Chronic heat stress in Iranian fat-tailed ram lambs: clinical and paraclinical parameters

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Abstract:

BACKGROUND: The effect of heat stress on physiological parameters has been well documented. However, there are reports of a genetic based heat tolerance in some sheep breeds. **OBJECTIVES:** The aim of the present study was to monitor the physiological responses of an Iranian fat-tailed breed against heat stress and an acute stress insult. **METHODS:** A total number of 15 fall-born ram lambs were selected and subjected to direct summer solar radiation throughout the day (May 2009 to February 2010). Ten lambs were semi-castrated in October to see the physiologic response of animals to an acute stress. The general parameter as well as blood chemistry and cortisol were analyzed during the study. **RESULTS:** The results showed that all physiologic parameters had significant changes; however, their values were in normal range. The fact that the animals in the present study experienced their first exposure of heat stress and a significant increase in serum cortisol concentration in semi-castrated lambs in October, compared to the respected values in the hottest months of the experiment, may indicate a genetic based adaptability of the breed in Iran. **CONCLUSIONS:** The present study shows the dynamic changes of general and biochemical parameters in response to chronic heat stress and an acute stress that raises a possible tolerance of the breed against heat stress.

Introduction

The impact of heat stress on animal physiology has been well documented (Elder et al., 1996). The physiologic responses of animals to heat stress include reducing the internal body temperature via sweating, peripheral vasodilation, and increasing the respiration rate, which in turn lead to some biochemical changes in serum. Primary alkalosis and electrolytes imbalance are the consequences of long-term exposure to heat stress. Serum Na, K, and CL are excreted through sweating and panting, and they lead to the reduction of the ions in the serum. Long-term exposure to heat stress also induced permanent functional changes of some endocrine glands mainly thyroid (Kolumanand Daskiran, 2011; Ross et al., 1985). The clinical picture of heat exposed lambs could be related to the disorders of kidney, heart, and respiratory tracts (Sula et al., 2012).

Mechanisms of heat adaptation depends on species (Can et al., 2008), breeds (Maurya et al.,), and type of rearing systems (Aggarwaland Singh, 2008).

There are many reports indicating a heat tolerance among some breeds of sheep in different parts of the world (Alhidary et al., 2012; Srikandakumar et al., 2003). There is an extensive review on effects of heat stress on sheep physiological parameters (Marai et al., 2007); however, there is no report about the native breed of Iran, especially in tropical parts of the country, which may have some potential genetic merits in heat tolerance.

Materials and Methods

Experimental location: The experiment was conducted from the beginning of May 2009 to the end of February 2010 at the veterinary medicine hospital, Shahid Chamran University in the Ahvaz city of the Khuzestan province of Iran. The region was an arid area with an annual rainfall of 224.83 mm and mean temperature of 26.6^{o}C with values daily ranging from 0 to 38.8 mm and 3 to 50^{o}C, respectively.

Surgical procedures: Semi and complete castration was performed using Turner and McIlwraith's method (Turnerand Mcilwraith, 1995). The animals were located in dorsal recumbency position. The area around the base of scrotum was locally anesthetized using lidocaine hydrochloride (Lurocaine®, Veto-quinol, France), and the ventral surface of scrotum was shaved and swabbed with antiseptics. Then, the scrotum was grasped and a horizontal incision was made through skin and fascia on the right or left scrotum. The testis was removed and scrotal incision was left to heal by second intention. The incision was sprayed with an Oxytetrtacycline solution (Vetaque®, Iran), and systemic antibiotic therapy was undertaken for 5 consecutive days.

Experimental design: The fall-born (September and November) ram lambs (n=15; 19.9±0.79 kg LW; 6.2±1.12 months) were purchased from a local farm (Iranian native Bakhtiari breed, a fat-tailed breed) and subjected to outdoor condition to experience chronic heats stress condition from May to September (temperature range of 31 to 50^{o}C). At the end of September, 10 lambs (18.7±1.19kg LW) had undergone semi-castration as an acute stress (Hensch et al., 2011), and the remaining five lambs were retained intact by the end of the experiment. The entire period of study lasted 10 months (from May 2009 to February 2010). The animals were kept in a conventional open shed under direct solar radiation. All lambs received a ration comprised of wheat straw, Lucerne hay, and concentrate. The animals of both groups were weighted in May, September, and February, and they had free access to water throughout the study.

Measurements: Daily maximum environmental temperatures, relative humidity, and temperature humidity index (THI) were recorded throughout the experiment. Rectal temperature (RT), Respiratory rate (RR), and Heart rate (HR) for each animal was measured once a week, twice in a day (0800 and 1400). On the first day of each month, blood samples were collected from jugular vein and allowed to stand for 20 min and centrifuged at 3000 rpm for 10 min. Then serum was separated and stored at -20^{o}C till it was convenient to assay Na, K, CL, Ca, Glucose, and cortisol concentrations.

Assays: Minerals, electrolytes, and Glucose were measured using Ziestchem Diagnostics kits, Tehran Iran. The serum Ca assay was measured based on O.Cresphetaleine complexone (CPC) procedure. The Glucose measurement was based on enzymatic/colorimetric GOD/PAP procedure. The serum CL was assayed using colorimetric/ thiocyante assay. Na and K were measured using flame photometer. The serum cortisol concentration was measured with Radioimmunoassay procedure (CORTISOL-RIA-CT; KIPI28000, B-1400 Nivelles, Belgium). Intra- and inter assay coefficient of variation were 5.2-7.7 (5 replicate) and 8.7-15.1 (8 replicate), respectively.

Statistical analysis: The mixed model analysis was applied to find the effect of the month and groups of experiment on Na, K, CL, Ca, and Glucose concentrations. The male number was considered as random effects. The changes in RT, BT, and RR in different days and months were analyzed using mixed model procedure of SAS. Animals were considered as random effect. A multiple regression analysis was applied to find any correlation between RT, RR, and HR. The data was expressed as least square of means±standard error of mean (SE).

Results

Figure 1 shows monthly changes of the environmental temperature, relative humidity, and

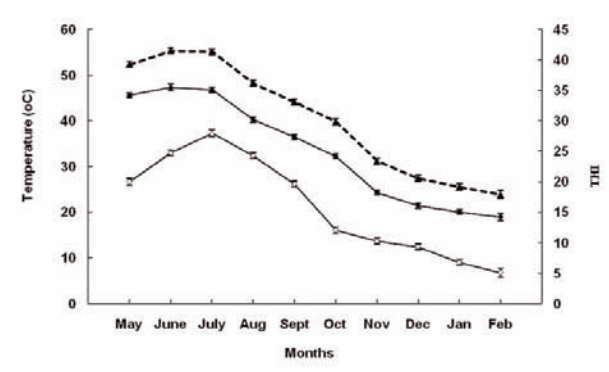

Figure 1. The monthly least square mean (±SE) of THI and maximum and minimum ambient temperatures throughout the experiment. —◆— Max Temp —◇— Min Temp - -▲- - THI

temperature-humidity index throughout the experiment. The mean maximum THI was recorded from May to July, followed by a slow decreasing trend to the end of the experiment. Based on the results, the animals experienced a mild chronic heat stress from May to August.

Body weight changes: Mean body weights in two groups at the start of the experiment and at the end of the experiment were not significantly different (Table 1; p>0.05). Mean growth rate was not significantly different (p=0.68) between intact (2.3±0.19; 2.16-3.38 kg/month) and castrated (2.4±0.19; 1.7-3. 8 kg/month) males. However, mean monthly weight gain (kg/month) during the period of heat stress (May-Sep; 1.96±0.16) was significantly less (p=0.0002) than thermo-neutral period (Oct-Feb: 2.93±0.16).

Clinical assessment: The changes within RT, RR, and HR were significantly differed throughout the months of the experiment; however, all changes were within documented normal values. The order of physiologic responses of the animals was carefully evaluated throughout this study.

RT (°C) was significantly affected (Figure 2; p<0.0001) by the months of the experiment: the highest mean RT was in August (39.3±0.003) and September (39.27±0.003). Mean RT was higher (p<0.0001) at 1400 (39.3±0.03) than 0800 (39.1±0.03) h during the experiment. A significant regression model was shown among RT, HR, and RR (Figure 3):

RT (°C)=38.731+0.0008HR+0.007RR; p<0.0001

Mean RR (per min) was higher at 1400 (60.7±1.3) than 0800 (53.8±1.3) h (p<0.0001). There was a significant variation in RR during different months of the experiment; mean RR in August (81.1±1.6) and September (73.9±1.6) was higher (p<0.0001) than those of the other months (Figure 2). There was no interaction between time of day and months of the experiment in the RR (Figure 2; p=0.75).

Mean Heart rate (per min) was significantly influenced by time (0800 h: 83.9±1.00 or 1400 h: 92.5±1.00; p<0.0001) and the month of experiment. The maximum mean Heart rate (p<0.0001) was recorded in October (99.1±1.3) and the minimum was recorded in May (66.2±1.3). There was not any interaction between the time and months of experiment in mean Heart rate (Figure 2; p=0.96).

Para clinical assessment: All of the biochemical values were within the documented normal values range. However, significant monthly changes were seen in all of the parameters. Castration operation did not influence the monthly pattern of the changes in the serum biochemical parameters.

Mean serum CL concentration (mEq/L) was significantly changed over the months of the experiment (Table 2; p<0.0001). The maximum of serum CL concentration was detected in September assay (130.7±3.27). Mean serum CL concentrations (mEq/L) of intact (117.4±1.88) and castrated (120.1±1.34) males were similar (p=0.24).

Mean serum K concentration (mEq/L) was affected throughout the experiment (Table 2; p<0.0001). The highest concentration of serum K was recorded in May (4.7±0.08) and June (4.8±0.08), and the lowest values were in January and February (3.8±0.09) assays. Mean serum K (mEq/L) concentration of two groups was similar (Intact males: 4.28±0.05 vs. castrated males: 4.22±0.03; p=0.36).

Significant monthly changes in serum Na concentration (mEq/L) were recorded throughout the experiment (Table 2; p<0.0001). Maximum concentration of serum Na was recorded in June (149.1±0.76) and the minimum concentration was assayed in February (133.5±0.78). Mean serum Na (mEq/L) concentrations were similar between intact (139.7±0.42) and castrated (140.4±0.30) males (p=0.15).

Mean monthly serum Glucose concentration

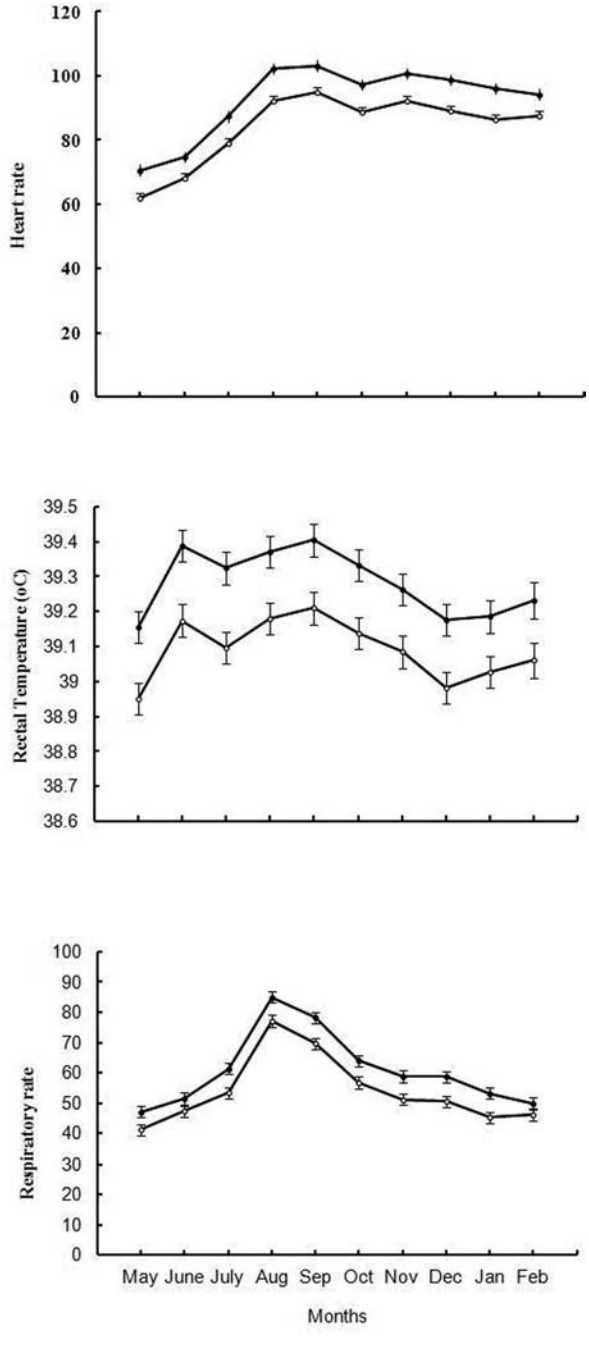

Figure 2. Monthly changes (LSmean±SE) of Heart Rate (HR), Rectal Temperature (RT) and Respiration rate (RR) of developing ram lambs during chronic heat stress (from May to September) throughout chronic and after acute induced stress (semi-castration in October). —○— 0800 —●— 1400

(mg/dl) significantly changed over the months of the experiment (Table 2; $p<0.0001$). The highest level of serum Glucose was recorded in August (80.1±2.82) and the lowest one was detected in January (49.2±2.92). There was not any significant difference ($p=0.098$) in serum Glucose concentrations between intact (65.8±1.65) and castrated males (62.4±1.18).

Significant variation was detected in serum Ca concentration throughout the months of the experiment (Table 2; $p<0.0001$). Means Ca concentration (mg/dL) was not significantly different between the intact (10.6±0.17) and castrated (10.55±0.12) males ($p=0.67$).

Table 3 shows a higher serum Glucose level in the castrated males in September compared with intact animals ($p>0.05$). While CL concentration was decreased in September in both groups, its levels continued to decline only in castrated animals in the next month assay ($p<0.05$). The changes of other elements were not significant among intact and castrated animals in months before and after the castration ($p<0.05$).

Significant monthly variation (all within normal value range) was seen in serum cortisol concentrations (nmol/L) throughout the experiment (Table 3; $p<0.0017$); two peaks of cortisol concentrations were in June and October in both treatment groups. The October values of serum cortisol in semi-castrated and intact animals were 81.3±9.30 and 65±12.47, respectively. The value of serum cortisol in June, for all animals, was higher than that of the other months. The cortisol concentration of surgically stressed group was not significantly higher than that of intact rams. However, overall mean cortisol concentration of semi-castrated (49.1±4.12) and intact (54.5±2.9) animals were not significantly ($p=0.29$) different.

Discussion

In the present study, the animals faced the first heat stress of their lives from May to September; the mean maximum ambient temperature was more than 45°C, and they were within thermo-neutral conditions up to the end of the experiment. The results of the present study also revealed some clinical and biochemical reactions of animals to chronic heat stress. All of the assayed parameters were in the documented normal value ranges. However, the significant monthly variations were seen in their values within the normal range value (Radostits et al., 2007).

The results of the present study showed that the first reaction of body to the chronic heat stress was

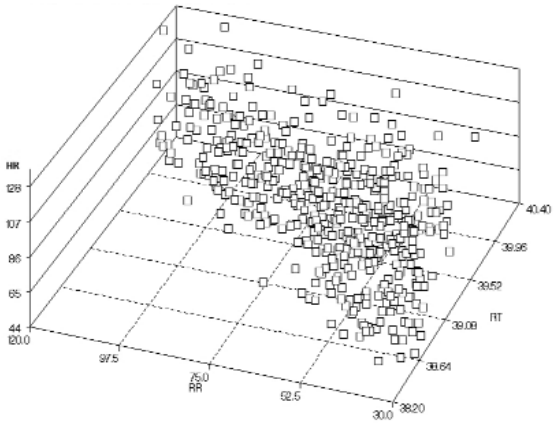

Figure 3. Three dimensional scatter plot that shows the relationship between rectal temperature (RT), respiratory rate (RR), and Heart rate (HR) of heat exposed ram lambs during the experiment. RT (^{0}C)=38.731+0.0008 HR+0.007RR;p<0.0001.

Table 1. Body weight changes (kg) of ram lambs throughout the experiment (LSmean±SE).

	no.	May	February	Growth rate (kg/month/ lamb)
Intact	5	23.6±1.03 (20.5-26)	46.6±1.3 (43.5-50)	2.3±0.19 (2.16-3.38)
Castrated lamb rams	10	22.4±0.58 (19.5-25)	48.4±1.88 (28-36)	2.6±0.19 (1.7-3.8)

increasing rectal temperature, i.e. while the highest environmental temperature was (48.5±0.54°C) in June, the rectal temperature began to rise from that time and remained constant for some consecutive months. Two months after rectal temperature elevation in heat stress condition, the animals started to show an increase in their RR and HR, which coincided with the serum K decline, lasting till the end of experiment. However, at the time of the peak rectal temperature, the serum concentrations of Na and CL were the highest, and then they started to decrease till the end of the experiment. The mean maximum RT was 39.4±0.0045°C, which was slightly higher than that of the normal values (39.1°C) for this species (Radostits et al., 2007). The monthly mean maximum Heart rate was higher than the normal value range (70-80 b/m) from June to the end of the experiment. The RR was considerably higher than the reported normal values (16-34 /min) in this breed throughout the experiment.

A decreasing trend of serum K and Na concentrations were noticed from August, i.e. three months after the beginning of heat exposure, toward the end of the experiment. Nonetheless, the serum chloride concentration rose during the first four months of the experiment significantly and then dropped. The animals did not experience levels of chloride lower than the normal values throughout the experiment. However, serum CL concentration significantly decreased one month after the castration. Although serum Ca concentration showed a significant variation, all of the changes were in the range of normal values. Two peaks of the serum Glucose concentration were noticed; the first was in the beginning of the experiment_ the animals experienced their first heat stress in their lives, and the second was at the time of significant changes in the environmental temperature, in October. Surprisingly, the castration just increased the values of cortisol and in turn the serum Glucose concentration in a non-significant pattern. The later may indicate a suppressive effect of chronic stress on overall stress response of animals.

The results of present study on weight gain was in line with the other studies (Dixon et al., 1999; Ogunjimi et al., 2008); the results showed that repeated exposure to heat stress caused a decrease in weight gain and food intake (Harikai et al., 2003). However, stress of castration operation did not significantly change weight gain in the present study.

Plasma cortisol levels are used as a general indicator of the degree of strain experienced by the body. Repeated exposure of mice to acute heat stress slightly increases the concentration of corticosterone, which is an indicator of stress in that species (Harikai et al., 2003). In a previous study, the serum cortisol concentration, followed by the exposure of ram lambs to the heat stress conditions, similar to the present study, was higher than that of the animals that were maintained in the thermo neutral environments (Rasooli et al., 2010). In the present study, two non-significant peaks of cortisol concentrations were noticed; the first was at the time of high-elevated temperature and the second was at the time of entering the thermoneutral condition (October). However, both of peaks were not out of normal range of cortisol concentration in the species (42-82 nmol/L (Radostits et al., 2007)). Although, the first peak of cortisol concentration was in May and June, the significant increase of Glucose concentration happened in August. In spite of the highest cortisol

Table 2. Monthly changes of biochemical parameters (LSmean±SE) in developing lamb rams. [abcdef] Values with different superscript within columns significantly differ ($p<0.05$).

Month	K (mEq/L)	Na (mEq/L)	Ca (mg/dl)	Glucose (mg/dl)	CL (mEq/L)
May	4.7±0.07[a]	146.8±0.6[a]	.		.
June	4.8±0.09[a]	149.1±1.11[b]	9.9±0.22[af]	67.8±2.48[ae]	115.7±2.73[a]
July	4.7±0.09[a]	145.8±0.54[a]	10.4±0.36[acdf]	75.6±2.71[bc]	127.01±3.92[bcd]
August	4.3±0.09[b]	139.8±1.26[c]	11.7±0.20[be]	80.1±2.52[c]	125.4±4.02[cd]
September	4.3±0.104[b]	135.1±0.67[d]	11.2±0.33 c[b]	65.9±3.14[ae]	130.7±3.60[cd]
October	4.05±0.1[c]	138.86±0.69[cef]	11.03±0.22 d[b]	61.2±3.03[e]	109.7±2.29[a]
November	4.01±0.06[c]	138.07±0.46[c]	11.9±0.53[e]	69.28±2.98[ab]	114.7±2.92[a]
December	3.9±0.07[cd]	139.1±0.51[c]	9.9±0.22[af]	52.9±2.39[d]	118.9±3.18[bcd]
January	3.8±0.09[cd]	135.85±0.65[e]	9.2±0.15[a]	49.2±3.21[d]	117.0±2.28[a]
February	3.8±0.09[d]	133.5±0.83[f]	10.01±0.34[af]	49.7±3.06[d]	113.8±4.09[a]

Table 3. The effect of castration (September is the time of castration) on the serum biochemical parameters of chronic heat exposed ram lambs (LSmean±SE). [ab] values with different superscript significantly differ within rows ($p<0.05$).

Biochemistry assay	Group	Months of experiment around castration time		
		August	September	October
CL (mEq/L)	Intact rams (n=5)	128.8±5.65[a]	125.6±5.65[a]	104.96±5.65[b]
	Castrated rams(n=10)	124.2±3.9[a]	133.3±3.9[a]	112.03±3.9[b]
Glucose (mg/dl)	Intact rams (n=5)	80.4±4.96[a]	68.04±4.96[a]	67.4±4.96[a]
	Castrated rams(n=10)	80±3.51[a]	65±3.51[b]	58.1±3.51[b]
Na (mEq/L)	Intact rams (n=5)	139.2±1.3 3[a]	134.4±1.33[b]	138±1.33[a]
	Castrated rams(n=10)	140.1±0.99[a]	135.4±0.99[b]	139.3±0.99[a]
K (mEq/L)	Intact rams (n=5)	0.48±0.21	0.52±0.21	0.88±0.21
	Castrated rams(n=10)	0.49±0.18	0.54±0.18	0.71±0.18
Ca (mg/dl)	Intact rams (n=5)	12±0.53	11.00±0.53	11.22±0.53
	Castrated rams(n=10)	11.5±0.38	11.3±0.38	10.9±0.38

Table 4. Monthly serum cortisol concentrations in intact and castrated rams (LSmean±SE). [abc] values with different superscript within columns differ ($p<0.05$). [AB] values with different superscript within rows differ ($p<0.05$).

Month	Cortisol (nmol/L)	
	Intact rams (n=5)	Castrated rams (n=10)
May	66.7±12.5[acA]	64.7±9.3[acA]
June	74.25±12.5[acA]	60.4±9.9[abcA]
July	59.1±12.5[abA]	57.9±8.82[abA]
August	37.35±12.5[abcA]	45.7±9.3[aA]
September	38.3±12.5[abcA]	39.94±9.3[aA]
October	65.0±12.5[acA]	81.3±9.3[cA]
November	57.75±16.11[aA]	53.6±8.82[abA]
December	26.3±12.5[bA]	37.3±9.87[abA]
January	33.3±13.95[abcA]	43.9±10.5[abA]
February	33.24±12.5[abcA]	60.3±9.3[abcA]

concentration in castrated rams in October, there was no change in the serum Glucose concentrations at this time.

The serum concentrations of all electrolytes except CL were within normal values range, with a significant variation between months. The Na and K concentrations began to decrease from August; when animals experienced higher respiration and Heart rates. The CL concentration had an ascending trend from the beginning of the study untill September, when CL concentration started a decreasing trend up to the end of the study. A significant variation was also seen between months according the serum Ca concentrations.

In conclusion, the variations of different biochemical, endocrinologic parameters may indicate the genetic potential of the breed against heat stress. The physiological response of animals to chronic heat stress, including increasing rectal temperature, also showed that the rectal temperature of animals during the hottest times of a day did not rise up to critical temperature (40°C), and the animals experienced a RT of slightly higher than normal range.

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استرس حرارتی مزمن در بره قوچ‌های دنبه دار ایرانی: برخی پارامترهای بیوشیمیایی و بالینی

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چکیده

زمینه مطالعه: اثرات تنش حرارتی بر شاخص های فیزیولوژیک به خوبی مورد بررسی قرار گرفته است. با این حال، گزارشاتی از مقاومت ژنتیکی به حرارت بالای محیطی در گوسفند وجود دارد. **هدف:** هدف از مطالعه حاضر پاسخ های فیزیولوژیک گوسفند نژاد دنبه دار ایرانی در مواجهه با استرس حرارتی و یک عامل موجد استرس حاد می باشد. **روش کار:** تعداد ۱۵ رأس بره قوچ متولد پاییز انتخاب و در طی اردیبهشت ۱۳۸۹ تا بهمن ۱۳۸۹ در معرض حرارت مستقیم خورشید در طول روز قرار گرفتند. در مهرماه ۱۰ رأس نیمه اخته شده تا اثرات استرس حاد جراحی بررسی شود. پارامترهای عمومی مانند بیوشیمی خون و سطح کورتیزول خون آنالیز گردید. **نتایج:** نتایج نشان داد که شاخص های فیزیولوژیک و بیوشیمیایی خون دچار تغییرات قابل ملاحظه در طول آزمایش گردید با این حال این تغییرات در دامنه نرمال اعلام شده برای گونه بوده است. این واقعیت که حیوانات مورد آزمایش در مطالعه حاضر اولین تجربه استرس حرارتی زندگی خود را داشته اند و افزایش قابل توجه سطح کورتیزول در پی اخته سازی در مقایسه با داغ ترین ماه سال نشان از سازش پذیری ژنتیکی این نژاد در ایران به استرس حرارتی دارد. **نتیجه‌گیری نهایی:** مطالعه حاضر تغییرات دینامیک شاخص های فیزیولوژیک و بیوشیمیایی در پاسخ به استرس حرارتی و یک استرس حاد را نشان داد که امکان مقاومت این نژاد به استرس حرارتی را تقویت می کند.

واژه‌های کلیدی: معاینه بالینی، استرس حرارتی، بره قوچ دنبه دار ایرانی، بیوشیمی سرم

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