RESPONSES OF HEMATOLOGY, BLOOD METABOLITES, MINERAL IONS AND HORMONAL PROFILE TO HEAT STRESS FOR EGYPTIAN BUFFALO-CALVES

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Abstract

This study was carried out at the Experimental Farm of the Animal Physiology Research Lab, Faculty of Agriculture, Cairo University, during the summer season of 2004. The objective was to assess the relevant physiological responses and growth performance of Egyptian buffalo-calves to artificial constant severe heat stress. Eight buffalo-calves were randomly divided into lab A and lab B, their age and live body weight were 6 months and about 119 kg, respectively. All calves were assessed under two constant thermal conditions, heat stress (40 °C and 87.5% RH, lab A) and comfort state (25 °C and 64.5% RH, lab B). Two trials were conducted, each one continued for one month in each lab. They were interrupted by 15 days under natural climatic outdoor conditions.

Values of Ht, Hb and RBCs were reduced by heat stress during the two trials. The white blood cells count (WBCs) were significantly increased (around 50%). Levels of total protein (TP), albumin (Alb), total lipids (TL), triglycerides (TG), total cholesterol (TC), glucose (Glu) and blood urea nitrogen (BUN) were decreased by heat stress during the two trials, while, the globulin (Glo) level was not affected. In the two trials, heat stress caused slight increase (4 to 8 %) in sodium (Na) and potassium (K), and increase (47 to 58 %) in phosphorus (P) level, and calcium (Ca) was decreased by 20%. In both trials, heat stress decreased insulin (Ins), increased glycogen (Glg) and both triiodothyronine (T_3) and thyroxine (T_4) were dropped by about 50%.

It can be concluded that buffalo-calves were able to maintain their lives with some chemical and physical changes that led to the delay in growth when exposed to high temperatures.

INTRODUCTION

Suitability of Egyptian buffaloes to hot climate is achieved by morphological, anatomical and physiological characteristics (Shafie, 1958, 1985, 1993 a, b, Ashour *et al.*,2000, 2004 and Omran, 1999, 2008). The tolerance capacity of Egyptian buffaloes was determined in Egypt under natural seasonal conditions and under artificial laboratory conditions of heat stress (Omran, 1999, 2008 and Ashour *et. al.*, 2000 and 2004).

The present work was carried out to identify the relevant physiological responses of Egyptian buffalo-calves under exposure to artificial heat stress (40°C) and comfort (25°C) as affecting changes in blood hematological picture, blood plasma metabolites, mineral and hormones.

MATERIALS AND METHODS

This study was carried out at the Experimental Farm of the Animal Physiology Research Lab, Animal Production Department, Faculty of Agriculture, Cairo University. The experimental procedures and data collection were executed during the summer season of 2004. The present work was executed to assess the relevant physiological responses of buffalo-calves to artificial constant severe heat stress. Eight buffalo-calves (*Bubalus bubalis*) were available for this study from Mehalet Mousa Experimental Farm, Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, Egypt. On the beginning of the experiment, the age of each calf was 6 months and its live body weight (BW) ranged between 118.0 - 119.3 kg. All animals appeared healthy normally. They were divided into lab A and lab B.

This work was carried out under two constant thermal conditions (CTC), heat stress (HS) 40°C, lab A and comfort state (CS) 25°C, lab B. Two trials, each of which continued one month in both labs and were interrupted by 15 days under outdoors conditions.

Feeding a commercial concentrate ration was offered in surplus amount daily to determine the free - well fed intake (FI). The roughage feeds, wheat straw and Berseem (Trifolium alexandrinum) hay were also delivered in surplus. The concentrate ration consisted of a starter during the first month of experimentation (6-7 months), then, followed by a growth concentrate till the end of experimental work at the 9th month.

Lab A was equipped by 4 heaters controlled by highly sensitive digital thermostat alongside ceiling and suction fans. Thus, the ambient temperature (AT) in this lab was fixed and maintained exactly at 40°C, the relative humidity (RH) was 87.3 – 87.7 %, and the temperature-humidity indexes (THI) were 101.2±0.1–100.0±0.1 at the 1st trial and 100.8±0.1-100.7±0.1 at the 2nd trial. Lab B was equipped by air conditioner and ceiling fan to maintain the AT at 25°C, the RH at 61.3 – 67.9 % and the THI were 74.0±0.3–75.0±0.6 at the 1st trial and 75.8±0.5-76.7±0.2 at the 2nd trial.

The blood sampling was carried out on the 1st, 2nd, 3rd days, as well as on the 15th day, and at the end of each period, the mean value expressed the data of the five measurements.

Blood samples were collected at 08:00h prior to morning feeding and watering in heparinzed tubes from the external Jugular vein. Directly, a portion of blood sample was used for determining hemoglobin (Hb, g/dl) conc., hematocrit value (Ht, %), red blood cells count (RBCs, $X10^6/mm^3$) and white blood cells count (WBCs, $X10^3/mm^3$). Alongside, a blood smear was prepared for differential counts of leukocyte types. Another portion of blood sample was centrifuged for 30 min at 3000 rpm to collect clear plasma sample. The plasma samples were drawn into micro-cuvetts and stored at -20°C until assaying the conc. of relevant, hormones, organic compounds and mineral ions.

The tested organic compounds were: total protein (TP, g/dl), albumin (Alb, g/dl), globulin (Glo, g/dl), total lipids (TL, mg/dl), triglycerides (TG, mg/dl), total cholesterol (TC, mg/dl), glucose (Glu, mg/dl) and blood urea nitrogen (BUN, mg/dl). The assessed minerals (mg/dl) were: sodium (Na), potassium (K), calcium (Ca) and phosphorus (P). The considered hormones were, triiodothyronine (T_3), thyroxine (T_4), insulin (Ins) and glucagon (Glg). Relevant statistical analysis of data was carried out applying the Statistical Analysis System (SAS, 2000).

RESULTS AND DISCUSSION

Blood parameters

1. Hematological picture

Table 1 shows that the values of Ht, Hb and RBCs counts were significantly reduced (-20 %) by heat stress in the two trials. On the contrary, the WBCs count was significantly increased (+50 %) in response to this stress.

Several investigators reported that (Hb and Ht) were decreased in heat stressed animals (Rowlands *et. al.*, 1974, Bond *et. al.*, 1984, Omran, 1999, 2008 and Ashour *et. al.*, 2004). This reduction was attributed to the destruction of erythrocytes and / or to hemodilution (Shebaita and Kamal, 1975 and Shaffer *et. al.,* 1981). In addition, Shafie and Badreldin (1962) stated that thermal stress of direct solar radiation in Egypt caused a decrease in Hb conc. in buffaloes and cattle breeds. They postulated that these animals reduced the Hb content in their blood to check the metabolic rate, thus, reducing production of metabolic heat.

Responses of leukocyte cells types % to heat stress are shown in Table 2. The monocytes, neutrophils and Neu / Lym significantly increased, while, the other types significantly showed clear drop in their percentages. The ratio neutrophils /

lymphocytes (Neu / Lym) was increased by heat stress. Values of neutrophils and lymphocytes are in agreement with those of Omran (1999 and 2008) and Ashour *et. al.* (2004) under heat stress at 40°C in lab.

2. Concentration of relevant plasma organic compounds

The results presented in Table 3 indicated that the levels of TP and Alb were significantly decreased by heat stress during the two trials, while, Glo conc. was not affected. Shafie and Badreldin (1962) found that the total protein in buffaloes exposed to direct solar radiation in Egypt was decreased by 11.9 %. The same trend was reported by several workers who found that, serum protein conc. usually was decrease under heat stress by about 10 % (Kamal *et. al.,* 1962 and 1989, Abouel-Naga, 1987, El-Masry, 1987, Habeeb, 1987 and Ashour *et. al.,* 2000).

The levels of TL, TG and TC were significantly decreased by heat stress during the two trials, however, with greater response in the second trial (Table 3). Similar results were reported in ruminants with prolonged exposure to high AT for TL (Noble *et. al*., 1973, O'Kelly, 1973, Daader *et. al.,* 1989 and Kamal *et. al*., 1989), and TC (Shaffer *et al*., 1981, Abdel-Samee, 1987, Abouel-Naga, 1987, El-Masry, 1987 and Ashour *et. al.,* 2000).

Glucose conc. was significantly reduced due to heat stress in the two trials. Shaffer *et. al*. (1981) identified a highly sig. effect of seasonal temperature on blood glucose levels. The level of BUN was also significantly lowered in group A than in group B during the two trials in response to HS. El-Masry (1987) reported that BUN level was decreased due to heat stress condition in Friesian cows and calves.

3. Concentration of relevant plasma minerals

Table 4 shows that both K and Na conc. were increased significantly by HS, (4 - 8 %) in the two trials. P conc. significantly showed greater increase by heat stress (47 and 58 %) in the two trials, meanwhile, Ca was significantly decreased (-20%) in the two trials.

4. Concentration of the studied hormones

Table 5 indicates that both T_3 and T_4 conc. were dropped by HS, around 50 % in each hormone in the two trials. The present result is in agreement with Omran (1999 and 2008) and Ashour *et. al.* (2004) under HS at 40°C. The stress caused decrease in Insulin (Ins) conc. (-60 and -57 %) and increase in glycogen (Glg) conc. (+29 and +20 %) in the two trials, respectively. The increasing of plasma glycogen may by due to decreases of plasma glucose (Table 3) and reduced feed intake under HS. El-Masry and Habeeb (1989) reported that high AT caused drop in the conc. of Ins. Thompson (1973) concluded that acclimatization and/or acclimatization to heat stress causes an increase in BT and decrease in thyroid activity.

Table 1. Hematological picture, Ht, %, Hb, g/dl, RBC's X106/mm3 and WBC's X103/mm3 in calf groups (A and B) under two CTC (Mean ±SE).

Items	**1st trial**		**2nd trial**	
	(A) 40°C	**(B) 25°C**	**(A) 40°C**	**(B) 25°C**
Ht, %	28.35±0.41 [b]	35.20±0.66 [a]	28.80±0.85 [b]	37.00±0.91[a]
Hb, g/dl	8.84±0.14 [b]	13.80±0.36 [a]	10.00±0.21 [b]	13.35±0.21 [a]
RBC's, X10^6/mm^3	4.65±0.07 [b]	7.19±0.06 [a]	5.00±0.16 [b]	7.02±0.10 [a]
WBC's, X10^3/mm^3	3.50±0.11 [a]	2.11±0.06 [a]	3.00±0.07 [a]	2.12±0.08 [a]

In the same row means with different superscripts are significantly different (P<0.05).

Table 2. Leukocyte cells types (%) of the buffalo-calf groups (A and B) under two CTC (Mean ±SE).

	1st trial		**2nd trial**	
Types	**(A) 40°C**	**(B) 25°C**	**(A) 40°C**	**(B) 25°C**
Neutrophils	53.7±0.68 [a]	47.5±0.33 [b]	52.0±0.65 [a]	46.0±0.52 [b]
Lymphocytes	35.4±0.54 [b]	42.4±0.4 [a]	36.9±0.85 [b]	43.4±0.78 [a]
Eosinophils	4.0±0.18 [a]	5.0±0.12 [a]	4.5±0.23 [a]	5.13±0.23 [a]
Basophils	0.1±0.07 [a]	0.23±0.09 [a]	0.43±0.11 [a]	0.37±0.11 [a]
Monocytes	6.8±0.17 [a]	4.9±0.09 [b]	6.2±0.21 [a]	5.1±0.13 [b]
Neutophils/lymphocytes	1.52±0.20 [a]	1.12±0.10 [b]	1.41±0.21 [a]	1.06±0.15 [b]

In the same row means with different superscripts are significantly different (P<0.05).

Table 3. Concentrations of the tested blood plasma organic compounds of the buffalo-calf groups (A and B) under two CTC (Mean ±SE).

	1st trial		2nd trial	
Items	**(A) 40°C**	**(B) 25°C**	**(A) 40°C**	**(B) 25°C**
Total protein (g/dl)	8.10±0.20 [b]	9.90±0.03 [a]	8.30±0.09 [b]	9.90±0.04 [a]
Albumin (g/dl)	3.70±0.19 [b]	5.50±0.10 [a]	3.80±0.07 [b]	5.40±0.10 [a]
Globulin (g/dl)	4.40±0.14 [a]	4.40±0.09 [a]	4.60±0.09 [a]	4.40±0.08 [a]
Total lipid (mg/dl)	227.00±12.10 [c]	670.00±33.30 [b]	210.00±10.10 [c]	770.00±16.10 [a]
Triglycerides (mg/dl)	64.52±1.70 [c]	146.60±9.40 [b]	70.30±1.40 [c]	185.20±3.60 [a]
Total cholesterol (mg/dl)	43.70±2.96 [c]	86.39±3.90 [b]	36.80±1.70 [c]	103.40±4.30 [a]
Glucose (mg/dl)	47.01±3.40 [b]	78.80±1.50 [a]	40.00±2.10 [b]	75.00±2.50 [a]
Urea (mg/dl)	31.61±1.21 [b]	47.71±0.49 [a]	28.90±0.79 [c]	48.50±0.30 [a]

In the same row means with different superscripts are significantly different (P<0.05).

Table 4. Concentrations of tested blood plasma minerals of the buffalo-calf groups (A and B) under two CTC (Mean ±SE).

	1st trial		2nd trial	
Items	**(A) 40°C**	**(B) 25°C**	**(A) 40°C**	**(B) 25°C**
Sodium (mg/dl)	358.98±8.00 [a]	333.55±4.40 [bc]	344.08±8.50 [b]	329.60±7.20 [c]
Potassium (mg/dl)	20.67±0.20 [a]	19.11±0.20 [b]	20.67±0.30 [a]	19.11±0.30 [b]
Calcium (mg/dl)	10.20±0.34 [c]	12.80±0.20 [a]	9.10±0.16 [d]	11.40±0.14 [b]
Phosphorus (mg/dl)	7.20±0.05 [a]	4.90±0.20 [b]	7.10±0.07 [a]	4.50±0.30 [b]

In the same row means with different superscripts are significantly different (P<0.05).

Table 5. Concentrations of the studied hormones of the buffalo-calf groups (A and B) under two CTC (Mean ±SE).

	1st trial		2nd trial	
Hormone	**(A) 40°C**	**(B) 25°C**	**(A) 40°C**	**(B) 25°C**
Triiodothyronine (ng/dl)	114.73±5.02 [b]	233.64±15.77 [a]	121.43±2.24 [b]	228.01±14.08 [a]
Thyroxine (µg/dl)	2.59±0.20 [c]	6.03±0.16 [a]	2.49±0.10 [c]	5.38±0.24 [b]
Insulin (µlU/ml)	19.37±1.17 [b]	48.30±2.25 [a]	20.39±0.74 [b]	47.08±2.16 [a]
Glycogen **(pg/ml)**	86.44±3.06 [a]	56.82±2.17 [b]	80.57±9.14 [a]	67.42±8.44 [b]

In the same row means with different superscripts are significantly different (P<0.05).

CONCLUSION

The blood picture for buffalo-calves showed clear drop in Ht, Hb conc. and RBCs count. This decrease may be, in part, due to hemodilution by excess of WI. However, the drop in Hb and RBCs was more than the decrease in Ht which indicated other mechanism imposing drop in Hb and RBCs to reduce oxidation activity of metabolism, thus, subsequent drop in metabolic heat production. On the contrary, the WBCs count was increased as indication of immunological activity. This immunological reaction was fortified by increase in Neu % against decreases in Lym % leading to increase in the ratio Neu/Lym.

The response of the studied blood plasma metabolites to heat stress showed great decrease in TL, TC and TG followed by Glu . These reductions indicated hamper of metabolic activities subsequently less product of metabolic heat. The drop in TP suggests effect of hemodilution along with drop in buildup of plasma proteins. The increase in Glb against drop in Alb indicates control in colloid osmotic pressure to maintain proper blood circulation.

Heat stress caused increasing in conc. of k, Na around 10 % opposite to 20 % decrease in Ca, in both trials. The P showed more than 50 % increase in both trials of stress. This increasing should be, particularly, due to hemoconcentration by activated water vaporization from the animals surface and increased respiratory vaporization. Nevertheless, the difference in the rate of increase between K, Na and P indicated active physiological arrangements concomitant with the hemoconcentration. The opposite trend by reduction in Ca, equal in the two treatments, emphasizes effective physiological interference.

Heat stress caused reduction in insulin (Ins) conc. (-60 and -57 in the two trials), coincidentally, the glycogen (Glg) conc. increased (+29 and +20 in the two trials). This opposite trend in the conc. of these hormones indicates hormonal control on critical drop in Glu conc. in blood plasma under HS, (47 and 40 mg/dl) in comparison to (79 and 75 mg/dl) under CS.

It is clear that T3 and T4 hormone conc. were decreased more than 50 % in response to HS, almost with equal levels in both trials, devoting depression in general metabolic activity, thus, metabolic heat production.

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استجابة هيماتولوجيل الدم ومكوناته والمعادن والهرمونات للإجهاد الحراري في عجول الجاموس المصري

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أجريت هذه الدراسة بالمزرعة التجريبية بمعمل فسيولوجيا الحيوان – كلية الزراعة جامعة القاهرة بالجيزة خلال موسم صيف عام 2004. و كان الهدف من هذه الدراسة هو تقدير الإستجابة الفسيولوجية الخاصة بعجول الجاموس للإجهاد الحرارى الشديد و الثابت (40 درجة مئوية) ، و الظروف المثلي (25 درجة مئوية) و كذلك الظروف المناخية الطبيعية و تأثير ذلك على هيماتولوجي الدم ومكوناته.

وأستخدم فى هذة الدراسة ثمانية عجول جاموسى (4 في كل معمل) عمر كل منها ستة شهور ومتوسط الوزن 118 الى 119.3 كجم و تم قياس إستجابة العجول تحت نوعين من الظروف الحرارية هما الإجهاد الحرارى داخل معمل (أ) حيث كانت درجة الحرارة ثابتة و مستمرة (40 درجة مئوية) بينما كانت الرطوبة النسبية 87.5 ٪ و كذلك داخل معمل (ب) تحت الظروف الم ثلي لفترة درجة الحرارة (25 درجة مئوية) والرطوبة النسبية (65.5 ٪) وأجريت تجربتان كل منهما لمدة شهر داخل هذة المعامل يفصل بينهما فترة 15 يوم عرضت فيها الحيوانات الى الظروف المناخية الطبيعية خارج المعمل.

و يمكن تلخيص أهم نتائج هذة التجربة فيما يلى:

- انخفضت قيم كل من الهيماتوكريت ، الهيموجلوبين ، عدد كريات الدم الحمراء بسبب الإجهاد الحرارى فى كلتا التجربتين و كان الإنخفاض واضح اً فى قيم كل من الهيموجلوبين، عدد كريات الدم الحمراء تقريباُ ضعف الإنخفاض فى قيم الهيماتوكريت. وفى المقابل زادت عدد كريات الدم البيضاء معنوياُ (حوالى 50 ٪). و زادت نسبة الكريات الأحادية و المتعادلة بسبب الإجهاد الحرارى ، بينما أظهرت النسب المئوية للأنواع الأخرى إنخفاضاً واضحاً مما أدى الى زيادة النسبة بين الكريات المتعادلة و الليمفاوية.
- ادي الاجهاد الحراري الي انخفاض مستويات كل من الدهون الكلية،والكوليستيرول الكلي والجلسريدات الكلية والبروتين الكلي،والالبيومين والجلوكوز ونيتروجين اليوريا خلال التجربتين مع ان الاستجابة الاكبر كانت في التجربة الثانية بينما لم يتأثر مستوي الجلوبيولين.

- في كلتا التجربتين ادي الاجهاد الحراري الي زيادة طفيفة (4–8 ٪) في تركيز كل من الصوديوم والبوتاسيوم بالبلازما والي زيادة كبيرة (47–58 ٪) في تركيز الفوسفور بينما نخفض تركيز الكالسيوم ا بوضوح بحوالي 20 ٪.
- في كلتا التجربتين ادي الاجهاد الحراري الي انخفاض مستوي هرمون الانسولين بحوالي 57–60 ٪ (حوالي60 ٪) بينما أزداد هرمون الجليكوجين بحوالي 29–20 ٪ (حوالي 25 ٪) في حين ان تركيز هرموني T_3 وT_4 انخفض انخفاضاً شديداً بنسبة 50 ٪ من قيمة كل منهما.

وعليه يمكن إستنتاج أن عجول الجاموس أستطاعت المحافظة علي حياتها مع إحداث بعض التغيرات الكيميائية والفيزيائية التي أدت إلي تأخير في النمو وذلك عند تعرضها للحرارة العالية. ومع توفير البيئة المناسبة للعجول أدى ذلك إلي تحسن في النمو.