

## The role of L-Tyrosine to relieve Barki sheep of physiological drawbacks resulted from short-term exposure to solar radiation

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**Abstract:** Exposure of rams to a heat stress has an adverse effect on behavioral and physiological responses. In this study, we examined the ability of exogenous L-Tyrosine, dopamine amino acid precursor to protect rams from developing these neurochemical and behavioral changes when exposed to heat stress. This experiment was carried out in Mariout station, Desert Research Center, Egypt. Fourteen mature Barki rams were divided into two groups, exposed to sunrise for three hours from 12am to 3pm. The first group (control (C)) was exposed to sunrise and before exposure was given an oral dose of normal saline (NaCl 0.9%, (0.45 ml/kg bodyweight)). The second group (treated group) was exposed to sunrise and given an oral dose of L-Tyrosine 100mg/kg. Our results clearly showed that L-Tyrosine supplementation (100mg/kgb.wt.) decreased the effect of heat stress on some physiological responses. There was a significant decrease in both skin temperature  $ST^{\circ}C$  and respiration rate RR, moreover a decrease in rectal temperature  $RT^{\circ}C$ . Furthermore, a significant increase in total protein due to elevation of total amino acids and Magnesium levels in serum that indicated a decrease in stress in the tyrosine-treated group.

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### 1. Introduction

One of the greatest challenges facing grazing and transportation of small ruminants is the exposure to solar radiation in the desert, where shade is absent or limited. Solar radiation adversely affects animal performance. Environmental factors such as heat stress and humidity have direct and indirect effects on animals. Direct effects include reduced performance and reproductive ability. Indirect effects as changes in pathogen level, animal behavior, reduced feed intake, growth efficiency, and reproduction are all recognized results of heat stress. Animals that are acutely stressed exhibit neurochemical and behavioral changes. In certain brain regions, turnover of norepinephrine increases and its absolute level declines. When these changes occur, the animals interact less with their environment and seem debilitated (Stone, 1975). Several attempts have been made to use tyrosine to ameliorate the clinical, behavioral, biochemical and other signs of various types of stress in desert sheep (Ali, *et al.*, 2001). A large body of evidence demonstrated that Tyrosine supplementation has beneficial effects against stress in domestic animals (Sanhouriet *al.*, 1991 and Ali and Al-Qarawi, 2002).

Tyrosine is a large neutral amino acid found in dietary proteins and is the precursor of norepinephrine, dopamine, and epinephrine (Wurtman, *et al.*, 1981). Some of the behavioral deficits caused by acute stress may result from

depletion of norepinephrine, and perhaps dopamine, in catecholaminergic neurons. L-Tyrosine has previously been shown to be effective as an anti-stressor agent in rats (Reinstein *et al.*, 1985) and human (Banderet and Liberman, 1989). Thus, L-Tyrosine may protect against the adverse behavioral effects of acute stress by preventing depletion of norepinephrine in such neurons. Treatment of desert sheep with tyrosine ameliorates some of the clinical, biochemical and hematological adverse effects of acute stress encountered during transportation (Ali *et al.*, 2001).

Tyrosine administration, before exposure to physical and/or environmental stressors, reduces the adverse behavioral, physiological and neurochemical consequences of the exposure (Harris *et al.*, 2005). Tyrosine supplementation is associated with increased endurance capacity in the heat in moderately trained subjects in human and reduced plasma tyrosine and phenylalanine (tyrosine precursor) is associated with impaired exercise capacity in the heat (Tumilty *et al.*, 2011 and 2013). The aim of this study is to evaluate the role of oral L-Tyrosine in relief of Barki sheep of physiological drawbacks resulted from exposure to summer solar radiation.

### 2. Materials and Methods

The study was carried out in Mariout Animal Breeding Station, Desert Research Center. The station is located in a semi-arid region in

Alexandria region, Egypt. The ambient temperature in summer (August) ranged between 30°C to 37°C.

#### Experimental design:

Ten healthy mature rams with live bodyweight (B. wt) ranged between 38 to 45 Kg were divided randomly into two equal groups. The L-Tyrosine (C<sub>9</sub>H<sub>11</sub>NO<sub>3</sub>) used in this study was 3-Nitro-L-tyrosine ≥ 99%, USP, f. d. Biochemie, Molecular Weight: 181.19, Article number: T207.2 (Carl Roth GmbH + Co. 76185 Karlsruhe, Germany). Briefly, 100gm tyrosine was dissolved in 450 ml normal saline solution (0.9 g NaCl/L). The first experimental group served as Control group (C) and was given a vehicle at a dose of 0.45ml/Kg saline solution orally. The second experimental group (T) was given (0.45 ml/Kg (Bwt.) saline solution containing 100mg L-Tyrosine (V/W)) orally. Two groups were exposed to heat stress for three hours (from 12pm to 3pm). Blood samples were collected from the two groups at 12 pm before animals were exposed to heat stress, and the second set of samples were drawn at 3pm after exposure to heat stress for three hours.

#### Climatic measurements:

Estimation of the severity of heat stress was done using both ambient temperature and relative humidity as temperature-humidity index (THI). The formula used was  $THI = Ta - [(0.31 - 0.31 * RH) * (Ta - 14.4)]$ , where: Ta = Ambient temperature (°C) RH = Relative Humidity (%). Scores of THI were as follow: <22.2 = absence of heat stress; 22.2 to <23.3 = moderate heat stress; 23.3 to <25.6 = severe heat stress and more = extreme severe heat stress (Marai *et al.*, 2001)

#### Experimental procedure:

The experiments were conducted in August during summer season. Ambient temperature (°C) and relative humidity (%) were recorded simultaneously, using mercury centigrade thermometer and hygrometer.

Rectal temperature (RT°C) was measured by digital thermometer, respiration rate (RR b.p.m [bpm: The number of both inhalation and exhalation (breathing) per minute]) was determined and skin temperature (ST°C) was measured by applying a thermometer to cleaned shaved regions in the right and left hips of the animal at 12pm and 3pm in the two experimental groups. Blood was collected from the jugular vein. Two samples were taken per animal, the first sample was collected into vials containing (EDTA) as an anticoagulant to determine the hematological parameters (packed cell volume (PCV %) and Hemoglobin (Hb g/dl). The second blood samples were centrifuged at 3500 rpm for 20 minutes to obtain serum, serum samples were stored at -20°C until assayed. The following serum constituents were spectrophotometrically determined;

the glucose level (mg/dl) was immediately determined according to Trinder (1969), serum total proteins (TP (g/dl)) according to Henry (1974) and serum total cholesterol (mg/dl) was according to Richmond (1973). Meanwhile, serum micro-elements (Ca (mg/dl) and Mg (g/dl)) were measured by atomic absorption spectrophotometer. The amino acids were extracted by acid hydrolysis and determined by the method of Spackman *et al.* (1958) using a Beckman 119 CL amino acid analyzer. Data were computed automatically (Cavins and Friedman, 1968). Amino acids were determined at 3pm from three animals in each group (after exposure to heat stress).

#### Veterinary care:

Throughout the experimental period, the animals were regularly examined and proved to be free from internal and external parasites and any pathogenic diseases.

#### Statistics analysis:

The data obtained from the physiological and blood parameters were analyzed using the generalized linear model of SAS (2004). Duncan's multiple range test was used to determine the significant difference between treatment and control in different time (Duncan, 1955, Snedecor and Cochran, 1989).

### 3. Results and Discussion

The temperature and relative humidity during exposure time (from 12 pm to 3pm) were 31.4 to 32.8°C and 62 to 52% respectively. According to equation of Marai *et al.*, 2001, temperature-humidity index (THI) from 12 pm up to 3pm was 29.4 & 30.1 respectively. Therefore, the rams were under extreme heat stress during the study. Fatigue of animal is possible with prolonged exposure and continuing activity that could result in heat cramps.

In the current work, we demonstrated that tyrosine is one of the advanced treatments in management strategies as it alleviates the effects of heat stress on the performance of the animal during the hotter seasons. However, the negative effects of heat stress will become more apparent in the future (Nardone, *et al.*, 2010). Hyperthermia during exposure to heat stress in semi-arid is the result of increased humidity and elevated ambient temperature which decreasing evaporation cooling mechanism.

As in table (1), there was a highly significant decrease in rectal temperature (RT) at the 3pm (39.28°C) than in control group (39.87°C). The increase in rectal temperature indicated that heat gain was exceeding heat loss. This result agreed with (Silanikove, 2000). At the same time, the heat load, when calculated according to (NRC, 1981), was equal to the margin between skin and rectal temperature, therefore the heat load in treated group

was decreased than in control group (0.8 vs 1.8) respectively. The respiration rate (RR) and RT<sup>0C</sup> have been shown to be good indicators of the thermal stress and may be used to assess the adversity of the thermal environment. There was a highly significant decrease in RR in L-tyrosine groups (77.4bpm vs control group 2002.2bpm). Skin temperature showed a highly significant increase in control group at 3pm (39.92°C±0.668) and a further increase in heat load when exposed to heat stress (14.5%) than l-tyrosine supplemented group (37.66°C±0.293), the increase in heat load was about (7.6%). Therefore, the rams in the tyrosine treated group (T) became more comfortable and less heat-stressed. The animal has a variety of temperature sensors at various locations in the body such as skin, mucous surfaces of the buccal cavity, and in regions of the spinal cord to regulate body temperature (Bligh, 1998), sensors rely

information to the hypothalamus which then initiates mechanisms to either increase or decrease heat loss or production. The exposure to heat stress causes warming of the hypothalamus immediately to initiate heat-losing mechanisms (Robinson, 2002), thus it was shown that there is an increase in ST<sup>0C</sup>, RT<sup>0C</sup>, and RR in heat-stressed animals. There are few studies that demonstrate how tyrosine decreases the thermoregulation parameters. Hsegawa, *et al.*, 2000 mentioned that dopamine has a well-documented role in heat loss mechanisms in the preoptic anterior hypothalamus area, which is the main thermoregulatory center in the brain. Also, Boulant, 2000 used drugs which augment dopamine metabolism in the preoptic area and have a vasodilator effect, these caused a fall in core and brain temperature.

**Table (1):** The Thermoregulation Parameters between treatments in different periods

Treatment	Period	Thermoregulation Parameters					
		RT <sup>0C</sup>		RRbpm		ST <sup>0C</sup>	
		Mean	+SE	Mean	+SE	Mean	+SE
Control group(C) Exposure to Sun)	12pm	39.08	0.107 <sup>b</sup>	54.40	1.600 <sup>b</sup>	34.86	0.199 <sup>c</sup>
	3pm	39.78	0.066 <sup>a</sup>	202.20	16.889 <sup>a</sup>	39.92	0.668 <sup>a</sup>
Treated group(T) treated with Tyrosine)	12pm	39.20	0.114 <sup>b</sup>	54.00	1.673 <sup>b</sup>	35.00	0.530 <sup>c</sup>
	3pm	39.28	0.193 <sup>b</sup>	77.40	3.586 <sup>b</sup>	37.66	0.293 <sup>b</sup>
S.O.V.		**		**		**	

RT: Rectal temperature RR: Respiration Rate bpm: breaths per minute ST: Skin temperature  
SE: standard Error. Means with the same letter in the same column are not significantly different  
\* Significant at  $P < 0.05$ . \*\*Highly Significant at  $P < 0.01$ . n = No significant at  $P > 0.05$

**Table (2):** The mean values of hematological and biochemical Parameters between treatment in different periods

Item	Control group(C) Exposure to Sun)				Treated group(T) treated with Tyrosine)				S.O.V.
	12pm		3pm		12pm		3pm		
	Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE	
Hb (g/dl)	7.86	0.481 <sup>b</sup>	16.22	1.890 <sup>a</sup>	7.45	0.356 <sup>b</sup>	14.80	0.800 <sup>a</sup>	**
PCV (%)	34.40	1.122 <sup>a</sup>	35.60	1.400 <sup>a</sup>	34.40	0.400 <sup>a</sup>	35.00	0.707 <sup>a</sup>	n
Glucose (mg/dl)	52.28	1.327 <sup>b</sup>	60.57	0.902 <sup>a</sup>	50.96	0.766 <sup>b</sup>	51.84	0.877 <sup>b</sup>	**
Total protein (g/dl)	5.91	0.232 <sup>bc</sup>	5.57	0.205 <sup>c</sup>	6.27	0.085 <sup>b</sup>	6.96	0.169 <sup>a</sup>	**
Total cholesterol (mg/dl)	67.74	1.428 <sup>b</sup>	71.71	0.573 <sup>a</sup>	67.40	1.111 <sup>b</sup>	64.50	1.588 <sup>c</sup>	**
Mg (g/dl)	3.73	0.264 <sup>ab</sup>	2.87	0.219 <sup>b</sup>	3.39	0.467 <sup>ab</sup>	4.73	0.696 <sup>a</sup>	*
Ca (mg/dl)	7.20	0.490 <sup>b</sup>	8.00	0.632 <sup>b</sup>	7.60	0.400 <sup>b</sup>	9.60	0.400 <sup>a</sup>	*

SE: standard Error. Means with the same letter in the same row are not significantly different TRT: between treatments \* Significant at  $P < 0.05$ . \*\*Highly Significant at  $P < 0.01$ . n = No significant at  $P > 0.05$  Ty: treated with Tyrosine

Bernabucci *et al.* (2002) & (2010) reported that the exposure to heat stress cause change in some of the biochemical parameter. The authors illustrated the role of l-tyrosine supplementation in small dose 100mg/kg as anti-stress treatment. In treated group, the hemoglobin concentration was numerical decrease (14.80mg/dl) but statistically non-significant at 3pm. While in heat stress group, hemoglobin Hb concentration increased at 3pm (16.22mg/dl) due to affect with heat stress. This result was confirmed by

Al-Haidary (2000), therefore the increase in Hb concentration could be part of a general stress response (Marai *et al.*, 2007), by taking tyrosine supplement would decline the Hb concentration (14.80±0.080 mg/dl vs 16.22±1.89mg/dl). The exposure to heat stresses in short period had no effect on packed cell volume (PCV). This result agreed with (El-Nouty *et al.*, 1990) Table 2.

The level of serum glucose in treated group was within the normal value (51.84mg/dl) at 3pm.

therefore, the Barki ram that were given tyrosine and exposed to heat stress had no changes in energy metabolism. Nonetheless, the glucose concentration in control group was significantly increased at 3pm (60.57 mg/dl). During hot climate, the change in glucose level was related in part to the decrease in concentration of insulin which is correlated closely to the decrease in energy metabolism. The hyperglycemia may be a secondary effect of the hypercortisolamia and/or may be due to increased production of glucose from the liver (Thompson, 1973) as Table (2).

Total protein concentration was significantly declined in control group at 3pm 5.57g/dl, than treated group 6.96g/dl. When the body temperature increased, the protein synthesis was degraded and also be altered (Horowitz and Kodesh, 2010). Moreover, the decrease in total protein concentration resulted by providing an efficient way of transferring the heat from inside the body to the outer surface in the skin for heat dissipation by non-evaporation process, since it holds an adequate percentage of water in the intra-vascular fluids to maintain the vascular fluids and viscosity of the blood (Kamal *et al.*, 1962) as in Table 2.

In Table (2), the tyrosine group (T), there was maintained level of the cholesterol concentration when exposed to heat stress (64.50mg/dl), at the same time; there was a significant increase in cholesterol concentration at 3pm (71.71mg/dl) in control

group caused by increased lipolysis in the blood due to exposure to heat stress. Bansal and Jaswal, 2009 mentioned that total cholesterol serum is really an indicator of the amount of the free radical damage in the body, therefore; tyrosine may be used as antioxidant dietary supplement.

At 3pm, plasma magnesium was significantly increased (4.73g/dl) in the tyrosine group than in the control group (2.87g/dl). Mg is an indicator of the stress response in different avian and mammalian species. Some magnesium salts can ameliorate stress (Ali *et al.*, 2001; Horowitz and Kodesh, 2010). Henrotte, 1986 mentioned that in humans deficiency of Mg enhances catecholamine secretion and sensitivity to heat stress. Furthermore, increased catecholamine causes intracellular Mg depletion and eventually increases urinary losses of Mg. These results were in an agreement with our results in the present report, in our control group there was a significant decrease in Mg in plasma but, the tyrosine group showed a significant increase as in Table (2). Mg also has an antagonistic effect on calcium release from the sarcoplasmic reticulum of skeletal muscles, therefore, may be reducing the neuromuscular contractile stimulation (Classen, 1987 and Murck, 2002) so there was a clear relation between Mg and Ca. However, in the control group there was a decrease in both Mg and Ca as in l-tyrosine supplementation group.

**Table 3:** Effects of heat stress and l-tyrosine on plasma amino acid concentrations in Barki ram.

Amino acids types	Heat stress group		Tyrosine group		s.o.v
	Mean	±SE	Mean	±SE	
Lysine (μmol/L)	3.12	0.017 <sup>b</sup>	3.90	0.029 <sup>a</sup>	*
Arginine (μmol/L)	2.51	0.017 <sup>b</sup>	3.79	0.029 <sup>a</sup>	**
Histidine (μmol/L)	1.60	0.017 <sup>b</sup>	2.23	0.029 <sup>a</sup>	*
Isoleucine (μmol/L)	1.51	0.017 <sup>b</sup>	2.30	0.029 <sup>a</sup>	*
Leucine (μmol/L)	2.32	0.017 <sup>b</sup>	3.47	0.029 <sup>a</sup>	**
Valine (μmol/L)	2.04	0.017 <sup>b</sup>	2.43	0.029 <sup>a</sup>	*
methionine (μmol/L)	0.40	0.017 <sup>a</sup>	0.69	0.029 <sup>a</sup>	n
Phenylalanine (μmol/L)	2.31	0.017 <sup>a</sup>	2.46	0.029 <sup>a</sup>	n
Tyrosine (μmol/L)	1.43	0.017 <sup>b</sup>	1.82	0.029 <sup>a</sup>	*
Alanine (μmol/L)	2.66	0.017 <sup>a</sup>	2.68	0.029 <sup>a</sup>	**
Aspartic acid (μmol/L)	3.27	0.029 <sup>b</sup>	4.07	0.017 <sup>a</sup>	*
Glutamic acid (μmol/L)	3.51	0.023 <sup>b</sup>	3.28	0.029 <sup>a</sup>	*
Serine (μmol/L)	2.18	0.023 <sup>b</sup>	2.62	0.023 <sup>a</sup>	**
Glycine (μmol/L)	1.19	0.017 <sup>b</sup>	2.21	0.029 <sup>a</sup>	**
Proline (μmol/L)	1.75	0.017 <sup>b</sup>	2.21	0.029 <sup>a</sup>	*
Therionine (μmol/L)	2.41	0.017 <sup>b</sup>	3.10	0.029 <sup>a</sup>	*
Total Amino Acid (μmol/L)	34.21		43.25		

SE: standard Error. Means with the same letter in the same row are not significantly different

\* Significant at  $P \leq 0.05$ . \*\* Highly Significant at  $P \leq 0.01$ . n = No significant at  $P > 0.05$

As in Table 3, plasma concentrations of several individual amino acids (AAs) were modified by dietary tyrosine (T group) with a significant increase in some essential amino acids (EAAs) which were polar and positive charged as lysine which is a necessary building block for all proteins, Lysine also plays role in calcium absorption; Arginine enhances the immune system. Non-polar aliphatic branches chain amino acids (BCAAs) as Isoleucine; Leucine and valine could increase protein synthesis rate in ruminant (Schaefer *et al.*, 1986). Otherwise no change in plasma sulfur amino acids (SAAs) as methionine was seen in the tyrosine-treated group. Aromatic non polar Phenylalanine amino acids concentration in plasma was not changed in the tyrosine-treated group, it may be due to phenylalanine that has been converted to tyrosine in the treated group. However, nonessential Tyrosine was significantly increased in the plasma of the treated animals (1.82  $\mu\text{mol/L}$ ) than control group (1.43  $\mu\text{mol/L}$ ). Alanine, Aspartic acid, Glutamic acid, Serine, Glycine and Proline were increased. In the present study exposure of rams to heat stress decreased the concentration of plasma amino acids in particular EAA, BCAA and AAA, These findings substantiate previous evidence that BCAA, threonine, Lysine and glutamine concentrations were decreased in chickens exposed to heat stress at 4 weeks of age (Geraert *et al.*, 1996b).

Pretreating of animals with tyrosine prior to stress caused NE norepinephrine feedback inhibition ACTH by suppressing hypothalamic corticotrophin – releasing factor. Reinstein *et al.*, (1985) recorded that increased dietary tyrosine prevented behavioral depression and suppressed the rise in corticosterones in stressed rats.

#### General conclusion:

In this study we showed that treatment of rams with tyrosine prior to exposure to heat stress for three hours, can alleviate the adverse effect of heat stress. The study agrees with the findings of Tumilty *et al.*, 2011, who reported that supplementary tyrosine maintains dopamine metabolism in the hypothalamus thus reducing the rate of increase of RT in human. The present study also clearly demonstrated that 100mg/kg of L-Tyrosine decreased the  $RT^{0C}$ , skin temperature ( $ST^{0C}$ ) and respiration rate (RR). At same time, tyrosine improved tolerance to heat stress by increasing the levels of Mg, total protein concentration and amino acids, the necessary building block for all proteins and also increased the energy content in muscles.

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