

# INFLUENCE OF BETAINE AND ZINC SUPPLEMENTATION ON MILK PRODUCTION AND ITS COMPONENTS THERMOREGULATORY RESPONSES, BLOOD CHARACTERISTICS AND REPRODUCTIVE PERFORMANCE OF LACTATING COWS IN ARID SUBTROPICAL REGIONS

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## SUMMARY

The present study was conducted to investigate the impact of betaine (Bet) and Zinc sulphate (Zn) supplementation on milk production, blood parameters, and reproductive efficiency of cows during heat stress (HS). Cows were categorized into three groups as control group, Zn and Bet groups each group have six animals. Control group was fed a basal diet, whereas Zn and Bet groups were supplemented with either 200 mg/h/d Zn or 50 g/h/d Bet, respectively. Physiological parameters such as respiration rate (RR), pulse rate (PR), and body temperature (BT) were recorded. Blood samples were collected to estimate hemato-biochemical parameters and total anti-oxidant capacity (TAC). Milk yield and its chemical composition was recorded and analyzed weekly. The first service of conception rate, number of services per conception (NCR) and conception rate (CR) were recorded. Estradiol (E2) and progesterone (P4) hormones were measured. Results showed Bet and Zn groups led to decrease rectal temperature (RT), BT, RR and PR. However, Hemoglobin concentration (HB) increased significantly in treated cows, while hematocrit (HCT) elevated ( $P < 0.05$ ) by Zn supplementation. Bet and Zn groups increased significantly total proteins (TP) and globulin, while decreased ( $P < 0.05$ ) alanine aminotransaminase (ALT) and aspartate aminotransaminase (AST). The TAC in Zn and Bet groups was increased significantly compared to control group. Our study showed a significant increase in CR, E2 and P4 hormones in cows treated with Bet and Zn compared to the control. It was concluded that supplementation of Zn and Bet in the diet of cow's results in significant improvement in lactation and reproductive performance during HS.

**Keywords:** Heat stress, Blood parameters, Reproduction, Betaine, Zinc sulphate

## INTRODUCTION

Under thermal stress, dairy cattle are unable to removal body heat to prevent overheating (Zhang *et al.*, 2014). The summer season in the New Valley is characterized by high temperature-humidity index (THI) and intensity of solar radiation. Therefore, cattle are under such extreme heat stress (HS) for nearly 180 days of the year. In general, the reproductive performance of dairy cattle is affected by a decrease in feed consumption when the THI exceeds 72 (Armstrong, 1994). High THI affects productivity as it leads to decreased feed intake, feed efficiency, and milk production, leading to economic losses in dairy cattle (Collier *et al.*, 2008). Animals dissipate body temperature transfer heat to the environment out of their core surfaces (Zimbelman *et al.*, 2010). Through the environmental modification method, it is possible to increase the productive

performance of farm animal, but its cost is expensive. Feed additives may be a cost-effective way to reduce the effect of HS in beef and dairy cattle and can improve animal performances.

Betaine is an amino acid composed of glycine and three methyl groups that acts as a methyl donor in many metabolic pathways (NCBI, 2021). Additionally, Betaine is a recent compound that has found to ameliorate HS in cattle (Kondiba *et al.*, 2023, and Soliman *et al.*, 2023) and poultry (Chitrio *et al.*, 2023, and Sun *et al.*, 2023). Betaine has the ability to improve the performance of steers (DiGiacomo *et al.*, 2014), and it reduces heat shock protein (HSP) expression in goats exposed to HS (Dangi *et al.*, 2016). These studies indicate that betaine has the ability to reduce HS by reducing energy expenditure. Thus, maintaining osmotic balance and reducing metabolic heat production in animals facing HS.

Zinc is one of the most important trace elements that involved in a wide range of activities.

The main sources of zinc for animal feed are inorganic salts such as zinc oxide (ZnO), zinc chloride (ZnCl<sub>2</sub>), and zinc sulphate (ZnSo<sub>4</sub>) (Grace and Knowles, 2012). Alterations in the physiological functions and metabolism of cattle can help improve their productivity and welfare during HS (Lakhani et al., 2020). By adding Zn, we can adjust the metabolic status of cows to mitigate HS. Adding Zn to ruminant diets improves the reproductive performance of cows during HS (Kassab et al., 2020). Furthermore, Zn is an essential micronutrient, important for maintaining vital body functions such as metabolism and immunity and therefore important for animal health and growth performance (NRC, 2021).

There is an urgent need to propagate and improve climate resilient breeds to alleviate the effects of HS on reproductive and productive efficiency of dairy cows. However, research on the effects of Zn and Bet supplementation on physiological, productive performance and antioxidant status of native cows under HS in New Valley are poor. Therefore, this study was conducted to investigate the effect of dietary Zn and Bet supplementation on hematological and biochemical parameters, antioxidant status, milk production, and reproductive traits of native cows in arid subtropical regions subjected to HS.

## MATERIALS AND METHODS

This study was performed on a traditional farm in the El-Kharga oasis (25°27'36.0"N 30°32'49.8"E), this area is a part of New Valley governorate, Egypt.

### *Animals feeding system and experimental design:*

Eighteen postpartum multiparous lactating cows (Crossbred: Native and Frisian cows) with an average weight of 365.17 ± 2.95 kgs was used in this study. Cows were housed in open sheds. At 45 d postpartum, animals were divided into 3 groups (6 in each) of which the first group was served as a control (G1) without treatment. Cows in G2 and G3 were orally treated with zinc sulphate at a rate of 200 (mg/h/d) and 50 (g/h/d) betaine Hcl, respectively. All cows were fed on ration of concentrate mixture (CFM), fresh berseem (*Trifolium alexandrinum*; 2<sup>nd</sup> cut) and wheat straw (WS). While fresh water was freely available throughout the experiment. The contents of CFM were 15 % soybean meal, 27% wheat bran, 55% yellow corn, 1% NaCl and 2% limestone. Chemical analysis on dry matter basis of CFM, fresh berseem and wheat straw were shown in Table 1. Feeding period lasted approximately after 150 days.

**Table 1. Chemical composition of different feedstuffs used in feeding experimental cows**

Item	DM%	OM%	CP%	CF% %	Fat%	Ash%	NFE%
CFM	89.33	91.31	16.24	5.56	3.84	8.69	65.67
Fresh berseem	18.63	89.90	13.31	27.48	3.40	10.10	45.71
Wheat straw	91.85	92.71	2.61	42.64	1.66	7.29	45.80

CFM: Concentrate Feed Mixture, DM: Dry Matter, OM: organic matter, CP: Crude Protein, CF: Crude Fiber, NFE: Nitrogen Free Extract

### *Measurement of temperature and humidity index (THI):*

During the experiment, the temperature and humidity were recorded using automatic temperature and humidity recorders at 08:00 am and 02:00 pm. THI was calculated as followed:  $THI = (0.8 \times Ta) + [(RH/100) \times (Ta - 14.4)] + 46.4$  (Mader et al., 2006). Where; Ta °C is the temperature (°C), and RH is the relative humidity.

### *Experimental procedures:*

#### *Body temperature measurements:*

The thermoregulatory responses, RR and PR were determined every two weeks before measuring the body temperature; RR was measured by counting flank movements at 15 s and multiplying by 4 to obtain breaths/min (Al-Qaisi et al., 2020). RT, °C was recorded using a digital thermom). Skin °C was measured by portable infrared thermometer (IRT).

#### *Blood samples:*

Two blood samples were collected from the jugular vein, The first blood sample (2 ml/cow) was

collected in a clean tube containing EDTA for hematological analysis by jugular venipuncture in the morning (before feeding) during the experimental periods. Whole blood was analyzed for white blood cell (WBC) count, red blood cell (RBC) count, HB, HCT, mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin (MCHC) and platelet (PLT) concentrations were calculated by an automatic blood cell counter (*Dirui Bcc-3600*).

The 2<sup>nd</sup> blood samples (6 ml/cow) were collected in a clean tube and left to clot, then centrifuged at 3500 rpm for 10 minutes to separate serum which was stored at -20 °C until used for analytical procedures. Serum were analyzed for TP and albumin, glucose, total cholesterol, urea, creatinine, ALT and AST. The serum antioxidant index including TAC was also determined. All biochemical analyses were assay using spectrophotometer commercial kits (Bio di-agnostics, Cairo, Egypt). All chemicals used in this study were of analytical grade. Hormonal essay (estrogen and progesterone) were determined by enzyme immunoassay for the

quantitative determination of E2 and P4 concentrations, Biocheck, Inc. Foster City, CCA 94404 U.S.A.

**Milk samples:**

Cows were milked twice daily at 8.00 am and 6.00 pm. Cows were hand milking; daily milk production was recorded individually and fat-corrected milk (4%) was calculated according to the following equation of Gaine (1928):  $FCM \% = actual\ milk\ yield\ (kg) \times 0.4 + 15 \times fat\ yield\ (kg)$ .

Milk samples were analyzed to determine its composition using Milk-Scan (Model 133 B).

Calorific value (Kcal/100g) was calculated using the following equation:

$$Kcal/100g = (protein \% \times 4) + (fat \% \times 9) + (lactose \% \times 4)$$

(Salman et al., 2014).

**Estrus synchronization and Reproduction parameters:**

All of the cows received the double intramuscular injected by synthetic prostaglandin analogue PG named Estrumate® (Each ml of Estrumate contains 0.263 mg Cloprostenol Sodium.) Two doses 2 ml of PG, the first given on day 0 and the second administration 12 day later (Figure 1).

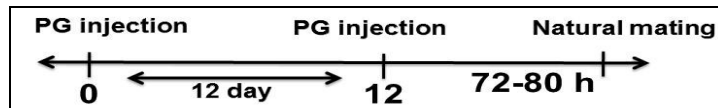


Figure 1: Estrous synchronization using prostaglandin.

Cows showed estrus have been mated with an intact bull at the appropriate time (12 hrs after the appearance of estrus signs). For two successive estrous cycles till pregnancy occur. Sixty days after successful last mating, cows were examined for pregnancy by using transrectal ultrasonography (ECM, Noveko International, Inc., Angouleme, France). The reproduction parameters studied during this experiment were, percentage of estrus, first service conception rate, CR, and number of services per conception (NSC). Estrus was detected and blood sample was taken to assay estradiol hormone. At day 12 (midestrus phase) blood sample was taken to assay progesterone hormone.

**Statistical analysis:**

Data were statistically analyzed using SAS v.9.1 (SAS, 2004). The differences among treatments were tested using Duncan's Multiple Range Test (Duncan, 1955).

The model used was  $Y_{ij} = \mu + A_j + e_{ij}$

$Y_{ij}$  = Observation traits,

$\mu$  = Overall mean,

$A_j$  = Experimental treatment

$e_{ij}$  = Random error

Conception rate and NSC were tested using Chi square test

**RESULTS**

**Meteorological measures:**

Ambient temperature °C, RH percent and THI through the trial periods were shown in Table (2) and Figure (1). The average AT °C through the trial periods ranged from 23.2° C to 29.2 ° C at 08:00 AM and from 40.8° C to 45.3° C at 02:00 PM. Moreover, the average RH% was 15% and 28% at 08:00 AM and from 12% to 16% at 02:00 PM. Furthermore, the THI values were 67.42 to 71.98 at 08:00 AM and 79.20 to 82.77 at 02:00 PM through the trial period.

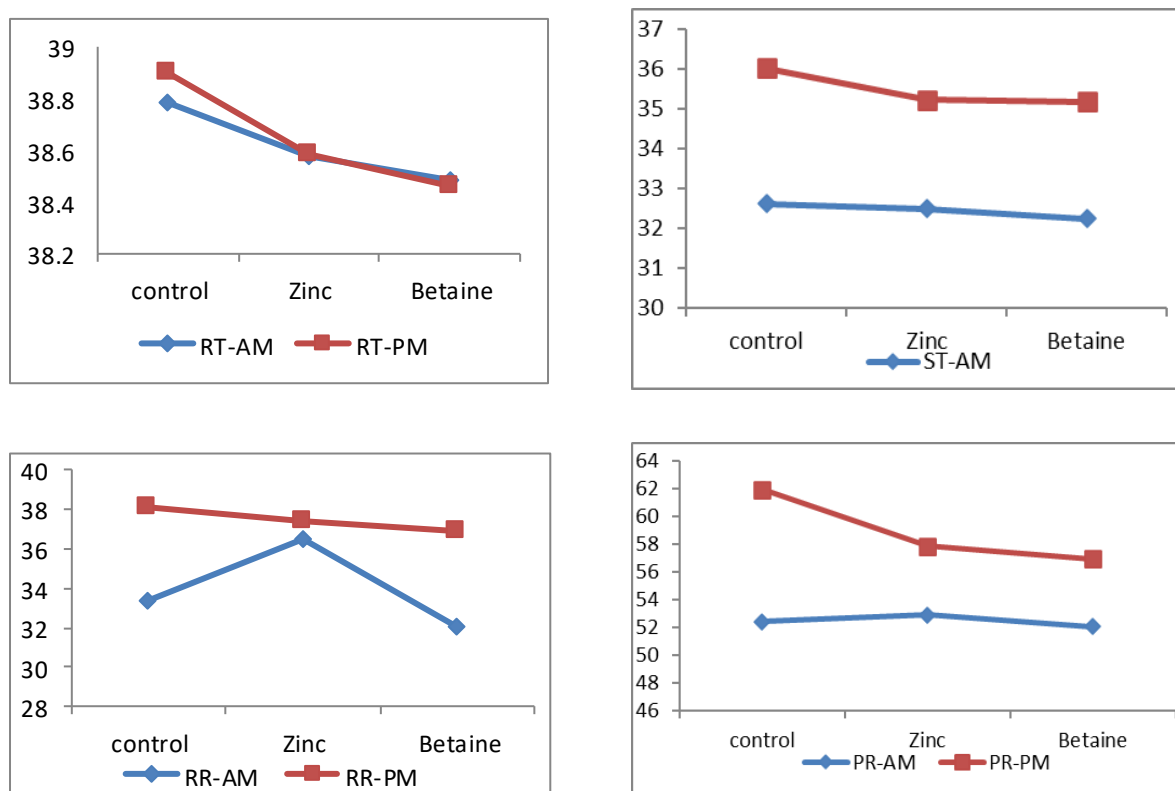
Table 2. Means of AT °C, RH %, and THI during the experimental period

Month	Time of day					
	08:00 AM			02:00 PM		
	AT, °C	RH, %	THI	AT, °C	RH, %	THI
May	23.2	28	67.42	43.5	16	81.36
	27.8	22	71.59	42.3	14	80.38
June	28.1	18	71.35	44.2	12	81.88
	27.6	16	70.59	45.3	13	82.77
July	29.2	15	71.98	45.0	12	82.52
	28.5	17	71.60	44.6	14	82.22
August	27.8	19	71.19	42.5	13	80.53
	28.2	15	71.03	42.3	12	80.36
September	28.0	18	71.25	41.5	14	79.74
	25.7	19	69.11	40.8	16	79.20

**Thermoregulatory response:**

Figure (2) showed that at 08:00 AM Bet and Zn groups led to decrease RT, ST, RR and PR. While caused a significant decrease (P<0.05) in RT and PR

at 02:00 PM in the treated animal compared to control. Our results show that Bet and Zn can improve thermoregulation in cows subjected to heat stress.



**Figure 2.** Effects of zinc and betaine supplementation on thermoregulatory responses of the experimental cows.

The blood indices of HB concentration, RBCs count, HCT, MCV, MCH, MCHC values, WBC, and platelet count are shown in Table (3). Concentration of HB increased ( $P < 0.05$ ) in cows treated with Bet and Zn. The percent of HCT elevated ( $P < 0.05$ ) by

zinc supplementation. On the other hand, RBCs, WBCs, erythrocytic values (MCV, MCH, and MCHC), and platelets were not affected by Zn and Bet treatments.

**Table 3.** Effect of zinc and betaine supplementation on hematological parameters of cows during the experimental period

Parameter	Experimental rations			SEM	P-values
	Control	Zinc	Betaine		
HB (g/dl)	10.52 <sup>b</sup>	11.91 <sup>a</sup>	12.08 <sup>a</sup>	0.166	<b>0.009</b>
RBC ( $10^6/\mu\text{l}$ )	6.82	7.45	7.18	0.095	0.171
HCT %	32.48 <sup>b</sup>	36.02 <sup>a</sup>	34.24 <sup>ab</sup>	0.399	<b>0.036</b>
MCV (fl)	46.99	46.87	46.04	0.460	0.829
MCH (p g)	17.23	17.26	16.70	0.209	0.705
MCHC (g/dl)	36.68	36.43	35.65	0.256	0.516
WBC ( $10^3/\mu\text{l}$ )	11.82	10.46	10.13	0.306	0.260
PLT	320.22	323.56	340.11	9.675	0.835

<sup>a,b</sup> Means in the same row lacking a common superscript difference ( $P < 0.05$ ). HB, hemoglobin concentration; RBC, red blood cell; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; WBC, White blood cells; PLT, Platelets

#### **Serum biochemical parameters:**

The present results indicated that Bet and Zn increased ( $P < 0.05$ ) concentrations of TP and globulin, while decreased ( $P < 0.05$ ) ALT activity in blood serum of treated cows (Table 5). AST activity exhibited a significant decrease ( $P < 0.05$ ) in the Bet

supplemented group as compared of those of Zn and control groups. On the other hand, no effect was observed on the concentrations of albumin, glucose, total cholesterol, urea-N and creatinine (Table 4). The result of the TAC of dairy cows are mentioned in (Table 4). The serum concentration of TAC in Zn

and Bet groups was significantly increased ( $p < 0.05$ ). In this regards, Bet and Zn supplementation improved ( $P < 0.05$ ) antioxidant activity in comparison

with the control. Zn treatment showed the highest impact of antioxidant status of cows compared with other groups.

**Table 4. Effects of zinc and betaine supplementation on blood biochemical parameters and antioxidant enzyme activity**

Parameter	Experimental rations			SEM	P-values
	Control	Zinc	Betaine		
Total protein (g/l)	7.23 <sup>b</sup>	7.44 <sup>a</sup>	7.60 <sup>a</sup>	0.033	<b>0.002</b>
Albumin (g/dl)	3.37	3.42	3.54	0.037	0.444
Globulin (g/dl)	3.60 <sup>b</sup>	4.04 <sup>a</sup>	3.98 <sup>a</sup>	0.051	<b>0.025</b>
Glucose (mg/dl)	55.89	58.11	59.56	0.788	0.431
Total Cholesterol (mg/dl)	174.55	167.18	169.76	1.792	0.515
<b>Liver function:</b>					
AST (U/l)	47.33 <sup>a</sup>	45.11 <sup>ab</sup>	43.89 <sup>b</sup>	0.369	0.021
ALT (U/l)	20.33 <sup>a</sup>	18.22 <sup>b</sup>	18.44 <sup>b</sup>	0.243	0.019
<b>Kidney function :</b>					
Urea-N (mg/dl)	25.57	25.20	25.39	0.309	0.951
Creatinine (mg/dl)	1.83	1.73	1.75	0.028	0.576
<b>Antioxidant index:</b>					
TAC (mmol/L)	0.642 <sup>b</sup>	0.803 <sup>a</sup>	0.775 <sup>a</sup>	0.019	0.035

<sup>a,b</sup> Means in the same row lacking a common superscript difference ( $P < 0.05$ ). AST, aspartates aminotransferase; ALT, alanine aminotransferase TAC, total antioxidant capacity

**Lactation performance:**

The total dry matter intake (TDMI), total solids (TS), milk fat and calorific value (CV) of Zn and Bet were significantly higher ( $p < 0.05$ ) than those of the

control group (Table 5). Compared to Zn and control groups, Bet had significantly increased ( $P < 0.05$ ) milk yield, FCM, solid not fat (SNF) and milk protein.

**Table 5. Effects of betaine and zinc supplementation on milk production and constituents of the experimental cows**

Parameter	Experimental rations			SEM	P-values
	Control	Zinc	Betaine		
Total number of cows	6	6	6	--	--
Average body weight, kg	362.67	363.67	369.17	2.951	0.670
<b>Average daily DM intake, kg /d:</b>					
CFM	4.25	4.25	4.25	--	--
Egyptian clover	2.80 <sup>b</sup>	2.91 <sup>ab</sup>	2.97 <sup>a</sup>	0.025	<b>0.023</b>
Wheat straw	3.23	3.32	3.29	0.022	0.242
TDMI	10.28 <sup>b</sup>	10.48 <sup>a</sup>	10.51 <sup>a</sup>	0.037	<b>0.022</b>
<b>Milk yield and Milk composition:</b>					
Milk yield, kg	7.950 <sup>b</sup>	8.175 <sup>ab</sup>	8.625 <sup>a</sup>	0.112	<b>0.046</b>
Milk yield 4% FCM, kg	7.170 <sup>b</sup>	7.897 <sup>ab</sup>	8.283 <sup>a</sup>	0.168	<b>0.017</b>
Total Solids, %	11.67 <sup>b</sup>	12.37 <sup>a</sup>	12.70 <sup>a</sup>	0.158	<b>0.018</b>
Fat, %	3.35 <sup>b</sup>	3.73 <sup>a</sup>	3.77 <sup>a</sup>	0.079	<b>0.055</b>
Solids not fat, %	8.31 <sup>b</sup>	8.6 <sup>ab</sup>	8.97 <sup>a</sup>	0.109	<b>0.050</b>
Protein, %	3.1 <sup>b</sup>	3.37 <sup>ab</sup>	3.5 <sup>a</sup>	0.067	<b>0.043</b>
Lactose, %	4.33	4.4	4.51	0.054	0.420
Ash, %	0.83	0.88	0.95	0.035	0.441
Calorific value (Kcal/100g)	59.88 <sup>b</sup>	64.97 <sup>a</sup>	65.67 <sup>a</sup>	0.949	<b>0.019</b>

<sup>a,b</sup> Means in the same row lacking a common superscript difference ( $P < 0.05$ ). CFM: concentrate feed mixture, TDMI: total dry matter intake, FCM: fat corrected milk

**Reproductive parameters:**

Date in table 6 showed that, Bet and Zn administration improved ( $P < 0.05$ ) the reproductive performance of cows in terms of increasing first service conception rate, CR percent and NSC in

comparison with control. Compared to control and Zn, bet group had significantly increased ( $p < 0.05$ ) serum concentrations of E2 and P4 hormones. In this respect, bet administration showed the highest impact on sexual hormones of cows.

**Table 6. Effects of betaine and zinc supplementation on reproductive traits and sexual hormones (estradiol and progesterone) of the experimental cows**

Parameter	Experimental rations		
	Control	Zinc	Betaine
Number of animals	6	6	6
Percentage of estrus %	100	100	100
First service of conception rate %	50 <sup>b</sup>	83.33 <sup>a</sup>	83.33 <sup>a</sup>
Conception rate %	83.33 <sup>b</sup>	100 <sup>a</sup>	100 <sup>a</sup>
Number of services per conception	1.67 <sup>a</sup>	1.17 <sup>b</sup>	1.17 <sup>b</sup>
<b>Reproductive hormones:</b>			
Estradiol concentration (pg/ml)	20.650 <sup>b</sup>	20.785 <sup>ab</sup>	21.648 <sup>a</sup>
Progesterone level (ng/ml)	12.588 <sup>b</sup>	12.933 <sup>ab</sup>	13.152 <sup>a</sup>

<sup>a,b</sup> Means in the same row lacking a common superscript differ.

## Discussion

### *Meteorological measures:*

THI values of less than 72 are normal, whereas, THI values from 72 to 78 are alarming, a THI values from 78 to 82 are dangerous and the values THI more than 82 are emergency (Du Preez et al., 1990). THI values in our study agree with Soliman et al. (2022) who found that THI during the period from May to September in the New Vally recorded from 79.43 to 85.46 at 02:00 pm, and these results indicate that animals are under HS. In addition, the mean THI values were found from 67.4 to 71.1 at 08:00 AM and from 76.5 to 80.53 at 02:00 PM during summer season in the New Valley (Kassab et al., 2023). The observed THI clear that cows were suffered from HS during the entire study period because it exceeded the upper critical limit for cows specially at evening according to (Du Preez et al., 1990).

### *Thermoregulatory parameters:*

The effect of Zn and Bet on heat-stressed cows had a clear impact when the AT was increased, at 02:00 pm as compared with at 8.00 am. In our study, supplementation of Bet and Zn helped to reduce HS in lactating cows. Moreover, results showed that Bet treatment recorded the highest impact of thermoregulatory responses of cows compared with Zn and control groups. These findings are consistent with those reported by Shankhpal et al. (2019) who found that RT, PR and RR were significantly lower at 02:00 pm in Bet group as compared to control group. They argued that physiological indicators stated that the buffaloes in the control group were more susceptible to HS compared to the supplemented groups. Hence, we accepted the hypothesis that Zn and Bet could reduce reverse the deleterious effects of HS on reproductive performance and improve the thermoregulatory responses to HS in animals.

In addition, the cows in the control group were unable to compete heat efficiently due to the higher THI, and the increase in RT. Although the mechanism by which Zn or Bet reduced RT and PR in the treated cows was not investigated in this study, it may be through an antioxidant mechanism by

enhancement TAC by 20% and 25% in Bet and Zn groups, respectively than a control group (Table 4), indicating Zn and Bet relieved the severity of oxidative stress in cows.

Fascinatingly, the RR and PR were significantly reduced by Bet, which may be explained by effects of antioxidant that are present in Bet on the cardiac autonomic nervous system. Whereas cows supplemented with Bet had decreased ST which reduces the acuteness of HS. The results showed that body temperature was significantly decreased with Bet and Zn supplementation. This trend may be related to the function of Bet and Zn helping animals relieve HS.

### *Blood hematological parameters:*

Hematological parameters are important for understanding the physiological and environmental interplay between environmental influences and blood characteristics (Ovuru and Ekweozor, 2004). In the present work, dietary Bet and Zn significantly ( $P < 0.05$ ) increased HB concentration. The present results are consistent with those reported by Kassab et al. (2021) who found that Bet increased ( $P < 0.05$ ) blood HB level in Angus cows. Similar result was observed by Abd-Elsattar (2018) in growing lambs.

The current results illustrated that, the percent of HCT elevated ( $P < 0.05$ ) by Zn supplementation. Whereas, RBCs, WBCs, MCV, MCH, MCHC, and platelets were not affected by Zn or Bet treatments within the normal range. These results are in agreements with those reported by Kassab et al. (2021) who observed that dietary Bet did not affect WBCs count and erythrocytic values (MCV, MCH, and MCHC), and platelets of cows. The improvement of the hematological parameters with Bet and Zn supplement will be reflected on the health status of animals and better nutrient utilization.

### *Serum biochemical parameters:*

Blood metabolites can offer important details about the healthy and physiological status. This study observed an improvement in protein metabolism, in terms of increasing TP concentration in cows as affected by Bet and Zn treatments. And also found a significant reduction in AST and ALT enzymes

activity of cows, as a marker of normality in liver function, whereas Zn and Bet also kept intact hepatic structure through the non-significant changes in serum hepatic injury biomarkers. In addition, a slight reduction was found in blood cholesterol in treated cows indicated that Zn and Bet had a positive effect on lipid profile by decreasing the levels of total cholesterol of cows in our study.

The results of the TAC of cows are mentioned in Table (4). TAC concentrations in Bet and Zn was increased ( $p < 0.05$ ) significantly by 20% and 25%, respectively compared with a control group (Table 4), indicating of Bet and Zn supplementation reduced the severity HS in treated cows. These results are in line with those reported by Kassab et al. (2020) who found an enhancement in glutathione peroxidase and TAC after inclusion of Zn in cows diet. In addition, Soliman *et al.* (2023) revealed that, bet supplementation improved TAC in supplemented groups as compared to control which might be due to fact that Bet enhances antioxidant activity and protect the body defense system against excessively produced free radical and stabilize health status of the animal. Low TAC values would suggest either a deficiency in nutrients, especially antioxidants, and/or an environmental stress and disease challenge that have overwhelmed the cow's readily mobilized antioxidants

#### **Milk production and its constituents:**

In the present study, DMI, milk yield, and milk composition increased with supplementation with Bet and Zn in HS conditions. Compared to Zn and control groups, Bet had significantly increased ( $P < 0.05$ ) milk yield, FCM, SNF, and milk protein. Shah *et al.* (2020) found that, DMI, yield and composition of milk increased with Bet supplementation (15 g/d) under HS conditions. In addition, Ghoneem and El-Tanany (2023) reported that Bet supplementation increased milk yield and milk fat in lactating goats under thermoneutral conditions.

Increased milk yield in cows treated with Bet may be due to improve the nutritional digestibility as reported by Ghoneem and El-Tanany (2023). This may be due to the fact that Bet is methyl donor and osmolyte, which reduces HS, maintains rumen pH, and also improves the microbial community in rumen (Eklund *et al.*, 2006). Peterson *et al.* (2012) demonstrated that Bet is metabolized in rumen and converted into acetate, which may play an important role in fat synthesis.

#### **Reproductive trials:**

Supplementation of Bet and Zn ameliorated ( $P < 0.05$ ) the reproductive performance of cows by increased first service of conception rate, CR and reduced the services per conception during HS conditions. Those results are in line with Patel *et al.* (2017) who found that Zn supplementation reduced the number of services to pregnancy and enhanced

the CR in cows. Soliman *et al.* (2023) added that the supplementation of Bet to Aberdeen Angus cattle reduced the number of services per pregnancy and increased the CR under HS conditions. The present results are in parallel with Raheja *et al.* (2018) who reported that Bet supplementation results in a significant reduction in services per conception and significant ( $P < 0.05$ ) increase in CR cows during hot humid season. Our results regarding the effect of Bet supplementation in reducing the number of inseminations that required to pregnancy may be associated good uterine health in treated cows. Furthermore, improved the reproductive measurements in cows received Bet and Zn in the diets may be due increasing TAC by 20% and 25%, respectively compared with a control (Table 4) and elevating E2 and P4 assay by 31% in both cows treated with Bet and Zn supplementation.

## **CONCLUSION**

The present study highlight the important of supplementation of zinc and betaine in the ration of native cows for improved productive, reproductive performance, milk production as well as its component and also helped in maintaining the physiological responses of cows during HS conditions. Further research will be needed with a large sample size to precisely validate the role of dietary Bet and Zn in improved the reproductive performance of dairy cattle.

#### **Ethical statement:**

All the experimental procedures and the study protocol have been approved by the research ethical committee of Arish University, Egypt. Ethical Committee protocol number: (ARU/Agri.20).

#### **Availability of data:**

All data generated or analyzed during this study are included in this published article.

#### **Conflict of interest:**

The authors declare no competing interests.

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### تأثير اضافة البيتاين والزنك على إنتاج اللبن ومكوناته والتنظيم الحراري وخصائص الدم والأداء التناسلي للأبقار في المناطق شبه الاستوائية القاحلة

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اجريت هذه الدراسة بهدف تقييم استخدام البيتاين والزنك على إنتاج اللبن ومكوناته وخصائص الدم والكفاءة التناسلية للأبقار تحت ظروف الاجهاد الحراري. تم تقسيم ١٨ بقرة الي ثلاث مجموعات عشوائيا تحتوي كل مجموعة على ٦ حيوانات. المجموعة الاولى للمقارنة (ضابطة) والمجموعة الثانية قدم لها كبريتات الزنك بمعدل (٢٠٠ ملجم / راس / يوم) مع العليقة والمجموعة الثالثة قدم لها البيتاين بمعدل (٥٠ جم / راس / يوم) مع العليقة.

تم قياس معدل التنفس، ومعدل النبض، ودرجة حرارة الجسم وجمع عينات الدم خلال التجربة. تم تسجيل إنتاج اللبن وتحليل مكوناته أسبوعيا. تم تسجيل عدد التلقيحات لكل حمل (NCR) ومعدل الحمل (CR). أظهرت النتائج أن مجموعات البيتاين والزنك أدت إلى انخفاض درجات حرارة الجسم ومعدل النبض والتنفس بينما ارتفع تركيز الهيموجلوبين (HB) بشكل ملحوظ في الأبقار المعاملة، في حين ارتفع تركيز الهيماتوكريت في مجموعة الزنك. أدت اضافة البيتاين والزنك الي حدوث زيادة معنوية في البروتينات الكلية (TP) والجلوبيولين، في حين إنخفض تركيز انزيمات الكبد ALT وAST في مجموعتي الزنك والبيتاين. ارتفع تركيز مضادات الاكسدة الكلية في مجموعتي الزنك والبيتاين مقارنة بالمجموعة الضابطة. أظهرت الدراسة زيادة معنوية في معدلات الحمل ومستوي هرمون الاستروجين والبروجسترون في الأبقار المعاملة بالبيتاين والزنك مقارنة مع بالمجموعة الضابطة. وقد استنتج أن اضافة الزنك والبيتاين في علائق الأبقار يؤدي إلى تحسن كبير في إنتاج اللبن والأداء التناسلي تحت ظروف الاجهاد الحراري.