

Metabolic and Endocrine Responses of Crossbred Dairy Cows in Relation to Pregnancy and Season Under Tropical Conditions

Ahmed Omer Alameen and Abdalla Mohamed Abdelatif

Department of Physiology, Faculty of Veterinary Medicine, University of Khartoum, Sudan

Abstract: The aim of this study was to evaluate effects of season and stage of pregnancy on metabolic and endocrine responses of crossbred dairy cows under tropical conditions. The effects of season (summer vs winter) on serum constituents were evaluated in 4 groups of cows; a group of empty cows and 3 groups representing early, mid- and late pregnancy. Serum levels of cholesterol, triglycerides and urea were higher during summer compared to respective winter values. Plasma glucose levels decreased with the advance of pregnancy in winter. During summer, the activity of serum aspartate aminotransferase (AST) was higher while alanine aminotransferase (ALT) was lower compared to winter value. Serum calcium (Ca) level was higher and serum inorganic phosphorus Pi level was lower in summer. With the advance of pregnancy, there was a decrease in Ca level and an increase in Pi level. Serum thyroid stimulating hormone (TSH) was elevated in summer compared to winter. Serum triiodothyronine (T_3) level was higher in summer and there was slight decrease with the advance of pregnancy in both seasons. Serum thyroxine (T_4) level was lower in summer. Serum cortisol level increased in response to summer heat load and it was higher during late pregnancy in both seasons. The results indicate the importance of thermal environment and physiological status in assessment of metabolic profile in crossbred dairy cows.

Key words: Dairy Cows • Pregnancy • Season • Blood Metabolites • Hormones

INTRODUCTION

Under tropical conditions, dairy cows are exposed to stressful climatic conditions associated with extended periods of high ambient temperature and solar radiation. Crossbred cows have been exploited for blending the adaptability of tropical cattle with the high milking potential of exotic breeds. The adaptation of dairy cows to tropical environment has been reduced as the locally genetically-adapted Butana and Kenana cattle are crossbred with high producing, non-adapted Holstein-Friesian breed of European origin. Genetic improvements that enhance production traits may increase the susceptibility to high thermal load as there is a close relationship between metabolic heat generation and the level of production [1]. Selection for milk yield reduces the thermoregulation ability during heat stress which magnifies the seasonal depression in productivity caused by climatic stress [2, 3]. The zebu (*Bos indicus*)

breeds have higher degree of thermotolerance compared with temperate (*Bos taurus*) breeds because of lower metabolic rate and greater sweating capacity [4, 5].

Exposure of dairy cows to hot environment produces reduction in the rates of metabolism, feed intake and an increase in respiratory rate and body core temperature that result in reduction of productivity during summer [6]. Also high yielding cows are more sensitive to hot environment than low yielding cows [7, 8]. Heat stress also reduces the length and intensity of oestrus [9, 10]; depression in conception rate in hot environment has been documented [11, 12].

The metabolic profile test that was adopted by Payne *et al.* [13], has been used for assessing metabolic status and diagnosis of metabolic disorders in dairy herds [14, 15]. The changes in blood constituents can reflect the physiological condition as well as nutritional and health status of cows. Acclimation to thermal stress also imposes physiological and metabolic adjustments

associated with reduction of performance and compromising of health [16]. The present study was undertaken to evaluate the effects of seasonal change in thermal environment and stage of pregnancy on blood metabolites, minerals and hormones of crossbred dairy cows under tropical conditions. The information generated can be utilized in adopting nutritional and environmental control strategies that can alleviate stress and improve productivity.

MATERIALS AND METHODS

Experimental Plan: The effect of season (summer vs winter) and physiological status on serum constituents were evaluated. In each season, 24 cows were assigned to 4 groups of 6 each: a group of empty cows served as control and 3 groups represented early, mid- and late pregnancy. The baseline data were obtained for all experimental groups of animals.

The blood samples were collected weekly at 9.00 to 11.00 a. m. for 9 weeks in each season. The serum samples harvested were used for the assessment of the concentrations of metabolites, enzymes, minerals and hormones.

Animals and Management: Clinically healthy, multiparous crossbred dairy cows (Butana X Friesian), aged 5-8 years were used in the study. The cows were selected from the herd of the governmental dairy farm at Khartoum North (15° 36' N; 32° 35' E). The animals were maintained on similar grazing programme under summer and winter conditions. Animals were kept in sheds located close to the milking parlor with appropriate facilities for feeding and watering. The non-grazing nutritional regimen comprised two types of feeds: roughages: alfalfa (*Medicago sativa*) and Abu 70 (*Sorghum bicolor*) and a computed concentrate. The roughages were offered twice daily at 10.00 a.m. and 5.00 p.m. and lactating cows were offered supplemental concentrate mixture twice daily,

before milking at 8.00 a. m. and at 11.00 p.m.. Table 1 projects the proximate analysis of the two types of roughage and the concentrate consumed by cows. All animals had free access to tap water during the experimental period.

Climatic Conditions: The climatic data were compiled from the Meteorological Station located approximately 5 km from the research site. The mean values of ambient temperature (Ta) and relative humidity (RH) recorded during the experimental period were used to calculate the respective mean values during summer and winter. Data were used to compute the composite climatic index, temperature-humidity index (THI) according to the formula of Ravagnolo *et al.* [17]:

$$THI = (1.8 \times T + 32) - [(0.0055 \times RH) \times (1.8 \times T - 26)]$$

where:

T = Air temperature (°C)

RH = Relative humidity (%)

Collection of Samples: Blood samples (10 mL) were collected aseptically by jugular venipuncture using plastic disposable syringes. 2 mL of blood was kept in a tube containing sodium fluoride and after centrifugation, the plasma sample was used for glucose determination. The rest of the blood sample was left at room temperature for 3 hrs at room temperature, then centrifuged and haemolysis-free serum samples were harvested and immediately frozen at -20°C for subsequent analysis.

Blood Metabolites: The plasma glucose concentration was determined by the enzymatic method using a kit (Randox Laboratories - London). Serum urea concentration was determined using a kit (Crescent diagnostic - MUSLCO - Saudi Arabia). Serum cholesterol level was determined using a kit (SPINREACT, S.A. Spain). Serum triglyceride concentration was determined

Table 1: The nutrient composition of roughages and concentrate (g/kg)

Ingredients	Alfalfa (<i>Medicago sativa</i>)	Abu 70 (<i>Sorghum bicolor</i>)	Concentrate
Dry matter	230.0	280.0	952.00
Crude protein	46.1	19.2	29.25
Crude fibre	70.0	108.0	35.30
Ether extract	3.3	8.4	3.99
Ash	28.2	23.9	7.88

using the enzymatic method (Liner Chemical-Spain). Serum concentrations of the transaminases, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined by the UV enzymatic method using kits (Liner chemical - Spain).

Serum Hormones: Serum concentrations of thyroid stimulating hormone (TSH) was measured by immunoradiometric assay kit MK - 432 (BEIJING ATOM HIGHTECH CO., LTD - China). Serum triiodothyronine (T_3) level was determined using radioimmunoassay kit - M (HTA CO. LTD, China), while serum thyroxine (T_4) level was determined using radioimmunoassay kit - IMK-437 (HTA CO. LTD, China). Serum cortisol level was measured by enzyme linked immunosorbent assay (ELISA).

Serum Minerals: Serum levels of calcium (Ca), phosphorus (P) and magnesium (Mg) were determined spectrophotometrically using kits (Liner Chemical- Spain).

Statistical Analysis: The experimental data obtained for 18 weeks covering two seasons (summer and winter) at different stages of pregnancy in dairy cows have been subjected to standard methods of statistical analysis. The mean values and standard deviations (Mean \pm SD) were calculated and the analysis was performed using General Linear Methods (GLM) procedure of Statistical Analysis System [18]. Analysis of variance (ANOVA) test was used to evaluate the effects of season and stage of pregnancy in dairy cows on serum metabolites, enzymes, minerals and hormones. The differences are considered statistically significant at $P < 0.05$.

RESULTS

Climatic Conditions: The mean values of ambient temperature (T_a), relative humidity (RH) and temperature-humidity index (THI) prevailing during the experimental period are shown in Table 2. The data indicate that the highest mean values of T_a ($^{\circ}$ C) were measured in dry summer while the minimum mean value was recorded during winter. The minimum mean value of RH (%) was measured in dry summer, whereas the highest mean value was recorded during winter. The computed mean temperature-humidity index (THI) indicates that the summer value was higher.

Plasma Glucose and Serum Organic Constituents: Table 3 illustrates the results of the effects of season and stage of pregnancy on the concentrations of plasma glucose and serum organic constituents. Winter data

indicate that there was a non-significant decrease in plasma glucose during late pregnancy. The serum cholesterol level was significantly higher in winter for empty cows ($P < 0.01$) and different stages of pregnancy ($P < 0.001$). In both seasons, the stage of pregnancy did not influence cholesterol level significantly. Serum triglycerides level was significantly ($P < 0.001$) higher in winter for all physiological states. The data exhibited a tendency for decrease in triglyceride level with the advance of pregnancy. Serum urea concentration was significantly higher in winter in empty, early pregnancy and late pregnancy states ($P < 0.01$) and mid-pregnancy ($P < 0.001$). The urea level tended to be slightly higher during early pregnancy in both seasons.

The season had no significant effect on serum ALT activity. However, in both seasons, the ALT activity increased significantly ($P < 0.001$) with the advance of pregnancy. The serum AST activity was not affected by season for all physiological states. The AST level was significantly higher during late pregnancy in summer ($P < 0.001$) and winter ($P < 0.05$).

Serum Minerals and Hormones: Table 4 depicts the results of the effects of season and stage of pregnancy on serum minerals and hormones. Serum calcium (Ca) level was not affected significantly by season and stage of pregnancy. However, in both seasons, there was a tendency for decline in Ca level in late pregnancy. Serum phosphorus (Pi) level was significantly lower in winter for empty, early pregnancy and late pregnancy ($P < 0.001$) as well as mid-pregnancy ($P < 0.01$) states. The Pi level was significantly ($P < 0.05$) lower in mid- and late pregnancy during summer. The serum magnesium (Mg) level was significantly higher during winter in empty ($P < 0.05$) and early pregnancy ($P < 0.01$) states. The stage of pregnancy had no significant effect on Mg level.

The serum level of triiodothyronine (T_3) was not affected significantly by season and stage of pregnancy. Serum thyroxine (T_4) level was significantly higher during winter in empty state ($P < 0.001$) and in all stages of pregnancy ($P < 0.01$). The serum T_4 level tended to be slightly lower with the advance of pregnancy in both seasons. Serum thyroid stimulating hormone (TSH) level was not affected significantly by experimental treatments. However, the general pattern indicates that the values of TSH were lower in winter.

The pattern of change in serum cortisol level (Table 4, Figure 1) shows that summer values were higher for all physiological states. The cortisol level was significantly higher during summer in early pregnancy ($P < 0.01$) as well as mid- and late pregnancy ($P < 0.001$).

Table 2: The mean values of ambient temperature, Ta (°C), relative humidity, RH (%) and temperature - humidity index (THI) during the experimental periods

	Ta (°C)			RH (%) (Mean)	THI (Mean)
	Max.	Min.	Mean		
Summer	40.3±3.0	25.7±2.3	32.9±1.7	43.4±19.8	80.92
Winter	27.6±2.5	14.8±1.9	21.3±1.9	33.1±5.3	65.75

Table 3: Effects of season and stage of pregnancy on the concentration of plasma glucose and serum organic constituents in dairy cows (Mean±SD)

Parameter		Pregnancy				P value
		Empty	Early	Mid	Late	
Glucose (mg/dL)	Winter	51.73±18.01	51.58±18.95	50.73±21.16	46.18±12.05	NS
Cholesterol (mg/dL)	Summer	137.92±30.08 ^a	128.75±17.53 ^a	127.14±31.10 ^a	125.18±38.66 ^a	NS
	Winter	157.67±39.77 ^b	149.73±30.82 ^b	146.33±36.27 ^b	142.60±35.12 ^b	NS
	P value	**	***	***	***	
Triglyceride (mg/dL)	Summer	33.10±16.99 ^a	33.4±13.71 ^a	30.56±13.63 ^a	27.15±16.42 ^a	NS
	Winter	49.14±10.76 ^b	48.52±10.62 ^b	51.70±15.76 ^b	45.20±15.55 ^b	NS
	P value	***	***	***	***	
Urea (mg/dL)	Summer	29.31±7.73 ^a	32.13±7.54 ^a	30.10±7.98 ^a	31.44±8.54 ^a	NS
	Winter	34.94±12.05 ^b	37.77±11.58 ^b	35.37±11.19 ^b	36.77±10.42 ^b	NS
	P value	**	**	***	**	
ALT (U/L)	Summer	10.25±3.86 ^A	10.81±4.25 ^A	12.78±6.42 ^B	14.46±7.50 ^B	***
	Winter	10.46±2.41 ^A	10.48±6.80 ^A	11.54±4.13 ^A	13.58±5.89 ^B	***
AST(U/L)	Summer	21.10±8.79 ^A	22.27±6.53 ^A	22.33±8.02 ^A	28.40±12.51 ^B	***
	Winter	19.39±8.02 ^A	20.23±6.05 ^A	21.55±8.96 ^A	24.93±9.93 ^B	***

** : P<0.01; ***: P<0.001

Table 4: Effects of season and stage of pregnancy on the concentration of serum minerals and hormones in dairy cows (Mean ±SD)

Parameter		Pregnancy				P value
		Empty	Early	Mid	Late	
Ca (mg/dL)	Summer	7.92±2.11	7.64±2.17	7.59±1.71	6.96±1.38	NS
	Winter	7.85±2.10	7.88±2.13	7.49±1.62	6.98±1.68	NS
Pi (mg/dL)	Summer	4.64±0.89 ^{aA}	4.43±0.98 ^{aB}	4.31±8.82 ^{aB}	4.15±0.76 ^a	*
	Winter	3.83±1.1.30 ^b	3.68±1.05 ^b	3.42±1.39 ^b	3.33±1.09 ^b	NS
	P value	***	***	**	***	
Mg (mg/dL)	Summer	1.71±0.44 ^a	1.77±0.43 ^a	1.82±0.64	1.70±0.61	NS
	Winter	1.88±0.37 ^b	1.90±0.36 ^b	1.87±0.25	1.82±0.32	NS
	P value	*	*	NS	NS	
T ₃ (nmol/L)	Summer	0.59±0.16	0.58±0.13	0.58±0.16	0.56±0.08	NS
	Winter	0.51±0.13	0.54±0.13	0.52±0.08	0.51±0.11	NS
T ₄ (nmol/L)	Summer	29.61±3.88 ^a	28.61±4.87 ^a	27.83±4.11 ^a	26.11±4.40 ^a	
	Winter	34.42±2.68 ^b	33.50±4.35 ^b	33.25±4.98 ^b	32.83±4.78 ^b	
	P value	***	**	**	**	
TSH (mLU/L)	Summer	0.73±0.35	0.76±0.29	0.71±0.31	0.78±0.30	
	Winter	0.64±0.27	0.68±0.21	0.69±0.24	0.66±0.17	
Cortisol (ng/ml)	Summer	21.36±4.11 ^a	23.67±4.75 ^{aB}	29.22±2.42 ^{aB}	31.82±4.54 ^a	***
	Winter	15.86±4.88 ^a	16.42±4.45 ^{aB}	17.32±4.86 ^b	^B 22.21±2.32 ^b	*
	P value	NS	**	***	***	

*: P<0.05; **: P<0.01; ***: P<0.001

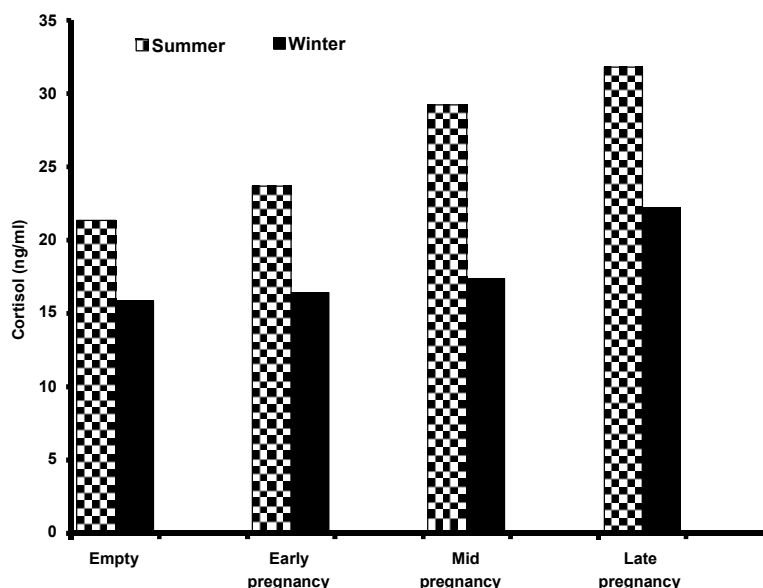


Fig. 1: Effect of season and stage of pregnancy on serum Cortisol concentration in dairy cows

There was significant increase in serum cortisol level with the advance of pregnancy in summer ($P < 0.001$) and winter ($P < 0.05$).

DISCUSSION

This study evaluated the effects of seasonal change in thermal environment and pregnancy on blood metabolites, minerals and endocrine responses in crossbred dairy cows. The climatic data (Table 2) indicate that the dairy cows were exposed to higher thermal load during summer. The THI value during summer (80.92) is considered to induce heat stress in cows; the value during winter (65.75) is considered to be comfortable to dairy cows. The THI is usually used as an indicator of thermal stress in animals; THI of 72 and below is considered as cool; 78-88 as moderate heat stress [6].

The reported decrease in blood glucose level with the advance of pregnancy in winter (Table 3) may be attributed to mobilization of glucose into fetal circulation through an active transport process as the conceptus energy demand in late pregnancy is mostly met by placental uptake of maternal glucose [19]. Tainurier *et al.* [20] attributed the decrease in glucose level to a higher energy requirement for fetal metabolism and/or progressive appearance of fetal insulin which was reported to pass into maternal blood. The reported decrease in plasma glucose level agrees with previous findings in ruminants [20, 21]. However, other reports indicated that an increase in glucose level during

pregnancy is associated with stress and increase in secretion of glucocorticoids and epinephrine which stimulate glycogenolysis in the liver [22, 23].

The data indicate that there was slight decrease in serum cholesterol level with the advance of pregnancy in both seasons (Table 3). The decrease in cholesterol level is probably attributed to the role in ovarian steroidogenesis [24] and to a decrease in food intake associated with alterations in lipid metabolism [25]. However, other studies reported marked reduction in lipogenesis during pregnancy [26, 27]. The significantly lower serum cholesterol levels in summer compared to winter values is probably related to metabolic disorder associated with exposure of cows to hot environment which may accelerate body fat catabolism. Abeni *et al.* [28] observed reduction in plasma levels of cholesterol and triglycerides in Friesian cows during the hotter season and attributed that to increase in lipid mobilization by peripheral tissues. A reduction in liver activity reported during heat exposure [29] could also explain the lower cholesterol level during summer.

The current results revealed insignificant reduction in serum triglyceride level with progress of pregnancy (Table 3). This response is related to decrease in dry matter intake or changes in endocrine status influencing lipid metabolism [25]. The decrease in serum triglycerides observed in this study is in accordance with increased concentration of these compounds in liver [30]. The significantly lower triglyceride levels obtained in summer could be related to decrease in voluntary food intake in hot season reported in previous studies [31, 32, 33].

The serum urea level was significantly lower in summer compared to the respective winter values in all experimental groups of cows (Table 3). This result is in agreement with previous studies in dairy cattle [32, 34, 35]. Kellaway and Colditz [36] reported that Friesian cows responded to heat stress by increasing N loss in urine and by decreasing N retention in relation to nitrogen intake. Lower urea levels in hot environment could also be related to recycling of urea from the blood to the rumen to compensate for decrease in ruminal ammonia-N that results from lower food intake [37]. However, other researchers have shown that heat stressed cows have increased plasma urea nitrogen levels compared with the thermoneutral controls [29, 38, 39] that could be attributed to higher utilization of amino acids as energy source. Previous studies reported that the concentration of blood urea is affected mainly by nutritional factors [40, 41]. The slight elevation of urea level reported during gestation in the current study could be related to enhancement of metabolic activities which induce an increase in protein catabolism. In late gestation, glucose availability for oxidation is supplemented by increased catabolism of amino acids at the expense of protein synthesis, thus increasing urea production [19]. In sheep, the plasma urea level started rising during week 10 of pregnancy and reached a peak at parturition [42]; this pattern was related to increase in the level of cortisol which induces catabolism of body proteins [43].

The significant increase in the activity of AST in late pregnancy in both seasons (Table 3) is probably associated with stress and negative energy status encountered in this situation [44]. However, previous studies reported irregular pattern of transaminase enzymes activity during pregnancy [20, 45]. Highr AST activity in summer was previously reported in cattle [46, 47].

The decline in serum Ca level in late gestation in both seasons (Table 4) is related to increase in movement of Ca out of blood which is not balanced by increase in rate of absorption from gut or mobilization from bone [48]. Furthermore, Blum *et al.* [49] indicated that changes in bovine Ca metabolism at parturition associated with an increased outflow of Ca to the udder may result in parturien hypocalcaemia and this effect increases towards the end of gestation. The decrease in Ca level in late pregnancy may also be associated with haemodilution which has been reported in cows [50]. However, Yokus and Cakir [51] reported increased Ca level during late pregnancy in cows and attributed this to increase of intestinal absorption of Ca and bone resorption because

of hormonal changes. The relative stability of Ca level despite change in thermal environment is consistent with the concept that Ca level is controlled mainly by a sensitive hormonal mechanism involving hydroxylated metabolites of vitamin D, parathyroid hormone and thyrocalcitonin [52]. However, decreased plasma Ca in hot environment was observed in dairy cows [53].

There was slight progressive decrease in Pi level with the advance of pregnancy in both seasons (Table 4). The decrease in mid- and late pregnancy during summer was significant. Braithwaite [54] attributed a similar decrease in Pi level during late pregnancy in sheep to an increase in the rate of mobilization of Pi out of maternal circulation into the foetus which was not balanced by increase in the rate of absorption of P from the gut or in the rate of resorption of Pi from the bone of dam. The significantly lower Pi level in winter in all physiological states could be associated with lower rate of synthesis of vitamin D₃ which increases extracellular levels of Ca and P. Serum vitamin D₃ concentrations in sheep have been associated with seasonal variations in solar radiation [55]. The present results are in accordance with Yokus and Cakir [51] who reported that in dairy cows reared under subtropical conditions, Pi varied only with seasonal but not physiological changes.

The data indicate insignificant decrease in serum Mg level during late pregnancy in both seasons (Table 4). Several factors associated with diet composition can influence utilization and bioavailability of minerals. Serum Mg concentration is influenced by dietary protein level as well as Ca and P in the diet [56]. The lower serum Mg level obtained during summer, particularly in empty and early pregnancy states is probably associated with decline in food intake in hot environment. During heat exposure, lower dry matter intake associated with increase in water content in the rumen, may lead to inadequate Mg in ruminal fluid, reducing Mg absorption [57].

In the current study, in both seasons there was no significant change in serum T₃ and T₄ levels in relation to pregnancy (Table 4). Similarly, Robertson and Falconer [58] indicated that there was no significant change in thyroid activity of ewes during pregnancy. In the present study, serum T₃ levels were slightly lower in winter compared to respective summer values. Other studies [59] indicated that T₃ level may be affected somewhat more by food intake than thermal stress. The decline in T₄ during late gestation could be related to reduction in food intake that occurs usually at this stage. The non-significant lower serum concentration of T₄ in pregnant cows as compared to non-pregnant (dry) cows could be related

to iodine uptake by the foetus through the placenta. This pattern could also be related to enzymatic activity of types 2 and 3 deiodinases which transform thyroid hormones into inactive metabolites during gestation [60].

The mechanisms by which hot environment influence reproduction and production of dairy cows seem to include alteration of the endocrine status and increased maintenance requirements [61]. The thyroid and glucocorticoid hormones assume a fundamental role in endogenous heat production and regulation [28]. The data indicate a significant decrease in T_4 level during summer at different stages of pregnancy (Table 4). This adaptive response is usually associated with decrease in food intake and metabolic heat production. In hot environments, heat acclimation and physiological adjustment by thermoregulatory centre induce a decrease in endogenous heat production influenced mainly by thyroid hormones [16]. The data indicate that serum TSH level was not influenced significantly by pregnancy and season (Table 4). However, the relatively lower TSH level in winter illustrates the negative feedback effects of thyroid hormones on TSH secretion. The maintenance of thyroid secretion depends on the feedback interplay of thyroid hormones with TSH and TRH [62].

The results indicate that gestation and seasonal change in thermal environment influenced serum cortisol level in dairy cows (Table 4). There was progressive increase in cortisol level with advance of pregnancy in both seasons, the increase was significant in late pregnancy during both seasons. This response indicates that pregnancy constitutes a physiological stress, particularly in the third trimester. Lacetera *et al.* [63] indicated that late pregnancy may constitute a source of additional stimulus that activates the hypothalamic-pituitary-adrenal axis causing increased secretion of cortisol in cows exposed to heat stress. Cortisol is significantly involved in various events during periparturient period including initiation of parturition [64].

The hot environment during summer induced a significant increase in serum cortisol level at different stages of pregnancy in crossbred cows (Table 4, Figure 1). This pattern indicates that crossbred dairy cows were exposed to thermal stress during tropical summer conditions. The blood cortisol level is generally considered as a reliable physiological index for determining animal response to stress, as indicated by assessment of glucocorticoid levels in cows under a variety of conditions [10]. The rise in cortisol level during summer in the present study is in agreement with

Bouraoui *et al.* [65] who reported higher plasma cortisol level in dairy cows in summer compared to values measured in spring. The authors reported a positive correlation between temperature-humidity index (THI) and cortisol concentration. Previous research showed that heat stress was associated with increase in blood cortisol level and elevation in secretion of cortisol and ACTH [43, 66]. Other researchers reported that glucocorticoid secretion was reduced in dairy cattle exposed to heat stress [10, 67]. However, Titto *et al.* [68] reported a significant increase in serum cortisol level during winter in Holstein dairy cows. The disparity could be attributed to experimental conditions which include specifications of thermal environment and the breed and physiological status of dairy cattle.

In conclusion, the data indicate the need for metabolic profile monitoring in dairy cattle in relation to season and physiological status. Reasonable nutritional strategies and modifications of thermal environment should be adopted in order to mitigate the negative effects of heat stress during summer.

ACKNOWLEDGEMENTS

The authors thank the technical staff of the Department of Physiology for assistance. This work received financial support from The Administration of Scientific Research, Ministry of Higher Education.

REFERENCES

1. Kadzere, C.T., M.R. Murphy, N. Silanikove and E. Maltz, 2002. Heat stress in lactating dairy cows: a review. *Livest. Prod. Sci.*, 77: 59-91.
2. Al-Katanani, Y.M., D.W. Webb and P.J. Hansen, 1998. Factors affecting seasonal variation in nonreturn rate of lactating dairy cows. *J. Dairy Sci.*, 81(Suppl. 1): 217 (Abstract).
3. Chaiyabutr, N., S. Champongsang and S. Suadson, 2008. Effect of evaporative cooling on the regulation of body water and milk production in crossbred Holstein cattle in a tropical environment. *Int. J. Biometeorol.*, 52: 575-585.
4. Blackshaw, J.K. and A.W. Blackshaw, 1994. Heat stress in cattle and the effect of shade on production and behaviour: A review. *Aust. J. Exp. Agric.*, 34: 285-295.
5. Hansen, P.J., 2004. Physiological and cellular adaptation of Zebu cattle to thermal stress. *Anim. Rep. Sci.*, 82-83: 349-360.

6. Armstrong, D.V., 1994. Heat stress interaction with shade and cooling. *J. Dairy Sci.*, 77: 2044-2050.
7. Igono, M.O., H.D. Johnson, B.J. Steevens, W.A. Hainen and M.D. Shanklin, 1988. Effect of season on milk temperature, milk growth hormone, prolactin and somatic cell counts of lactating cattle. *Int. J. Biometeorol.*, 32: 194-200.
8. West, J.W., 2003. Effects of heat stress on production in dairy cattle. *J. Dairy Sci.*, 86: 2131-2144.
9. Monty, D.E. Jr. and L.K. Wolff, 1974. Summer heat stress and reduced fertility in Holstein-Friesian cows in Arizona. *Am. J. Vet. Res.*, 35: 1495-1500.
10. Abilay, T.A., H.D. Johnson and M. Madan, 1975. Influence of environmental heat on peripheral plasma progesterone and cortisol during the bovine oestrous cycle. *J. Dairy Sci.*, 58: 1836-1840.
11. Ryan, D.P., J.F. Prichard, E. Kopel and R.A. Godke, 1993. Comparing early embryo mortality in dairy cows during hot and cool season of the year. *Theriogenology*, 39: 719-737.
12. De Rensis, F. and R.J. Scaramuzzi, 2003. Heat stress and seasonal effects on reproduction in the dairy cow. *Theriogenology*, 60: 1139-1151.
13. Payne, J.M., S.M. Dew, R. Manston and M. Faulks, 1970. The use of metabolic profile test in dairy herds. *Vet. Rec.*, 87: 150-158.
14. Cote, J.F. and B. Hoff, 1991. Interpretation of blood profiles in problem dairy herds. *The Bovine Practitioner*, 26: 7-11.
15. Kida, K., 2002. The metabolic profile test: Its practicability in assessing feeding management and periparturient diseases in high yielding commercial dairy herds. *J. Vet. Med. Sci.*, 64: 557-563.
16. Bernabucci, U., N. Lacetera, L.H. Baumgard, R.P. Rhoads, B. Ronchi and A. Nardone, 2010. Metabolic and hormonal acclimation to heat stress in domesticated ruminants. *Animal*, 4: 1167-1183.
17. Ravagnolo, O., I. Misztal and G. Nboom, 2000. Genetic component of heat stress in dairy cattle, development of heat index function. *J. Dairy Sci.*, 83: 2120-2125.
18. SAS User's Guide, 1996. Statistics, Version 6.11 Edition. SAS Inst., Inc., Cary, NC, USA.
19. Bell, A.W., 1995. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. *J. Anim. Sci.*, 73: 2804-2819.
20. Tainturier, D., J.P. Braun, A.G. Rico and J.P. Thouvenot, 1984. Variation in blood composition in dairy cows during pregnancy and after calving. *Res. Vet. Sci.*, 37: 129-131.
21. Mbassa, G.K. and J.S. Poulsen, 1991. Influence of pregnancy, lactation and environment on some clinical chemical reference values in Danish Landrace dairy goats (*Capra hircus*) of different parity. II. Plasma urea, creatinine, bilirubin, cholesterol, glucose and total serum proteins. *Comp. Biochem. Physiol. B.*, 100: 423-431.
22. Hill, R.M., W.M. Verniaud, R.L. Peter, L.M. Tennyson, G.M. Rettig Zion, T.E. Vorderman, A.L. Helms, P.G. McCulley and L.B. Hill, 1984. The effect of intrauterine malnutrition on the term infant. A 14-year progressive study. *Acta Paediatr. Scand.*, 73: 482-487.
23. Swenson, M.J., 1993. Physiological properties and cellular and chemical constituents of blood. In: *Dukes' Physiology of Domestic Animals*. 11th edition. (Edited by Swenson, M.J. and W.O. Reece), pp: 15-41 and 518-527 Cornell University Press, Ithaca and London.
24. Piccione, G. and G. Giannetto, 2009. Daily rhythms of 25 physiological variables in *Bos taurus* maintained under natural conditions. *J. Appl. Biomed.*, 7: 55-61.
25. Drackley, J.K., 2002. Transition cow management and periparturient metabolic disorders. In: M. Kaske, H. Scholz and M. Holtersshinken, (Eds.). *Recent Developments and Perspectives in Bovine Medicine*. Hannover, pp: 224-235.
26. Watson, T.D., L. Burns, C.J. Packard and J. Shepherd, 1993. Effects of pregnancy and lactation on plasma lipid and lipoprotein concentrations, lipoprotein composition and post-heparin lipase activities in Shetland pony mares. *J. Reprod. Fertil.*, 97: 563-568.
27. Nazifi, S., M. Saeb and S.M. Ghavami, 2002. Serum lipid profile in Iranian fat-tailed sheep in late pregnancy, at parturition and during the post-parturition period. *J. Vet. Med. Series A.*, 49: 9-12.
28. Abeni, F., L. Calamari and L. Stefanini, 2007. Metabolic conditions of lactating Friesian cows during the hot season in the Po valley. 1. Blood indicators of heat stress. *Int. J. Biometeorol.*, 52: 87-96.
29. Ronchi, B., U. Bernabucci, N. Lacetera, A. Verini Supplizi and A. Nardone, 1999. Distinct and common effects of heat stress and restricted feeding on metabolic status of Holstein heifers. *Zoot. Nutr. Anim.*, 25: 11-20.
30. Smith, R.W. and A. Walsh, 1975. The composition of liver lipids of the ewe during pregnancy and lactation. *Res. Vet. Sci.*, 19: 230-232.

31. McGuire, M.A., D.K. Beede, M.A. Delorenzo, C.J. Wilcox, G.B. Huntington, C.K. Reynolds and R.J. Collier, 1989. Effects of thermal stress and level of feed intake on portal plasma flow and net fluxes of metabolites in lactating Holstein cows. *J. Anim. Sci.*, 67: 1050-1060.
32. Marai, I.F.M., A.A. Habeeb, A.H. Daader and H.M. Yousef, 1995. Effects of Egyptian subtropical summer conditions and the heat-stress alleviation technique of water spray and a diaphoretic on the growth and physiological functions of Friesian calves. *J. Arid. Environ.*, 30: 219-225.
33. Nardone, A., N. Lacetera, U. Bernabucci and B. Ronchi, 1997. Composition of colostrum from dairy heifers exposed to high air temperatures during late pregnancy and the early postpartum period. *J. Dairy Sci.*, 80: 838-844.
34. Dhali, A., R.K. Mehla, S.K. Sirohi, K. Mech and M. Karunakaran, 2006. Monitoring feeding adequacy in dairy cows using milk urea and milk protein contents under farm conditions. *Asian-Aust J. Anim. Sci.*, 19: 1742-1748.
35. Khan, H.M., T.K. Mohanty, M. Bhakat, V.S. Raina and A.K. Gupta, 2011. Relationship of blood metabolites with reproductive parameters during various seasons in Murrah buffaloes. *Asian-Austr. J. Anim. Sci.*, 24: 1192-1198.
36. Kellaway, R.C. and P.J. Colditz, 1975. The effect of heat stress on growth and nitrogen metabolism in Friesian and F1 Brahman X Friesian heifers. *Austr. J. Agric. Res.*, 26: 615-622.
37. Alnaimy, A., M. Habeeb, I. Fayaz, M. Marai and T.H. Kamal, 1992. Heat stress. In: C. Phillips and D. Piggins, (Eds.), *Farm Animals and the Environment*. CAB International, Wallingford, UK, pp: 27-47.
38. Baumgard, L.H. and R.P. Rhoads, 2007. The effects of hyperthermia on nutrient partitioning. In: *Proceedings of Cornell Nutritional Conference For Feed Manufacturers* (Editor: T. Overton), pp: 93-104. Cornell University, New York.
39. Shwartz, G., M.L. Rhoads, M.J. Van Baale, R.P. Rhoads and L.H. Baumgard, 2009. Effects of supplemental yeast culture on heat-stressed lactating Holstein cows. *J. Dairy Sci.*, 92: 935-942.
40. Harris, B., 1995. MUN and BUN values can be valuable management tools. *Feedstuffs.*, 76: 14.
41. Westwood, C.T., I.J. Lean, J.K. Garvin and P.C. Wynn, 2000. Effects of genetic merit and varying dietary protein degradability on lactating dairy cows. *J. Dairy Sci.*, 83: 2926-2940.
42. El-Sherif, M.M.A. and F. Assad, 2001. Changes in some blood constituents of Barki ewes during pregnancy and lactation under semi arid conditions. *Small Rumin. Res.*, 40: 269-277.
43. Silanikove, N., 2000. Effects of heat stress on the welfare of extensively managed domestic ruminants - a review. *Livest. Prod. Sci.*, 67: 1-18.
44. Cebra, C.K., F.B. Garry, D.M. Getz and M.J. Fettman, 1997. Hepatic lipidosis in anorectic, lactating Holstein cattle: A retrospective study of serum biochemical abnormalities. *J. Vet. Intern. Med.*, 11: 231-237.
45. El-ghoul, W. and W. Hofmann, 2002. Influence of claw diseases of varying degrees on measurable stress reactions with special consideration of cortisol and lactate in bovine blood serum. *Praktische Tierarzt*, 83: 354-361.
46. Shaffer, L., J.D. Roussel and K.L. Koonce, 1981. Effects of age, temperature, season and breed on blood characteristics of dairy cattle. *J. Dairy Sci.*, 64: 62-70.
47. Sakatani, M., A.Z. Balboula, K. Yamanaka and M. Takahashi, 2012. Effect of summer heat environment on body temperature, oestrous cycles and blood antioxidant levels in Japanese Black cow. *Anim. Sci. J.*, 83: 394-402.
48. Sansom, B.F., K.J. Bunch and S.M. Dew, 1982. Changes in plasma calcium, magnesium, phosphorus and hydroxyproline concentration in ewes from twelve weeks before until three weeks after lambing. *Br. Vet. J.*, 138: 393-401.
49. Blum, J.W., C.F. Ramberg, K.G. Johnson and D.S. Kronfeld, 1972. Calcium (ionized and total), magnesium, phosphorus and glucose in plasma from parturient cows. *Am. J. Vet. Res.*, 33: 51-56.
50. Hassan, G.A. and F.D. El-Nouty, 1985. Thyroid activity in relation to reproductive status, seasonal variation and milk production in buffaloes and cow heifers during their first lactation. *Indian J. Dairy Sci.*, 38: 129-136.
51. Yokus, B. and U.B. Cakir, 2006. Seasonal and physiological variation in serum chemistry and mineral concentration in cattle. *Biol. Trace Elem. Res.*, 109: 255-266.
52. Hays, V.W. and M.J. Swenson, 1993. Minerals and bones. In: *Dukes' Physiology of Domestic Animals* (Editor: M.J. Swenson and W.O. Reece), pp: 517-535. Cornell University Press. Ithaca.
53. Sanchez, W.K., M.A. McGuire and D.K. Beede, 1994. Macromineral nutrition by heat stress interactions in dairy cattle: review and original researches. *J. Dairy Sci.*, 77: 2051-2079.

54. Braithwaite G.D., 1983. Calcium and phosphorus requirements of the ewe during pregnancy and lactation. II. Phosphorus. *Br. J. Nutr.*, 50: 723-736.
55. Smith, B.S.W. and H. Wright, 1981. Seasonal variation in serum 25-hydroxyvitamin D concentrations in sheep. *Vet. Rec.*, 109: 139-141.
56. Underwood, E.J. and N.F. Suttle, 1999. General Introduction. In: *The Mineral Nutrition of Livestock*. (Editor: Underwood E.J.), pp: 1-16. CAB International.
57. Calamari, L., F. Abeni, F. Calegari and L. Stefanini, 2007. Metabolic conditions of lactating Friesian cows during the hot season in the Po valley. 2. Blood minerals and acid-base chemistry. *Int. J. Biometeorol.*, 52: 97-107.
58. Robertson, H.A. and I.R. Falconer, 1961. Reproduction and thyroid activity. *J. Endocrinol.*, 22: 133-142.
59. McGuire, M.A., D.K. Beede, R.J. Collier, F.C. Buonomo, M.A. DeLorenzo, C.J. Wilcox, G.B. Huntington and C.K. Reynolds, 1991. Effects of acute thermal stress and amount of feed intake on concentrations of somatotropin, insulin-like growth factor (IGF)-I and IGF-II and thyroid hormones in plasma of lactating Holstein Cows. *J. Anim. Sci.*, 69: 2050-2056.
60. Kelly, G., 2000. Peripheral metabolism of thyroid hormones: a review. *Altern. Med. Rev.*, 5: 306-332.
61. Collier, R.J., L.H. Baumgard, A.L. Lock and D.E. Bauman, 2005. Physiological limitations, nutrient partitioning. In: *Yield of Farmed Species: Constraints and Opportunities in the 21st Century* (Editors: R. Sylvester-Bradley and J. Wiseman), pp: 351-377. Nottingham University Press, Nottingham, U.K.
62. Ganong, W.F., 2005. Review of Medical Physiology (International Edn), 21st edn, pp: 326-327, Appleton and Lange, Stamford, CT, USA.
63. Lacetera, N., U. Bernabucci, D. Scalia, B. Ronchi, G. Kuzminsky and A. Nardone, 2005. Lymphocyte functions in dairy cows in hot environment. *Int. J. Biometeorol.*, 50: 105-110.
64. Jacob, S.K., V. Ramnath, P.T. Philomina, K.V. Raghunandhan and A. Kannan, 2001. Assessment of physiological stress in periparturient cows and neonatal calves. *Indian J. Physiol. Pharmacol.*, 45: 233-238.
65. Bouraoui, R., M. Lahmar, A. Majdoub, A.M. Djemali and R. Belyea, 2002. The relationship of temperature - humidity index with milk production of dairy cows in a Mediterranean climate. *Anim. Res.*, 51: 479-491.
66. Dobson, H. and R. Smith, 2000. What is stress and how does it affect reproduction. *Anim. Reprod. Sci.*, 60-61: 743-752.
67. Ronchi, B., G. Stradioli, A. Verini Supplizi, U. Bernabucci, N. Lacetera, P.A. Accorsi, A. Nardone and E. Seren, 2001. Influence of heat stress or feed restriction on plasma progesterone, oestradiol-17B, LH, FSH, prolactin and cortisol in Holstein heifers. *Liv. Prod. Sci.*, 68: 231-241.
68. Titto, C.G., J.A. Negro, E.A. Titto, T.D. Canaes, R.M. Titto and A.M. Pereira, 2012. Effect of an evaporative cooling system on plasma cortisol, IGF-1 and milk production in a tropical environment. *Int. J. Biometeorol.* (in press). DOI 10.1007/s 00484-012-0554-6.