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The influences of seasonal variations on thyroid activity and some biochemical parameters of cattle

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Summary

Blood samples were taken from eighteen non-pregnant Holstein heifers to investigate the effects of heat stress on some serum biochemical parameters with emphasis on thyroid hormones. The entire period of study was classified into two seasons, from July to September with mean temperature of 35.5°C (Max: 51°C and Min: 19°C) and from January to March with mean environmental temperature of 14.5°C (Max: 30°C and Min: 2°C). In each season 4 samples were collected every 3 weeks intervals. A significant difference in the concentrations of serum total protein, albumin, glucose, cholesterol BUN, Calcium, T_3 and T_4, and activities of AST and CK was seen between hot and cold seasons ($P<0.05$), whereas the concentration of serum inorganic phosphorus was not significant difference between these two seasons.

The concentrations of BUN, total protein, albumin and activities of AST and CK in summer were higher than winter, in contrast, the concentrations of glucose, cholesterol, calcium, T_3 and T_4 were lower in summer than winter ($P<0.05$). A significant correlation between T_3 and serum glucose, cholesterol, AST and CK was noticed. Mean environmental temperature had a significant correlation with serum T_3, glucose, cholesterol, total protein, albumin, BUN, AST and CK. The results of this study showed that very hot conditions had a profound effect on thyroid activity and some serum biochemical parameters.

Key words: Heat stress, Biochemical parameters, Thyroid activity, Cattle

Introduction

Summer temperature and humidity conditions decrease milk production from 10 to 35% below yearly means (Johnson, 1987). This depression may be mediated by a reduction in feed intake, which is reported to occur as ambient temperature rises above 27°C (Schwab, 1983). An increase in body temperature usually follows the rise in ambient temperature and may be a primary stimulus for reductions in both feed intake and milk production. High ambient temperature and relative humidity compromise the ability of the cows to dissipate heat, and coupled with metabolic heat makes it difficult to maintain thermal balance, particularly for dairy cattle. Selection for milk yield reduces thermoregulatory ability in the face of heat stress (Berman *et al.,* 1985) and magnifies the seasonal depression in fertility caused by heat stress (Al-katanani *et al.,* 1998). The physiological responses of animals to environmental stress during the winter and summer and their energy balance, showed that seasonal heat and cold stress have profound effects on some serum biochemical parameters (Barakat and Abdel-Fattah, 1971; Rowlands *et al.,* 1979; Eldon *et al.,* 1988; Kataria and Bhatia, 1991; Kataria *et al.,* 1991, 1993; Soveri *et al.,* 1992; Bengoumi *et al.,* 1997; Nazifi *et al.,* 1999).

Thyroid gland is one of the most sensitive organ to the ambient heat variation. It has been shown that thyroid hormones are important modulators of developmental processes and general metabolism (Kaneko, 1989). Seasonal variations in the concentration of serum thyroxine (T4) and triiodothyronine (T_3) of cow, camel, goat, mare and dog have been reported (Yagil *et al.,* 1978; Ingraham *et al.,* 1979; Bojanowska *et al.,* 1991; Prakash and Rathore, 1991; Flisinska-Tuckova *et al.,* 1995; Nazifi *et al.,* 1999).

In Khoozestan province the temperature starts to increase from the beginning of spring and reaches its peak at the middle of summer and cows are subjected to high ambient temperature for a long time (about 7 months). For this reason, the very high temperature (above 50^{o}C) in this province is not comparable with any other part of the world. Therefore the following experiment was designed to evaluate the effect of the ambient temperature on thyroid function and some other serum biochemical parameters in cattle.

Materials and Methods

Eighteen non-pregnant Holstein heifers in dairy farm of Shahid Chamran University were randomly selected for this study. The entire period of investigation was classified in two seasons, from July to September (Average temperature 35.5^{o}C, max. temperature 51^{o}C, min. temperature 19^{o}C) and from January to March (Average temperature 14.5^{o}C, max. temperature 30^{o}C, min. temperature 2^{o}C). Blood samples were taken at 3 weeks intervals, 4 times in each season. All the samples were collected in the morning, before feeding, from a jugular vein directly into test tubes without any anticoagulant. The serum was separated following centrifugation for 15 min. at 3000 r.p.m. Serum samples were stored at -20^{o}C until analysed.

Estimation of T_3 and T_4 levels in serum were made by the standard Radio Immunoassay in Golestan medical center, Ahvaz, Iran. Activity of aspartate aminotransferase (AST) was determined by the colorimetric method of Reitman and Frankel and creatine kinase (CK) by the colorimetric method of Hughes. Serum total protein was measured by the Biuret method, albumin by bromcresol green (BCG), calcium by o-cresolphethalein, inorganic phosphorus by the ammonium molybdate method, urea nitrogen by the diacetyl monoxime method, glucose and cholesterol by enzymatic method.

The effective environmental temperature of each sampling time was determined by calculation of mean environmental temperature between different sampling times (3 weeks). In regions with high relative humidity, temperature-Humidity Index (THI) should be considered. The THI combines temperature and humidity into a single figure with a demonstrated usefulness for evaluating climate stress in cattle (Ingraham *et al.,* 1979; Richards *et al.,* 1995; Silanikove, 2000). Since the relative humidity in Ahvaz was low, therefore only mean environmental temperature was measured in this study (Table 1).

The data of this study were statistically analysed by nested design ANOVA and the pairwise comparison of sampling times were done by Tukey method. Relationships among thyroidal hormones, biochemical parameters and mean environmental temperatures were evaluated by correlation analyses (Montgomey, 1997).

Results

The means (±SE) of serum biochemical parameters of Holstein heifers in different times during summer and winter are presented in Tables 2 and 3, respectively. The means (±SE) of serum biochemical parameters in summer (hot) and winter (cold) seasons are presented in Table 4.

There was a significant difference in the concentration of serum T_3, T_4, albumin, total protein, glucose, calcium, cholesterol, blood urea nitrogen (BUN) and in the activities of AST and CK between hot and cold seasons ($P<0.05$), with the lower concentration of T_3, T_4, glucose, cholesterol and calcium during the summer season and by contrast, the higher concentration of total protein, albumin, BUN and the higher activities of AST and CK during this season ($P<0.05$), whereas the concentration of serum inorganic phosphorus was not significant difference between these two seasons.

T_3 level were significantly correlated with serum T_4 level ($P<0.01$, $r = +0.37$), glucose ($P<0.01$, $r = +0.43$), cholesterol ($P<0.01$, $r = +0.50$), AST ($P<0.01$, $r = -0.35$) and CK ($P<0.05$, $r = -0.18$).

Mean environmental temperature was significantly correlated with serum T_3

(P<0.01, r = -0.86), albumin (P<0.05, r = +0.83), total protein (P<0.01, r = +0.90), glucose (P<0.01, r = -0.91), cholesterol level (P<0.01, r = -0.88), AST (P<0.01, r = +0.96) and CK activity (P<0.01, r = +0.93).

Table 1: Minimum, maximum and mean effective environmental temperatures (^{o}c) of each sampling time

Season	Time	Mean temp.	Min. temp.	Max. temp.
Summer	1	34.4	24	45.8
	2	37.2	26.8	47.6
	3	38.3	28.1	48.1
	4	33.8	23.7	44
Winter	1	15.7	10.4	21.5
	2	10.5	4.6	16.4
	3	13.3	6.3	20.2
	4	15.5	8.1	23.4

Table 2: Mean (±SE) serum biochemical parameters of Holstein heifers during summer

Biochemical parameter	Time 1 (a)	Time 2 (b)	Time 3 (c)	Time 4 (d)
T_3 (nmol/l)	1.06±0.07	0.81±0.05	0.86±0.05	0.75±0.08
T_4 (nmol/l)	55.49±1.64^{d}	49.40±2.34	54.88±3.62	46.67±3.42^{a}
Albumin (g/l)	41.25±0.76	39.83±0.73	38.71±0.97	41.14±0.41
Total protein (g/l)	69.50±1.12	71.14±0.68	67.94±1.52	68.47±1.97
Glucose (mmol/l)	3.11±0.10^{bd}	2.55±0.11^{a}	2.69±0.09	2.55±0.08^{a}
Calcium (mmol/l)	2.38±0.06^{c}	2.48±0.06^{c}	2.11±0.06^{abd}	2.51±0.05^{c}
Inorganic phosphorus (mmol/l)	2.09±0.06	2.15±0.05	2.15±0.04	2.08±0.05
CK (U/l)	111.11±9.80	121.11±6.20	116.67±8.48	119.44±5.74
AST (U/l)	84.50±5.99	93.28±4.16	83.56±3.72	81.89±3.60
Cholesterol (mmol/l)	1.88±0.15	1.59±0.11	1.88±0.12	1.93±0.09
BUN (mmol/l)	1.89±0.05	2.02±0.08	1.73±0.12	1.71±0.08

n=18; T_3 , triiodothyronine; T_4 , thyroxine; CK, creatine kinase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; mean environmental temperature = 35.5 ^{o}C ; The letters a, b, c and d indicate different times and existence of each letter in each cell shows that there is significant difference between two times (P<0.05)

Table 3: Mean (±SE) serum biochemical parameters of Holstein heifers during winter

Biochemical parameter	Time 1 (a)	Time 2 (b)	Time 3 (c)	Time 4 (d)
T_3 (nmol/l)	1.35±0.07^b	1.99±0.17^{acd}	1.29±0.11^b	1.29±0.09^b
T_4 (nmol/l)	50.37±2.20^b	64.20±2.03^a	57.06±2.31	56.08±2.23
Albumin (g/l)	32.45±0.70	35.25±0.89	36.35±0.81	36.29±0.70
Total protein (g/l)	64.00±1.51	65.34±1.38	62.78±1.69	63.42±1.62
Glucose (mmol/l)	3.26±0.14	3.64±0.09	3.37±0.15	3.43±0.14
Calcium (mmol/l)	2.65±0.03	2.57±0.04	2.44±0.02	2.56±0.04
Inorganic phosphorus (mmol/l)	2.22±0.05	2.07±0.06	2.10±0.05	2.11±0.05
CK (U/l)	103.33±8.89	94.44±5.38	97.22±4.19	90.56±4.24
AST (U/l)	67.50±4.54	61.39±6.07	64.50±4.15	59.61±4.00
Cholesterol (mmol/l)	2.17±0.12^b	3.07±0.09^{acd}	2.43±0.09^b	2.43±0.07^b
BUN (mmol/l)	1.60±0.10	1.51±0.07	1.73±0.08	1.77±0.05

n = 18; mean environmental temperature=14.5°C. For abbreviations see Table 2

Table 4: Comparison between mean (±SE) of serum biochemical parameters of Holstein heifers in summer and winter

Biochemical parameter	Summer (hot)	Winter (cold)
T_3 (nmol/l)	0.87±0.03 ^{a}	1.48±0.06 ^{b}
T_4 (nmol/l)	51.61±1.47^a	56.93±1.22^b
Albumin (g/l)	40.23±0.38^a	35.09±0.42^b
Total protein (g/l)	69.26±0.70^a	63.88±0.77^b
Glucose (mmol/l)	2.73±0.05 ^{a}	3.42±0.06 ^{b}
Calcium (mmol/l)	2.37±0.03 ^{a}	2.56±0.02 ^{b}
Inorganic phosphorus (mmol/l)	2.12±0.02 ^{a}	2.12±0.02 ^{a}
CK (U/l)	117.08±3.81^a	96.39±2.98^b
AST (U/l)	85.81±2.25^a	63.25±2.36^b
Cholesterol (mmol/l)	1.82±0.06 ^{a}	2.53±0.06 ^{b}
BUN (mmol/l)	1.84±0.04 ^{a}	1.65±0.04 ^{b}

n = 18; different letters within the same row indicate significantly different results (P<0.05). For abbreviation see Table 2

This study reveals that very hot circumstances have serious effects on thyroid activity and some biochemical parameters of the blood.

Discussion

The present study shows that heat stress depressed thyroid activity and altered some serum biochemical parameters in Holstein heifers. In this study, the concentrations of serum T_3 and T_4 in summer were lower than winter (P<0.01). This finding was in accordance with other studies in sheep, buffalo and cow (Henneman *et al.,* 1955; Prakash and Sharma, 1975; Ingraham *et al.,* 1979; Pratt and Wettemann, 1986). Prakash and Rathore (1991) showed that in the goat a significant decrease in serum T_3 and T_4 levels from May to July was noticeable. They also reported that from summer to winter the serum levels of T_3 and T_4 were significantly increased. These authors believed that during summer the exposure of animals to the high environmental temperature depressed the functional activity of the thyroid gland and thereby caused a relatively lower concentration of thyroid hormones. Cold environment maybe a stimulus to increase the thyrotrophic hormone output thereby resulting in a higher concentration of thyroid hormones in serum. Although, there are some evidences to suggest conversely, that thermal exposure acts directly on the hypothalamic pituitary axis and causes a reduction in TSH secretion (Tal and sulman, 1973; EL-Nouty and Hassan, 1983). It has also been reported that in the dog, serum T_3 and T_4 levels are lowest in summer and their concentrations are at highest level in winter and autumn, respectively (Tuckova *et al*., 1995). In contrast to the above findings, Yagil *et al*., (1978) and Nazifi *et al.,* (1999) reported higher levels of serum T_3 and T_4 in summer in comparison to winter in the camel. Yagil *et al*., (1978) believed that in summer if enough water provided to camel, thyroid function is stimulated but in the absence of water, inhibition could be seen.

The results of this study showed that although T_3 and T_4 both decreased in summer, only T_3 had significant negative correlation with mean environmental temperature.

The comparison between different sampling times in the present study in winter showed that at the second sampling time, serum T_3 was significantly higher than the other times (Table 3), which was coincidence with the lowest environmental temperature in this season (Table 1). A comparison was also made between serum T_4 at different sampling times. As it is shown in Table 2, the prolonged hot weather in summer had a significant effect on T_4 supression. In winter when the environmental temperature was at the lowest degree (Table 1), the serum T_4 like T_3 was higher than the other sampling times, although for T_4 these differences were only significant between the first and second sampling times (Table 3).

There are some evidences to indicate that the factors that have inhibitory effect on thyroid function could reduce reproductive efficiency (Robinson *et al.,* 1996; Huszenicza *et al.,* 2000). In recent years it has become increasingly clear that an adequate level of circulating T_3 is of primary importance for ovarian function in laboratory rodents (Ortega *et al.,* 1990; Osorio *et al*., 1998). Furthermore, Spicer *et al*., (2001) reported a direct stimulatory effect of T_3 and T_4 on thecal cell steroidogenesis in cattle. By looking at the results of our study and we could express an acceptable explanation for the reduction of reproductive efficiency which is started with beginning of hot season in cattle in Ahvaz.

The serum glucose and cholesterol concentrations of heifers in this study were significantly lower in summer than in winter. These findings were similar to the observations of Kataria *et al.,* (1993) in Marwari goats. Soveri *et al.,* (1992) reported that in the reindeer calves serum cholesterol concentrations are higher in winter. Eldon *et al*., (1988) showed higher serum glucose concentrations of cows in winter. The serum T_3 had a significant positive correlation with the serum concentrations of glucose and cholesterol. The mean environmental temperature showed a significant negative correlation with glucose and cholesterol. In other word, cold environment could be a stimulus to augment thyroid hormones secretion to increase basal metabolic rate in order to maintain body temperature which accompanies with high levels of blood metabolites (Prakash and Rathore, 1991; Kataria *et al.,* 1993). In contrast to our findings, Sinha *et al.,* (1981) showed that the concentration of cholesterol in cattle was higher during hot season. On the other hand, some authors reported that in cattle and neonatal calves, season had no effect on serum cholesterol (Edfors-Lilja *et al.,* 1978; Kweon *et al.,* 1986; Chand and Georgie, 1989).

The comparison between serum glucose levels in different sampling times in summer showed that at the first sampling time serum glucose was significantly higher, which is in agreement with serum T_3 and T_4 levels in this season (Table 2). The concentration of cholesterol in second sampling time in winter was significantly at the highest level, which is in line with thyroid hormones level and the lowest environmental temperature (Tables 1 and 3).

The results of present study showed an increase in serum total protein and albumin during the summer season. These findings were similar to the data of Payne *et al.,* (1974) and EL-Nouty and Hassan (1983). The increment in serum protein concentration, noticed in this study, may indicate a loss of extracellular fluid due to heat exposure. The results of this investigation have also shown that environmental temperature has a significant positive correlation with serum total protein and albumin. In contrast, kataria *et al*., (1993) reported no significant rise in the plasma protein concentration in summer in Marwari goats and related it to maintaine blood circulatory volumes by the animals.

The higher concentration of blood urea in summer probably is the result of a loss of extracellular fluid due to heat exposure, although protein content of diet could alter serum urea concentration. Our result is in line with the findings of Payne *et al.,* (1973) and Rowlands *et al.,* (1979) in dairy cows, where they showed the augmentation of this metabolit in summer in comparison to winter, but differs from Soveri *et al*., (1992) in reindeer calves and also Eldon *et al*., (1988) in dairy cows.

The results of this study showed that serum AST and CK activities were higher in extremely hot season compared with cold season. The higher AST activity in summer was also reported by Georgie *et al.,* (1973) and Shaffer *et al*., (1981) in cattle, Kataria and Bhatia (1991) in camel, Kataria *et al.,* (1991 and 1993) in camel and Marwari goats.

Nazifi *et al.,* (1999) attributed it to increase heat adaptation. Increased activity in some enzymes with rising temperature maybe due to the fact that reactions simply are accelerated at higher temperatures (Shaffer *et al.,* 1981). Naqvi *et al.,* (1991) reported that higher values of AST and CK activity in Avikalin sheep than in Malapura sheep may be attributed to greater influence of thermal stress. In contrast to these findings, Shaffer *et al.*, (1981) showed that season had no effect on serum CK activity in cattle and Soveri *et al.,* (1992) did not see any effect of season on serum AST activity in camel. Nazifi *et al.,* (1999) indicated that in camel, serum CK activity was higher in cold condition, which could be attributed to greater acivity of the camel in winter months. Triiodothyronine had a significant negative correlation with serum AST and CK activites. The mechanism by which T_3 decreases AST and CK is uncertain. Mean environmental temperature had a significant positive correlation with the activity of these enzymes.

In the present study, serum calcium concentrations were higher during winter than summer. This finding was in agreement with the data of Fatemi Ardestani (1989) and Seifi (1997), who believed that usage of corn silage during winter, with pH below than 4 and high lactic acid content, simplifies absorption of dietary calcium. Also, Payne *et al.,* (1974) reported that in dairy cows, serum calcium concentrations were higher in winter. In contrast to these findings, Ross and Halliday (1976) showed that in summer, serum calcium concentration was high. Eldon *et al.,* (1988) also showed that the highest blood level of calcium was in the autumn after that animals had been grazing all summer on cultivated pasture and decreased from the autumn months to the summer season after animals were housed and fed hay, silage and concentrates. However, some authors showed that season had no effect on serum calcium concentration (Rowlands *et al.,* 1975; Shaffer *et al.,* 1981; McAdam and O'Dell, 1982).

Comparison between serum calcium levels in different sampling times in summer showed that at the third sampling time, serum calcium was significantly low. This could be due to the highest environmental temperature at this time and depression of the animals appetite owing to hot weather.

The result of our study showed that, hot and cold seasons had no effect on serum inorganic phosphorus concentration. Seifi (1997) reported that serum inorganic phosphorus concentration was highest in winter and lowest in spring and autumn. Also, Peterson and Waldern (1981) indicated that in winter serum inorganic phosphorus concentration was high. These authors believed that high serum inorganic phosphorus concentration was due to usage of high concentration in winter. In contrast, Fatemi Ardestani (1989) reported that mean serum inorganic phosphorus concentration decreased significantly in winter.

The results of this study showed that high environmental temperature in summer had a profound effect on thyroid activity and a significant difference in the concentrations of serum total protein, albumin, glucose, cholesterol, BUN, Calcium and activities of AST and CK was seen between hot and cold seasons.

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