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ASCORBIC ACID ADMINISTRATION AS ANTI-STRESS BEFORE TRANSPORTATION OF SHEEP

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SUMMARY

Currently in Egypt there is no regulation ruling over transportation of animals. The aims of the current study were to investigate the effects of ascorbic acid administration (125 mg/kg) before transportation in an open truck during summer season on live weight loss, and some physiological responses, in New Valley governorate, Egypt. Fourteen Farafra male sheep, 35.0 ± 3.86 kg body weight and aged 11-12 months were used in this study. Animals were divided into two equal groups (7/each group) as not-administered and administered ascorbic acid groups. Animals were transported in an open truck covering a distance of approximately 225 km for three hours. Before loading and after transportation, rectal temperature, respiration rate and pulse rate as thermal responses were recorded. In addition, two blood samples were collected before and after transportation from each animal. Complete blood samples were investigated to determine hematological parameters. Also, blood serum samples were analyzed for hormones (T3, T4 and cortisol) and some blood metabolites (total protein, albumin, globulin, sodium, potassium, chloride, urea, ALT, AST, creatinine, glucose, triglycerides and total cholesterol). The results indicated that transportation caused adverse changes as a results of stress in live body weight losses, hematological parameters and some blood metabolites. Ascorbic acid administration lowered effect of stress caused by transportation through modulating physiological responses. Therefore, administration of ascorbic acid in sheep before transport especially at high ambient temperatures can be recommended.

Keywords: Ascorbic Acid, sheep, transport, physiological responses, stress

INTRODUCTION

Transportation of livestock in open trucks between farms, markets and slaughterhouses is a routine practice in Egypt. Transportation of animals is generally recognized as a stressful event (Dantzer and Mormede, 1983, Kadim *et al.*, 2007, Minka and Ayo, 2011). In addition, adverse climatic conditions such as temperatures fluctuations and relative humidity are also additional stressors in animals during transportation. Haematological parameters have been demonstrated to be important indices of health, production and adaptability to prevailing environmental conditions in livestock (Adenkola *et al.*, 2009) and also as an indicator of stress in livestock (Adenkola and Durotoye, 2004 and Togun and Oseni, 2005). It has been established that transportation stress has an effect on leucocytosis, with associated lymphopenia, neutrophilia, and eosinopenia (Minka and Ayo, 2007 and Minka and Ayo, 2008). In addition, neutrophil/lymphocyte (N/L) ratio has been used as trustworthy indices in evaluating the immune response and adaptability of animals to various stress conditions (Nwe *et al.,* 1996 and Stanger *et al.,* 2005). Generally, majorities of sheep in tropical regions are reared under extensive management systems and are difficult to handle, and this may increase the level of stress encountered by sheep during rounding up, handling, and loading.

The current management strategies towards alleviation of road transportation stress in animals included the use of analgesic, neuroleptics, electrolytes, or supplementing one or more dietary elements, before, during, or after transportation. Some of these agents are difficult to obtain and apply by the farmers; others are counterproductive, with great consequential effects on both animals and humans, while some have no effect and lack consistency and efficacy (Schaefer *et al.*, 1997; Ali and Al-Qarawi, 2002; Ali *et al.*, 2006 and Ferguson and Warner, 2008). The identification of an additional agent that may be cheaper, nontoxic, with no withdrawal time, and easily to administer would be of value in the field of animal transport.

Ascorbic acid (vitamin C) is known as antioxidant agent and has been widely used to manage stress conditions in animals. (Pardue *et al.*, 1985) reported that under specific environment and physiological conditions, the amount of ascorbic acid produced by the animal may be insufficient to meet its requirement. Under heat stress, free radicals are generated in the body in such a large quantity that the natural antioxidant defense systems of the body are overwhelmed. Therefore, antioxidant supplementation may provide beneficial effect against stress induced tissue damage (Sen, 2001). There is a reasonable body of evidence supporting the use of ascorbic acid in reducing different kind of stresses in animals (Ayo *et al.,* 2006; Ali and Al-Qarawi, 2002 and Powers and Jackson, 2008).

A little work has been carried out to use or investigate factors that alleviating effects of stress in transported sheep under Egyptian conditions. The aim of the current study was to determine the beneficial effect of administration of ascorbic acid as an alleviation suggested factor against road transportation stress in Egyptian sheep.

MATERIALS AND METHODS

This experiment was carried out in the new valley governorate during summer season. New Valley governorate in Upper Egypt in western desert between 25º; 42& 30º; 47 E longitude, 22º 30& 29º 30N latitude and lies 77.8m altitude above the sea level. The climate of this area is arid and dry, essentially that of the desert. Rainfall is almost negligible and the ambient temperature ranges from 46°C during summer days to 8 °C in the chilly winter nights.

Animals and management:

Fourteen Farafra (local breed in the new valley) male healthy sheep weighting 35.0±3.86 kg and aged 11-12 months were used in this study. The sheep were living in a standard sheep pen at a stocking rate of 1.25 m2/ sheep. The sheep were not tied inside the pen. The sheep were herded out and grazed daily on a sown pasture from 08:00 a.m to 16:00 p.m. Animals was given access to drinking water ad-libitum. The sown pasture contains trees to provide shade against sunlight.

Experimental design:

On transportation day, the sheep were numbered, and body weight, rectal temperature, respiration and pulse rate as thermal responses were recorded. Rectal temperature was recorded using a clinical thermometer (Dong E Digital thermometer, Model No: BT-AIICN; LOT NO: 0911 Mad). Respiration rate was recorded with the help of flank movement. Pulse rate was recorded from the coccygeal artery with the help of a stethoscope. Two blood samples before and after transportation from each animal were collected. Thereafter, the sheep were divided into group I oral administered with 125 mg/kg with ascorbic acid and group II non- administered with ascorbic acid (ADWIC, Pharmaceutical Chemicals Co., Egypt). This dose was slightly higher than that (100 mg/kg) given by Saidu *et al.* (2012). The dose was dissolved in 10 mL of sterile water. At the same time, group II were only administered 10 mL of sterile water orally. Immediately after transportation, the previous thermal responses (rectal temperature, respiration and pulse rate) were recorded and the second blood samples were taken. The obtained both blood samples were analyzed for hematological parameters and some blood metabolites.

The handling, loading, and transportation of sheep were performed humanely in accordance with the guidelines governing animal transport welfare by road (Farm Animal Welfare Council, 2003). The sheep were stocked at a rate of 0.27 m^2 per animal inside the vehicle. All the handling and loading of animals were conducted between 11:0 a.m. to 12:0 noon. The floor of the truck was provided with beddings (wood shavings) covered with rubber mats for secured footing. The journey started by 12:00 a.m. and was terminated at 15:00 p.m. The vehicle travelled for 3.00 h. for about 225 km.

Recording air temperature and humidity:

Air temperature (°C) and humidity (%) were recorded during the study using Lutron HA-701 DM-6016 Multimeter (Taiwan), and hygrometer (GH Zeal Ltdature 8 Deer Park Road Merton London SW19 3UU UK), respectively. Data are presented in Table (1).

Table 1. Air temperature and humidity recoded before and after transportation.

Items	Air temperature (°C)	Humidity (%)
Inside the barn	35.0	39.5
Before transportation	44.0	30.4
After transportation	42.0	18.1

Blood sampling analysis:

About 10 mL of blood via jugular venipuncture was collected from each sheep at each period of blood sampling and decanted into two sterile test, one tube with 0.14% anticoagulant (EDTA K3, Pty Ltd., Adelaide, SA, Australia) for determination hematological parameters, and the other one without anticoagulant for obtaining serum. The collected blood samples were quickly kept in ice pack and sent to the laboratory. Whole blood samples were analyzed for complete blood analysis, while serum samples were obtained by centrifugation of blood samples for 10 minutes at 3,000 r.p.m, then dispensed into 1.5 ml Eppendorf tubes and stored at -20 °C for blood metabolites analysis.

Complete blood analysis were determined using sysmex xp-300 (Japan). Serum samples of all the animals were assayed for cortisol, free triiodothyronine (T3) and free thyroxine (T4) concentrations using radioimmunoassay (RIA) Technique. Total protein, albumin, sodium, potassium, chloride, urea, AST, ALT, glucose, creatinine, cholesterol and triglycerides concentrations were determined using appropriate commercial test kits. The concentrations were measured using standard protocols (Photometer 5010 v5+).

Statistical analysis:

Data were analyzed using General Linear Model (GLM) procedure of SAS (SAS, 1998) according to the following model:

$Yij = \mu + Ti + eij$

Where: μ = Mean, Ti = Effect of treatment and eij= Standard error

Duncan's multiple range test (1955) was used to compare between means of the control and treated groups.

RESULTS

Body weight loss:

After 3-h transportation, live body weight losses were -1.22±0.18 and -0.47±0.20 in the two groups. Ascorbic acid administration alleviated live weight losses from 3.52 % to 1.33 % in without ascorbic acid and with ascorbic acid groups, respectively but, the differences between the two groups were not significant (Table 2).

Thermal responses:

Rectal temperature and respiration rate were increased significantly (P< 0.05), while pulse rate was increased non-significantly as a results of transportation (Table 3).

Hematological parameters:

After the 3-h. transportation, complete blood parameters were changed in the two groups but ascorbic acid administration led to increase red and white blood cells compared to other group (Table 4). Moreover, neutrophils / lymphocyte ratio was non-significantly decreased upon ascorbic acid administration.

Blood metabolites:

Animals after transportation had significantly (P<0.05) or not significantly increases in the concentrations of cortisol, T3, total protein, potassium, chloride, globulin, urea, creatinine. At the same time Animals after transportation had significantly (P< 0.05) or not significantly decreases on the concentrations of T4, AST, ALT, glucose, triglycerides and cholesterol concentrations than before transportation in both groups (Table 5). Generally, the data indicated that values of blood metabolites in ascorbic acid administration group were lower than other groups Thus, ascorbic acid administration alleviated stress effects of transported sheep through changes in serum metabolites, hormones concentrations.

Table 2. Effect of ascorbic acid administration (mean±SEM) on live body weight loss of sheep

Items	Without ascorbic		With Ascorbic	
	Before	After	Before	After
No. of animals	7	7	7	7
Body weight, Kg	34.68 ± 4.75	33.83 ± 4.72	35.37 ± 3.02	34.16 ± 3.05
Body weight loss, Kg	-	1.22 ± 0.18	-	0.47 ± 0.20

Table 3. Effect of ascorbic acid administration (mean±SEM) on thermal responses of sheep

Items	Before transportation	After transportation	
		Without ascorbic	With ascorbic
No. of animals	14	7	7
Rectal temperature, °C	39.3 ± 0.32 b	39.7 ± 0.16 a	39.4 ± 0.28 b
Respiration rate/ min	74.2 ± 4.04 b	96.5 ± 10.7 a	84.1 ± 15.4 ab
Pulse rate/ min	85.2 ± 12.2	91.0 ± 15.9	89.5 ± 12.1

a,b: Values with the different superscripts on the same column differ at P<0.05

Table 4. Effect of ascorbic acid administration (mean±SEM) on hematological parameters of sheep

Items	Before transportation	After transportation	
		With Ascorbic	Without Ascorbic
No. of animals	14	7	7
Red blood cells, 10^6/µl	9.88 ± 2.44	10.62 ± 3.61	9.18 ± 3.28
Hematocrit (PCV) %	9.2 ± 4.5	10.38 ± 4.66	8.57 ± 3.22
Hemoglobin (g/dl)	8.8 ± 2.44	10.0 ± 0.61	9.27 ± 0.38
Mean cell volume (MCV)	37.0 ± 1.22	37.83 ± 4.11	37.0 ± 1.41
Mean cell volume (MCH)	40.4 ± 16.81	41.33 ± 14.22	45.0 ± 17.66
Mean corpuscular hemoglobin concentration, MCHC g/dl	109.8 ± 49.19	112.66 ± 46.31	122.25 ± 52.74
White blood cells, 10^3/µl	9.58 ± 2.82b	12.48 ± 1.91a	13.62 ± 0.70a
Neutrophils (%)	38.6 ± 4.61	39.66 ± 4.62	42.35 ± 9.90
Lymphocyte (%)	56.0 ± 4.74	54.5 ± 4.56	53.65 ± 5.55
Neutrophils / lymphocyte ratio	0.698 ± 0.14	0.73 ± 0.13	0.80 ± 0.23
Monocyte (%)	3.4 ± 0.54	4.0 ± 2.19	2.25 ± 0.5
Eosinophil (%)	1.8 ± 0.44	1.83 ± 0.40	1.75 ± 0.5
Basophil (%)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

a,b: Values with the different superscripts on the same column differ at P<0.05

Table 5. Effect of ascorbic acid administration (mean±SEM) on some blood metabolites of sheep

Items	Before transportation	After transportation	
		With ascorbic	Without ascorbic
No. of animals	14	7	7
Cortisol, µg/dl	13.28 ± 1.10 b	14.11 ± 1.42 b	16.50 ± 1.62 a
T3, µg/dl	1.46 ± 0.35	1.56 ± 0.33	1.81 ± 0.47
T4, µg/dl	4.81 ±1.09 a	3.46 ± 1.18 b	4.30 ± 0.98 ab
Total protein, g/dl	6.7 ± 0.95	6.83 ± 0.53	6.87 ± 0.31
Albumin, g/dl	3.1 ± 0.14	3.14 ± 0.14	3.19 ± 0.15
Globulin, g/dl	3.61 ± 1.00	3.69 ± 0.52	3.66 ± 0.43
Sodium, mmol/l	133.87 ± 5.02	131.65 ± 7.34	132.65 ± 6.70
Potassium, mmol/l	4.85 ± 0.43	5.50 ± 0.48	5.03 ± 0.52
Chloride, mmol/l	102.37 ± 4.42	102.70 ± 6.59	103.97 ± 5.36
ALT, U/l	49.55 ±7.94 a	34.32 ± 7.22 b	32.21 ± 8.67 b
AST, U/l	155.55 ± 4.25 a	139.5 ± 3.11 b	137.83 ± 3.65 b
Urea, mg/dl	29.73 ± 4.35	30.81 ± 5.71	30.10 ± 6.52
Creatinine, mg/dl	0.90 ± 0.047 b	0.93 ± 0.018 b	1.09 ± 0.133 a
Glucose, mg/dl	79.25 ± 5.21 b	68.65 ± 6.38 ab	65.30 ± 4.53 a
Cholesterol, mg/dl	73.79 ± 7.22	72.38 ± 6.70	72.07 ± 5.78
Triglyceride, mg/dl	47.06 ± 8.51 a	37.96 ± 9.33 b	31.97 ± 4.06 b

a,b: Values with the different superscripts on the same column differ at P<0.05

DISCUSSION

After the 3-h. transportation, body weight loss and hematological blood parameters and blood metabolites, values were changed. Generally, ascorbic acid administration alleviated stress of transported sheep. Antioxidants such as ascorbic acid are free radical scavengers which protect the body defense system against excessively produced free radicals and stabilize health status of the animal. Ascorbic acid is a water soluble, extra-cellular, natural antioxidant and is involved in a number of oxidation and reduction reactions in the body (Sen, 2001 and Aya *et al.,* 2006).

After the 3-h transportation, live weight loss was observed in the two groups but ascorbic acid alleviated live weight loss. Loss of live weight during transportation is most probably due to loss of water (dehydration) and deprivation of feed. High temperatures (42-43±1°C) during transportation might likely cause weight loss through loss of moisture from the respiratory tract. Warriss (1993) reported that animals can lose weight when they are subjected to greater energy demands, such as those needed to maintain balance or to thermoregulate in transport. Thermoregulation may involve greater loss of body water through sweating or panting. Average live weight losses in sheep ranged between 0.09 to 0.34% per hour (Thompson *et al.,* 1987, and Warriss *et al.,* 1987). In this study, live weight loss was greater than the previous studies which might be due to high ambient temperature.

Rectal temperature and respiration and pulse rates are recognized as important measures of physiological status (Lefcourt *et al.,* 1986) as well as ideal indicators for assessment of stress in animals. The decreased rectal temperature and respiratory rates in ascorbic acid supplemented group compared with non-supplemented one indicated that supplementation of ascorbic acid ameliorated the heat stress in sheep. Ascorbic acid directly alters thermal set point by decreasing prostaglandin output, whose turnover increases during stress (Hadden *et al.,* 1987) and which has a direct effect on the hypothalamic thermoregulatory zone (Ganong, 2001). Therefore, by affecting the prostaglandin output, this vitamin may have an ameliorating effect upon heat stressed animals.

After the 3-h transportation, values of hematological parameters were changed in the two groups but ascorbic acid group had highest red and white blood cells (Table 4) as a results of ascorbic acid administration. The increase in RBC count in the present study may be due to changes in erythrocyte osmotic fragility upon ascorbic acid administration. The result of Adenkola *et al.* (2009) and Adenkola *et al.* (2010) indicated that ascorbic acid protect membrane integrity of erythrocyte of livestock during stress. Consequently, exposure of sheep to heat stress (summer season) in this study resulted in an increase in packed cell volume (PCV). WBC counts after transportation was increase still within the normal range values in both groups. The change in WBC counts may be as a result of the release of Glucocorticoids during transportation, which is responsible for the trafficking and release of WBC from the bone marrow (Stanger *et al.,* 2005, and Urban-Chmiel *et al.,* 2009). Insignificant change in neutrophil/lymphocyte ratio obtained in the present study as a results of administered ascorbic acid after 3-h transportation showed that ascorbic acid ameliorated the stresses induced by transportation in sheep. Ascorbic acid’s inhibitory role on cortisol. Also, ascorbic acid has been reported to be a chain-breaking antioxidant, involved in the prevention and restriction of free radical chain formation and propagation, consequently, protecting blood cells, including neutrophils and lymphocytes from oxidative damage (Powers and Jackson, 2008 and Urban-Chmiel *et al.,* 2009).

In the present study, transportation led to increased cortisol, T3, total protein, globulin, urea, creatinine, and lower T4, AST, ALT, triglycerides and cholesterol concentrations than before transportation in both groups. Three hours transportation decreased plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in sheep, but the decreases the values these enzymes are within the normal values (Duncan and Prasse, 1994). The decreases in these enzymes suggest that there is no liver damage but rather a slowdown of the function of the liver when the animals were subjected to transportation.

The increase of urea value found following 3-h. transportation in the present study might be due to feed deprivation and elevated cortisol concentration as established by several authors (Odore *et al.,* 2004), and also as a result of increased protein degradation caused by hypoglycaemia. It has been shown that heat stress induces ascorbic acid depletion in the adrenal glands, and this is associated with corticosterone release (Sahota and Gillani, 1995).

The present results indicated that glucose concentrations values in the blood of sheep decrease as a results of transportation Sauberhich (1994) reported that glucose concentrations in animals subjected to stressful conditions, especially during road transportation recorded lower values.

Our results indicated that total cholesterol and triglycerides values were decreased after 3-h. transportation. In other studies, percentage reduction changes of total cholesterol and triglycerides upon vitamin C supplementation was 4.5 and 8.8%, respectively (McRae, 2007 and 2008). In this study, percentage reduction of total cholesterol was 3.26 and 13.17% and triglycerides was 11.8 and 15.63% of vitamin C supplemented and non-supplemented groups, respectively. It has been found that vitamin C supplementation led to lowers serum low-density lipoprotein cholesterol and triglycerides (McRae, 2008). This means that body fat reserves are mobilized to provide energy during heat stress.

Non-significance differences in the values of sodium, potassium and chloride ions post 3-h transportation in ascorbic acid supplemented and non-supplemented sheep indicated that ascorbic acid administration as well as 3-h road transportation did not affect these important ions in sheep.

Generally, ascorbic acid administration alleviated stress effects of transported sheep through changes serum metabolites, values to normal values recorded before transportation

CONCLUSION

The results of the current study revealed that valuable effects of ascorbic acid administration on some physiological responses such as thermal and blood paramaters of sheep transported by road during the hot season. Therefore, ascorbic acid administration as anti-stress before transportation specially under hot season of sheep can be recommended.

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تجريع الأغنام بفيتامين ج المضاد للإجهاد قبل النقل

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لا يوجد فى مصر حاليا قواعد منظمة لنقل الحيوانات. الهدف من هذه الدراسة هو بحث تاثير تجريع أغنام الفرافرة باستخدام فيتامين ج (125مجم/كجم) قبل النقل بواسطة عربة نقل (شاحنة مفتوحة) خلال فصل الصيف على الفقد فى الوزن وكذلك بعض الاستجابات الفسيولوجية فى محافظة الوادى الجديد- مصر. استخدم فى هذه الدراسة اربعة عشر ذكر اغنام الفرافرة (سلالة أغنام محلية) تتراوح أعمارها من 11-12 شهر وكان متوسط اوزانها35.00 ± 3.86 كجم. قسمت هذه الحيوانات الى مجموعتين متساويتين تشتمل كل مجموعة على 7 ذكور. حيوانات المجموعة الاولى تم تجريعها باستخدام فيتامين ج بينما حيوانات المجموعة الثانية لم يتم تجريعها. تم تحميل الحيوانات فى عربة النقل المكشوفة واستغرقت مدة رحلة النقل 3 ساعات تم خلالها قطع مسافة 225كم. وقبل تحميل الحيوانات على عربة النقل وبعد النقل مباشرة تم قياس درجة حرارة الجسم, ومعدل التنفس, ومعدل النبض فى كل الحيوانات وكذلك تم اخذ عينتين من الدم قبل وبعد النقل لكل حيوان قدر فيها بعض مكونات الدم الخلوية كما تم تقدير بعض مكونات الدم الاخرى ,من هرمونات (الكورتيزون-الثيروكسين-الترای ايودوثيرونين), وكذلك محتويات السيرم من البروتين الكلى ,الالبيومين, الجلوبيولين,الصوديوم, البوتاسيوم, الكلوريد, اليوريا , الكرياتينين, وAST , ALT و الجلوكوز , والتراى جلسريد, الكلوسترول الكلى.

اظهرت النتائج ان النقل يتسبب فى تغيرات سلبية فى وزن الجسم ومكونات الدم الخلوية وكذلك بعض مكونات الدم كنتيجة للاجهاد. كما اشارت النتائج الى ان التجريع باستخدام فيتامين سى يقلل من تاثير الاجهاد عن طريق التحسن فى بعض الاستجابات الفسيولوجية. وبناء على ذلك يمكن التوصية بتجريع الاغنام بفيتامين سى قبل النقل وخصوصا عند ارتفاع درجات الحرارة.