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Seasonal Variations in Hematological Values of High-and Average-yielding Holstein Cattle in Semi-arid Environment

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Abstract. A total of thirty cows divided into two equal groups were used in the experiment. During each season fifteen non-pregnant Holstein cows, including 5 dry cows, 5 high-yielders and 5 average-yielders, were used to study the effects of season of the year and milking intensity on blood cellular and non-cellular constituents. The rise in Temperature-Humidity Index from 66.6 in winter to 81.9 in summer was associated with a 28% decline in the milk production of the high-yielding cows. Hot summer also resulted in a significant reduction in blood hemoglobin (Hb), packed cell volume (PCV), mean cell volume (MCV) and mean cell hemoglobin (MCH) values, but had no significant effect on red blood cell (RBC) counts, mean cell hemoglobin concentration (MCHC), white blood cell (WBC) counts and serum proteins.

Lactating cows had lower Hb, PCV, MCV, MCH, total protein (TP) and globulin (G) compared with dry cows. Among the two lactating groups, the high-yielders had significantly lower TP and G and significantly higher Hb compared with the average-yielders. The significant interactions between season and productivity revealed that lactating cows had significantly higher albumin (A) than the dry ones during summer, but showed an opposite trend during winter. High - yielders had higher WBC counts and lower TP and G in summer compared with winter indicating their susceptibility to high environmental temperature compared with average-yielding cows.

Introduction

The numbers of specialized dairy farms in Saudi Arabia increased considerably during this decade. Statistics released by the Ministry of Agriculture and Water [1] indicated that the relative increase in the numbers of specialized dairy farms, cows and milk production during 1981 - 1986 were 73%, 104%, 172%, respectively. The breed of dairy cattle raised in most of these farms is the exotic temperate-evolved Holstein cows. The environmental temperature prevailing in the country remains above the thermoneutral temperature of lactating Holstein (21°C) for at least 8 months of the year.

Lactation and environmental conditions, mainly seasonal variations in air temperature, are considered to be physiological stressors which affect the animal's biological system. Both stressors are known to exert significant influence on blood hematological characteristics of dairy cattle [2-4]. In this regard, high milk yielders may be more affected than animals with low potential for milk production or dry cows [2,5,6, pp 3-16]. Therefore, this study was conducted to evaluate the effect of hot summer and moderate winter seasons on blood cellular and non-cellular constituents of high - yielding , average - yielding and dry Holstein cows.

Materials and Methods

This study was conducted at Al-Kharj Agriculture Project, Al-Hassa Irrigation and Drainage Authority, Ministry of Agriculture and Water during the summer of 1987 and the winter of 1988. A total of thirty cows divided into two equal groups were used in the experiment. During each season, 15 non-pregnant Holstein cows including 5 dry cows, 5 high-yielding and 5 average-yielding, were chosen to be used in the experiment. Average daily milk yield of the high - yielding cows during the experimental periods were 24.1±0.45 and 33.5±0.77 kg/day during summer and winter seasons, respectively. The corresponding values for the average-yielding cows were 19.2±0.41 and 18.4±0.66 kg/day. The animals had an average body weight of 699.6±9.74 kg. All the lactating cows were in their third to fourth lactations and were matched for stage of lactation (60-110 days) and general body conformation.

Animals were housed in an open shaded barn and water was available at all times in drinking basin. They were fed according to the standard regime practiced in the farm on commercially formulated dairy concentrates (13.4% digestible protein and 72% total digestible nutrients) and roughage. The roughage was provided *ad libitum* and consisted of 80% green fodder (*Medicago sativa*) and 20% alfalfa hay. The concentrates mixture was offered to the animals according to their actual requirements and was given at 4.30 and 15.30 hr. when the animals were machine-milked.

The experiment was started in summer 1987 (August) and lasted for two weeks. The first week was considered as a preliminary period and was followed by a one week experimental period. Daily maximum and minimum ambient air temperatures and relative humidities were recorded during the 6 - day experimental period. Also dry and wet bulb temperatures were measured twice a day at 9.00 and 16.00 hr. Three blood samples were obtained from each animal every other day during the 6-day period. Blood samples were collected from the external Jugular vein using 10 ml vacutainer tubes and were placed immediately on ice. Ethylene diamine-tetra-acetic acid (EDTA) was used as anticoagulant but, in part of sample, it was withheld to obtain serum. Plasma or serum was obtained by centrifugation of blood at 860 xg for 20 min, and were stored at -20°C until analyzed.

Whole blood was analyzed shortly after collection for hemoglobin (Hb), packed cell volume (PCV), red blood cell (RBC) counts and leucocyte (WBC) counts. Total protein (TP) and albumin (A) were determined in stored serum. Blood Hb concentration was determined using Drabkin's reagent Hemotrol (bioMerieux, France). RBC were counted on AO Bright line hemocytometer using a light microscope at 450 × magnification. Blood samples were diluted 200 times with physiological saline (0.9% sodium chloride solution) before counting. WBC were counted on AO Bright line hemocytometer using a light microscope at 100 × magnification after diluting blood samples to 20 times with diluting fluid that consisted of 1% acetic acid solution and little of methyl violet 2B [7, pp. 33-35]. Mean cell volume (MCV), mean cell hemoglobin (MCH) and mean cell volume hemoglobin concentration (MCHC) were calculated using the formulae proposed by Schalm [8]. Total serum proteins were measured by the Biuret method as described by Armstrong and Carr [9] and albumin (A) concentration was determined according to the method of Doumas *et al.* [10]. Globulin (G) concentration was calculated as the difference between TP and A, and A/G ratio (A/G) was calculated. The previously described experimental protocol was carried out again during February 1988 (winter season), using the other 15 non-pregnant Holstein cows.

Data were subjected to statistical analyses at King Saud University Computer Center according to Goodnight *et al.* [11]. The least-Squares Means (LSMEANS) procedure was applied to the data. The following model was used:

$$Y_{ijkl} = u + S_i + P_j + SP_{ij} + C_k\,(SP_{ij}) + e_{ijkl}$$

where, Y_{ijkl} is 1st observation of the k th cow of the j^{th} production level during the i^{th} season; u is the overall mean; S_i is the effect of the i^{th} season (i=1 and 2); P_j is the effect of the j^{th} level of production (j=1,2 and 3); SP_{ij} and C_k (SP_{ij}) are the interaction effects, and e_{ijkl} is the residual term. Overall simple correlation coefficients between milk production and blood constituents were also calculated.

Since repeated measurements were taken on the same animal during a particular season, some assumptions of the analyses might have been violated. However the separate analyses carried out for each day and for the pooled data showed similar pattern of significancy, therefore the analysis was made on the pooled data.

Results and Discussion

During the experimental periods, the average maximum ambient air temperatures were 38.7 and 24.2°C during summer and winter seasons, respectively. The corresponding minimum values were 20.7 and 12.1 °C. Average maximum relative humidity was 48.8% during summer and 78.2 % during winter, with corresponding minimum values of 27.5 and 30.0%. The calculated Temperature-Humidity Index, THI [THI = T_{db} + 0.36 T_{dp} + 41.2°C, where: T_{db} = dry bulb temperature (°C) and T_{dp} = dew point temperature (°C)] was 81.9 during the summer period and 66.6 during the winter period. This index is used throughout the world, especially in the hotter regions of the world, to generally characterize climatic effects on animal performance. The upper critical THI for the high producing temperate-evolved Holstein cows, above which milk production declines, was reported to be 72 [6, pp. 3-16]. In the present study, the calculated THI was above the upper critical limit during summer and below it during winter. This explains the 28% decline in the milk yield of the high producing cows during summer compared with winter (24.1 VS 33.5 kg/day).

The rise in ambient temperature during summer was associated with lower hematological values of the Holstein cows than those values during the moderate winter season (Table 1). These seasonal variations were significant for Hb, PCV ($p<0.01$), MCV and MCH ($p<0.05$) but not for RBC or MCHC values. The decline in Hb and PCV with elevated ambient temperature during summer agrees with previous reports [2-4, 12-15]. In the present study, heat induced depression of blood Hb and PCV without any significant alterations in circulating erythrocytes. Some workers [4,16] verified the depression in blood Hb and PCV of cattle subjected to elevated ambient temperature to the hemodilution effect where more water is transported in the circulatory system for evaporative cooling. Our data showed that the reduction in Hb and PCV in hot summer was mainly due to the significant decrease in both MCV and MCH, suggesting that the depression is related to reduction in cellular oxygen requirements in order to reduce metabolic heat load and, consequently, to compensate for elevated environmental heat load [3].

The lactating cows had significantly lower blood Hb, PCV ($p<0.01$) and MCH ($p<0.05$) when compared with dry cows. MCV was also lower in lactating cows, but the difference was statistically insignificant. RBC counts and MCHC were similar in the two groups (Table 1). The lactation-induced depression of blood Hb and PCV was reported in cattle [2, 12, 17], and goats [18]. This decline in blood Hb and PCV was apparently due to the reduction in MCH and MCV (Table 1) as a result of the rise in mammary tissue requirements for milk synthesis and a concomitant rise in blood flow to the mammary gland. Among the two lactating groups, the high-yielders possessed higher ($P<0.05$) blood Hb compared with the average-yielders and both had lower values than the dry cows (Table 1). Although lactation affected blood Hb concentrations in the present study, the correlation between milk yield and blood Hb was not significant ($r=0.22$). Few studies were conducted on hematological values of high and low-milk producing animals. Blum *et al.* [5] working with various breeds of dairy cattle during a full lactation noted that Hb and PCV were not correlated with milk yield. Meanwhile, El-Nouty *et al.* [2] in their study on Holstein cows in Egypt reported that the high-yielders had lower PCV values than the low-yielders. This discrepancy may be related to the degree of acclimatization and/or the differences in milk production level of the studied animals. The high blood Hb observed

Table 1. Least square means and standard errors of blood hemoglobin (Hb), packed cell volume (PCV), red blood cell (RBC) counts, mean cell volume (MCV), mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) as influenced by season and milk productivity in Holstein cows.

	n	Hb (gm %)	PCV (%)	RBC ($\times 10^6$/mm^3)	MCV (U^3)	MCH (pq)	MCHC (%)
Season(s)		**	**	ns	*	*	ns
Summer (Su)	45	9.46±0.08^{a}	29.47±0.25^{a}	5.55±0.18	55.51±1.64^{a}	17.80±0.54^{a}	32.15±0.28
Winter (W)	45	10.26±0.08^{b}	31.62±0.25^{b}	5.32±0.18	60.63±1.64^{b}	19.69±0.54^{b}	32.47±0.28
Productivity(P)		**	**	ns	ns	*	ns
Dry(D)	30	10.59±0.13^{Aa}	32.53±0.37^{Aa}	5.43±0.21	60.85±1.97	19.81±0.66^{Aa}	32.52±0.38
High(H)	30	9.66±0.10^{b}	29.73±0.31^{b}	5.37±0.22	57.27±2.01	18.58±0.66^{ab}	32.56±0.34
Average(A)	30	9.33±0.10^{c}	29.37±0.31^{b}	5.50±0.22	56.10±2.01	17.86±0.66^{b}	31.84±0.34
Lactating(L)$^{+}$	60	9.49±0.09^{B}	29.55±0.26^{B}	5.43±0.15	56.68±1.40	18.22±0.47^{B}	32.20±0.27
S×P		ns	ns	ns	ns	ns	ns
Overall mean		9.86±0.06	30.54±0.02	5.43±0.13	58.07±1.16	18.75±0.38	32.31±0.20
C.V.		5.59	5.52	21.99	19.00	19.26	5.83
R^2		0.842*	0.811**	0.226	0.302	0.352	0.461*

Means in the same column within the same effect bearing different superscripts differ ($p<0.05$); small letters are used to compare dry, high - and average - yielders; capital letters are used for comparing dry versus lactating cows.
* $P < 0.05$
** $P < 0.01$; ns = nonsignificant.
\+ LSMEANS of high and average producers.

in the high-producing cows may be ascribed to their fast metabolic process necessary for sustained milk production and respiratory enhancement. This view is supported by the slightly higher MCV and MCH observed in the high-producers. The correlation coefficients between milk yield and MCV and MCH were 0.12 and 0.14, respectively.

Although WBC counts were not significantly affected by season or productivity, least square means revealed that the high-yielders had significantly higher WBC counts compared with the dry cows. The interaction between season and productivity exerted significant influence ($P<0.01$) on WBC counts (Table 2). Regardless of productivity level, lactating animals in both seasons had higher WBC counts compared with the dry ones, but the differences were statistically insignificant. Similar

Table 2. Least square means and standard errors of white blood cell (WBC) counts and serum proteins as affected by season and productivity in Holstein cows.

	n	WBC ($\times 10^3/mm^3$)	TP (gm%)	A (gm%)	G (gm%)	A/G Ratio
Season(S)		ns	ns	ns	ns	ns
Summer(Su)	45	4.57±0.20	7.52±0.04	4.21±0.03	3.31±0.04	1.33±0.02
Winter(W)	45	4.64±0.20	7.55±0.04	4.20±0.03	3.35±0.04	1.32±0.02
Productivity (P)		ns	**	ns	**	**
Dry(D)	30	4.20±0.29	7.69±0.07^{Aa}	4.20±0.06	3.49±0.09^{Aa}	1.26±0.05^{Abc}
High(H)	30	5.03±0.25	7.36±0.04^{c}	4.23±0.04	3.13±0.05^{b}	1.40±0.03^{a}
Average(A)	30	4.59±0.25	7.56±0.04^{b}	4.18±0.04	3.37±0.05^{a}	1.31±0.03^{b}
Lactating(L)$^+$	60	4.81±0.20	7.46±0.05^{B}	4.21±0.04	3.25±0.06^{B}	1.35±0.04^{A}
S × P		**	**	**	**	**
Su × D	15	4.03±0.41^{Ab}	7.63±0.10^{ABa}	4.03±0.08^{Bb}	3.60±0.13^{Aa}	1.15±0.07^{Bb}
Su × H	15	5.76±0.35^{a}	7.27±0.06^{b}	4.23±0.05^{a}	3.04±0.07^{d}	1.42±0.04^{a}
Su × A	15	3.94±0.35^{b}	7.66±0.06^{a}	4.36±0.05^{a}	3.30±0.07^{bc}	1.40±0.04^{a}
Su × L$^+$	30	4.89±0.29^{A}	7.47±0.07^{B}	4.30±0.06^{A}	3.17±0.09^{B}	1.41±0.05^{A}
W × D	15	4.37±0.41^{Abc}	7.74±0.10^{Aa}	4.36±0.08^{Aa}	3.38±0.13^{ABbc}	1.37±0.07^{Aa}
W × H	15	4.31±0.35^{bc}	7.45±0.06^{c}	4.24±0.05^{a}	3.21±0.07^{cd}	1.37±0.04^{a}
W × A	15	5.24±0.35^{ac}	7.45±0.06^{c}	4.00±0.05^{b}	3.45±0.07^{ab}	1.21±0.04^{b}
W × L$^+$	30	4.78±0.29^{A}	7.45±0.07^{B}	4.12±0.06^{B}	3.33±0.09^{AB}	1.29±0.05^{AB}
Overall mean		4.61± 0.14	7.53± 0.26	4.20± 0.02	3.33± 0.03	1.32± 0.03
C.V.		29.46	3.25	4.97	7.92	20.72
R^2		0.581**	0.807**	0.830**	0.872**	0.536**

Means in the same column within the same effect bearing different superscripts differ ($p<0.05$); small letters are used to compare dry, high -and average- yielders; capital letters are used for comparing dry versus lactating cows.

* $P < 0.05$

** $P < 0.01$; ns = nonsignificant.

\+ LSMEANS of high and average producers.

observations were reported on dairy cattle [2] sheep and goats [19]. The leucocytosis obsreved in lactating animals can be attributed to their higher levels of blood glucocorticoids compared with the dry ones, since glucocorticoids are known to stimulate milk synthesis and are released by the stressful response caused by milk secretion [20,21].

During the summer season, WBC count in high-yielding cows was markedly higher than in the average ones, a situation which was reversed in winter. The reason for this, however, is unclear. It may be assumed that the rise in WBC counts in high-yielding group during summer is due to the combined stressful effects of high milk yield and high environmental temperature. Lee *et al.* [3] reported a rise in WBC counts of lactating Holstein from intermediate to the hot temperature-season. Meanwhile, the decline in WBC count in the high-yielding group during the winter season compared with the average ones can be ascribed to the rise in WBC excretion into the milk as a consequence of the increase in blood flow to the mammary gland [22]. It should be stated that the high-yielding cows were producing 28% more milk in winter than in summer, and that WBC counts were correlated negatively with milk yield ($r = -0.12$).

Season caused no significant effect on serum TP or various protein fractions, but productivity significantly ($P<0.01$) influenced TP, G and A/G ratio. TP and G were higher in dry cows than in the lactating ones. Accordingly A/G ratio was higher in the lactating cows, but this increase was statistically insignificant. Among the two lactating groups, the high-yielders possessed significantly lower TP and G ($p<0.01$), and higher A/G ratio ($P<0.05$) than the average-yielders (Table 2). The non-significant effect of season on serum proteins agrees with results by Rowlands *et al.* [17]. On the other hand, several investigators reported a slight increase in serum TP during temperature-season progression [4, 13]. This discrepancy may be ascribed to managerial factors, i.e. studies involved range cattle versus stall-fed cattle. It would be logic to assume that quality of the range may be affected by temperature-season. The lactation-induced depression of serum proteins was reported earlier for lactating Holstein [23]. This observed decline in TP can be partly attributed to the lactation-induced increase in total body water [24], blood volume [25] and/or to the utilization of serum protein for milk biosynthesis. The above changes may be of greater magnitude in the high - yielding cows causing further decline in TP and G when compared with the average - yielders.

There were significant interactions ($P<0.01$) between season and productivity on TP and the various protein fractions. Lactating cows had significantly higher A than the dry ones in summer, with an opposite trend in winter. This rise in blood A of lactating cows during summer may be explained by their greater need for maintenance of expanded extracellular fluid volume relative to the dry cows. In winter, A is secreted directly to the milk which is produced in high amount, resulting in low concentration of blood A in lactating cows. In summer, the high - yielders had lower TP and G compared with the average-yielders. During the winter season both groups had similar TP, but the high-yielding cows had higher A and lower G. These changes can be attributed to the heat-induced depression of feed intake and the accompanied alterations of water and hormonal balances.

In conclusion, the rise in THI above the upper critical level for lactating Holstein cows altered most blood cellular and non-cellular constituents. These alterations, which were more pronounced in the high-yielding cows, included a rise in WBC counts and a decline in TP and G, and resulted in a 28% decline in their milk yield compared with their production in winter.

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الاختلافات الموسمية في مكونات دم أبقار الهولشتين عالية ومتوسطة الإدرار في البيئة شبه الجافة

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ملخص البحث. استخدمت في هذه الدراسة ٣٠ بقرة هولشتين غير حامل قسمت إلى مجموعتين متساويتين. ١٥ منها خلال كل موسم مقسمة كما يلي: ٥ أبقار جافة و٥ أبقار عالية الإدرار و٥ أبقار متوسطة الإدرار وذلك لدراسة تأثير الفصل من السنة والإنتاجية من الحليب على صفات الدم الخلوية وغير الخلوية. ولقد أظهرت النتائج أن ارتفاع دليل الحرارة والرطوبة من ٦٦,٦ في الشتاء إلى ٨١,٩ في الصيف صاحبه إنخفاض إنتاج الحليب في الأبقار عالية الإدرار بنسبة ٢٨٪. وأدى ارتفاع درجة الحرارة في الصيف إلى إنخفاض معنوي في كل من هيموجلوبين الدم ونسبة المكونات الخلوية ومتوسط حجم الكرة الحمراء ومتوسط محتوى الخلية الحمراء من الهيموجلوبين، ولم يكن له تأثير على عدد كرات الدم الحمراء والبيضاء وعلى متوسط تركيز الخلية الحمراء من الهيموجلوبين وتركيز البروتينات الكلية في مصل الدم.

الحيوانات الحلابة كانت أقل في هيموجلوبين الدم ونسبة المكونات الخلوية ومتوسط حجم الكرة الحمراء ومتوسط محتوى الخلية الحمراء من الهيموجلوبين والبروتينات الكلية والجلوبيولين مقارنة بالحيوانات الجافة. كما لوحظ أن البروتينات الكلية والجلوبيولين كانت أقل في الأبقار عالية الإدرار بينما كان هيموجلوبين الدم بها أعلى من الأبقار متوسطة الإدرار.

التأثير المعنوي للتداخل بين الموسم والإنتاجية أظهر أنه خلال فصل الصيف كانت الحيوانات الحلابة أعلى في الألبيومين من الحيوانات الجافة والعكس لوحظ في الشتاء. كما اتضح أن الأبقار عالية الإدرار خلال فصل الصيف كان عدد كرات الدم البيضاء بها أعلى والبروتينات الكلية أقل مقارنة بمستوياتها خلال فصل الشتاء مما يدل على أنها تكون أكثر تأثرًا بارتفاع درجة حرارة الجو مقارنة بالأبقار متوسطة الإدرار.