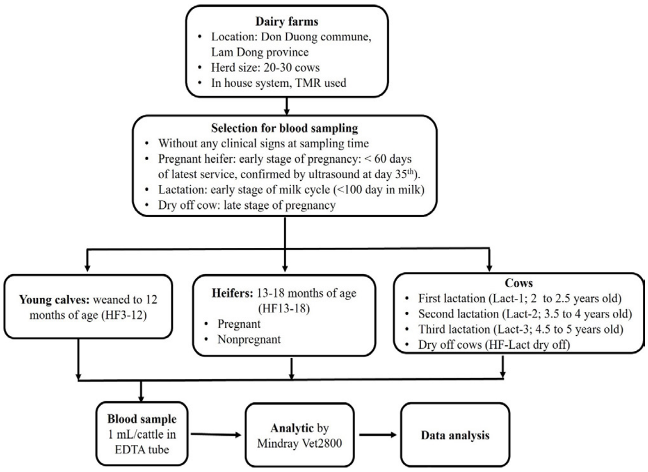

Figure 2: Flow chart of research process.

The heifers were then classified into non-pregnant and pregnant groups within 60 days after the last artificial insemination, which are confirmed by ultrasound at the Day 35 post the last service. Similarly, the lactating cows (within first 100 days in milk – DIM) were separated into first lactating cows (HF-Lact1) with the age about 2 to 2.5 years old, second lactating cows (HF-Lact2) with the age about 3.5 to 4 years old, third lactating cows (HF-Lact3) with the age about 4.5 to 5 year old and dry off cows – HF(Lact- dry off) with the age about > 3 to 6 years old depend on parity for further data analysis. The workflow chart is added in the Figure 2.

Samples and hematological analysis

Blood sample collection

Whole blood samples were collected from the coccygeal vein by a professional technician, blood samples were collected in a blood collection tube containing EDTA (ethylenediamine tetra-acetic acid) as anticoagulant after the animal was carefully restrained. The procedures for sampling adhered to the standard of animal care in Vietnam, following guidelines based on EU directive 2010/63 for the best practice of using animals in research. The collected samples were stored at 4°C on ice and transported to the laboratory. The analysis was run immediately after the blood samples were delivered to the Lab.

Hematological analysis

An automated hematology analyzer (Mindray BC-2800 Vet; Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, China) was used to perform complete blood cell count: WBC: White Blood cell (10^9/L), Lym# (×10^9/L), Mon# (×10^9/L), Gran# (×10^9/L), Lym (%), Mon (%), Gran (%); RBC: Red Blood Cell (10^{12}/L), HGB: Hemoglobin (g/L), HCT: Hematocrit (%), MCV: Mean corpuscular volume (fL), MCH: Mean Corpuscular Hemoglobin (pG), MCHC: Mean Corpuscular Hemoglobin Concentration (g/L), RDW: Red Cell Distribution Width (%); PLT: Platelet Count (10^9/L), PDW: Platelet Distribution Width (%), MPV: Mean Platelet Volume (%), and PCT: Procalcitonin (%). Results were compared with calibrated Mindray reference and for Holstein cattle according to Chen *et al*. (2022).

Data analysis

To obtain the mean such as standard deviation (SD), standard error of the mean (SEM), 95% confident interval (95% CI), the descriptive statistics were applied. All obtained data were subjected to one way ANOVA followed by Tukey´s test (Minitab) for multiple comparison, percentage data were transformed to arcsine before being subjected to statistical analysis. Data are presented as mean ± SEM/SD, and significance level was set at $P<0.05$. Severely anemia thresholds were established using erythrocyte data derived from young calves, heifers and lactating cows of current study, defined as the mean minus two SD according to Gonzalez-Garduno *et al*. (2025).

RESULTS AND DISCUSSION

The hematological profile of HF young calves, heifers, and lactating cows

The white blood cell count and other related parameters

An automatic measurement analyzer of individual samples was performed, and the data of WBC and other related WBC parameters were analyzed and presented in Table 1. Data from Table 1 showed that the WBC (10^9/L) value of HF3-12 group was lower than that of the HF13-18 and

Table 1: The WBC count and other related WBC parameters in young calves, heifers and lactating cows.

Parameter	Group	N	Mean ± SD	Min - Max	Q1	Q3	Reference range	
							Generic manufacturer*	Chen et al. (2022)
WBC (×10^9/L)	HF3-12	35	$8.12^b \pm 3.84$	1.60 - 21.70	5.50	10.40	5.00 -16.00	5.99 – 20.47
	HF13-18	44	$11.47^a \pm 4.76$	4.10 - 25.50	8.08	13.85		
	HF-Lact	83	$12.80^a \pm 6.14$	3.60 - 36.90	8.60	14.10		
Lym# (×10^9/L)	HF3-12	35	$3.24^b \pm 1.64$	0.70 - 7.60	2.20	3.90	1.50 - 9.00	2.24 – 13.01
	HF13-18	44	$5.83^a \pm 3.45$	1.10 - 17.20	3.40	7.38		
	HF-Lact	83	$5.64^a \pm 4.66$	1.00 - 26.40	2.80	7.20		
Mon# (×10^9/L)	HF3-12	35	$1.07^b \pm 0.72$	0.20 - 4.10	0.60	1.50	0.30 - 1.60	0.26 – 1.19
	HF13-18	44	$1.48^a \pm 0.69$	0.60 - 3.30	1.00	1.88		
	HF-Lact	83	$1.30^{ab} \pm 0.67$	0.30 - 4.50	0.80	1.70		
Gran# (×10^9/L)	HF3-12	35	$3.81^b \pm 2.30$	0.70 - 10.90	2.00	5.80	2.30 - 9.10	1.65 – 6.80
	HF13-18	44	$4.09^b \pm 2.24$	1.00 - 12.00	2.70	4.55		
	HF-Lact	83	$5.86^a \pm 2.74$	1.30 - 14.40	4.10	7.00		
Lym (%)	HF3-12	35	$41.68^b \pm 12.50$	16.60 - 66.30	30.80	51.00	20.00 - 60.30	26.96 – 72.40
	HF13-18	44	$50.45^a \pm 15.31$	14.90 - 73.50	39.10	64.48		
	HF-Lact	83	$41.18^b \pm 17.14$	12.80 - 72.60	28.50	55.40		
Mon (%)	HF3-12	35	$12.90^a \pm 3.30$	7.40 - 19.90	9.70	15.10	4.00 - 12.10	2.74 – 10.70
	HF13-18	44	$12.96^a \pm 1.85$	9.50 - 18.00	11.70	14.43		
	HF-Lact	83	$10.38^b \pm 2.92$	5.70 - 24.20	8.50	11.70		
Gran (%)	HF3-12	35	$45.42^a \pm 11.99$	22.80 - 68.70	38.20	51.40	30.00 - 65.00	18.20 – 60.10
	HF13-18	44	$36.58^b \pm 14.37$	16.50 - 72.20	24.23	45.03		
	HF-Lact	83	$48.45^a \pm 16.69$	16.10 - 79.30	35.80	61.00		

*Reference ranges from manufacturer's set up. Chen *et al*. (2022): 95% Reference Interval from mean; Q1: first quartile; Q3: third quartile; Within colunm and the same parameter, the value with different superscript letter differs (P<0.05). Data are presented as Mean±SD.

Links Researchers

Table 2: The RBC count and other related RBC parameters in young calves, heifers and lactating cows.

Arameter	Group	N	Mean ± SD	Min – Max	Q1	Q3	Reference range	
							Generic manufacturer*	Chen et al. (2022)
RBC ($\times10^{12}$/L)	HF3-12	35	$8.75^a \pm 1.88$	5.16 - 11.65	7.26	10.52	5.00 - 10.10	4.81 - 7.54
	HF13-18	44	$7.20^b \pm 1.76$	3.76 - 11.88	6.17	8.23		
	HF-Lact	83	$6.15^c \pm 0.77$	4.06 - 7.83	5.60	6.68		
HGB (g/L)	HF3-12	35	$106.31^a \pm 19.96$	64.00 - 137.00	89.00	125.00	90.00 - 139.00	86.00 - 120.00
	HF13-18	44	$105.70^a \pm 25.24$	41.00 - 171.00	92.25	123.75		
	HF-Lact	83	$98.18^a \pm 13.97$	77.00 - 150.00	89.00	106.00		
HCT (%)	HF3-12	35	$30.53^a \pm 5.70$	18.80 - 40.00	26.40	36.00	28.00 - 46.00	24.50 - 33.90
	HF13-18	44	$30.32^a \pm 6.86$	12.90 - 47.20	26.45	34.88		
	HF-Lact	83	$28.32^a \pm 4.88$	5.10 - 39.40	25.15	31.40		
MCV (fL)	HF3-12	35	$35.45^b \pm 4.32$	30.00 - 47.70	32.10	37.80	38.00 - 53.00	39.74 - 57.36
	HF13-18	44	$43.95^a \pm 6.93$	33.30 - 79.10	40.78	45.93		
	HF-Lact	83	$46.03^a \pm 5.57$	18.40 - 60.10	42.53	49.33		
MCH (pG)	HF3-12	35	$12.25^b \pm 1.34$	10.50 - 16.60	11.40	13.00	13.00 - 19.00	14.50 - 20.10
	HF13-18	44	$15.16^a \pm 2.03$	11.80 - 25.00	14.13	16.00		
	HF-Lact	83	$16.21^a \pm 4.36$	12.50 - 53.10	14.70	16.70		
MCHC (g/L)	HF3-12	35	$347.77^a \pm 9.13$	329.00 - 368.00	341.00	356.00	300.00 - 370.00	338.00 - 375.00
	HF13-18	44	$347.32^a \pm 10.17$	317.00 - 370.00	341.00	353.75		
	HF-Lact	83	$340.94^b \pm 17.43$	288.00 - 430.00	333.00	348.00		
RDW (%)	HF3-12	35	$19.40^a \pm 2.61$	16.20 - 30.80	17.70	20.10	14.00 - 19.00	17.90 - 23.60
	HF13-18	44	$17.71^b \pm 1.06$	15.20 - 20.30	17.03	18.35		
	HF-Lact	83	$17.21^b \pm 1.29$	14.40 - 21.90	16.30	17.63		

*Reference ranges from manufacturer's set up. Chen *et al.* (2022): 95%; Q1: first quartile; Q3: third quartile; Reference Interval from mean; Within colunm and the same parameter, the value with different superscript letter differs ($P<0.05$). Data are presented as Mean ± SD.

HF-Lact groups (8.12 vs 11.47 and 12.80, $P<0.05$), with no significant difference between HF13-18 and the HF-Lact group. The value of Lym# ($\times10^9$/L) was lower in the HF3-12 group compared to the HF13-18 or HF-Lact groups (3.24 vs. 5.83 or 5.64; $P<0.05$). The value of Mon# ($\times10^9$/L) was 1.07 in HF3-12, 1.48 in HF13-18, and 1.30 in the HF-Lact group, and the significant difference was found between HF3-12 and the HF13-18 or HF-Lact groups.

The Gran# ($\times10^9$/L) value was 3.81 in HF3-12, 4.09 in HF13-18, and 5.86 in the HF-Lact groups, the significant difference was found between HF-Lact and the HF3-12 or HF13-18 groups. The value of Lym (%) was significantly lower in HF3-12 and HF-Lact compared to those in the HF13-18 group (41.68 and 41.14 vs. 50.45; $P<0.05$). The value of Mon (%) was 12.90 in HF3-12, 12.96 in HF13-18, and 10.38 in HF-Lact. The value of Gran (%) was 45.52 in HF3-12, 36.58 in HF13-18, and 48.45 in HF-Lact, the significance was found between HF13-18 and HF7-12 or HF-Lact groups.

In the current study, the results indicate that variation in WBC count and related parameters was observed at different stages of age in young calves, heifers, and lactating cows examined, and similar trends have also been reported in previous studies in calves with or without sepsis (Unal and Uslu, 2024), in cattle infected or non-infected parasite diseases (Nguyen *et al.*, 2023; Kim *et al.*, 2024; Ramabu *et al.*, 2024), in case of iron supplementation (Budny-Walczak *et al.*, 2023) and in normal physiological conditions of beef cattle (Dau *et al.*, 2025) or HF young calves (Bui *et al.*, 2025). In Pulikulam cattle, Srinivasan and Sathiamoorthy (2021) reported that the WBC ($\times10^3\mu$L) and MCH values were significantly higher in calves as compared to heifers, and no significant difference was found between heifers and other stages of lactation in cows or bulls.

The red blood cell count and other related parameters

The data of RBC value and other related RBC parameters according to the stages of age of HF such as young calves, heifers and lactating cows were obtained and presented in Table 2. Based on the data from Table 2, the RBC ($\times10^{12}$/L) value was 8.75 in HF3-12, 7.20 in HF13-18, and 6.15 in HF-Lact, and the significant difference was found among the groups of cattle examined. The HGB (g/L) ranged from 98.18 to 106.31 among groups (P>0.05). Similarly, the value of HCT (%)ranged from 28.32 to 30.53, and no difference (P>0.05) among groups of animals was found. The MCV (fL) was lower in HF3-12 as compared to HF13-18 or HF-Lact (35.34 vs 43.95 or 46.03; P<0.05). The value of MCH (pg) was significantly low in HF3-12 as compared to those in HF13-18 or HF-Lact (12.25 vs 15.16 or 16.11, respectively; P<0.05). The MCHC (g/L) value was 347.77 in HF3-12, 347.32 in HF13-18, and 340.94 in HF-Lact, however, the significant difference was only found between HF-Lact and HF3-12 or HF13-18 groups. Similarly, the RDW(%) value was 19.40 in HF3-12, 17.71 in HF13-18 and 17.21 in the HF-Lact group, and the significance was found in HF3-12 compared to HF13-18 or HF-Lact.

The results in our current study correspond to the results that are obtained in beef cattle by Dau *et al.* (2025) or HF young calves by Bui *et al.* (2025). However, in Pulikulam cattle, the MCV (fL) and MCH (pG) were significant lower in calves compared to heifers and other stages of lactating cow or bulls, meanwhile, the RBC ($\times10^6\mu$L) or Hb (g/dL) values were not affected by the physiological stage of development (Srinivasan and Sathiamoorthy, 2021). Besides this, in ruminant, several studies also noted that fetuses and neonates have greater amounts of RBC than adults (Herdt and Hoff, 2011; Wood, 2022; Budny-Walczak *et al.*, 2023) or male has a higher value than female (de Souza *et al.*, 2018). One study in HF dairy cows from 3-6 years at the peak of lactation reported that the groups of dairy cows with copper deficiency at different levels had the lower RBC count than the dairy cow group without copper deficiency (Abramowicz *et al.*, 2019).

The low value of MCV and MCH in young calves might be due to under stress of mineral deficiency such as iron, copper or subclinical parasites, especial in blood parasites, Beside this, Etim at al. (2013) mentioned that by the way automated analyzers used to count red blood cells, a high MCHC (greater than about 300g/L) may indicate the blood is from an animal with cold agglutination or the sample stored at low temperature that means when the blood gets colder than 37°C, it begins to gather together. Consequently, the analyzer may incorrectly report a low number of very dense red blood cells for blood samples in which agglutination has occurred. From this mention, it can suggest the technicians that the blood samples need to be warmed until the cells separate from each other, and then quickly put through the machine while still warm due to the blood samples usually picked up to the laboratory in low temperature. Form these findings, it also indicates the potential risks on anemia by mineral deficient in calves or parasites in heifers and adult cattle. Beside this, the technicians pay attention for operating the automated analyzer with cold samples and double check the sample when the data report fall into lower reference range.

The platelet cell count and other related parameters

Furthermore, the platelet cell count and other related parameters of HF calves and heifers were calculated and presented in Table 3. According to the presented data in Table 3, the PLT ($\times10^9$/L) value was higher in HF3-12 or HF-Lact groups (390.80 or 397.50) as compared to in HF13-18 group (361.80) but no significance was found. The MPV (fL) was lower (P<0.05) in HF3-12 group (5.33) compared to those in HF13-18 group (6.02) or in HF-Lact group (5.96). The value of PDW (%) was 15.75 in

Table 3: The PLT count and other related PLT parameters in young calves, heifers and lactating cows.

Parameter	Group	N	Mean ± SD	Min - Max	Reference range			
					Q1	Q3	Generic manufacturer*	Chen et al. (2022)
PLT ($\times10^9$/L)	HF3-12	35	390.80^a ± 189.70	40.00 - 831.00	235.00	486.00	120.00 – 820.00	273.15 - 980.70
	HF13-18	44	361.80^a ± 157.20	148.00 - 991.00	273.50	447.50		
	HF-Lact	83	397.50^a ± 162.60	119.00 - 870.00	270.00	506.00		
MPV (fL)	HF3-12	35	5.33^b ± 0.51	4.20 - 6.30	4.90	5.80	3.80 - 7.00	8.70 - 19.20
	HF13-18	44	6.02^a ± 0.52	5.10 - 7.50	5.70	6.30		
	HF-Lact	83	5.96^a ± 0.62	4.10 - 7.50	5.60	6.30		
PDW (%)	HF3-12	35	15.75^b ± 0.30	15.30 - 16.60	15.50	15.90	-	
	HF13-18	44	16.28^a ± 0.67	15.10 - 18.30	15.90	16.47		
	HF-Lact	83	16.49^a ± 0.67	14.20 - 19.80	16.20	16.80		
PCT (%)	HF3-12	35	0.21^a ± 0.16	0.02 - 0.67	0.08	0.30	-	
	HF13-18	44	0.21^a ± 0.11	0.02 - 0.59	0.14	0.27		
	HF-Lact	83	0.22^a ± 0.10	0.01 - 0.43	0.15	0.28		

*Reference ranges from manufacturer's set up. Chen *et al.* (2022): 95% Reference Interval from mean; Q1: first quartile; Q3: third quartile; Within colunm and the same parameter, the value with different superscript letter differs (P<0.05). Data are presented as Mean ± SD.

HF3-12, 16.28 in HF13-18, and 16.49 in HF-Lact, the difference was only found in HF3-12 compared to HF13-18 or HF-Lact group. The PCT (%) value ranged from 0.21 to 0.22 among groups. The present results were lower than the results that reported by Ramabu *et al.* (2024) in both dairy and beef cattle, however, they are similar to the PLT value in HF dairy cattle (Yang *et al.*, 2024), young calves HF (Bui *et al.*, 2025) or beef cattle (Motta *et al.*, 2023; Dau *et al.*, 2025). The low value of MPV and MDW in HF3-12 group can indicate they are in anemia status due to feeding with suboptimal nutrition, need to pay more attention for feeding regime of young calf's post weaning.

Taken together, we found that most of critical hematological indices fall into normal range, however, some value of individual blood parameters was out of minimal reference range which should be considered the factor(s) affecting to the results in the further studies. Taken the data from Tables 2 and 3 we can imply that the young calves may be under suboptimal feeding resulted in fall down the value of MCV, MCH, MPV and PDW that indicates anemia status without clinical sings in young calves.

The hematological parameters of pregnant and non-pregnant HF heifers, different parity of lactating cows

Classified data of hematological parameters between pregnant and non-pregnant heifers from the HF (13-18) group, including the WBC, RBC, and PLT counts, were calculated and presented in Table 4. The data in Table 4 indicated that no significant variation in blood parameters between pregnant or non-pregnant heifers was found and the trend of data in current report was similar to our previous published data by Bui *et al.* (2025). From this result, this could be due to the early stage of gestation of heifers examined, and it can be considered for further studies.

Similarly, classified data of hematological parameters among the different parities of lactating cows as well as dry off cows, including the value of WBC, RBC and PLT count was calculated and presented in Table 5.

According to the data in Table 5 it was indicated that no significant variation in WBC and RBC count among lactating cows was found. The value of PLT was 318.50 in HF(Lact-dry off), 495.40 in HF(Lact-1), 399.90 in HF(Lact-2) and 374.20 in HF(Lact-3) groups. The significance was found between groups of HF(Lact-1) and HF(Lact-dry off) or HF(Lact-3), it is speculated that the group of HF(Lact-1) may be under physiological stress of first parturition.

Table 4: The effect of pregnancy status of heifers on critical values of blood cell count.

Parameter	Non-pregnant heifer (n=24)	Pregnant heifer (n=20)
WBC ($\times10^9$/L)	$10.44^a \pm 5.98$	$12.36^a \pm 3.35$
RBC ($\times10^{12}$/L)	$7.34^a \pm 2.48$	$6.79^a \pm 1.01$
PLT ($\times10^9$/L)	$326.80^a \pm 190.20$	$353.90^a \pm 152.80$

Within the same row, the value with different superscript letter differs (P<0.05). Data are presented as Mean ± SD.

Mekroud *et al.* (2023) noted that the RBC level was lowest in the 2nd third of gestation or in the postpartum period in comparison to the 1st third and 3rd third of gestation in Holstein cows. The same observation was reported for HCT and MCV, which were highest in dry cows, meanwhile, the MCH and MCHC were highest in cows that were in the postpartum period. In contrast to our findings, Chen *et al.* (2022) reported in the China HF that the WBC value was lower in 2 years old of cows compared to 3 to 5 years old cows, the RBC value was higher in 2 years cows compared to 3 to 5 years old of cows, and PLT value was low in the 2 to 4 years old of cows compared to group of cows in 5 years old.

Estimation of anemia threshold in HF young calves, heifers, and different parity of lactating cows

Further analysis of RBC and related parameters to estimate the anemia threshold of cattle examined, including the normal value of the mean with 95% Confident Interval (CI) for μ and the lower threshold, as defined by mean minus 2 SD, was recorded, and the results are presented in Table 6.

The normal value of RBC ($\times10^{12}$/L) ranged from 6.11 in the HF-Lact to 8.75 in the HF3-12 group. The lower threshold was 3.67 in HF13-18, 4.42 in HF-Lact, and 4.50

Table 5: The effect of parity on critical values of blood cell count.

Parameter	HF(Lact-dry off) n=21	HF(Lact-1) n=22	HF(Lact-2) n=17	HF(Lact-3) n=23
WBC ($\times10^9$/L)	$11.04^a \pm 3.42$	$14.68^a \pm 7.07$	$14.26^a \pm 5.83$	$11.52^a \pm 6.29$
RBC ($\times10^{12}$/L)	$5.94^a \pm 1.76$	$6.18^a \pm 0.82$	$5.55^a \pm 1.54$	$6.22^a \pm 0.54$
PLT ($\times10^9$/L)	$309.40^b \pm 99.40$	$495.80^a \pm 182.70$	$370.20^{ab} \pm 203.80$	$359.70^{ab} \pm 171.70$

HF(Lact-dry off) dried off cows; HF(Lact-1): first parity lactating cows, HF(Lact-2): second parity lactating cows HF(Lact-3): third parity lactating cows. Within the same row, the value with different superscript letter differs (P<0.05). Data are presented as Mean ± SD.

Table 6: Estimated Red Blood Cell count for anemia threshold in HF cattle at different physiological stage of development.

Parameter	Group	N	Mean (95% CI for μ)	SD	Threshold	
					Lower limit (Mean – 2SD)	Higher limit (Mean + 2SD)
RBC ($\times 10^{12}$/L)	HF(3-12)	35	8.75 (8.10, 9.39)	1.88	4.50	12.50
	HF(13-18)	44	7.20 (6.66, 7.74)	1.76	3.67	10.73
	HF(Lact)	83	6.11 (5.93, 6.30)	0.85	4.42	7.81
HGB (g/L)	HF(3-12)	35	106.31 (99.46, 113.17)	19.96	66.39	146.23
	HF(13-18)	44	105.70 (98.03, 113.38)	25.24	55.22	156.18
	HF(Lact)	83	98.18 (95.11, 101.25)	13.97	70.24	126.12
HCT (%)	HF(3-12)	35	30.53 (28.58, 32.49)	5.70	19.14	41.92
	HF(13-18)	44	30.32 (28.23, 32.40)	6.86	16.60	44.04
	HF(Lact)	83	28.32 (27.25, 29.39)	4.88	18.57	38.07
MCV (fL)	HF(3-12)	35	35.45 (33.96, 36.93)	4.32	26.81	44.09
	HF(13-18)	44	43.95 (41.84, 46.06)	6.93	30.09	57.81
	HF(Lact)	83	46.03 (44.81, 47.25)	5.57	34.89	57.17
MCH (pG)	HF(3-12)	35	12.25 (11.79, 12.71)	1.34	9.57	14.93
	HF(13-18)	44	15.16 (14.54, 15.78)	2.03	11.10	19.22
	HF(Lact)	83	16.21 (14.54, 15.78)	4.36	7.49	24.93
MCHC (g/L)	HF(3-12)	35	347.77 (344.64, 350.91)	9.13	329.51	366.03
	HF(13-18)	44	347.32 (344.23, 350.41)	10.17	326.98	367.66
	HF(Lact)	83	340.94 (337.11, 344.77)	17.43	306.08	375.80
RDW (%)	HF(3-12)	35	19.40 (18.51, 20.30)	2.61	14.19	24.61
	HF(13-18)	44	17.71 (17.39, 18.04)	1.06	15.59	19.83
	HF(Lact)	83	17.21 (16.92, 17.49)	1.29	14.64	19.78

μ: population mean of RBC ($\times 10^{12}$/L), HGB (g/L), HCT (%), MCV (fL), MCH (pg), MCHC (g/L), RDW (%); CI: confidence interval.

in the HF3-12 group. The normal value of HGB (g/L) ranged from 98.18 in HF-Lact to 106.3 in the HF3-12 group. The lower threshold was 55.52 in HF13-18, 66.39 in HF3-12, and 70.24 in the HF-Lact group. The normal value of HCT (%) ranged from 28.32 in HF-Lact to 30.53 in the HF3-12 group. The lower threshold was 16.60 in HF13-18, 18.57 in HF-Lact, and 19.14 in the HF3-12 group. The normal value of MCV (fL) ranged from 35.45 in HF3-12 to 46.03 in the HF-Lact group. The lower threshold was 26.81 in HF3-12, 30.09 in HF13-18, and 34.89 in the HF-Lact group. The normal value of MCH (pG) ranged from 12.25 in HF3-12 to 16.21 in HF-Lact group. The lower threshold was 7.49 in HF-Lact, 9.57 in HF3-12, and 11.10 in the HF13-18 group. The normal value of MCHC (g/L) ranged from 340.94 in HF-Lact to 347.77 in the HF3-12act group. The lower threshold was 306.08 in HF-Lact, 326.98 in HF13-18 and 329.51 in HF3-12 group. The normal value of RDW (%) ranged from 17.21 in HF-Lact to 19.40 in the HF3-12 group. The lower threshold was 14.19 in HF3-12, 14.64 in HF-Lact, and 15.59 in the HF13-18 group.

According to the report by Piotr and Marta (2024), it was noted that MCH ranged from 15.55 to 18.97 pG in primiparous cows and from 16.05 to 20.94 pG in multiparous cows, and that MCHC ranged from 33.73 to 38.23 ± 0.51 g/dL in primiparous cows and from 32.43 to 37.67 g/dL in multiparous cows. The MCHC value is regarded as more important than MCH, and a decrease in MCHC is quite common in hypochromic anemia and the MCHC values above the upper limit may indicate adverse effects of pre-analytical factors during blood testing.

Several studies have reported the confidence intervals for hematological parameters indicative of anemia in calves as follows: HCT <20.1%, HGB <6.1 g/dL, and RBC count $<5.9\times10^6$ cells/μL (Katsogiannou *et al*., 2018; Wood, 2022; Golbeck *et al*., 2023; Unal and Uslu, 2024; Gonzalez-Garduno *et al*., 2025). In current study, several parameters are lower than suggested value, this discrepancy needs to be investigated in the further research to figure out the reasonable factors, including subclinical infections of parasites, mineral deficient such as iron as well as other nutritional factors, especial in young calves.

Combination data from Table 3 for Q1 as well as Q3 value and threshold from Table 6, we suggest that the RBC and other related parameters fail into around lower threshold can be considered as severe anemia and the data within Q1 and Q3 can be acceptable for normal data in the condition of current study.

CONCLUSION

Under smallholder farm conditions, for the first time, hematological parameters of HF young calves, heifers, and lactating cows were measured, and variations of some critical parameters at different stages of age were found. The basic of hematological indices established in the current study could be used in the diagnosis of different diseases or nutritional disorders in HF dairy cattle, especially in determination of anemia in cows and calves, the data are also meaningful for academic purposes. The findings from current study also suggest the farmers for considering the potential risks of anemia in young calves of their farms due to mineral deficient or parasites. More studies are required to better understand the variability of HF cattle hematological profiles for further applications in diagnostics, health, as well as productivity performance assessment.

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NOVELTY STATEMENT

Our results increase knowledge on hematological profiles of Holstein Friesian cattle at different stages of development under practical field conditions, especially in Vietnam. The findings on normal hematological values established in this study could be helpful in the diagnosis of different diseases or nutritional disorders in HF dairy cattle especially in determination of anemia in cows and calves, the data are also meaningful for academic purposes.

AUTHORS CONTRIBUTION

All the authors contributed to designing the experiments and reading as well as approving the manuscript at each step. NNT covered all the research, writing the research proposal and as the corresponding author evaluated the manuscript, checked for plagiarism and revised manuscript. BTA has contributed to process the samples, analyzing the data.

Generative AI and AI-assisted technology statement

The authors declare that no Genrative AI was used in the creation of this manuscript.

Conflicts of interest

We certify that there are no conflicts of interest with any financial, personal, or professional organization regarding the material discussed in this manuscript.

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