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ASSESSMENT OF BETAINE AS A FEED ADDITIVE TO IMPROVE THE METABOLIC STATUS AND REPRODUCTIVE PERFORMANCE OF ABERDEEN ANGUS COWS IN ARID SUBTROPICAL REGIONS

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SUMMARY

This work studied the effect of betaine (BET) supplementation on the metabolic, hormonal and reproductive efficiency of Aberdeen Angus cattle under heat stress (HS) conditions. Twelve cows were assigned randomly into two equal groups (6 cows each). Treatments included: control group (CG) and BET group supplemented with 30 g BET/h/d. Total antioxidant capacity (TAC) and biochemical parameters were measured monthly for 150 days from the start of treatment till pregnancy occurs. The results showed a significant increase in albumin, globulin, T3, and T4 in the BET group compared to the CG. Whereas the concentrations of ALT, AST, and cortisol decreased (P<0.05) significantly in BET group than CG. TAC was significantly greater (P<0.05) in BET group than CG. The elevated conception rate was recorded for BET-treated cows (100%) compared with (83.33%) extent of CG. The number of services required per conception was lower in BET-treated cows (1.17) compared with CG (1.67). The presented results concluded that BET could enhance metabolic, antioxidant status and reproductive efficiency of Aberdeen Angus cows during HS conditions of summer season.

Keywords: Betaine; Aberdeen Angus; Reproductive performance; Antioxidant

INTRODUCTION

When cattle in New Valley are exposed to HS, for instance under summer heatwaves, it can incur unfavorable effect on animal welfare and efficiency. New Valley is a desert governorate located in the western Egypt between 25º; 42& 30º; 47 E longitude, 22º 30& 29º 30N latitude. This area is dry and arid. The ambient temperature (AT) ranges between 43-48°C in summer to 8°C during winter (Soliman *et al.*, 2022). Regardless of the adaptation shown by the native breeds, with the beginning of summer, a decline in production is observed, leading to a shortage of fresh milk and necessitating the import of powdered milk, which requires huge foreign exchange.

According to estimates, 5-20% and 10–40% declines in feed consumption and production of dairy cattle, respectively, are attributable to HS conditions (Babinszky *et al.*, 2011). Additionally, HS is the major factor contributing to poor fertility by causes decreased estrus expression, decreased fertilization rate, and increased embryonic mortality in cows during HS which leads to an economic deficit in dairy production (Kassab *et al.*, 2020). HS can adversely affect pregnancy rates in cows inseminated during HS (Turk *et al.*, 2015).

Betaine, tri-methylglycine is a recent compound found to ameliorate HS in cows (Kassab *et al.*, 2021;Williams *et al.*, 2021, and Kondiba *et al.*, 2023), buffalo (Shankhpal *et al.*, 2019; Deshpande *et al.*, 2020; Shakkarpude *et al.*, 2022, and Hussain *et al.*, 2023), goat (Ghoneem and El-Tanany., 2023), sheep (Tsiplakou1 *et al.*,2017, and DiGiacomo *et al.*, 2023), and in poultry (Al Sulaiman *et al.*, 2023, and Al-Garadi *et al.*, 2023).BET has several activities that may reduce the impact of HS on cattle and improve its performance. Initially, BET is a modified amino acid composed of glycine with three methyl groups that acts as a methyl donor in many metabolic pathways (NCBI, 2021). According to Wen *et al* (2020), BET was found to be involved in inhibiting oxidative stress generated during several stressors. Furthermore, it has been found to mitigate the harmful effects of HS in livestock and poultry, increasing milk yield, carcass quality, and lean meat production (Singh *et al.*, 2022).

We hypothesize that BET supplementation will reduce the effect of HS on animal reproduction and improve cellular thermo-tolerance by increasing heat shock protein. Furthermore, this improvement in thermo-tolerance would result in greater production of cows. Thus, the study aims to investigate the impact of BET on blood metabolites, hormonal profile, and reproductive efficiency in Aberdeen Angus cows.

MATERIALS AND METHODS

The study was performed from May to September 2019 at an animal production experimental farm, Faculty of Agriculture, New Valley University. The ambient temperature (AT,°C), relative humidity (RH%) and THI during the experimental period were shown in Table (1).

Table 1. Means of AT,°C, RH% and THI during the experimental period

day	Time of day					
	08:00 AM			02:00 PM		
	AT, °C	RH, %	THI	AT, °C	RH, %	THI
0	28.4	18	71.64	40.2	15	78.71
15	29.3	17	72.37	43.8	12	81.56
30	30.1	16	72.99	45.6	13	83.01
45	31.5	13	73.82	44	10	81.70
60	30.2	18	73.40	43.2	10	81.06
75	31.6	18	74.78	42.5	12	80.52
90	31.4	15	74.07	44	11	81.71
105	29.1	18	72.33	39	13	77.73
120	28.5	17	71.6	38.2	13	77.09
135	27.4	17	70.53	38.1	13	77.01
150	27.2	18	70.46	37.8	14	76.78

$THI = (0.8 \times Ta) + [(RH/100) \times (Ta - 14.4)] + 46.4$ (**Mader *et al.*,2006**).

Experimental design:

Twelve multiparous non-milking Aberdeen Angus cows, with an average body weight (BW) of 480 ± 10.45 kg, and their age ranging from 5 to 6 years, were distributed into two identical groups (6 animals each) according to their BW. Cows were randomly given one of the experimental rations. All cows were fed 60% of their nutrient requirements as a Concentrate Feed Mixture (CFM) based on NRC (2000) guidelines, while wheat straw (WS) was given *ad lib* to cover the rest of the requirements. The contents of CFM were 55% yellow corn, 27% wheat bran, 15 % soybean meal, 2% limestone and 1% NaCl. The chemical composition of CFM and WS are presented in Table (2). Cows in CG were fed their rations without BET. While BET group were supplemented with 30 g BET /head/day. The betaine additive was mixed with CFM daily before feeding.

Table 2. Chemical Composition of Concentrate Feed Mixture (CFM) and Wheat Straw

Item	% On dry matter basis						
	DM	OM	CP	CF	Fat	Ash	NFE
CFM	**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, OM: Organic Matter, CP:Crude Protein, CF: Crude Fiber, NFE: Nitrogen Free Extract

Blood sampling and analytical methods:

Blood samples were drawn monthly at 08:00 am via jugular vein in to clean tubes. Blood sera were separated by centrifugation at 3500 rpm for 15 min., and thensera were frozen at -18 °C for further analysis.

Serum analysis was done using jenway spectrophotometer. Serum albumin was estimated by Doumas *et al.* (1971). Total serum protein (TP)and creatinine concentrations were measured according to Tietz (2006). Urea, AST, ALT, glucose, and cholesterol were measured according to Reed (2013). TAC was analyzed calorimetrically by jenway spectrophotometer, by Bio-diagnostic kits. Direct radioimmunoassay (RIA) technique was performed for determination of thyroid hormones. The concentrations of thyroxine (T4, µg/dl)and triiodothyronine (T3, ng/ml) were measured according to Barker and Silverto (1982). Cortisol concentration (ng/ml) was measured by ELISA method as described by Munro and Lasley (1988).

Estrus synchronization and Reproduction parameters:

All the cows received the double intramuscular injection by synthetic prostaglandin analogue PG named Estrumate® (0.263 mg Cloprostenol Sodium/ ml estrumate.) Two doses 2 ml of PG, the first given on day 0 and the second administered at12 days later.

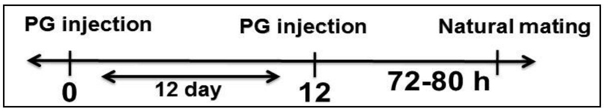

Figure 1. Estrous synchronization using prostaglandin.

Cows shown to be in estrus were mated with a healthy bull at the favorable time (12 hours after signs of estrus) for two consecutive cycles until pregnancy occurred. The reproductive indicators studied during this experiment were percentage of estrus, first service conception rate, number of services per conception (NSC), and conception rate (CR).

Statistical analysis:

Data were statistically analyzed by one- way ANOVA using software **(**SAS, 2009). The following statistical model was used: $Y_{ij}=\mu+R_i+e_{ij}$ where: μ is the overall mean of Y_{ij}; R_i is the effect of treatment; e_{ij} is the experimental error

Percentage of estrus, first service conception rate, number of services per conception, and conception rate were analysed by chi-square test.

Duncan's new multiple range test (Duncan, 1955) was used to calculate differences between means.

RESULTS AND DISCUSSION

Metabolic and biochemical parameters:

Table (4) showed that the addition of BET led to a significant decrease (P < 0.05) in the concentration of globulin, cholesterol, AST, and ALT and a significant increase (P < 0.05) in the concentration of albumin and TAC. However, there was no effect of BET supplementation on total protein, glucose, and urea-N and creatinine concentrations in Aberdeen Angus cows. In agreement with the present results, no effect was observed in TP and glucose concentrations (Ghoneem and El-Tanany, 2023), urea-N (Parker *et al.*, 2007) and creatinine (Fernández *et al.*, 2009).

Table 4. Effects of betaine supplementation on biochmical parameters

Parameter	Experimental groups		P-values
	Control	Betaine	
Total protein (g/l)	7.50±0.07	7.44±0.06	0.373
Albumin (g/dl)	3.10^{b}±0.09	3.23^{a}±0.06	0.017
Globulin (g/dl)	4.40^{a}±0.15	4.22^{b}±0.11	0.041
Glucose (mg/dl)	56.53±1.41	57.73±0.83	0.494
Total Cholesterol (mg/dl)	173.82^{a}±2.34	157.93^{b}±2.22	0.001
AST (U/l)	47.27^{a}±2.57	40.53^{b}±2.04	0.009
ALT (U/l)	17.87^{a}±0.40	16.07^{b}±0.43	0.011
Urea-N (mg/dl)	24.77±1.41	24.55±1.24	0.252
Creatinine (mg/dl)	1.78±0.07	1.80±0.10	0.367
TAC (mmol/L)	0.65^{b}±0.02	0.87^{a}±0.02	0.001

^{a,b} : values with different superscripts within the same row are significantly different at (P<0.05). AST, aspartate aminotransferase; ALT, alanine aminotransferase TAC, total antioxidant capacity

The decrease in serum cholesterol concentrations may be explained by increased lipid mobilization which may confirm the role of BET as a methyl donor (Davidson *et al.*, 2008). Ghoneim and Tanani (2023) found that adding 4 g BET/kg DM to goat diets resulted in a decrease in cholesterol concentration. In the other trend, BET supplementation resulted in a significant (P < 0.05) reduction in AST concentrations in cows. The reduction in liver enzymes (ALT and AST) might be due to the lipotropic effects of BET, which in turn may decrease the liver fat content, indicating better liver function (Xu and Feng, 1998).

Endogenous free radical formation in cows increases under HS conditions and reduces the animal's antioxidant activity. The TAC of BET-supplemented cows were higher (P < 0.05) than that of CG, indicating that BET enhances antioxidant activity and reduces homocysteine to prevent oxidative stress.

Moreover, an *in vitro* study suggested the action of BET in glutathione synthesis, which may have a helpful impact on the antioxidant activity of animals (Zhang *et al.*, 2016). Current blood metabolites indicated no negative impact on cow health, as blood parameter values were within normal limits.

Hormonal profile:

Results in Table (5) cleared that BET supplementation caused a significant increase (P < 0.05) in T3 and T4 compared to CG. Indicating improvement in the metabolic and nutritional status in treated cows. While cortisol levels in cows decreased significantly due to BET supplementation. Since cortisol is a good indicator of stress, BET supplementation (30 g/day) might be beneficial for alleviating oxidative stress and HS in farm animals.

Table 5. Effects of betaine supplementation on hormonal profile

Parameter	Experimental groups		P-values
	Control	Betaine	
T3 (µg/dl)	1.74^{b}±0.01	1.98^{a}±0.03	0.001
T4 (µg/dl)	4.20^{b}±0.03	4.66^{a}±0.07	0.001
Cortisol (ng/dl)	15.65^{a}±0.11	15.04^{b}±0.13	0.001

^{a,b} : values with different superscripts within the same row are significantly different at (P<0.001).

In this study, serum T3 and T4 concentrations were increased in cows treated with BET, but the mechanism underlying the effect of BET in modulating the concentration of thyroid hormones is required to be determined. The glycine binding site is located in the hypothalamic N-methyl-D-aspartate (NMDA) receptor (Johnson and Ascher, 1987), It is generally believed that glycine is required for NMDA receptor activation (Kleckner and Dingledine, 1988). BET is glycine analogue; A possible illustration may lie in the fact that BET is also a co-agonist of the NMDA receptor and may stimulate the secretion of hypothalamic thyrotropin-releasing hormone (TRH) by NMDA receptor activating, thereby increasing the of TSH in the anterior pituitary gland. This led to an increase in the levels of T3 and T4 in the blood.

Cortisol *(ng/ml)* concentrations was significantly (P<0.05) lower in BET group. These results agreed with those of Raheja *et al* (2019), who found that concentrations of cortisol were significantly lower (P<0.05) in BET treated-cows (4.91±0.68 ng/ml) as compared to CG (6.89±0.49 ng/ml) in Karan Fries cows during HS. Similarly, Shakkarpude *et al* (2022) noted that cortisol levels of lactating buffalo during HS was significantly reduced in BET groups compared to CG. This indicates that in the BET-supplemented group, the stress level was significantly lower in comparison with CG that lacking the supplement.

Reproductive performance:

The results regarding the effect of BET supplementation on reproductive efficiency in Aberdeen Angus cattle are presented in Table (6). Results demonstrated that cows showed no difference in their estrus synchronization responses due to the treatments. As all cows taken in each treatment were cyclic, all the cows showed normal estrus so all animals in these groups were naturally inseminated at the appropriate time for estrus observed according to signs of estrus.

Table 6. Effect of betaine on reproductive performance of Aberdeen Angus cows

	Experimental groups	
Items	**Control**	**Betaine**
Number of animals	6	6
Percentage of estrus %	100	100
First service conception rate %	50 (3/6) [b]	83.33 (5/6) [a]
Second service conception rate %	33.33 (2/6) [b]	16.67 (1/1) [a]
Conception rate %	83.33 (5/6) [b]	100(6/6) [a]
Number of services per conception	1.67 (10/6) [a]	1.17(7/6) [b]

[a,b] : values with different superscripts within the same row are significantly different at (P<0.05).

The pregnancy rate at first service and CR were lower in cows of CG as compared to BET supplemented cows. These results agree with Raheja *et al.* (2018) who noted that the first service conception rate in BET group was higher than CG. Likewise, the first service conception rate was significantly (P<0.05) higher in Aberdeen Angus cows treated with antioxidant when compared to CG under New Valley hot conditions (Kassab *et al.*, 2020).BET supplementation during HS of summer season is an effective strategy to enhancing the reproductive efficiency and antioxidant activity of Aberdeen Angus cows under HS conditions. In a retrospective study, Amundson *et al.* (2006) observed a significant decrease in the CR in cows during HS (62%) when the THI values were higher than 72. Moreover, Nanas *et al.* (2021) observed high embryo mortality rate during HS, which infer the devastating effects of HS on cows' fertility. Cows supplemented with BET showed a higher CR which may be due to the dilution properties of BET and thus improved CR during the adverse effect of HS.

The NSC was lower in the BET group (1.17) than control (1.67). Likewise, A reduction in NSC was observed in BET group (1.2 ± 0.1) as compared to CG (1.8 ± 0.1) under HS (Fedota *et al.*, 2017) Also, Raheja *et al.* (2018) found a lower NSC in BET group (2.00 ± 0.24) as compared with control (2.89 ± 0.39) under HS. The present findings relating to the effect of BET supplementation in lowering the NSC with good uterine health in BET-group and they also confirm the results of Fedota *et al.* (2017) who indicate a positive effect of BET in increasing the CR with minimal number of services.

CONCLUSION

Supplementation of BET had a significant effect on antioxidant capacity and reproductive efficiency during HS of summer season. In conclusion, BET supplementation during HS could be an effective strategy to enhance the reproductive efficiency and antioxidant status without negative impact on the health of Aberdeen Angus cows under HS conditions.

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تقييم البيتايين كإضافة علفية لتحسين التمثيل الغذائى والأداء التناسلي لأبقار الابردين أنجس في المناطق شبه الاستوائية القاحلة

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أجريت هذه الدراسة بمزرعة الإنتاج الحيواني التجريبية بكلية الزراعة جامعة الوادي الجديد بهدف تقييم تأثير أضافة البيتايين على التمثيل الغذائى والاداء التناسلي والمؤشرات البيوكيميائية. قسمت ١٢ بقرة عشوائيا إلي مجموعتين متساويتين (٦ بقرات لكل منها). المجموعة الأولي للمقارنة (كنترول)، المجموعة الثانية (BET) قدم لها البيتايين بمعدل (٣٠جم/رأس/يوم) مع العليقة. تم قياس المؤشرات البيوكيميائية وحالة مضادات الأكسدة خلال فترة التجربة.

وقد أظهرت النتائج زيادة معنوية في الألبومين والجلوبيولين وT3 وT4 في مجموعة البيتايين مقارنة بمجموعة CG. في حين إنخفضت تراكيز ALT وAST والكورتيزول بشكل ملحوظ ($P<0.05$) في مجموعة البيتايين مقارنة بالكنترول. بالاضافة الي ذلك كان تركيز مضادات الأكسدة الكلية (TAC, mmol/l) أعلى معنويا ($P <0.05$) في المجموعه المعاملة بالبيتايين مقارنة بالمجموعة الكنترول. كما ارتفع معدل الحمل في الأبقار المعالجة بالبيتايين (١٠٠٪) مقارنة بالكنترول (٨٣,٣٣٪). كما إنخفض عدد التلقيحات اللازمة لحدوث الحمل في مجموعة البيتايين (١,١٧) مقارنة بـ (١,٦٧) في المجموعة الكنترول.

بناء على النتائج المتحصل عليها فإن إستخدام البيتايين يمكن أن يؤدي إلي تحسين الاداء التناسلي وصفات الدم البيوكيميائية وحالة مضادات الأكسدة لأبقار الأبردين أنجس تحت ظروف الاجهاد الحراري خلال موسم الصيف في المناطق شبة الاستوائية.