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Impact of Betaine Supplementation as Anti Stress on Some Hematological Parameters and Thermoregulatory Responses of Aberdeen Angus Cows in Arid Subtropical Regions

Kassab A.Y. [1], H. A., Hamdon[1], H. A. Daghash[2], and A.S.H. Soliman[1*]

[1] Department of Animal Production, Faculty of Agriculture, Univ. of New Valley, Egypt.
[2] Department of Animal Production, Faculty of Agriculture, Univ. of Assiut, Egypt.

* Corresponding author

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Abstract

This study aimed to investigate the effects of betaine supplementation on some hematological and physiological parameters of Aberdeen Angus cows. Twelve cows were divided randomly into two equal groups (6 cows each) and each group was treated for 150 successive days. All experimental animals were fed 60% of their requirements as a concentrate mixture and the rest of other requirements was covered from wheat strew *ad-libitum*. In addition to basal diet, animals in experimental group were supplemented with 30 g betaine hydrochloride per day. Air temperature and relative humidity were recorded during the experimental days to calculate the current temperature–humidity index (THI). Blood samples were collected during experimental period from jugular vein. Rectal temperature (RT), pulse rate (PR), respiration rate (RR), skin temperature (ST) and hair temperature (HT) were recorded during the experimental days. The obtained results showed that the average values of THI were between 71.6 to 74.78 at 08:00 am and 77.09 to 83.01 at 02:00 pm during the experimental period which indicates exposure of animals to heat stress. There were no significant differences among groups in WBC, HCT, MCV, MCH, MCHC and PLT. Moreover, RBC and HGB was higher ($P < 0.05$) in betaine group than control. adding betaine had no significant decrease in RR, ST, HT, and ET. While significant decrease in RT and PR was observed at 02:00 pm of experimental animal. It is concluded that betaine may be improved some hematological parameters and thermoregulatory responses of Aberdeen Angus cows under New Valley arid areas.

Keywords: Betaine; hematological parameters; Thermoregulation; Aberdeen Angus.

Introduction

According to World Meteorological Organization, World Health Organization and the United Nations Environmental Program, global warming would be a higher frequency and longest duration of exposure to higher temperatures, especially during the summer (Habeeb et al., 2018). New Valley governorate in Upper Egypt in western desert between 25º; 42& 30º; 47 E longitude, 22º 30& 29º 30N latitude and lies 77.8 m altitude above the sea level. The climate of this area is arid and dry, essentially that of the desert. Rainfall is almost negligible and the ambient temperature ranges from 46°C during summer days to 8 °C in the chilly winter nights (Kassab and Mohammed, 2014; Nasreldin *et al.,* 2020). Under subtropical countries, climatic characteristic is the major constraint on animal production. Growth, reproductive performance, and milk production are impaired as a result of the drastic physiological changes caused by thermal stress. The summer in New Valley is characterized by high ambient temperature, low relative humidity, and intense solar radiation. Accordingly, farm animals are exposed to severe climatic stress for about 6 months of the year.

Betaine is a modified amino acid consisting of glycine with three methyl groups that serves as a methyl donor in several metabolic pathways (NCBI, 2021). Betaine, also known trimethyl glycine is a recent compound and has been found to alleviate thermal stress in cows (Raheja et al.,2018; Lakhani et al., 2020; Shah et al., 2020), buffalo (Sachin et al., 2019; Shankhpal et al., 2019), goat (Fernandez et al., 2009; Dangi et al., 2016), sheep (DiGiacomo et al., 2016; Cai et al., 2021), and rabbit (Elsawy et al., 2017). Furthermore, betaine serves as a good antioxidant and found to be implecated in handicap of oxidative stress produced during several stress conditions (Alirezaei et al., 2014: Wen et al., 2020). Betaine gradually decreased heat shock protein expression in goat during thermal stress acclimation (Dangi et al., 2016). The objective of this study was to elucidate the effects of dietary betaine supplementation on some hematological parameters and thermoregulatory responses of Aberdeen-Angus cows under hot climatic conditions in New Valley governorate.

Materials and Methods: This experiment was carried out at the animal production experimental farm, Faculty of Agriculture, New Valley university from May tell September 2019.

Experimental design: A total number of 12 healthy Aberdeen Angus cows about 5-6 years of age and an average body weight of 460-520 kg were used in study. The animals were divided randomly into two equal groups (6 animals each). The first group was fed on the basal diet consists of 60% concentrate mixture and the rest was covered from wheat straw and served as a control. The treatment group received the same basal diet with supplemented betaine hydrochloride at rate 30 g/ day. All calves were fed 60% of their nutrient requirements as a concentrate mixture based on NRC (2000) guidelines, while wheat straw was given ad lib to cover the rest of the requirements. Ingredients of concentrate feed mixture presented in Table (1). The chemical composition of concentrate feed mixture and wheat straw are shown in Table (2).

Meteorological measures: Air temperature and humidity were recorded during experimental days using (Temperature /Humidity Temperature thermometer) at 2 pm and 10 pm. Temperature Humidity Index (THI) was calculated according to Mader et al. (2006) as following THI = $(0.8 \times Ta) + [(RH/100) \times (Ta - 14.4)] + 46.4$ Where; Ta °C is the ambient temperature (°C), and RH is the relative humidity (RH %) /100.

Blood hematological parameters: About 2 ml blood was collected from each cow in clean and dry ependroph tube containing anticoagulant (EDTA) for the hematological studies through jugular venipuncture in the morning (before watering and feeding) at day 0, 30, 60, 90, 120 and 150 of the experimental periods. Anti-coagulated blood was analyzed shortly for the number of white blood cell (WBC) count, lymphocytes (LYM), neutrophils (NEUTR), monocytes (MID), red blood cell (RBC) count, hemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelets (PLT), were calculated by an automatic hematology cell counter (Dirui Bcc-3600).

Thermoregulatory responses: Respiration rate (RR) was determined per 15 days by counting the flank movements for a minute (breath / min). Pulse rate (PR) was determined per 15 days. The pulse rate and respiration rate were counted before measuring the body temperature. Rectal temperature (RT, ^{o}C) was measured using a clinical thermometer inserted gently into the rectum for one minute. Skin temperature (ST, ^{o}C), hair temperature (HT, ^{o}C) and ear temperature (ET, ^{o}C) were measured using portable infrared thermometer produced by RadioShack company designed for temperature measurements.

Statistical Analysis: The data were analyzed using a completely randomized design with the GLM procedure of the statistical program SAS/STAT 9.1 (SAS, 2004). The differences among treatments were tested using Duncan's Multiple Range Test (Duncan, 1955).

The model used was $Yij = U + A_j + E_{ij}$

Y_{ij} = Observation traits, U = Overall mean, A_j = Experimental treatment and E_{ij} = Random error

Results

Meteorological measures: The average values of THI were between 70.08 to 74.78 at 08:00 am and 75.33 to 83.01 at 02:00 pm during the experimental period (Figure 1). The present data indicated that animals were under heat stress according to Du Preez, (1990).

Blood hematological parameters: The blood indices of HGB concentration, RBCs count, HCT, MCV, MCH, MCHC values, WBC, and platelets count are shown in Table (3). adding betaine did not significantly affect the HCT, MCV, MCH, MCHC values, WBC, LYM, NEUTR, MID and platelets of Aberdeen-Angus cows throughout the experimental period. However, dietary betaine significantly increased ($P<0.05$) the HGB concentration and RBCs count.

Thermoregulatory responses: Data in Table (4) showed that at 08:00 am betaine supplementation led to decrease rectal temperature, skin temperature, hear temperature, ear temperature, respiration rate and pulse rate, but the differences were not significant. While caused a significant decrease ($P<0.05$) in rectal temperature and pulse rate at 02:00 pm in the treated animal. These results demonstrate that betaine supplementation alleviated the thermoregulatory responses to heat stress in Aberdeen Angus cows.

Discussion

Meteorological measures: Temperature humidity index (THI) values of less than 70 is normal, while the THI value of 70 to 78 is alarming, a THI values of 78 to 82 is considered as dangerous and THI values of 82 or above is considered as emergency (Du Preez et al., 1990). The THI values in our study agree with Kassab et al. (2017) who reported that the THI values from May to September in New Valley recorded between 75.39 to 82.12, these results indicating that animals under heat stress. Likewise, the average values of THI were between 79.43 to 85.46 at 02:00 pm and 69.04 to 74.14 at 10:00 pm in the new valley governorate during summer season indicating that animals under severe stress zone at 2:00 pm and heat stress at 10:00 pm (Kassab et al., 2020). The present data indicated that animals were under heat stress according to (Du Preez et al., 1990).

Blood hematological parameters: Haematological parameters are important for understanding the interaction between blood properties and the environment effects from both a physiological and an environmental standpoint (Ovuru and Ekweozor, 2004) Erythrocytes (RBCs) serve as a carrier of HGB. that reacts with oxygen carried in the blood to form oxyhaemoglobin during respiration (Chineke et al., 2006). Also, RBCs are involved in transportation of carbon dioxide and oxygen in the body (Isaac et al.,2013). In our results, dietary betaine significantly increased ($P<0.05$) the HGB concentration and RBCs count in both cows and bulls, Similarly, Abd-Elsattar (2018) found that betaine increased ($P<0.05$) blood HGB and RBCs in growing lambs. Moreover, betaine supplementation had a significant ($P<0.05$) effect on RBCs count in Saidi lambs (Hussein et al., 2021).

The results of our study indicated that dietary betaine did not effect on WBCs count of cows. Also, the percentage of blood LYM, NEUTR and MID in treated group was in normal rang. The improvement of the hematological picture with betaine supplement will reflected on the health status of animals and better nutrient utilization.

Thermoregulatory responses: The positive effect of supplement betaine to cows on thermal stress was clear at 2.00 pm when the temperature was increased as compared with at 8.00 am, which lowering the values of rectal temperature and pulse rate. Hence, we accepted the hypothesis that betaine can reverse some of the adverse effects of thermal stress on productive performance and improve some physiological responses to thermal stress in farm animal. Dunshea et al. (2013) stated that the prediction of heat stress through web-based tools or meteorological bureau data will greatly increase the ability to use dietary manipulations to ameliorate the effects of thermal stress. The general homeostatic responses to thermal stress in mammals included elevated respiration rate and heart rate, reduced feed intake, profuse sweating, panting, and drooling of saliva (Silanikove, 1992). The results of our study in in rectal temperature and pulse rate were agreement with reported by Shankhpal et al. (2018) who reported that supplemental betaine (30 g/day) significantly decreased the rectal temperature and pulse rate at 14:00 hr. as compared to control group in crossbred cows. Similar decrease in rectal temperature and pulse rate by betaine supplementation was reported by Shankhpal et al. (2019) in buffaloes and DiGiacomo et al. (2016) in sheep. In addition, animals in the control group were unable to dissipate heat efficiently due to the high THI, result an increase in RT. Although the mechanism by which betaine lowered RT in the treated animals was not investigated in this study, it may be through antioxidant mechanism involving the enhancement of synthesis of glutathione (Newsholme et al., 2003 and Hsu et al., 2012), a very potent endogenous antioxidant molecule. Interestingly, the heart rate was significantly reduced by the betaine supplementation, which may be explained by the beneficial effects of betaine on the cardiac autonomic nervous system. Moreover, supplement of betaine to cows slightly decreased respiration rate, skin temperature and ear temperature which participate on decreasing the severity of thermal stress.

Conclusions: In conclusion, dietary betaine supplementation may be improved some hematological parameters and thermoregulatory responses of Aberdeen Angus cows. In addition, ameliorate the harmful effects of thermal stress.

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Conflicts of Interest/ Competing interest: The authors declare that they have no competing interests.

Ethical statement

All the experimental procedures and the study protocol have been approved by the National Animal Care and Use Committee, and the experiments were performed in accordance with the internationally accepted standard ethical guidelines for animal use and care.

Table 1: Ingredients of concentrate feed mixture.

Items	%
Yellow Corn%	55
Wheat bran%	21.5
Soyabean meal%	20
Limestone %	1.5
Dicalcium phosphate%	0.5
Yeast %	0.2
Bicarbonate %	0.3
Sodium chloride %	1

Table 2: Chemical composition of concentrate mixture and wheat straw (on DM basis).

Item	DM	OM	CP	CF	Fat	Ash	NFE
Concentrate mixture	88.76	93.79	15.76	14.12	2.39	6.21	61.52
Wheat straw	90.35	89.05	1.79	38.71	1.12	10.95	47.43

DM: Dry Matter, CP: Crude Protein, CF: Crude Fiber, NFE: Nitrogen Free Extract

Table 3: Effects of betaine supplementation on hematological parameters.

Parameter	Control Means+ *SEM*	Betaine Means+ *SEM*	Sign
HGB (g/dl)	10.44 ± 0.17^{b}	11.87 ± 0.20^{a}	*
RBC (10^6/µl)	6.35 ± 0.15^{b}	7.04 ± 0.13^{a}	*
HCT %	32.11±1.91	30.61±0.90	NS
MCV (f l)	47.44±1.05	46.49±1.66	NS
MCH (p g)	17.13±0.45	16.91±0.60	NS
MCHC (g/dl)	36.07±0.65	35.33±0.72	NS
WBC (10^3/µl)	8.93±0.29	9.01±0.27	NS
LYM (%)	66.24±0.94	64.09±0.73	NS
NEUTR (%)	23.52±0.81	25.01±0.67	NS
MID (%)	10.24±0.22	10.90±0.20	NS
PLT	256.60±6.00	248.73±6.09	NS

a-b The means placed at the rows with different superscript letters are significantly different (P > 0.05); SEM, standard error of the means. RBC, red blood cell; HGB, hemoglobin concentration; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; HCT, hematocrit; MCV, mean corpuscular volume; WBC, white blood cell; LYM, Lymphocytes; NEUTR, Neutrophils; MID, Monocytes; PLT, platelets.

Table 4: Effects of betaine supplementation on thermoregulatory responses.

Time	Parameter	Control Means+ *SEM*	Betaine Means+ *SEM*	Sign
08: 00 AM	RT (ºC)	38.67±0.04	38.62±0.03	NS
	ST (ºC)	33.07±0.14	33.09±0.16	NS
	HT (ºC)	32.19±0.14	32.22±0.14	NS
	ET (ºC)	35.38±0.11	35.30±0.11	NS
	RR (breath/min)	36.51±0.47	36.49±0.041	NS
	PR (beat/min.)	59.80±0.71	60.84±0.75	NS
02: 00 PM	RT (ºC)	38.86±0.05[a]	38.72±0.05[b]	*
	ST (ºC)	35.77±0.20	35.75±0.17	NS
	HT (ºC)	34.90±0.19	34.94±0.17	NS
	ET (ºC)	36.47±0.17	36.28±0.16	NS
	RR (breath/min)	36.27±0.45	35.98±0.40	NS
	PR (beat/min.)	62.98±0.78[a]	61.36±0.70[b]	*

a-b The means placed at the rows with different superscript letters are significantly different (P > 0.05); SEM, standard error of the means. RT, Rectal temperature; RR, Respiration rate; PR, Pulse rate; ST, Skin temperature; HT, Hair temperature; ET, Ear temperature

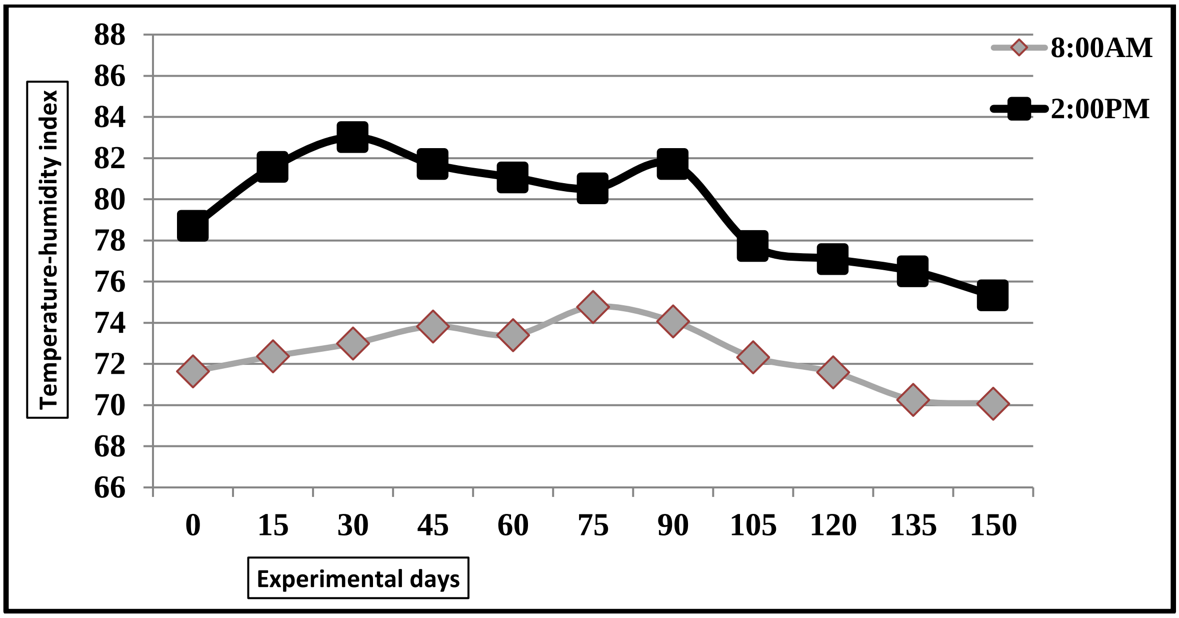

Figure 1: Temperature humidity index (THI) during the experimental period.

Abbreviations
ET, Ear temperature
HCT, hematocrit
HGB, hemoglobin concentration
HT, Hair temperature
LYM, Lymphocytes
MCH, mean corpuscular hemoglobin
MCHC, mean corpuscular hemoglobin concentration
MCV, mean corpuscular volume
MID, Monocytes
NEUTR, Neutrophils
PLT, platelets.
PR, Pulse rate
RBC, red blood cell
RR, Respiration rate
RT, Rectal temperature
ST, Skin temperature
THI, Temperature humidity index
WBC, white blood cell

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