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THE EFFECT OF GIVING ASCORBIC ACID ON SOME PHYSIOLOGICAL AND HEMATOLOGICAL PARAMETERS OF SUCKLING LAMBS EXPOSED TO SOLAR RADIATION AND EXERCISE

(With 4 Tables and 9 Figures)

By

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تأثيراضافة حمض الأسكوربيك على بعض المقاييس الفسيولوجيه وبعض مقاييس الدم فى الحملان الرضيعة عند تعرضها لأشعة الشمس والتمارين

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شملت الدراسه عدد سبعة وأربعون حمل من خليط الكيوس عند عمر أسبوع قسمت الى مجموعتين الأولى ضابطة لم تعطى حمض الأسكوربيك، والثانية أعطيت حمض الاسكوربيك (بواقع ٢ جم لكل حمل كل يومين). عند عمر ٩ أسابيع عرضت الحيوانات لأشعه الشمس المباشره، الرياضه بالمشى ساعه (٤ كم/ساعه) تحت أشعه الشمس. وتم تقدير بعض المقاييس الفسيولوجية وكذلك بعض مقاييس الدم. أدى التعرض لأشعه الشمس والتمارين الى زياده معنويه ($P< 0.01$) فى معدل التنفس ودرجة حراره المستقيم والجلد والصوف فى الحملان. كما أدى حامض الاسكوربيك فى الغذاء الى انخفاض فى معدل التنفس ودرجة حرارة المستقيم. وقد اتضح من البحث أن التعرض لأشعه الشمس والاجهاد التمرينى قلل من تركيز كل من الهيموجلوبين، تعداد كرات الدم الحمراء والبيضاء وكذلك محتوى السيرم من الجلوكوز وهرمون الثيروكسين بينما أدى الى زيادة مكونات السيرم من الألبيومين والكولستيرول وهرمون الكورتيزول. كذلك تسبب اعطاء حامض الأسكوربيك فى الغذاء فى تقليل التغيرات فى المكونات الخلوية للدم والهيموجلوبين ومحتوى السيرم من البروتين الكلى نتيجة التعرض للشمس والاجهاد التمرينى. وأخيرا فان اعطاء حامض الأسكوربيك فى الغذاء أدى الى زيادة هرمون الثيروكسين وقلل من هرمون الكورتيزول فى السيرم. من كل هذه النتائج يتضح ان حامض الأسكوربيك عند الاجهاد الحرارى والاجهاد التمرينى يحسن من مقاييس صورة الدم والمقاييس الفسيولوجية فى الحملان الرضيعة.

SUMMARY

A total number of 47 one week age suckling Chios crosses lambs were assigned to two groups, a control group receiving no ascorbic acid supplement and the other received supplemental ascorbic acid (2 grams per animal every other day). At the age of 9 weeks animals were exposed to direct sunlight and direct sunlight with exercise with walking for one hour (4 km/h) under solar radiation. Some physiological and hematological parameters were measured. Lambs exposed to solar radiation and exercise showed highly significant P(0.01) increase in respiratory rate and temperatures of rectum, skin and wool. Ascorbic acid treated lambs had lower respiratory rate and rectal temperature than control lambs. Exposure to solar radiation and exercise decreased the concentrations of hemoglobin (Hb), packed cell volume (PCV) %, total count of RBC's and WBC's /mm^3blood, serum glucose and thyroxin (T4), while the concentrations of serum albumin, cholesterol and cortisol hormone were increased. Dietary ascorbic acid reduced the changes in PCV, % , Hb and serum total protein due to sun exposure and exercise. Dietary ascorbic acid increased serum T4 concentration and decreased serum cortisol hormone. These results means that dietary ascorbic acid could improve heat tolerance and blood profile in suckling lambs.

Key words: *Suckling lambs - Physiology and Haematology - Ascorbic acid.*

INTRODUCTION

It is generally considered that ruminants can synthesis enough ascorbic acid for normal growth and development, thus no dietary requirement of ascorbic acid has been recommended for the adult ruminants (Itzeova, 1984). However, suckling animals may have a marginal ascorbic acid deficiency (Wegger and Moustgaard, 1982). Dietary ascorbic acid improved the performance and blood profile of suckling buffalo calves (Kobeisy and Abd El-All, 1993). Dietary ascorbic acid improved heat tolerance and blood profile of suckling jersey calves exposed to direct sun light under Upper Egypt condition (kobeisy, 1994). As there is no available data on the effect of ascorbic acid on suckling lambs, this study was carried out to determine this effect on physiological and hematological responses to heat stress.

MATERIALS and METHODS

Forty seven suckling Chios crosses lambs one week age were used where, 3/4 Chios x 1/4 Ossimi (31) and 1/2 Chios x 1/2 Ossimi (16) were classified to two groups, a control group receiving no ascorbic acid supplement and an ascorbic acid treated group received supplemental ascorbic acid (2 grams ascorbic acid per animal every other day). The experiment lasted for 8 weeks, from the first to the ninth week of age,during summer season. Animals were subjected to exposure to solar radiation and exercise during the 8 th and 9 th week of age, respectively. Exposure to solar radiation was for two hours, from 12.00 to 14.00 hours and exercise was by walking for one hour (4 km/h) from 12.00 to 13.00 hours under solar radiation. Average ambient air temperature and relative humidity during sun exposure and exercise were 34.9, 35.4 °C and 47.8, 49.3 %, respectively. Respiratory rate and temperatures of rectum, skin and wool were recorded. Blood samples were taken just before and after exposure to both treatments where blood samples transferred to two dry, clean and sterilized vials one without heparin and the other contained heparin (1 gm/1000 blood). Serum was then separated from the whole blood by centrifugation at 4000 r.p. for 15 min., frozen and stored at -20° C until subsequent analyses. Total red blood cells and total white blood cells (count/mm^3 blood) were determined by using hemocytometer. Hemoglobin (Hb) concentration in g/dl was determined by using kits supplied by Diamond Diagnostics (Egypt). Packed cell volume percent (PCV, %) was measured according to Schalm (1986). Serum total protein was determined using kits supplied by Sclavo diagnostics (Italy). Serum glucose, cholesterol, aspartic aminotransferase (AST) and alanin aminotransferase (ALT) concentrations were determined using test kits supplied by Diamond Diagnostics (Egypt). Serum albumin concentration was determined using test kits supplied by biomerieux (France). Serum thyroxine (T4), triiodothyronine (T3) and cortisol concentrations were determined using a commercial enzyme immunoassay kits supplied by diatech diagnostics incorporation. (USA).

Data were statistically analyzed using general linear model (GLM) procedure of SAS (1982).

RESULTS and DISCUSSION

1- Thermorespiratory responses

Sun exposure and exercise increased ($P< 0.01$) respiratory rate (RR), rectal temperature (RT), skin temperature (ST) and wool temperature (WT) (Table 1 and Figs. 1 to 3). Animals exposed to exercise showed always

higher values of RR and RT than that exposed to solar radiation (Table 1 and Figs. 1 to 3). This effect was mainly attributed to accumulation of heat in the body due to the increase in heat gain by either radiation or muscular activity, consequently increased core body temperature and/or rectal temperature, which stimulates the mechanism of heat dissipation through skin and respiration. In fact, the mainly way of heat loss in heat stressed sheep is panting (Ulberg, 1971). In the present study respiratory rate increased by 3.9 and 3.4 folds in control and 3.4 and 3.8 in treated lambs due to sun exposure and exercise, respectively, Similar results were found by Khalifa (1982) and Shalaby (1996) in sheep.

Dietary ascorbic acid decreased RR and RT, ST and WT during both experiments, sun exposure and exercise. (Table 1 and Figs. 4 & 5). Similarly, Kobeisy (1994) found that ascorbic acid treated calves maintain their respiration efficiency by increasing the depth of air exchange rather than increasing their respiratory rate. This results means that dietary ascorbic acid may be useful in heat stressed suckling lambs.

2- Hematological response.

Exposure to solar radiation or exercise under direct sunlight decreased Hb concentration, total RBC`s, WBC`s (count/mm^3 blood) and PCV,%, most of such differences were significant (Table 2 and Fig. 6). This may be due to hemodilution effect occurred during exposure to heat stress as an adaptive mechanism to replace the water lost by evaporative cooling and to increase the blood volume (Paape et al. 1972). Also the higher destruction of RBC`s occurred during heat stress may be involved (Khalifa, 1982 and Kobeisy, 1994).

Ascorbic acid treated lambs tended to have higher Hb, RBC`s WBC`s and PCV, % (Table 2). Higher Hb, RBC`s and PCV,% values in ascorbic acid treated lambs may be related to the involvement of ascorbic acid in iron metabolism and subsequently Hb and RBC`s formation by: 1)Enhancing its absorption (Nandi et al., 1977). 2) Increasing bioavailability of nonheme iron by reducing the ferric ions in the stomach and forming complex compounds available for absorption by mucosal cells of duodenum (Volker et al. 1984).

Dietary ascorbic acid significantly increased ($P< 0.01$) the total WBC`s count/mm^3 blood (Table 2). Such effect may be related to the protected effect of ascorbic acid on leukocytic membrane from autooxidation (Jaffe, 1984). Similar results were reported in Guinea pigs (Mahan and Saif, 1983) and buffalo calves (Kobeisy and Abd-El-All, 1993).

3- Serum metabolites

Control lambs exposed to both direct solar radiation and exercise had significantly higher ($P< 0.01$) serum total protein and albumin (Table 3). Such higher serum albumin concentration during heat stress is due to either solar radiation or solar radiation with exercise, is a useful physiological response, which maintain higher plasma volume, since the significant increase of serum albumin is responsible for controlling the osmotic pressure and flow of water between blood and tissue fluids to compensate the loss of water occurred during exposure to heat stress (Khalifa, 1996).

Ascorbic acid treated lambs tended to have a higher serum total protein, albumin and globulin concentrations, particularly before sun exposure and exercise, than controls (Table 3), This indicate the biological activity of ascorbic acid in the metabolism of amino acid and protein synthesis through its availability of protein molecule (Schonheyder and lyaglye, 1962) and stimulating enzymes responsible for metabolism of protein (Cesh and Battacharya, 1966). However after sun exposure and exercise the change in serum albumin concentration in control lambs was higher than treated ones (Table 3), This means a higher effort exerted by the control lambs to increase serum total protein and its fraction, particularly albumin, to control the osmotic pressure of blood and compensation the loss of water occurred during evaporative cooling mechanisms. On other words, control animals may be severely affected by heat stress than ascorbic acid treated ones.

Serum glucose concentrations significantly decreased ($P< 0.01$) after both sun exposure and exercise where in control animals it decreased by 6% and 10 % due to solar radiation and exercise, respectively (Table 3 and Fig. 7). This decrease may be related to the rapid utilization of blood glucose by respiratory muscles due to a high ($P< 0.01$) respiration rate (Table 1). Hemodilution occurs during evaporative cooling mechanism may be involved. Ascorbic acid treated lambs tended to have a higher serum glucose level, both before and after sun exposure and exercise (Table 3).

Serum cholesterol concentration increased after sun exposure and exercise (Table 3). This effect may be due to the lipolytic effect of cortisol hormone (Hadly, 1984), which was higher after exposure to both direct sun light and exercise (Table 4). However the significant decrease ($P< 0.01$) of serum cholesterol concentration in ascorbic acid treated lambs (Table 3) was mainly due to the effect of ascorbic acid on degradation of cholesterol to bile salts and decrease its deposition in blood vessels (Ginter, 1975).

4- Serum transaminases (AST and ALT).

Lambs exposed to both direct sun light or exercise exhibited slight insignificant increase in serum AST and ALT (Table 4), which suggested increased their activity with rising temperature that accompanied with acceleration of metabolic reactions (Shaffer et al., 1981). No significant effect of ascorbic acid was observed on AST and ALT (Table 4).

5- Thyroid hormones (T3 and T4) and cortisol.

Suckling lambs exposed to direct sunlight and exercise significantly had a lower ($P< 0.01$) serum T4 concentration and higher ($P< 0.01$) serum cortisol (Table 4 and Figs. 8 & 9). However no significant changes in serum T3 concentration after sun exposure and exercise (Table 4). The inverse relationship between thyroxin secretion and air ambient temperature is useful as a compensatory adjustment to decrease the difference between heat production and heat loss to a lower limit as could as possible, while the increase of cortisol level is very important during heat stress, to provide more metabolic substrates necessary for survival in the absence of hepatic glycogen (Hadly, 1984).

Ascorbic acid treated lambs had a higher serum T4 either before or after sun exposure and exercise. The higher serum T4 in treated lambs indicated the positive effect of dietary ascorbic acid on thyroid weight (Al-Janabi et al., 1988), and its important role in the formation of thyroid metabolites which lead to a higher thyroid activity. Abdel Wahab et al. (1975) revealed that improvement of thyroid hormone in ascorbic acid treated animals may be due to the increase of thyroid I uptake. In fact a significant increase in body weight of buffalo calves (Kobeisy and Abdel-All, 1993) and Chicken (Esa, 1992) supplemented with ascorbic acid may be due to the increase in thyroid hormones.

Ascorbic acid treated lambs subjected to solar radiation and exercise had relatively lower serum cortisol level than control ones, except after exercise (Table 4). This may be attributed to the effect of ascorbic acid on reduced glucocorticoids synthesis by the adrenal cortex (Thaxton and Pardue, 1984).

Conclusion

Dietary ascorbic acid, as an antioxidant nutrient can be used to solve heat stress problem in suckling lambs. It releases or decreases heat stress through different physiological effects. These are: 1) Decrease of rectal temperature and respiratory rate, 2) Increase of blood cells (RBC`s and WBC`s), hemoglobin concentration and PCV,%. 3) With the increase of serum albumin concentration, plasma volume will be increased, 4) Increase of cortisol hormone to provide extra energy of glucose for different bodily

reactions. 5) Increase of serum thyroxin concentration to avoid the adverse effect of heat stress on animal performance.

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Table 1. Effect of sun exposure and exercise on respiratory rate (breath./min.) and rectal, skin and wool temperatures (° C) of suckling lambs fed ascorbic acid.

Item *	Control		Ascorbic acid **		S. E. of
	A	B	A	B	L. S. M.
			Sun exposure		
Respiratory rate	41.6 ^{c}	102.1 ^{de}	38.6 ^{c}	130.0 ^{df}	4.66
Rectal temperature	39.5 ^{c}	40.6 ^{d}	39.4 ^{c}	40.5 ^{d}	0.06
Skin temperature	37.9 ^{c}	39.7 ^{d}	37.6 ^{c}	39.6 ^{d}	0.12
Wool temperature	36.6 ^{c}	38.9 ^{d}	36.3 ^{c}	38.7 ^{d}	0.17
			Exercise		
Respiratory rate	53.5 ^{c}	180.4 ^{de}	42.6 ^{c}	161.8 ^{df}	4.32
Rectal temperature	39.7 ^{c}	41.4 ^{d}	39.5 ^{c}	41.3 ^{d}	0.08
Skin temperature	37.6 ^{c}	39.8 ^{d}	37.3 ^{c}	39.7 ^{d}	0.17
Wool temperature	36.0 ^{c}	38.8 ^{d}	35.6 ^{c}	38.7 ^{d}	0.21

* Values are least-squares means ± standard error of L. S. M.
A = Before exposure, B= After exposure.
** Ascorbic acid, animals fed 2 gm ascorbic acid/ animals every other day.
c,d; e,f (P< 0.01).

Table 2. Effect of sun exposure and exercise on some hematological parameters of suckling lambs fed ascorbic acid.

Item *	Control		Ascorbic acid **		S. E. of
	A	B	A	B	L. S. M.
			Sun exposure		
Hemoglobin, g/dl	10.38 ^{c}	9.44 ^{dg}	10.49 ^{c}	9.88 ^{dh}	0.16
Total RBC's count/mm ^{3}blood	12.40 ^{c}	10.50 ^{d}	13.09 ^{c}	11.47 ^{d}	0.25
Total WBC's count/mm^{3}blood	8.48 ^{c}	7.47 ^{di}	9.08 ^{cd}	8.68 ^{dj}	0.10
PCV %	32.50	30.39 ^{fi}	32.37	30.68 ^{j}	0.79
			Exercise		
Hemoglobin, g/dl	9.91 ^{g}	9.32 ^{he}	10.23	9.74	0.18
Total RBC's count/mm ^{3}blood	11.80 ^{g}	10.77 ^{he}	12.70 ^{g}	11.51 ^{h}	0.35
Total WBC's count/mm^{3}blood	8.25 ^{ci}	8.03 ^{di}	9.00 ^{cj}	8.62 ^{dj}	0.09
PCV %	32.39	30.95	32.67	31.03	0.86

* Values are least-squares means ± standard error of L. S. M.
A = Before exposure, B= After exposure.
** Ascorbic acid, animals fed 2 gm ascorbic acid/ animals every other day.
c,d; i,j (P< 0.01), g,h (P< 0.05).

Table 3. Effect of sun exposure and exercise on some blood metabolities of suckling lambs fed ascorbic acid.

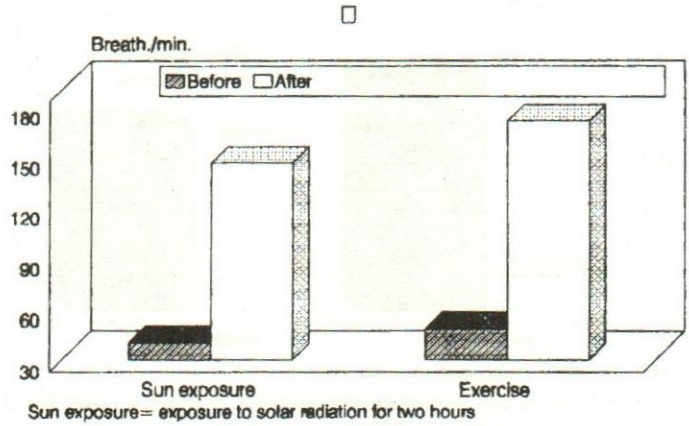
Item *	Control		Ascorbic acid **		S. E. of L. S. M.
	A	B	A	B	
			Sun exposure		
Total protein, g/dl	7.66	7.95	8.11	8.22	0.17
Albumin, g/dl	3.56 ^{c}	3.94 ^{de}	3.65 ^{e}	3.80 ^{f}	0.05
Globulin, g/dl	4.11	4.01	4.46	4.42	0.15
Glucose, mg/dl	59.28 ^{c}	55.49 ^{d}	65.72 ^{c}	58.14 ^{d}	1.85
Cholesterol, mg/dl	132.47 ^{c}	137.31 ^{c}	107.95 ^{d}	111.95 ^{d}	2.37
			Exercise		
Total protein, g/dl	7.73 ^{e}	8.22 ^{f}	7.99	8.08	0.17
Albumin, g/dl	3.46 ^{c}	3.88 ^{d}	3.57	3.63	0.04
Globulin, g/dl	4.22	4.34	4.39	4.45	0.14
Glucose, mg/dl	60.48	54.84	71.57 ^{c}	63.71 ^{d}	2.07
Cholesterol, mg/dl	133.03 ^{ec}	141.14 ^{fc}	105.10 ^{ed}	113.55 ^{fd}	2.75

* Values are least-squares means ± standard error of L. S. M.
A = Before exposure, B= After exposure.
** Ascorbic acid, animals fed 2 gm ascorbic acid/ animals every other day.
c,d (P< 0.01), e,f (P< 0.05).

Table 4. Effect of sun exposure and exercise on serum transaminases (GOT and GPT), thyroid hormones (T3 and T4) and cortisol of suckling lambs fed ascorbic acid.

Item *	Control		Ascorbic acid **		S. E. of L. S. M.
	A	B	A	B	
			Sun exposure		
AST, u/l	36.36	35.46	33.80	35.30	1.71
ALT, u/l	15.16	16.45	15.81	15.12	0.66
T3, ng/l	1.33	1.11	1.25	1.26	0.10
T4, ng %	3.91 ^{ci}	3.08 ^{dk}	4.89 ^{cj}	3.62 ^{d}	0.20
Cortisol, ng/dl	1.98 ^{c}	3.78 ^{di}	1.78	2.54 ^{i}	0.28
			Exercise		
AST, u/l	36.03	37.31	33.52	33.98	1.78
ALT, u/l	14.84	15.55	15.22	15.11	0.64
T3, ng/l	1.12 $_{c\,i}$	1.59	1.22	1.15	0.26
T4, ng %	4.06 ^{cj}	3.13 ^{d}	4.64 ^{cj}	3.47 ^{d}	0.16
Cortisol, ng/dl	1.95 ^{g}	3.02 ^{h}	1.89 ^{c}	3.25 ^{d}	0.33

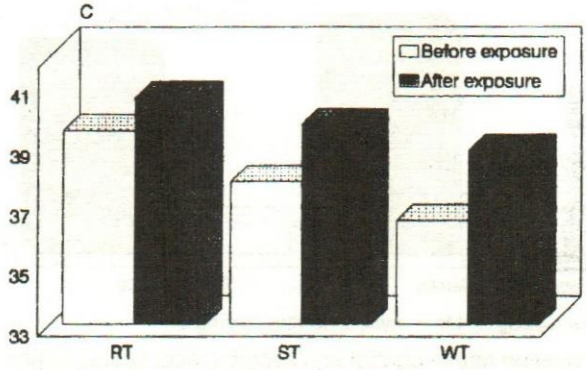
* Values are least-squares means ± standard error of L. S. M.
A = Before exposure, B= After exposure.
** Ascorbic acid, animals fed 2 gm ascorbic acid/ animals every other day.
c,d; i,j (P< 0.01), g,h (P< 0.05).



Sun exposure= exposure to solar radiation for two hours

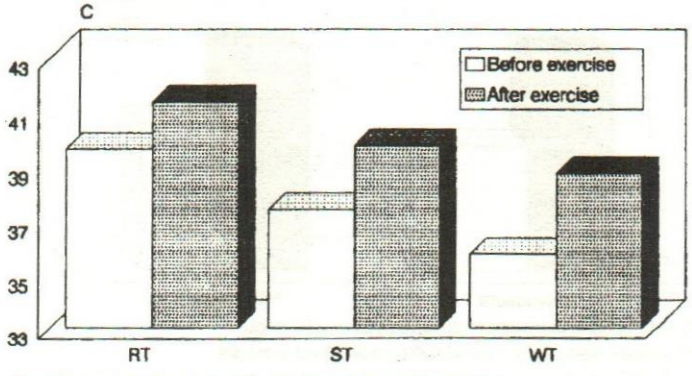
Exercise= walking for 1 hr, 4 km/hr, under direct sun light.

Fig. 1. Effect of sun exposure and exercise on respiration rate of suckling lambs.



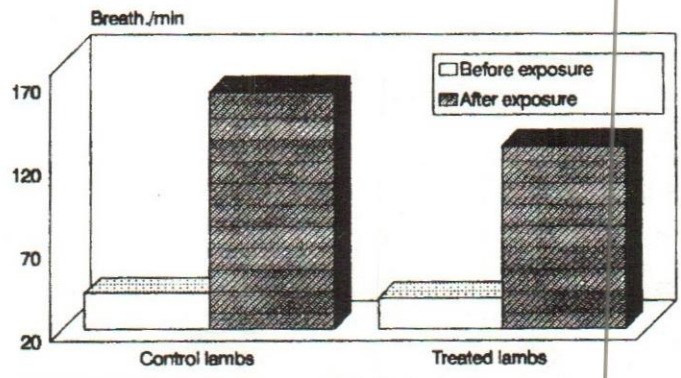
Sun exposure= exposure to solar radiation for two hours

Fig. 2. Effect of sun exposure on rectal (RT), skin (ST) and wool (WT) temperatures of suckling lambs.



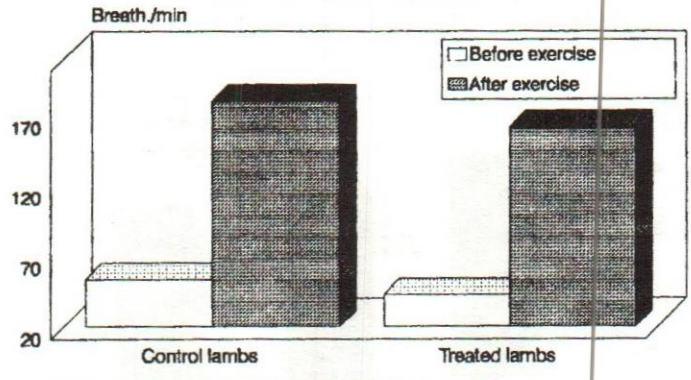
Exercise= walking for 1 hr, 4 km/hr, under direct sun light.

Fig. 3. Effect of exercise on rectal (RT), skin (ST) and wool (WT) temperatures of suckling lambs.



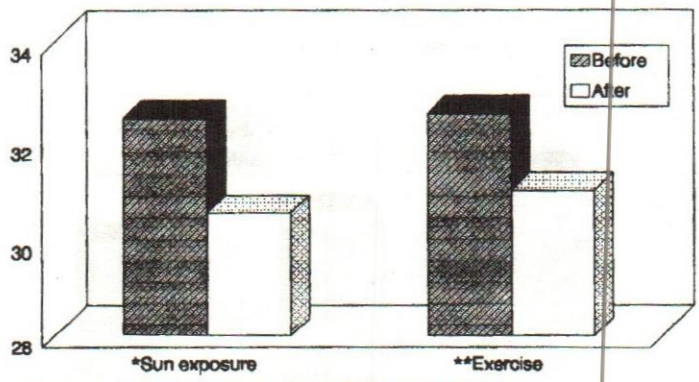
Sun exposure= exposure to solar radiation for two hours.

Fig. 4. Respiration rate in control and ascorbic acid treated lambs as influenced by sun exposure.



exercise= walking for 1 hr, 4 km/hr, under direct sun light.

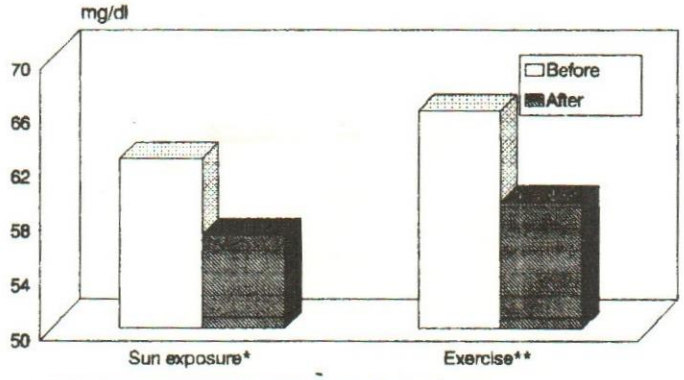
Fig. 5. Respiration rate in control and ascorbic acid treated lambs as influenced by exercise.



*Sun exposure= exposure to solar radiation for two hours

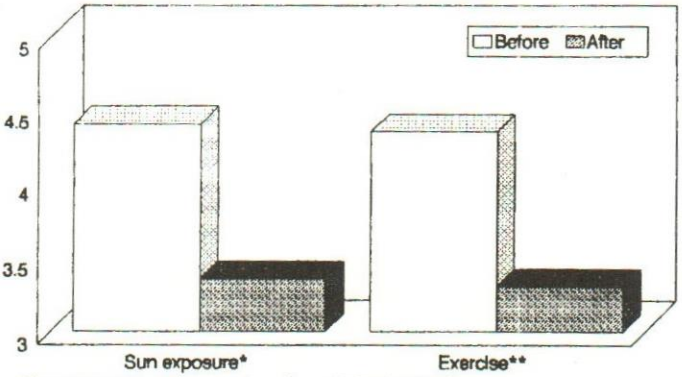
**Exercise= walking for 1 hr, 4 km/hr, under direct sun light.

Fig. 6. Effect of sun exposure and exercise on packed cell volume (PCV,%) of suckling lambs.



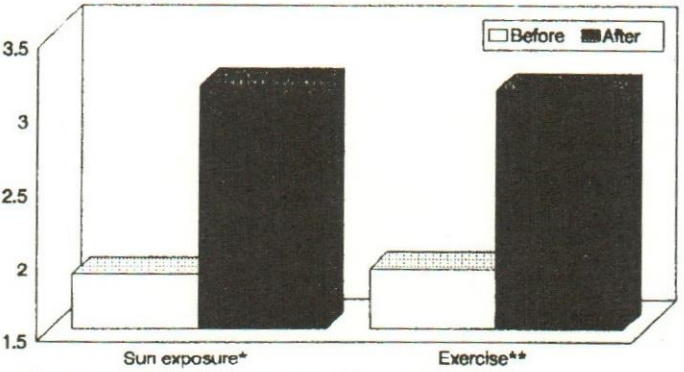
*Sun exposure= exposure to solar radiation for two hours
**Exercise= walking for 1 hr, 4 km/hr, under direct sun light.

Fig. 7. Serum glucose concentration of suckling lambs as influenced by sun exposure and exercise.



*Sun exposure= exposure to solar radiation for two hours
**Exercise= walking for 1 hr, 4 km/hr, under direct sun light.

Fig. 8. Serum thyroxine hormone (T4) concentrations of suckling lambs subjected to sun exposure and exercise.



*Sun exposure= exposure to solar radiation for two hours
**Exercise= walking for 1 hr, 4 km/hr, under direct sun light.

Fig. 9. Serum cortisol concentration of suckling lambs subjected to sun exposure and exercise.