IMPACT OF ASCORBIC ACID AND PROBIOTICS FOR IMPROVEMENT OF CONCEPTION RATE OF EWES DURING SUMMER SEASON

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ABSTRACT

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This study was performed to compare the influence of premating supplementation of ascorbic acid (Vit C) and probiotics (live dried yeast, DY) (Yea-Sacc 1026) (Saccharomyces cerevisiae) on conception rate, thermo-respiratory activities, serum antioxidants, hormonal response and body weight change of heat stressed breeding ewes. The trial was performed on twenty four ewes which were selected and divided into three groups (8 ewes in each) with a completely randomized design. (1) control group: received basal diet without additives, group (2): received basal diet plus (75/mg/kg body weight/day) of vitamin C (Vit C) and group (3): received basal diet plus live dried yeast (DY) (5/g/head/day). The treatments were initiated 2 weeks premating and continued for 4 weeks then after. Blood samples were collected at 2 weeks intervals, starting from the 2^{nd} week of treatment till the 6^{th} week of treatment (June-August). All ewes were weighed and observed for thermo-respiratory activity before the beginning of study and at 1 month and 2 month after the treatment. The results revealed that there was a significant ($P < 0.01$) increase in conception rate in group 2 supplemented with Vit C as compared with that supplemented with DY or the control group. Vit C and DY treated groups had a significantly ($P < 0.01$) lower rectal temperature and respiratory rate values as compared with the pre-treatment values and that of the control group. There is a significant increase in Vit C and glutathione -S- transferase levels in the treated groups than those of the control one. Serum level of T3 was elevated ($P<0.05$) significantly throughout the 2^{nd} and the 6^{th} weeks in Vit C group and throughout the 2^{nd} week in DY group as compared with the control one. Serum cortisol level was significantly ($p<0.05$) decreased throughout the trial in Vit C treated group, and only during the 2^{nd} week in DY treated group. Also, Vit C supplementation lead to a significant ($P < 0.05$) increase in the body weight as compared with the control one; meanwhile, DY supplementation had a nonsignificant effect on body weight. Results of a premating Vit C supplemented ewes showed a trend towards improving conception rate, body weight, thermo-respiratory activity, antioxidant level, cortisol concentration and thyroid hormones response. So, it could be concluded that premating dietary Vit C supplementation at a level of 75/mg/kg BW/d may offer a thermo-protective role to lessen the destructive effects of heat stress on breeding ewes and may be more economically attractive than DY dietary supplementation under heat stress in summer season.

***Key words**: vitamin C, probiotics, ewes, summer season, antioxidants, hormones, conception rate.*

INTRODUCTION

Heat stress is a very common in livestock especially in tropical countries and sub-tropical areas. Indigenous sheep tends to breed throughout the year, although the sexual activity is restricted to a certain extent to the summer months (Marai *et al.*, 2004). In such regions, a high ambient temperature is the major constraint on animal productivity (Marai *et al.*, 2000; Shelton, 2000). This effect is aggravated when heat stress is accompanied by high ambient humidity (Abdel-Hafez, 2002). Exposure of sheep to elevated temperatures results in a decrease of body weight, average daily gain (ADG), growth rate and body total solids which is reflected by impaired reproduction (Shelton, 2000 and Abdel-Hafez, 2002). Moreover, stressful conditions. such as exposure to heat .Leads to increased free radicals production (Loven, 1988).

The elevation of free radicals affects strongly all body systems including reproductive performances of both male and female. Free radical have been suggested to be partly responsible for the detrimental effect of elevated temperature on cellular membrane integrity, and for compromising the cellular function of steroidogenic tissues and embryos, as these have been found to be sensitive to free radical damage (Hansen 1997; Arechiga *et al.*, 1998). A major strategy to reduce the effect of heat stress on animals is to alter the environment through the use of sheds ‹fans or evaporative cooling (Bucklin *et al.*, 1991). Such practices are not possible in semi-intensive systems as sheep are grazed in the open during most of the day. This necessitates other strategies to counteract the adverse effects of heat stress such as supplementation of antioxidants. An antioxidants such as Vit C is a free radical scavenger, that protects the body defense system against excessively produced free radicals during heat stress and stabilizes the health status of the animal. Although ruminants can synthesize Vit C (McDowell, 1989), a great reduction in plasma Vit C concentration was reported in heat- stressed cows (Padilla *et al.*, 2006). Vit C may also be able to regenerate other antioxidants such as Vit E (Bruno *et al.*, 2006). The manipulation of rumen microbial activity including dietary antibiotics and probiotics has been widely studied during the last 20 years. The probiotics (bacterial and yeast cultures) are live microbial feed supplements that have been used as growth promoters to replace the widely used antibiotics and synthetic chemical feed supplements (Sumeghy, 1995 and Strzetelski, 1996). Dawson (1990) reported that, yeast culture increased ruminal cellulose digestion and consequently improved feed efficiency and gain and increased the microbial growth in rumen and enhanced the microbial protein synthesis. Dawson (1994) showed that, yeast or yeast culture are rich source of vitamins, enzymes and other important nutrients and co-factors which make them attractive as digestive enhancers as a basic source of nutrients. El-Shaer (2003) reported that, all nutrients digestibility were increased for sheep. Also, improve nitrogen balance (El-Ashry *et al.*, 2003). Abdel-Latif (2005) found an increase in total VFA due to adding yeast culture in sheep diets. Saccharomyces cerevisiae were reported to balance the energy and the acid-base metabolism in sheep that resulted in a significantly higher milk production and increasing milk yield of ewes which is an important factor for the production of robust lambs at weaning (Helal and Abdel-Rahman, 2010). During heat stress, sheep performance was improved by adding YC, El-Shaer, (2003) detected a decrease in rectal and skin temperatures as well as pulse and respiration rates. Also, an improvement in reproductive performance was also obtained by Abdel-Khalek (2003) in Friesian cows and by Ebrahim (2004) in Egyptian buffaloes due to yeast culture supplementation.

Aim of the work:

It was conducted to assess whether supplementation of vitamin C(Vit C) or live dried yeast (DY/probiotics) could alleviate heat stress drastic changes in ewes or not.

MATERIALS and METHODS

The present study was carried out at the experimental farm belonging to Animal Reproduction Research Institute (ARRI). Twenty four sexually mature Barki ewes of 2-3 years of age and average weight 30-34 kg confirmed by ultrasound examination(knorton, 6.5MC, sigma 300, France) that they are non pregnant before the start of the trial. Ewes were housed under conditions of natural day light and temperature in semi-shaded well ventilated pens. Ewes were offered basal ration according to management of ARRI and water was offered ad libitum. A second ultrasound scanning for pregnancy detection was performed one month after ram exposure (natural mating). Ewes were randomly divided into 3 groups, each of 8 animals. The control group fed the basal ration without any supplementation [group (1)]. In treatments viamin C (Vit C) was given at 75/mg/kg BW/d (powder supplied by the Egyptian Arabian Co. of feed additives and vitamins) [group (2)]. Live Dried Yeast (DY) was supplemented at 5/ g/head/day (Yea-Sacc 1026, Saccharomyces Cerevisiae) [group (3)]. Vit C and DY were supplemented to the animals orally through the period of the experiment. The treatments were given 2 weeks premating and lasted for 4 weeks postmating during the summer season (June to August) where the highest temperature level recorded in Egypt (figure 1) describes temp /humidity level in this period.

All ewes were weighed before and after the end of the treatment. Respiration rate (RR) and rectal temperature (RT) of the ewes were recorded in the three groups in the period before the treatment and then regularly at 2 weeks intervals till the end of the treatments. Rectal temperature was measured using digital thermometer. After 2 weeks from the beginning of the experiment all ewes were subjected to estrus detection 2-times daily by good vasectomies ram. Conception rate of ewes in the three groups was recorded by ultrasonography at 4 weeks post examination.

Blood samples were collected from all ewes regularly at 2 weeks intervals from the beginning of the trial (on the second, fourth and six week), ten ml of blood was collected from jugular vein before feeding. Blood was collected in two vaccutainer tubes one containing EDTA for plasma and the other plain for serum separation. Serum and plasma were obtained by centrifugation at 3000 rpm/15 min samples aliquoted and frozen at -20 C until analysis. Blood samples were analyzed colorimetrically by commercial kits for: Total antioxidant capacity (TAC) (koracivic *et al.*, 2001), ascorbic acid (Vit C) (Harris and Ray, 1935), Glutathione - S- transferase (GST) (Habig *et al.*, 1974). Free radicals: Lipid peroxidase [malondialdehyde (MDA)] (Ohkawa *et al.*, 1975) and Nitric oxide (NO) (Rajaraman *et al.*, 1998).

All hormones were performed using ELISA microwells kits (Monobind Inc. Lakeforest, CA 92630.USA): Total triiodothyroxine (T_3): Braverman, (1996), total thyroxin (T_4): Muzzaffari and Gharib (1998), cortisol: Burtis and Asweed (1994).

Statistical analysis:
All data were subjected to statistical analysis according to Snedecor &Cochran (1982) and results were expressed as Means ± standard error (SE). Differences between means in different groups were tested for significance using T test as independent t test for all parameters except for thermo-respiratory activities were tested for significance using paired "t" test.

RESULTS

The overall conception rate of control and treated groups are illustrated in Figure 2, that showed that there is a highly significant ($P < 0.01$) increase in conception rate (100%) due to Vit C supplementation as compared with that of control one (62.5%). Meanwhile the same figure clearly indicated that conception rate was not affected by DY supplementation (62.5 %) as compared with that of control group.

The results of thermo-respiratory activities are shown in Table (1), revealed that the Vit C and DY treated groups had a significantly ($P \leq 0.01$) lower rectal temperature and respiratory rate values as compared with the pre-treatment values. On the other hand, there was a nonsignificant change in rectal temperature and respiratory rates in the control group throughout the trial, also, average rectal temperature and respiratory rates values before treatment were similar in the control and treated groups

The data in Table (2) showed that there is a significant ($P < 0.05$) increase in Vit C and glutathione -S- transferase (GST) levels in treated groups (1&2) than the control one. While, total antioxidant capacity (TAC) serum level showed a significant ($P < 0.01$) increase in both treated groups during the 6^{th} week only when compared with that of the control one.

The data in Table (3) showed that there is no difference between all experimental groups in nitric oxide (NO) serum level all over the trial; however, there was a significant decrease in malondialdehyde (MDA) level in both supplemented groups after the 6 th week of the treatment as compared with that of the control one.

With regard to thyroid hormones and serum cortisol levels, data in Table (4) clearly indicated that the level of serum T_3 in Vit C group was elevated ($P<0.05$) significantly throughout the 2^{nd} and the 6^{th} weeks and was elevated nonsignificantly throughout the 4^{th} week of the trial compared to the control one. Meanwhile, its level in DY treated group was elevated ($P<0.05$) significantly throughout the 2^{nd} week and nonsignificantly throughout the 2^{nd} and the 6^{th} weeks of the trial when compared with the control one. In respect to serum T_4 level which was elevated ($P<0.01$) significantly throughout the 2^{nd} and the 4^{th} weeks and was elevated nonsignificantly throughout the 6^{th} week of the trial in Vit C and DY treated groups as compared with the control one.

In respect to serum cortisol level which was significantly ($p<0.05$) decreased in Vit C treated group throughout the trial, meanwhile the decrease in serum cortisol level due to DY supplementation was significantly ($P<0.05$) only during the 2 nd week and nonsignificantly during the 4 th and 6^{th} weeks of the treatment as compared with control group.

The change in body weight due to supplementation and heat stress in the present experiment are presented in Table (5). Data analysis showed that supplementation of Vit C to heat stressed ewes prevent weight loss due to heat stress and significantly ($P < 0.05$) increased body weight of the treated group throughout the trial as compared with the control one. In the same table the results revealed that DY minimized weight loss due to heat stress and did not improve body weight of the treated ewes as their initial weight was unaltered with DY supplementation, and there was a nonsignificant difference between the DY supplemented group and the control one in respect to body weight throughout the trial.

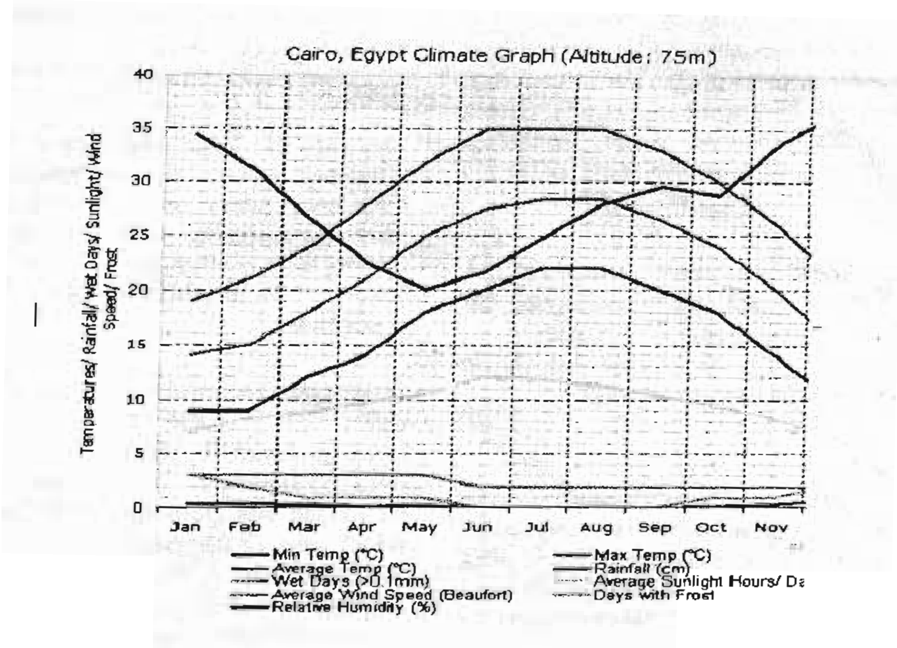

Figure 1: Cairo,Egypt climate showing high temperature and humidity in summer season.

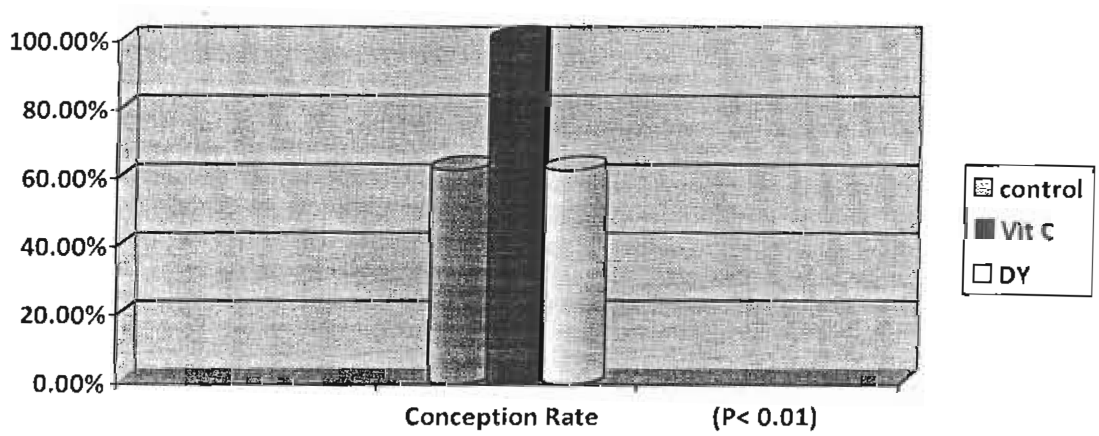

Figure 2: Conception rate of all ewes after 6 weeks of the trial.

Table 1: Means ± SE of thermo-respiratory activities of the control and treated groups.

Parameters	Rectal Temperature (RT) (C)°		
	Before treatment	1 Month After ttt	2 Month After ttt
Control	40.00 ± 0.02	40.31 ± 0.02	40.27 ± 0.03
Vit C	40.75 ± 0.02	39.80 ± 0.01**	39.80 ± .02*
DY	40.75 ± 0.02	40.00 ± 0.02**	40.08 ± 0.01***
	Respiratory Rate (RR) (breaths /min)		
Control	58.375 ± 0.26	58.375 ± 0.23	58.250 ± 0.36
Vit C	58.63 ± 0.33	48.13 ± 0.24***	41.75 ± 0.23***
DY	54.00 ± 0.34	47.50 ± 0.29***	42.88 ± 0.32***

* Significant at P <0.05, ** Significant at P <0.01, *** Significant at P <0.001, ttt: treatment

Table 2: Means ± SE of serum vitamin C (Vit C), total antioxidant Capacity (TAC) and glutathione-s-transferase (GST) concentrations of the control and treated groups.

Parameters		Control	Vit. C	DY
Vit C (mg/L)	2W After ttt	11.1 ± 0.5 [a]	14.34 ± 0.73 [b]	12.93 ± 2.4 [b]
	4W After ttt	10.7 ± 0.6 [a]	15.27 ± 0.4 [b]	13.60 ± 0.65 [c]
	6W After ttt	11.44 ± 0.7 [a]	15.45 ± 0.38 [b]	13.20 ± 0.24 [c]
TAC (mmol/L)	2W After ttt	0.37 ± 0.01	0.38 ± 0.007	0.36 ± 0.007
	4W After ttt	0.38 ± 0.003	0.42 ± 0.01	0.48 ± 0.01
	6W After ttt	0.36 ± 0.003 [a]	0.47 ± 0.01 [b]	0.48 ± 0.007 [c]
GST(U/dl)	4W After ttt	1.31 ± 0.04 [a]	1.71 ± 0.05 [b]	1.78 ± 0.1 [b]
	6W After ttt	1.24 ± 0.03 [a]	1.71 ± 0.08 [b]	1.84 ± 0.1 [b]

Means with different superscripts letters in the same row are significantly different at P<0.05 and P<0.01, ttt: treatment, no: nonsignificant

Table 3: Means ± SE of serum Nitric oxide (NO), Malondialdehyde (MDA) concentrations of control and treated group.

Parameters		Control	Vit. C	DY
NO (µg/l)	2W After ttt	5.94 ± 0.55	6.15 ± 0.54	5.29 ± 0.31
	4W After ttt	6.98 ± 0.10	5.64 ± 0.37	5.95 ± 0.66
	6W After ttt	5.70 ± 0.50	6.69 ± 0.50	6.33 ± 0.69
MDA (nmol/ml)	2W After ttt	6.53 ± 0.63	5.64 ± 0.58	6.38 ± 0.54
	4W After ttt	6.66 ± 0.45	5.85 ± 0.35	5.78 ± 0.55
	6W After ttt	6.89 ± 0.45 [a]	4.97 ± 0.37 [b]	5.79 ± 0.65 [ab]

Means with different superscripts letters in the same row are significantly different at P<0.01.

Table 4: Means ± SE of serum triiodothironine (T_3), thyroxin (T_4) and cortisol levels of the control and treated groups.

Parameters		Control	Vit C	DY
T_3(ng/ml)	2W After ttt	2.51± 0.21 [b]	3.50 ± 0.26 [a]	3.10 ± 0.13 [ab]
	4W After ttt	2.47 ± 0.24 [b]	3.03 ± 0.21 [ab]	3.70 ± 0.18 [a]
	6W After ttt	2.46 ± 0.11 [b]	3.18 ± 0.20 [a]	2.82 ± 0.16 [ab]
T_4(µg/dl)	2W After ttt	5.90 ± 0.35 [b]	8.68 ± 0.56 [a]	8.18 ± 0.57 [a]
	4W After ttt	3.33 ± 0.49 [b]	5.68 ± 0.35 [a]	5.78 ± 0.55 [a]
	6W After ttt	7.21 ± 0.55 [a]	8.93 ± 1.16 [a]	8.71 ± 1.09 [a]
Cortisol (µg/dl)	2W After ttt	27.25 ± 1.80 [a]	19.75 ± 2.10 [b]	18.12 ± 2.41 [b]
	4W After ttt	25.87 ± 2.05 [a]	16.88 ± 2.25 [b]	15.63 ± 1.94 [ab]
	6W After ttt	20.12 ± 2.04 [a]	13.63 ± 1.35 [b]	16.12 ± 1.87 [ab]

Means with different superscripts letters in the same row are significantly different at P<0.05 and P<0.01, ttt: treatment, ns: nonsignificant.

Table 5: Means ± SE of body weight (kg) of the control and treated groups.

Parameters	Control	Vit C	DY
Before ttt	32.87 ± 0.60	35.38 ± 1.22	34.23 ± 1.42
1 M after ttt	31.43 ± 0.63 ^{a}	35.75 ± 1.16 ^{b}	34.00 ± 1.32 ^{ab}
2 M after ttt	30.68 ± 0.63 ^{a}	36.25 ± 1.32 ^{b}	33.88 ± 1.50 ^{ab}

Means with different superscripts letters in the same row are significantly different at P<0.05, ttt: treatment, M: month.

DISCUSSION

Our results was clearly indicated that premating dietary Vit C supplementation to ewes during summer season (heat-stressed) significantly improved conception rate as compared with control ewes. This results were in agreement with those obtained by (Haliloglu and Serpek, 2000) who reported that Vit C supplementation to the sheep during the breeding season improved their fertility, that also were in constant with Marai *et al.* (2004) who found a negative relationship between conception rate and ambient temperature in sheep. Such improvement of conception rate in Vit C supplementation group may be attributed to the increment of progesterone secretion and/or protecting the embryos from the harmful effect of free radical produced with heat stress and allowing them to bypass the period when they are most sensitive to elevated temperature (i.e., in 2 nd days after breeding), low progesterone level associated with enhanced secretion of PGF2α which may induce luteolysis of the corpus luteum and termination of pregnancy (Santos *et al.*, 2004), Vit C content in the CL is at its maximum when the CL is fully mature, remains high during pregnancy, and decreases as the CL regresses (Petroff *et al.*, 1997). One of the proposed mechanisms to explain the luteolytic effect of PGF2α is the generation of the oxidative radicals, and the cytotoxic effect (Niswender *et al.*, 2000). And it was suggested that the oxidative radicals may be fundamental in the initiation of luteolysis and the consequent inhibition of the progesterone synthesis (Tanaka *et al.*, 2000 and Pepperell *et al.*, 2003). Moreover, it was found that Vit C supplementation to embryo culture medium (IVF) followed by incubation at 20% oxygen level resulted in significantly higher rates of cleavage, morula and blastocyst formation and blastocyst total cell count in sheep, which reflect the protective effects of Vit C against the oxidative damage to DNA (Natarajan *et al.*, 2010). Meanwhile, there is no significantly difference in conception rate between DY supplemented group and the control one. These results were agreed with those obtained by Abd Rahman *et al.* (2012) and Mousa *et al.* (2012) who reported that supplementation of yeast had no significant effect on conception rate.

The mean of ambient temperature and relative humidity during the experimental period were illustrated in (figure 1) which was considered as heat stress condition. The exposure to high ambient temperatures, is a major constraint on animal productivity as it evokes a series of drastic changes in the animals' biological functions, Such changes resulted in the impairment of productive and reproductive performance. The effect of heat stress is aggravated when it is accompanied by high humidity (Marai *et al.*, 2007). Poorly sweating animals like sheep (due to very thick wool fleece) attempt to maintain the heat balance by increasing their respiratory activity, losing more heat through evaporation from the respiratory tract. Our results, revealed that Vit C supplementation minimized the elevation of rectal temperature due to heat stress and significantly decrease rectal temperature of treated ewes than before treatment. These results were in agreement with Sivakumar *et al.* (2010) who found that Vit C supplementation to heat stressed goats significantly decreased the rectal temperature and respiratory rates. Vit C directly alters the thermal set point by decreasing the prostaglandin output, especially of PGE series, whose turnover increases during stress and has a direct effect on the hypothalamic thermoregulatory zone (Ganong, 2001). Similarly, our results indicated that there is a significant decrease in RT, RR values for the DY supplemented group as compared to the control one. These findings are in accordance with those obtained by Abdel Rahman *et al.* (2012) on sheep who indicated that DY supplementation significantly decreased RT and RR. That may due to minerals content of DY, especially selenium which has a favorable effect on body thermoregulation.

Heat stress stimulates excessive production of free radicals (superoxide anion, hydroxyl, hydrogen peroxide and singlet oxygen) (Bernabucchi *et al.*, 2002) that can damage healthy cells if they are not eliminated. Heat production is directly controlled by the nervous and endocrine systems through the modifications of appetite, digestive process and, indirectly, by alterations of the activity of respiratory enzymes and protein synthesis (Yousef, 1985). As shown in Table (2), dietary supplementation of DY and Vit C during heat stress caused a higher level of total antioxidant that only were significant statistically in Vit C group ($p < 0.05$). While, glutathione-S-transferase was significantly higher in both treatments. The results of the present study suggested that Vit C supplementation may be beneficial to ewes under heat stress which may alleviate its adverse effects, that agreed with Matsui, (2012); Abdul Kareem (2011) and Padilla *et al.* (2006) who explained the positive role of Vit C in the oxidative stress as antioxidant by protecting the Red Blood Corpuscles (RBC) membrane (consist of phospholipids) against the harmful oxidation processes and thus reduce the RBC haemolysis via increasing the flexibility of the cell membranes (so, it maintain the oxygen level and so, the respiratory rate). Dietary supplementation of ewes with DY during heat stress condition can improve oxidant / antioxidant balance under hot conditions that agreed with Aït-Belgnaoui (2009) and Messaoudi *et al.* (2011) who reported that the oral intake of certain live dry yeast may act in a direct way on the animal well-being by reducing the physiologic response related to stress. They added that there is a two-fold link between probiotics and animal well-being: not only probiotics allow to treat stress consequences (flora balance, transit regulation, strengthening of the ruminal ecosystem, etc.) but they also they enable to reduce directly the animal stress perception and improving the well-being feeling of the animal. This is all more important as animal well-being is not only a moral and social necessity but it's an essential condition to have optimal zoo technical performances. Moreover, Maziar *et al.*, 2007 found that the addition of probiotics to diets benefit the host animal by stimulating appetite, improve intestinal microbial balance, stimulate the immune system, decreased pH and release the bacteriocins. In the last few years, the detection of free radicals damage and the protection against it has become very important in the studies related to ruminant production/reproduction as the level of lipid peroxidation (malondialdehyde, MDA) and antioxidant status give complementary information about the metabolic status of the animal rather than metabolic parameters alone (Castillo *et al.*, 2003).

In the present investigation, it was found that probiotics, and Vit C addition decreased lipid peroxidation biomarker (MDA) significantly in both supplemented groups. This is may be referred to vitamins dual effects: (a) control of metabolic pathways or gene expression and (b) Reactive Oxygen Species (ROS) trapping activity (Aurousseau *et al.*, 2006). In addition, Vit C helps to scavenge the oxygen radicals throughout the female reproductive tract (Goud *et al.*, 2008). Although cattle can synthesize Vit C in the liver, a large reduction in plasma Vit C concentration was reported in heat stressed cows (Padilla *et al.*, 2006). The supplementation of ascorbic acid has been shown to enhance the plasma ascorbic acid concentration and immune response of the cattle subjected to environmental stressors (Padilla *et al.*, 2007).

Concerning serum T_3 and T_4 levels in Vit C supplemented ewes there was a significant increase in serum T_3 and T_4 at all weeks of the trial except at the 4th week which showed a non-significant increase in serum T_3 level and at the 6th week which showed a non-significant increase in serum T_4 as compared with the control ewes. Marai *et al.* (2000) confirmed that T_3 concentration in blood plasma was significantly lower in summer season than in winter season in sheep. Decreased thyroid hormone levels during heat stress are an adaptive response and also might be an attempt to reduce metabolic rate and heat production, as thyroid and glucocorticoid hormones assumes a fundamental role in endogenous heat production and regulation (Abeni *et al.*, 2007). This modification in thyroid activity due to heat stress is consistent with decrease in food intake and metabolic heat production (Alameen and Abdelatif., 2012). These results were consistent with those of Sivakumar *et al.* (2010) who noticed that Vit C supplementation significantly increased serum T_3 and T_4 levels in heat stressed goat and Al- Katib (2001) who found that Vit C cause an increase in thyroid gland weight and activity. On the other hand Usha *et al.* (2002) related the findings to the free radical H_2O_2 (hydrogen peroxide) that served as a substrate for the thyroperoxidase enzyme which catalyzed the synthesis of thyroid hormones. Production of more H_2O_2 under stress condition might have reduced the levels of thyroid hormones. Moreover, 5' mono deiodinase, an enzyme which converts T_4 to T_3, is affected by free radicals under heat stress (Brzezinska *et al.*, 2001). Increased levels of thyroid hormones in Vit C supplemented group in the present study might be due to protection of the above mentioned enzymes from free radicals. Thus, this increment in serum T_3 and T_4 levels due to dietary Vit C supplementation reflect the alleviation in heat-stress in the treated ewe. However, similar findings were observed and supported by Ghanem, (2005) who stated that Vit C supplementation may alleviate stress in Awassi sheep.

At the same time, the DY group showed an increase in serum T_3 level was significantly at the 4th week and non-significantly at the 2nd and the 6th week, meanwhile due to dietary DY supplementation a significant increase in serum T_4 at all weeks except at the 6th week which showed only a non-significant increase in serum T_4 level,. These results were agreed with Sarwar *et al.* (2010) who found that Lambs fed diets with probiotic had higher T_4 level than those fed diets without additives, and a higher T_3 level was also noticed in lambs fed diets containing probiotics than those fed ionophore.

Concerning serum cortisol level, the obtained data indicated there is a significant reduction in serum cortisol level in premating dietary Vit C supplementation group all over the trial, that may be due to the impact of Vit C to reduce the magnitude of hyperthermia caused by heat stress. This finding was in accordance with Sivakumar *et al.* (2010) who ststed that the supplementation of Vit C had a negative effect on cortisol levels during heat stress, which relived the severity of heat stress in goats. The blood cortisol level is generally considered as a reliable physiological index for determining the animal response to stress, as a consequence of the activation of the hypothalamic-pituitary-adrenal axis, heat stress causes an increase in cortisol production (Louise, 2003 and Silanikove, 2000). Bouraoui *et al.* (2002) found a positive correlation between temperature-humidity index (THI) and cortisol concentration that support the rise in cortisol level in the control group in the present study. On the same hand, DY supplementation decreased significantly the serum cortisol level during the 2 nd week and non-significantly at the 4th and the 6th weeks of the treatment. These results were in accordance with those reported by Messaoudi *et al.* (2011) who noticed that probiotics directly decreased the cortisol levels by improving the gut flora that affects the brain neurotransmitters that produced in the gut. There is a well established link between stress, central nervous system and gastrointestinal disease. Additionally, probiotis decreases the unhealthy pathogens in the gut helping to get rid of inflammation symptoms and inflammation biomarkers (C- reactive proteins) which lead to less depression (Messaoudi *et al.*, 2011).

In the present study dietary Vit C supplementation to heat stressed ewes prevent weight loss as compared with the control. This result was supported by Haliloglu and Serpek (2000) who found that Vit C supplementation increased the body weight of pregnant ewes and newly born lambs. A possible explanations for such improvement in body weight, is the elevation of glucose and protein levels Abdul Kareem (2011), that may be due to reduction of oxidative stress produced by heat stress (Bruno *et al.*, 2006). Also, Vit C increases food consumption and food conversion and causes an increase in thyroid gland secretion resulted in an increase metabolic rate and thus improves body weight (Al- Katib 2001). In contrast DY supplementation did not improve body weight of the treated ewes as their initial weight. These results are in accordance with those reported by Mukhtar *et al.* (2010) who reported that the initial, final and daily weight gain, were non significant due to yeast culture supplementation for growing lambs. An opposite trend was reported by komonna (2007) Helal and Abdel-Rahman (2010) who found that addition of yeast culture of diets of ewes during nursing period resulted in improving its feed utilization and resulted in satisfactory ewe live weight and lamb growth rate. The confliction in the results concerning the effect of DY on body weight and weight gain may be attributed to facts indicating that efficiency of probiotics differ depending upon the probiotic dose rate, species and age of animals, viable yeast cell number, diets composition, strains of yeast and the season of the treatment.

CONCLUSION

The results of the present study revealed that Vit C offers a thermo- protective role that lessen the destructive effects of heat stress in breeding ewes, and improved the conception rate, physiological parameters, antioxidant biomarkers and endocrine responses. So, Vit C can be used as anti-heat stressors to breeding ewes in the premating period as it more economically and favorable than live dried yeast under these conditions.

REFERENCES

Abdel-Hafez, M.A.M. (2002): Studies on the reproductive performance in sheep. Ph.D. thesis. Faculty of Agriculture, Zagazig University, Zagazig, Egypt.

Abdul Kareem, A.B. (2011): Effect of vitamin C on haematology and serum biochemistry in heat-stressed sheep. ROAVES, 1(11), 731-733.

Abdel-Khalek, A.E. (2003): Productive and reproductive performance of primiparous and multiparous Friesian cows fed rations supplemented with yeast culture (Yea-Sacc 1026). Egyptian J. Nutr. And Feeds, 6 (Special Issue): 1095–1105.

Abdel-Latif, M.A. (2005): Physiological and nutritional studies on reproduction in diary cattle. Ph.D. Thesis, Fac. Agric., Mansoura Univ.

Abdel Rahman, H.; Baraghit, G.A.; Abu El-Ella, A.A.; Omar, S.S.; Faten, F. Abo Ammo, and Kommona, O.F. (2012): Physiological responses of sheep to diet supplementation with yeast culture. Egyptian Journal of Sheep Goat Sciences, Vol.7 (1), P: 27-38.

Abeni, F.; Calamari, L. and Stefanini, L. (2007): Metabolic conditions of lactating Friesian cows during the hot season in the Po valley. 1. Blood indicators of heat stress. Int. J. Biometeorol., 52: 87-96.

Ait-Belgnaoui, A.; Eutamene, H.; Houdeau E.; Han, W.; Fioramonti, J.; Bueno, L. and Theodorou, V. (2009): Lactobacillus farciminis treatment attenuates stress-induced overexpression of FOS protein in spinal and supraspinal sites after colorectal distension in rats. Neurogastroenterol Motil; 21(5): 567-73.

Alameen, A.O. and Abdelatif, A.M. (2012): Metabolic and Endocrine Responses of

Crossbred Dairy Cows in Relation to Pregnancy and Season under Tropical Conditions. Am-Euras. J. Agric. & Environ. Sci., 12 (8): 1065-1074.

AL-Katib, S.R. (2001): Effect of vitamin C administration on serum cholesterol, triglyceride and thyroxin level in rats. Kufa Medical Journal, 4(1): 187-190.

Arechiga, C.F.; Vazquez-Flores, S.; Ortiz, O.; Hermandz-Ceron, J.; Porra, A.; McDowell, L.R. and Hansen, P.J. (1998): Effect of injection of beta-carotene or vitamin E and selenium on fertility of lactating dairy cows. Theriogenology 50, 65–76.

Aurousseau, B.; Gruffat, D. and Durand, D. (2006): Gestation linked radical oxygen species fluxes and vitamins and trace mineral deficiencies in the ruminant. Reprod. Nutr. Dev., 46: 601-620

Bernabucchi, V.; Ronchi, B.; Lacetera, N. and Nardone, A. (2002): Markers of oxidative status in plasma and erythrocytes of transition dairy cows during hot season. J. Dairy Sci. 85: 2173.

Bouraoui, R.; Lahmar, M.; Majdoub, A.; Djemali, A.M. and Belyea, R. (2002): The relationship of temperature-humidity index with milk production of dairy cows in a Mediterranean climate. Anim. Res., 51: 479-491.

Braverman, L.E. (1996): Evaluation Of thyroid status in patients with thyrotoxicosis.Clin.Chem.42.174-178.

Brzezinska-Slebodzinska, E. (2001): Fever induced oxidative stress: the effect on thyroid status and the 5'-monodeiodinase activity, protective role of selenium and vitamin E. J. Physiol. Pharmacologic. 52(2): 275-284.

Bruno, R.S.; Leonard, S.W. and Atkinson, J. (2006): Faster plasma vitamin E disappearance in smokers is normalized by vitamin C supplementation. Free Radical Biology and Medicine, 40 (4): 689-697.

Bucklin, R.A.; Turner, L.W.; Beede, D.K.; Bray, D.R and Hemken, R.W. (1991): Methods to relieve heat stress for dairy cows in hot, humid climates. Appl. Eng. Agric. 7: 241-247.

Burits, C.A. and Ashweed, E.R. (1994): Tietz Text book of clinical chemistry, 2nd Ed.W.B. Saunders Company. Philladiphia, pp 1825-27.

Castillo, C.; Hernandez, J.; López-Alonso, M.; Miranda, M. and Benedito, J.L. (2003): Values of plasma lipid hydro peroxides and total antioxidant status in healthy dairy cows: preliminary observations. Arch. Tierz., 46: 227-233.

Dawson, K.A. (1990): Designing the yeast culture of tomorrow. Mode of action of yeast culture for ruminants and non ruminants. In Biotechnology in Feed Industry. The Proc. of 6th Ann. Symp. of Alltech. Alltech Tech. Publ. Unit, Kentucky, USA, P: 59.

Dawson, K.A. (1994): Current and future role of yeast culture in animal production. A review of research over the last six years. Proc. Alltech's 8th Ann. Symp. (Suppl.) Alltech. Tech. Publ., Kentucky, USA.

Ebrahim, S.E.A. (2004): Physio-nutritional studies on Egyptian buffaloes. Ph. D. Thesis, Fac. Agric. Mansoura Univ., Egypt.

El-Ashry, M.A.; Fayed, A.M.; Youssef, K.M.; Salem, F.A. and Aziz, H.A. (2003): Effect of feeding flavomycin or yeast as feed supplement on lamb performance in Sinai. Egyptian J. Nutr. and Feeds, 6 (Special issue) : 1009–1022.

El-Shaer, E.K.H.I. (2003): Nutritional studies in ruminants. "Effect of yeast culture supplementation and concentrate: roughage ratio on performance of growing lambs." Ph. D. Thesis, Fac. Agric., Mansoura Univ., Egypt.

Ganong, W. F. (2001): Text book of review of medical physiology. Lange Medical books/Mc Graw hill Medical Publishing division, pp. 246.

Ghanem, A. (2005): The effect of vitamin C supplementation on some physiological and immunological indicators in water-deprived Awassi ewes. Thesis. American University of Beirut.

Goud, A.P.; Goud, P.T.; Diamond, M.P.; Gonik, B. and Abu-Soud, H.M. (2008): Reactive oxygen species and oocyte aging: role of superoxide, hydrogen peroxide, and hypochlorous acid. Free Radic. Biol. Med., 44: 1295-1304.

Habig, W.; Pabst, M. and Jakoby, W. (1974): Biological assay kit measures total GST activity.W.J.Biol.Chem. 249, 7130-7139.

Haliloglu, S. and Serpek B. (2000): The effects of plasma vitamin C and ceruloplasmin levels and exogen vitamin C supplementation on reproduction in sheep. Turk J Vet Anim Sci, 24, 403-411.

Hansen, P.J. (1997): Effects of environment of bovine reproduction. In: Young Quist RS (ed.), Current Therapy in Large Animal Theriogenology. W.B. Saunders, Philadelphia, pp. 403–415.

Harris, L.J. and Rays, N. (1935): Determination of Vit C Lanat 1(71): 462.

Helal, F.I.S. and Abdel-Rahman, K.A. (2010): Productive performance of lactating ewes fed diets supplementing with dry yeast and/or bentonite as feed additives. World J. Agric. Sci. 6(5): 489-498.

Kommonna, O.F.A. (2007): Phsiological and nutritional responses of sheep to some feed additives. Ph.D. Thesis, Fac. Agric., Minufiya Univ., Egypt.

Koracevic, D. and Koracevic, G. (2001): J clin. Pathol. 54:356-361.-Miller, J.E., J.A.

Loven, D.P. (1988): A role for reduced oxygen species in heat induced cell killing and the induction of thermo tolerance. Med Hypotheses 26, 39–50.

Louise, N. (2003): Postpartum changes in hormones and metabolites during early lactation in summer and winter calving Holstein cows. MS Thesis, Department of Animal Science, Graduate Faculty of North Carolina State University, Raleigh, NC, USA.

Marai, I.F.M.; El-Darawany, A.A.; Fadiel, A. and Abdel-Hafez, M.A.M. (2007): Physiological traits as affected by heat stress in sheep, a review, Small Ruminant Research 7, 1 1–12.

Marai, I.F.M.; El-Darawany, A.A.; Abou-Fandoud, E.I. and Abdel-Hafez, M.A.M. (2004): Reproductive traits and the physiological background of the seasonal variations in Tropical and Subtropical Agroecosystems, 8: 209–234.

Marai, I.F.M.; Bahgat, L.B.; Shalaby, T.H. and Abdel-Hafez, M.A. (2000): Fattening performance, some behavioural traits and physiological reactions of male lambs fed concentrates mixture alone with or without natural clay, under hot summer of Egypt. Annals of Arid Zone, 39: 449–460.

Matsui, T. (2012): Vitamin C Nutrition in Cattle .Asian-Aust. J. Anim. Sci.,25, NO.5:597-605

McDowell, L.R. (1989): Vitamin C Pages 365-387 in vitamins in Animal Nutrition. L.R. McDowell, ed. Academic Press, San Diego, CA.

Messaoudi, M.; Lalonde, R.; Violle, N.; Javelot H.; Desor, D.; Nejdi, A.; Bisson, J.F.; Rougeot, C.; Pichelin, M.; Cazaubiel, M. and Cazaubiel, J.M. (2011): Assessment of psychotropic-like properties of a probiotic formulation (Lactobacillus helveticus R0052 and Bifidobacterium longum R0175) in rats and human subjects. Br J Nutr. 2011 Mar; 105(5): 755-64.

Maziar Mohiti Asli, Seyed Abdollah Hosseini, Houshang Lotfollahian and Farid Shariatmadari (2007): Effect of Probiotics, Yeast, Vitamin E and Vitamin C Supplements on Performance and Immune Response of Laying Hen During High Environmental Temperature International Journal of Poultry Science 6 (12): 895-900.

Mousa, Kh.M.; El-Mallky, O.M.; Kommona, O.F. and Rashwan, S.E. (2012): Effect of some yeast and minerals on the productive and reproductive performance in ruminants. Journal of American Science, 8 (2), 291-303.

Mukhtar, N.; Sarwar, M.; Nisa, M.U. and Sheikh, M.A. (2010): Growth response of growing lambs fed on concentrate with or without ionophores and probiotics. Int. J. Agric. Biol., 12: 734–738.

Muzzaffari, E.L. and Gharib, H. (1998): Thyroxin suppressive therapy in patients with nodular thyroid disease. Ann Intern Med; 128, 386-394.

Natarajan, R.; Bhawani, S.M. and Munuswamy, D. (2010): Effect of L-ascorbic acid supplementation at different gaseous environments on in vitro development of preimplantation sheep embryos to the blastocyst stage. Anim. Reprod., v.7, n.1, p.21-28.

Niswender, G.D.; Juengel, J.L.; Silva, P.J.; Rollyson, M.K. and McIntush, E.W. (2000): Mechanisms controlling the function and life span of the corpus luteum. Physiological Reviews. 80 (1): 1-29.

Ohkawa, H.; Ohish, W. and Yag, I.K. (1975): Determination of malondialdehyde. A. nal Biochem. , 95:351.

Padilla, L.; Matsui, T.; Kamiya, M.; Tanaka, M. and Yano, H. (2006): Heat stress decreases plasma vitamin C concentration in lactating cows. Livest. Sci. 101: 300-304.

Padilla, L.; Matsui, T.; Ikeda, S.; Kitagawa, M. and Yano, H. (2007): The effect of vitamin C supplementation on plasma concentration and urinary excretion of vitamin C in cattle. J. Anim. Sci., 85: 3367-3370.

Pepperell, J.R.; Porterfield, D.M.; Keefe, D.L.; Behrman, H.R. and Smith, P.J.S.(2003): Control of ascorbic acid efflux in rat luteal cells: role of intracellular calcium and oxygen radicals. American Journal of Physiology-Cell Physiology. ,285 (3): C642-C651.

Petroff, B.K.; Dabrowski, K.; Ciereszko, R.E. and Ottobre, J.S. (1997): Total ascorbate and dehydroascorbate concentrations in porcine ovarian stroma, follicles and corpora lutea throughout estrous cycle and pregnancy. Theriogenology, 47 (6): 1265-1273.

Rajaraman, V.; Nonnecke, B.J.; Franklin, S.T.; Hammell, D.C. and Horst, R.L. (1998): Effect of vit A and E on nitric oxide production by blood mononuclear leukocyte from neonatal calf fed milk replacer .J.Dairy Sci., 81: 3278-3285.

Santos, J.E.P.; Thatcher, W.W.; Chebela, R.C.; Cerria, R.L.A. and Galvaoa, K.N. (2004): The effect of embryonic death rates in cattle on the efficacy of estrus synchronization programs. Animal Reproduction Science, 82(83): 513-535.

Sarwar, M.; Mukhtar, N.; Shahzad, M.A. and Nisa, M. (2010): Traditional versus high input feeding system: impact on nutrients intake, blood dynamics, hormonal profile, weight gain and economics in growing lamb. Eg. J. of Sh. & G. Sci., Vol. 5 (1), P: 127- 145.

Snedechor, G.W. and Cochran, W.G. (1982): In. Statistical Methods,8th Edition, Iowa state university press, Ames, Iowa -50010.

Shelton, M. (2000): Reproductive performance of sheep exposed to hot environments. In: Malik, R.C., Razzaque, M.A., Al-Nasser, A.Y. (Eds.), Sheep Production in Hot and Arid Zones. Published by the Kuwait Institute for Scientific Research, pp. 155–162.

Sivakumar, A.V.N.; Singh, G. and Varshney, V.P. (2010): Antioxidant supplementation on acid base balance during heat stress in goats. Asian- just J. Anim. Sci .11: 1462-1468.

Silanikove, N. (2000): Effects of heat stress on welfare of extensively managed domestic ruminant a review. Livest. Prod. Sci., 67: 1-18.

Strzetelski, J. (1996): Modern principles and methods of fattened cattle nutrition. Instytut Zootechniki biuletyn Informacyjny, 34: 45.

Sumeghy, L. (1995): Production – oriented veterinary management and its results on the Holstein. Friesian dairy farm of Mczohegyes. Stud-Estate Co. Magyar Allatorvosok Lapja, 50: 529.

Tanaka, M.; Miyazaki, T.; Tanigaki; S.; Kasai, K.; Minegishi, K.; Miyakoshi, K.; Ishimoto, H. and Yoshimura, Y. (2000): Participation of reactive oxygen species in PGF2α-induced apoptosis in rat luteal cells. Journal of Reproduction and Fertility.,120 (2): 239-45.

Usha, R. Deshpande; Joseph, L.J.; Patwardhan, U.N. and Samuel, A.M. (2002): Effect of Antioxidants (Vitamin C, E and Turmeric extract) on methimazole induced hypothyroidism in rats. Indian J. Exp. Biol. 40: 735-738.

Yousef, H.M. (1985): Studies on some environmental factors affecting production and reproduction in some farm animals. M.Sc. thesis. Faculty of Agriculture, Zagazig University, Zagazig, Egypt.

تأثير استخدام فيتامين سى أو الخمائر الجافة كوسيلة لتخفيف الاجهاد الحرارى على النعاج لتحسين معدل الإخصاب فى فصل الصيف

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أجريت هذه الدراسه للمقارنة بين تأثيرإستخدام كل من الخمائر الجافة وفيتامين سى كمضادات للأجهاد الحرارى أثناء فترة التناسل على معدل الخصوبه ومستوى كل من مضادات الأكسدة وهرمون الكورتيزول وهرمونات الغدةالدرقية ووزن النعاج المعرضه للإجهاد الحرارى. لهذا الغرض تم إستخدام عدد ٢٤ نعجه بالغة تم تقسيمهم عشوائياً على ٣ مجموعات المجموعه الأولى ضابطه: تتغذى على العليقه الأساسيه بدون إضافات وفى المجموعه الثانيه يضاف للعليقه ٧٥مللجرام من فيتامين سى لكل كيلوجرام من وزن كل نعجه يومياً لمدة أسبوعين قبل حدوث الشبق والتلقيح الطبيعى و٤ أسابيع بعده والمجموعه الثالثه يضاف للعليقه ٥مللجرام من الخميرة الحيه (البروبايوتك) (سكاروميزس سيرفيزى) يومياً لكل نعجه لمدة ٦ أسابيع بنفس النظام السابق. تم تجميع عينات الدم مره كل أسبوعين حتى نهاية التجربه. وزن الحيوانات قبل وبعد التجربه ومتابعة درجات الحراره ومعدل التنفس لكل المجموعات. أوضحت النتائج وجود إختلافات معنويه فى مجموعة النعاج المعالجه بفيتامين سى عن المعالجه بالخمائر والمجموعه الضابطه فى معدل الخصوبه والوزن وزيادة نسبة الجلوتاثيون ونقص مستوى الأجسام الشارده فى الدم. إلى جانب وجود إختلافات معنويه فى المجموعتين المعالجتين عن المجموعه الضابطه فى معدل التنفس ودرجة حرارة الجسم ونشاط الغدة الدرقيه ومستوى الكورتيزول فى الدم لذلك ننصح بإضافة فيتامين سى لعلائق للأغنام عند التعرض لدرجات الحراره العاليه وإرتفاع نسبة الرطوبه لمواجهة الآثار الضارة والمدمرة للحرارة على الوظائف الحيويه المختلفه والتغلب عليها وتحسينها.