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**Short Communication**

# Influence of Vitamins and Exogenous Enzymes Combination on alleviating Heat Stress in Lactating Ewes under Egyptian Summer Conditions

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## ABSTRACT

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The present experiment was designed to study the effects of vitamin mixtures and exogenous enzymes ZADO® in alleviaties heat stress in ewes in summer temperatures (25-44°C) compared with winter temperatures (8-22°C). Fifty lactating Ossimi ewes were divided randomly into 5 treatment groups. In summer, the experiment included four treatments: (i) control i.e., normal summer conditions, no supplements (control 2), (ii) ewes supplemented with 10 g/head/day of ZADO® mixed in the concentrate, (iii) ewes supplemented with vitamin $AD_3E$ (250 mg/kg BW) and C (45 mg/kg BW) mixture, and (iv) ewes supplemented with ZADO® and vitamin mixture as above. In winter, the treatment comprised of assessments only under normal winter condition (8 to 22°C) without any additives (control 1). Blood and milk samples from each ewe were collected biweekly throughout lactation. Results indicate that plasma total protein, total lipids and glucose, and milk production and composition were decreased ($P<0.01$) in summer compared with winter. Addition of ZADO® increased plasma total protein, glucose, milk production, protein and lactose as compared with summer control (control 2). Vitamin mixture increased ($P<0.01$) plasma total protein, milk production and milk protein, but had no effect on total lipids, glucose, milk fat and lactose. Combination of vitamin mixture with ZADO® addition increased the ability of lactating ewes to alleviate heat stress as evident by increased plasma parameters and milk production during the summer season.

**Key words:** Enzymes, Ewes, Milk composition, Milk yield, Vitamins.

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## INTRODUCTION

Heat stress is one of the limiting factors for dairy production in hot climate. Abdalla *et al*. (1993) reported that climatic heat is one of the major constraints for growth, milk production and reproduction which are impaired as a result of the drastic changes in biological functions caused by heat stress. Heat load conditions may induce declines in milk yield, milk fat and protein contentsin lactating sheep. Sevi *et al*. (2002) showed a marked increase in rectal temperature, a metabolic alteration, and a reduction of milk yield after ewes were exposed to daily temperatures of 35°C for short time or to 30°C for prolonged period. Johnson *et al*. (1987) concluded that a decline in voluntary feed intake was the key factor in heat-induced reduction of milk yield, milk fat and milk protein content in dairy cows. Olsson and Hydbring (1996) found that milk lactose concentration was not affected during heat stress, while Barash *et al*. *(*2001) indicated that, during the hotter months of the summer, high-producing cows are more affected by heat stress. Finocchiaro *et al*. (2005) found that a better phenotypic correlation of 0.93 between heat stress and high temperature with daily milk yield. In fact, as the temperature exceeded 30°C, the decline in yield was greatest when relative humidity was low. The aim of this study was to evaluate the effects of vitamin mixtures and an exogenous enzyme preparation (ZADO®) combination for alleviating heat stress in ewes during the Egyptian summer temperatures interms of milk production and composition.

## MATERIALS AND METHODS

The present study was carried out at the experimental research station located in Shalakan, Qalyobia governorate and the Laboratories of Animal Physiology and Nutrition at the Animal Production Department, Faculty of Agriculture, Ain Shams University, Cairo, Egypt. The mean ambient temperature ranged between 25°C and 44°C in the summer months and between 8°C and 22°C in the winter months. Overall mean temperature was 38.5°C in the summer versus 17.5°C in the winter. The relative humidity averaged 76 and 56.5% in the summer and winter, respectively.

Fifty lactating Ossimi ewes of 35 to 40 kg BW and in the 3$^{rd}$ lactation were divided randomly into 5 treatment groups, and used in the experiment during both winter and summer seasons. The experiment in winter season comprised of assessment of the studied traits only under normal winter condition (8 to 22°C) without any additives (Control 1). In summer, the experiment included four treatments; the first served as control under the normal summer condition (Control 2- without any supplements), the second treatment was supplemented with 10 g/head/day of ZADO® [a mixture of digesting enzymes such as alpha amylase (6l.5 unit/g), protease (29.1 unit/g), cellulase (7,1 unit/g) and xylanase (2.3 unit/g)]. The enzyme preparation ZADO® was mixed with the concentrate feed. The third treatment was supplemented with vitamins mixture ($AD_3E$ and C) and the dosage of vitamin C was 45 mg/kg BW and that of $AD_3E$ was 250 mg/kg BW. The vitamins powder was dissolved in 100 ml of water and orally administered weekly. The fourth treatment was supplemented with ZADO® and vitamins mixture. The data of treatment in winter season were considered as base to the information of the other treatments tested during summer.

All animals were fed a maintenance ration formulated according to NRC (1985) which contained (per kg) 520g corn, 150g wheat bran, 200g soybean meal, 100g beet pulp and 7g dicalcium phosphate, 10g sodium chloride, 3g mineral mix and 10g limestone. Feed was offered twice daily, while drinking water was available all time. The houses were sanitized every week.

Blood samples (10 ml) were collected biweekly in heparinized tubes from the jugular vein. These samples were centrifuged at 3000 rpm for 15 min to separate blood plasma and plasma glucose was determined according to Siest *et al.* (1981). The rest of blood plasma was transferred into clean dry glass vials and stored at -20ºC for subsequent analysis. Total protein was determined as described by Gomall (1949), and albumin according to the method of Doumas *et al.* (1971). Plasma globulin was calculated by subtracting albumin from total protein, and albumin globulin ratio calculated. The concentration of plasma total lipids was determined as described by Nelson and Freeman (1959).

Milk samples from each ewe were collected biweekly throughout the lactation period. Milk samples (20 ml) were taken at 08:00 hrs in the morning before feeding and watering and stored at -20°C for subsequent analysis. Samples were analysed for protein, fat, and lactose using a LactoScandevice (Meganetco, India) in the milk laboratory at Food Science Department, Faculty of Agriculture, Ain Shams University.

Milk energy was calculated based on measured production characteristics according to Tyrell and Reid (1965): milk energy (MJ/kg)=4.184 x [{(41.63 Fat) + (24.13 Protein) + (21.60 Lactose) – 11.72} / 1000] x 2.204; where, fat, protein and lactose in the milk were in g/kg milk.

The data were analysed using randomized complete design. To test the difference between treatments, a two-way analysis of variance (ANOVA) was performed. In case of any significance, means were separated by Duncan's Multiple Range Test (Duncan, 1955). The least square analysis of variance was performed on the data using the SAS (2001) general linear model (GLM). The proposed model used was: $Y_{ij} = \mu + G_i + e_{ij}$

Where, $Y_{ij}$=the observation of the $1^{th}$ animal in the ith treatment; $\mu$=the overall mean; $G_i$=the effect of $i^{th}$ treatment, i= 1, 2, 3, 4, 5; $e_{ij}$=the effect of random error associated with the $1^{th}$ individual assumed normal distributed.

## RESULTS

Total protein, albumin, globulin and albumin/globulin ratio decreased ($P<0.01$) in ewes under summer conditions (Control 2) in comparison with winter ewes (Control 1). In ewes supplemented with vitamin mixture exhibited increased ($P<0.01$) total protein, albumin, globulin and albumin/globulin ratio. Plasma protein increased due to the use of enzyme additives, while the addition of ZADO® product plus vitamin mixture did not affect on plasma total lipids when compared with the summer control (Control 2). The ewes treated with vitamin mixture showed increased total lipids was compared to the summer control ewes. The plasma glucose concentrations in the ewes found to be decreased ($P<0.05$) with vitamin mixture supplementation, while addition of ZADO® increased ($P<0.05$) glucose concentration in plasma compared with ewes under summer conditions (Control 2) and other treatments. Vitamin mixture with or without ZADO® did not alter glucose concentration (Table 1).

Milk production as well as its composition in terms of protein, fat and lactose decreased ($P<0.05$) in summer compared to winter. Milk production, milk protein, and lactose were however found to be increased ($P<0.05$) in ewes supplemented with

Table 1. Blood metabolites of Ossimi ewes supplemented with exogenous enzyme and/or vitamin mixture during lactation period in summer and winter seasons

| | Winter | Summer | | | | | |
|---|---|---|---|---|---|---|---|
| | Control 1 | Control 2 | ZADO® | Vitamin mixture | ZADO®+ Vitamin mixture | SEM | P value |
| Total protein (g/dl) | 8.1[a] | 6.5[c] | 7.4[b] | 8.1[a] | 8.3[a] | 0.03 | <0.01 |
| Albumin (A, g/dl) | 4.8[a] | 3.4[c] | 4.5[b] | 4.4[b] | 4.5[b] | 0.04 | <0.01 |
| Globulin (G, g/dl) | 3.2[b] | 3.1[b] | 2.9[c] | 3.8[a] | 3.8[a] | 0.03 | <0.01 |
| A:G ratio | 1.6[a] | 1.2[b] | 1.6[a] | 1.2[b] | 1.2[b] | 0.02 | <0.01 |
| Total lipids (mg/dl) | 433.9[a] | 284.9[c] | 294.0[c] | 333.1[b] | 297.4[c] | 0.48 | <0.001 |
| Glucose (mg/dl) | 86.5[a] | 58.5[c] | 87.4[a] | 75.5[b] | 75.4[b] | 0.19 | <0.05 |

[abc]Means with different superscripts in the same row are significantly different ($P<0.05$).

Control 1: Mean ambient temperature ranged between 8°C and 22°C.

Control 2: Mean ambient temperature ranged between 25°C and 44°C.

Table 2. Milk production and composition of Ossimi ewes supplemented with exogenous enzyme and/or vitamin mixture during lactation period in summer and winter seasons

| | Winter | Summer | | | | | |
|---|---|---|---|---|---|---|---|
| | Control 1 | Control 2 | ZADO® | Vitamin mixture | ZADO®+ Vitamin mixture | SEM | P value |
| Milk production (g/d) | 855.7[a] | 656.7[c] | 744.7[b] | 885.8[a] | 893.6[a] | 13.82 | <0.05 |
| Milk protein (g/l) | 38.4[a] | 28.9[c] | 31.4[b] | 37.7[a] | 37.9[a] | 0.184 | <0.01 |
| Milk fat (g/l) | 42.5[a] | 38.6[b] | 39.3[b] | 39.1[b] | 39.4[b] | 0.17 | <0.01 |
| Milk lactose (g/l) | 42.9[a] | 38.6[b] | 42.5[a] | 39.1[b] | 42.7[a] | 0.144 | <0.05 |
| Milk energy (MJ/kg) | 33.3 | 28.8 | 30.4 | 31.1 | 31.9 | - | - |

[abc]Means with different superscripts in the same row are significantly different ($P<0.05$).

Control 1: Mean ambient temperature ranged between 8°C and 22°C.

Control 2: Mean ambient temperature ranged between 25°C and 44°C.

ZADO® compared with Control 2. Milk fat was not affected by the addition of ZADO®. The addition of vitamin mixture increased ($P<0.05$) milk production, milk protein, milk energy compared with ZADO® supplementation and summer control (Control 2) but did not affect milk fat and lactose (Table 2).

## DISCUSSION

Total protein, albumin, globulin level and albumin-globulin ratio were decreased ($P<0.01$) in ewes plasma of summer conditions (Control 2) in comparison with winter ewes (Control 1). These results were similar to those found in similar conditions during study carried out by Ballantyne and Fleck (1973). In contrast, Antunovic *et al.* (2002) reported that in summer, higher blood concentrations of total proteins in ewes were detected compared with the ewes winter season. The increases in the total protein, albumin, globulin and albumin/globulin ratio in ewes treated with vitamin mixture are supported by other results carried out by McDowell (1989) and Sahin *et al.* (2002). A number of studies reported a beneficial effect of vitamin C supplementation on protein and albumin concentrations. It has also been reported that serum total protein, albumin, globulin concentrations were increased when dietary vitamin C and folic acid were supplemented (Kutlu and Forbes, 1993).

Total lipids concentrations in the plasma Ossimi ewes were decreased ($P<0.01$) by heat stress of summer conditions. Consistent results were obtained by Abdel-Samee (1996) who reported that total lipid in winter ewes were higher than those in the other seasons. Addition of ZADO® plus vitamin mixture did not show significant differences in plasma total lipids when compared with summer control ewes. Although in ewes supplemented with vitamin mixture the total lipids increased ($P<0.05$) compared with summer ewes (Control 2). Vitamin E plays an important role in preventing oxidation of polyunsaturated fatty acids in the phospholipids and unsaturated lipid materials within cells (Mladenov *et al.*, 2006). In contrast, Kotze *et al.* (1974) demonstrated that vitamin C suppress the activity of the lipoprotein lipase. An inverse association between vitamin D status and lipid profiles has been supported in a number of studies (Holick, 2007; Hypponen *et al.*, 2008).

Plasma glucose concentrations in the ewes was decreased ($P<0.05$) in summer control compared with other treatments. Similar results were obtained in sheep (Achmadi *et al.*, 1993), which is in contrast with Garriga *et al.* (2006); Rhoads *et al.* (2009) and Wheelock *et al.* (2010), who reported increased serum glucose concentration in animals exposed to heat stress in summer season. The decrease in blood glucose under hot climate (summer season) is mainly attributed to high respiration rate and high utilization of glucose by the respiratory muscles. Addition of ZADO® increased ($P<0.05$) the plasma glucose compared with summer control and the other treatments. Vitamin mixture with or without ZADO® failed to elevate glucose values to its level in winter, but the mean value of glucose increased ($P<0.01$) compared with ewes under summer conditions. Comparable results were reported by Appel *et* *al*. (2001) who found beneficial effect of vitamin C supplementation on glucose. The metabolic defect of glucose can be corrected nutritionally by vitamins E and C (Swislocki, 1993). In contrast, Sahin *et al*. (2002) reported that serum glucose concentrations decreased when both dietary vitamin C and vitamin E were increased.

Milk production decreased ($P<0.05$) in summer (Control 2) compared with winter (Control 1). Similar results were obtained by Barash *et al*. (2001); Sevi *et al*. (2002) and Finocchiaro *et al*. (2005); they reported that climatic heat is the major constraint on milk production and reproduction which are impaired as a result of the drastic changes in biological functions caused by heat stress.

Milk production in groups treated with ZADO® with or without vitamin mixture was increased compared with summer control. Consistent results were obtained by Gado *et al*. (2009) who reported when ZADO® was supplemented daily to the cow ration in early lactation, milk production was increased due to positive effects on nutrient intake and digestibility, extent of ruminal fermentation and microbial protein synthesis.

Vitamin mixture additives increased ($P<0.05$) milk production compared with ZADO® and summer control. McDowell (1989), Abo El-Nor (2000) and Kim *et al*. (2002) reported that raising the level of vitamin A in serum would be effective to increase milk production of low producing animals. Several studies showed that administering vitamin E had positive effects on milk production in cattle (Kincaid *et al*., 2003; Pechova *et al.,* 2008).

Milk contents of protein, fat and lactose decreased ($P<0.05$) in summer control (Control 2) compared with winter (Control 1). Hang *et al*. (1982) reported that milk protein was decreased by 6% due to hot conditions of summer season. Habeeb *et al*. (1992) reported that milk fat yield by Friesian cows was reduced by 26.45% with concomitant decrease ($P<0.05$) of milk lactose by 28% during summer season compared with winter conditions in Egypt. The reduction in milk fat is proportional to the reduction in daily milk yield, triglycerol synthesis from glycerol and fatty acids, and decreased daily milk lactose due to summer hot conditions that led to decrease in daily milk yield. Environment can play a significant role affecting milk synthesis as well as milk fat synthesis (McGuire and Bauman, 2002). The decreased availability of plasma glucose could be the possible cause of lower percentage of milk lactose in Friesian cows under hot conditions (Ahn *et al*., 2003), while Olsson and Hydbring (1996) found that milk lactose concentration did not change during heat stress.

Milk protein, and lactose were increased ($P<0.05$) in ewes supplemented with ZADO® compared with summer control. Milk fat was not improved by addition of ZADO®. Addition of ZADO® has been reported to cause an increase in digestibility of feed, absorption and utilization of protein which consequently improved the N balance and daily N retention (Gado *et al*., 2007). Addition of ZADO® increased availability of plasma glucose that could be the possible cause of the higher milk lactose in ewes. Glucose is the primary precursor for synthesis of lactose, which controls milk volume by maintaining the osmolarity of milk and glucose uptake in the mammary gland (Wheelock *et al.*, 2010).

Vitamin mixture additives increased ($P<0.05$) milk protein compared with ZADO® and control groups in summer but did not improved milk fat and lactose. This is in agreement with the results of Abo EL-Nor (2000) who found that fat soluble vitamins supplementation enhances DM intake and nutrients digestibility. Sahin *et al.* (2002) reported that vitamin E had positive effects on milk production and milk protein in cattle.

Addition of vitamin ($AD_3E$ and C) mixture is effective in improving ewes performance; a combination of the above vitamin mixture and the exogenous multienzyme (ZADO®) could be used to alleviate the effect of heat stress by impacting some blood biochemical responses, increases in milk production.

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