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Seasonal variations in hematological profile of Holstein dairy cows as an indicator for physiological status assessment

M. Mekroud[1], N. Arzour-Lakehal[1], N.A. Ouchene-Khelifi[1,2], N. Ouchene[1,2]*, A. Titi[1], A. Mekroud[1]

[1]PADESCA laboratory, Institute of Veterinary Sciences, Road of Guelma 25100 El Khroub, University of Mentouri Brothers, Constantine 1, Algeria
[2] Institute of Veterinary Sciences, University of Saad Dahlab Blida 1, Road of Soumaa, BP 270, 09000, Blida, Algeria

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Abstract. *This study aims to investigate, for the first time in Algeria, the effect of seasonal climatic changes on the hematological profile of Holstein dairy cows as an indicator for their physiological status assessment. The study was conducted between November 2018 and October 2019 and was performed on a lot of 14 dairy cows. Blood samples were collected quarterly during this study period. The data regarding seasonal changes in hematological parameters were not significant concerning red blood cell count (RBC), hemoglobin concentration (Hb) and mean corpuscular volume (MCV). However, variations were significant for hematocrit (HCT), mean corpuscular hemoglobin content (MCH), mean corpuscular hemoglobin concentration (MCHC) and blood platelets (PLT). The percentage of HCT was higher in autumn (27.99%) and spring (28.58%) compared to winter (26.52%) and summer (26.75%), ($p<0.05$). MCH, MCHC and PLT levels were higher in summer compared to the other seasons ($p<0.05$), ($p<0.01$) and ($p<0.001$), respectively. The highest number of WBC was generally observed in summer and autumn and the lowest in winter and spring ($p<0.01$). The ratio neutrophils/lymphocytes (N:L) was equal to 1.01, 0.79, 0.98 and 0.89 in autumn, winter, spring and summer, respectively of which this difference was not significant. This study might be helpful for providing base line information on the hematological profile of dairy cattle for the evaluation of physiological status.*

Keywords: changes, dairy cows, hematological parameters, seasons

Introduction

The evaluation of the physiological status is essential as a reference for the development of different livestock maintenance systems. The hematological profile is one of the physiological variables that can provide crucial information for the diagnosis, monitoring and prognosis of disease progression, production and welfare of livestock, which can have an impact on increasing livestock production and productivity (Bezerra et al., 2017).

For ruminants, hematological parameters depend on many factors such as animal's physiological status, age, management system (Brucka-Jastrzębska et al., 2007; Botezatu et al., 2014) and season (Farooq et al., 2017). The influence of seasonal variations on hematological parameters has been largely studied in various animal species; in dairy cows (Abdelatif and Alameen, 2012; Casella et al., 2013; Vallejo-Timaran et al., 2020), sheep (Rathwa et al., 2017), goats (Banerjee et al., 2015), Cholistani service bulls (Farooq et al., 2017) and Dromedary camel (Babeker et al., 2013).

Changes in hematological parameters are possible indicators to predict the potential resistance of cattle to different climatic conditions. Cattle resistant to dry and hot climates are expected to show the slightest changes in haematological parameters when exposed to high temperatures and feed shortages (Radkowska and Herbut, 2014; Dzavo et al., 2020). To determine the abnormal physiological situation in cattle, values from hematological analysis should be compared with ranges of standard values (Winnicka, 2008; George et al., 2010).

In Algeria, the cattle population represents about 6% of the total population (sheep, goats, cattle, camels and horses) with a total of 1.9 million individuals of which 52% are dairy cows (MADR, 2018). Three breeding systems are identified in Algeria: extensive, semi-intensive and intensive. The extensive system concerns 40% of milk production and 78% of meat production in the country (Nedjraoui, 2006).

Considering that the hematological profile is of great importance in monitoring the health status of dairy cows and that environmental conditions are considered as physiological stressors that affect the cattle's biological system, the objective of this investigation was to study seasonal variations in the hematological profile of

*e-mail: ouchenenaa@gmail.com

Holstein dairy cows as an indicator for their physiological status assessment. The obtained results, for the first time in Algeria, could be used as reference data until a large-scale study on a larger population of this breed is carried out.

Material and methods

Study area

The study was conducted between November 2018 and October 2019 at the Baaraouia pilot dairy farm in Constantine region, north-east Algeria (36°16'20" N; 6°40'19" E) (Figure 1). This region of 626 m altitude is recognized for its semi-arid climate characterized by a cold winter and a hot and dry summer. The temperature during the monitoring period was 27.5±7.5°C in summer, 18.5±9.5°C in autumn, 8.0±5.0°C in winter and 14.5±9.5°C in spring. Relative humidity was 53.8±28.8%, 63.4±27.8%, 73.8±20.6% and 64.8±27.8% in summer, autumn, winter and spring. The average annual precipitation was 630 mm (Constantine weather station, 2019).

Figure 1. Map of the study area

Animals

The study involved Holstein cows aged between 5 and 6 years of which milk production ranged from 19 to 20 liters cow/day. The cows' dry-off is done at the beginning of the 8th month of gestation. The insemination of the cows is artificial.

During the study period, all the cows were homogeneous in terms of health status, age groups and physiological stages in order to keep only one factor of variation, which is the season. Dairy cow selection criteria excluded clinically ill animals detected by veterinary clinical examination. This clinical visit was carried out on the day of the blood test.

The housing system of the cows is semi-intensive where the animals are kept on pasture for six hours per day and then receive hay and straw (8 kg/cow/day) in two daily rations with a distribution of barley silage between November and March. Dairy cows receive an additional 8-9 kg/cow/day of concentrate. Water is provided *ad libitum*.

Blood samples collection

The study was performed on lots of 14 dairy cows. Blood samples were collected quarterly during the study period, aseptically from the coccygeal vein, which is considered the most accessible vein (with the jugular vein) and the most commonly used for blood collection in cattle. The blood was mixed with the anticoagulant in the tube carefully. Individual blood samples were collected in the morning, before food distribution. Blood samples were taken into vacuum tubes containing EDTA and sent immediately to the laboratory in a cooler at +4°C. During the blood samples collection animals were calm to avoid changes in the blood parameters due to stress.

Laboratory analysis

The following hematological parameters were analyzed: red blood cell count (RBC, *10^6/µL), hemoglobin concentration (Hb, g/dl), hematocrit (HCT, %), mean corpuscular volume (MCV, μm^3), mean corpuscular hemoglobin content (MCH, pg), mean corpuscular hemoglobin concentration (MCHC, g/dl), blood platelets (PLT, *10^3/µL), and white blood cell count (WBC, *10^3/µL) including neutrophils (NEU, *10^3/µL), eosinophils (EOS, *10^3/µL), basophils (BAS, *10^2/µL), lymphocytes (LYM, *10^3/µL) and monocytes (MON, *10^3/µL). All these parameters were performed using a hematology automate (MINDRAY BC3000 Plus, Ref SKU-BC-3000Plus, China) and (BIOTA, Ref VABIO 360, Turkey).

The relative values of the leukocyte formula are established on the basis of microscopic observation of blood smears stained by the May-Grünwald Giemsa staining technique (kit RAL 555, RAL diagnosis, Martillac, France). Absolute values are then calculated from the total number of white blood cells for each cow. Mean values for each blood parameter were calculated for each season.

Statistical analysis

The statistical program used was R i386 3.0.2 for Windows GUI front-end. Chi square test and multiple range tests were used for the statistical analysis. The threshold value of different tests was $p<0.05$.

Results

The data regarding seasonal changes in hematological parameters in dairy cows are presented in Tables 1 and 2. The results showed that seasonal changes were not significant concerning RBC, Hb and MCV (Table 1). However, variations were significant for HCT, MCH, MCHC and PLA. The percentage of HCT was higher in autumn (27.99%) and spring (28.58%) compared to winter (26.52%) and summer (26.75%), ($p<0.05$). The MCH was higher in summer compared to the other seasons ($p<0.05$). The highest MCHC level in blood was observed in summer (37.20 g/dl) then this level decreased gradually in autumn (35.21 g/dl) and winter (34.21 g/dl) to the lowest level in spring (32.97g/dl) ($p<0.01$) The highest number of platelets was observed in summer (379.57*10^3/µl) and the lowest number in winter (330.93*10^3/µl), ($p<0.001$) (Table 1).

Table 1. Seasonal changes in hematological parameters of red blood cells in dairy cows

Parameters	Autumn (CI, 95%)	Winter (CI, 95%)	Spring (CI, 95%)	Summer (CI, 95%)	p-value
RBC (*10^6/µL)	5.78^a (5.26-6.30)	5.40^a (5.02-5.78)	5.80^a (5.32-6.28)	5.65^a (4.99-6.31)	
Hb (g/dl)	9.84^a (6.82-11.35)	9.09^a (8.44-9.74)	9.42^a (8.59-10.25)	9.96^a (8.78-11.14)	
HTC (%)	27.99^a (23.44-32.54)	26.52^b (24.62-28.42)	28.58^a (25.65-31.51)	26.75^b (23.38-30.12)	p<0.05
MCV (µm^3)	49.09^a (41.96-56.22)	49.30^a (46.21-52.39)	49.38^a (45.97-52.79)	47.46^a (44.31-50.61)	
MCH(pg)	16.92^a (15.37-18.47)	16.81^a (15.84-17.78)	16.21^a (15.44-16.98)	17.65^a (16.08-19.22)	
MCHC (g/dl)	35.21^b (33.22-37.20)	34.21^c (33.56-34.86)	32.97^d (32.01-33.93)	37.20^a (36.02-38.38)	p<0.01
PLT(*10^3/µL)	355.07^b (256.58- 453.56)	330.93^d (268.76-393.1)	346.93^c (281.84- 412,02)	379.57^a (362.51-396.63)	p<0.001

^{a,b,c,d} Values that have not the same letter in the same line are different at p<0.05;
Red blood cell count (RBC), hemoglobin concentration (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin content (MCH), mean corpuscular hemoglobin concentration (MCHC) and blood platelets (PLT).

The dendrogram shows that seasonal changes of the hematological parameters of RBC are divided into three groups (Figure 2). The first group includes the hematological results of spring and autumn; the second group includes the summer hematological results. Winter is the third group.

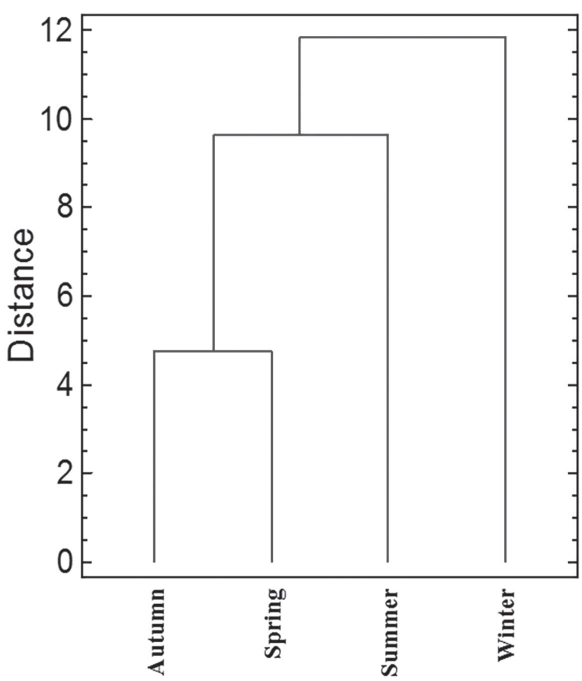

Figure 2. Dendrogram of the different hematological parameters of red blood cells in dairy cows according to the season

Results of the hematological analysis of WBC show a significant seasonal variation (p<0.01). The highest values were generally observed in summer and autumn and the lowest in winter and spring (Table 2). The ratio neutrophils/lymphocytes (N:L) was equal to 1.01, 0.79, 0.98 and 0.89 in autumn, winter, spring and summer, respectively. The differences between the seasons were not significant.

The dendrogram, presented in Figure 3, shows that seasonal changes in hematological parameters of WBC are divided into two groups. The first group includes hematological results of summer and autumn and the second group includes those of winter and spring.

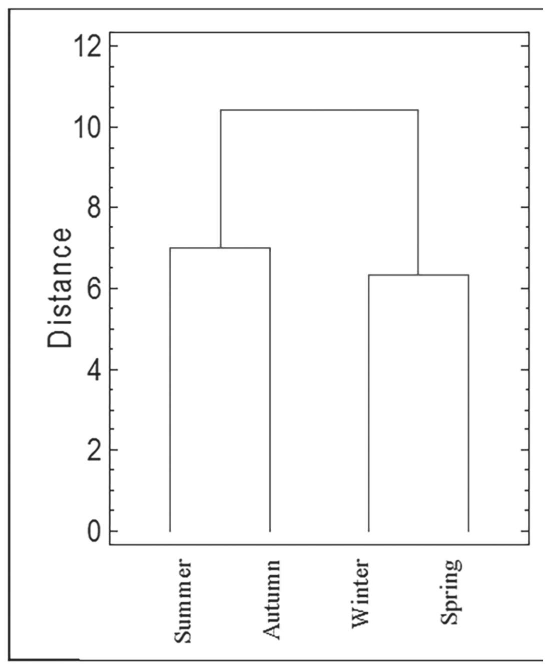

Figure 3. Dendrogram of the different hematological parameters concerning of white blood cells in dairy cows according to the season

Discussion

The hematological profile in clinically normal lactating cows is affected by many factors such as animal's physiological status, management system, stage of lactation, and season (Brucka-Jastrzębska et al., 2007; Farooq et al., 2017; Dzavo et al., 2020). In this study, we investigated seasonal variations in the hematological profile of Holstein dairy cows as an indicator for the evaluation of physiological status. The average results of the blood parameters, reported in the present study, were within the range of reference values for healthy dairy cows (Winnicka, 2008; George et al., 2010). The results obtained are consistent with other reports (Giri et al., 2016; Farooq et al., 2017).

The most common indications for RBC analysis are clinical anemia or hemorrhage. In the case of absolute anemia, RBC value decreases (Brockus, 2011). In this finding, no cases of hemorrhage or anemia were recorded as the RBC values remained comparatively high and unchanged throughout the year. This was confirmed elsewhere (Babeker et al., 2013). RBC values can be incorrect due to hemolysis or an inadequate blood sample/anticoagulant ratio, which can lead to dilution of RBC (Jones and Allison, 2007). All these remarks have been taken into consideration in our study.

Table 2. Seasonal changes in hematological parameters of white line or white blood cells categories in dairy cows

Parameters	Autumn (CI, 95%)	Winter (CI, 59%)	Spring (CI, 59%)	Summer (CI, 59%)	p-value
WBC	10.66^b	8.31^c	7.91^c	12.01^a	p<0.01
(*10^3/µL)	(5.25-16,07)	(4.01-12.61)	(2.82-13)	(6.4-17.62)	
LYM	4.18^b	3.89^c	3.09^d	4.99^a	p<0.01
(*10^3/µL)	(4.0-4.36)	(3.55-4.24)	(1.03-5.15)	(4.51-5.48)	
MON	0.26^b	0.25^b	0.17^c	0.53^a	p<0.01
(*10^3/µL)	(0.20-0.32)	(0.01-0.49)	(0.01-0.33)	(0.45-0.61)	
NEU	4.48^b	3.16^b	3.14^b	4.92^a	p<0.01
(*10^3/µL)	(4.33-4.62)	(2.54-3.79)	(1.12-5.16)	(4.27-5.57)	
EOS	1.67^b	0.96^d	1.47^c	1.53^a	p<0.01
(*10^3/µL)	(0.40-2.94)	(0.34-1.59)	(0.57-2.37)	(0.69-2.37)	
BAS	0.59^a	0.28^b	0.20^c	0.16^d	p<0.01
(*10^2/µL)	(0.42-0.76)	(0.24-0.31)	(0.16-0.23)	(0.12-0.20)	

^{a,b,c,d} Values that have not the same letter in the same line are different at p<0.05;
White blood cell (WBC), lymphocytes (LYM), monocytes (MON), neutrophils (NEU), eosinophils (EOS) and basophils (BAS).

In our study, no significant seasonal effects on Hb concentration were found, however, Hb concentration decreased insignificantly (p>0.05) during the winter compared to the other three warmer seasons. This result is consistent with the results of other studies (Kumar and Pachauri, 2000; Farooq et al., 2017). The mechanism of the effect of seasons on blood Hb concentration is still unclear (Farooq et al., 2017), but the energy shift during different seasons to maintain physiological processes controlling heat loss could be a credible justification (Scharf et al., 2010; Fadare et al., 2012).

In agreement with Rowlands et al. (1977) and Casella et al. (2013), HCT was significantly higher in spring and autumn than summer and winter. In summer the ambient temperature rises which causes an increase in the animal body temperature associated with an elevation in water consumption and peripheral vasodilatation leading to hemodilution resulting in a reduction in HCT values (Koubkova et al., 2002). In winter, the decrease in HTC value is related to the start of the cow's milk production cycle and the required recovery time after calving which occurred during the winter in our study (Rowlands et al., 1977; Fisher et al., 1980).

The non-significant variation in MCV and MCH during the different seasons in this study indicates that cows are adaptable to harsh climatic conditions without being stressed (Farooq et al., 2017). In agreement with our findings, previous studies have reported higher MCHC in cattle during the summer season (Giri et al., 2017; Dzavo et al., 2020).

PLT play an essential role in hemostasis. In practice, platelet counting may be indicated in cases of severe hemorrhage or increased tendency to hemorrhage (Russell, 2010). Decreased PLT content has been associated with increased summer temperature, which is the main environmental stressor (Casella et al, 2013). However, the contrary was observed in our study, which could be due to other factors that should be developed in other studies.

WBC (lymphocytes, monocytes, neutrophil, eosinophilic and basophilic) play an essential role in the body's immune defense and constitute a small percentage of the total number of blood cells (Webb and Latimer, 2011). In the present investigation, WBC values were higher in summer, confirming the results obtained in previous reports (Abdelatif et al., 2012; Giri et al., 2016). The rise in temperature in summer stimulates the release of corticosteroids or epinephrine hormones which, in turn, increased the number of WBC (Lateef et al., 2014). This elevation is also due to an increase in subclinical parasitic infection of animals in summer (Rutkowiak, 2001). However, in another report, a significant decrease in WBC during the summer season was described (Al-Shami, 2003) while another study found no seasonal effect on this parameter (McManus et al., 2009).

In this study, lymphocytes were the predominant leukocytes, followed by neutrophils, which is specific to ruminants (Kumar and Pachauri, 2000). Lymphocytes, monocytes, neutrophils and eosinophils were significantly more numerous during the summer. This is consistent with Farooq et al. (2017) and suggests that the overall increase in WBC during the summer was due to an increase in lymphocytes, monocytes, neutrophils and eosinophils. The number of monocytes in cattle is variable between individuals, which indicate that it is therefore not a relevant indicator for a specific disease (Jones and Allison, 2007).

In our survey, the number of basophiles varies significantly between seasons with a high number in autumn. The increase in basophils has often been linked to hyperlipidemia and to allergies, ulcerations and tick infestations (Tornquist and Rigas, 2010). However, it is not commonly reported in the literature because the number of basophils in the blood is very low (Tornquist and Rigas, 2010; Webb and Latimer, 2011).

This study showed that the season had no significant influence on the neutrophil to lymphocyte (N:L) ratio. This ratio is the most common indicator of stress and the adaptability of animals to the local environment, which generally increases under stress (Minka and Ayo, 2007).

Conclusion

It can be stated that although the hematological values obtained were comparable to the values considered normal for dairy cattle, the season can influence the hematological profile of the dairy cow. Blood parameters influenced significantly by the season are HCT, MCH, MCHC, PLA and WBC. Therefore, this study might be helpful for providing base line information on the hematological profile of dairy cattle for the evaluation of physiological status. This study needs to be further investigated with regard to differences in hematological parameters according to age and sex, with special attention to reproductive status.

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